Intersociety Meeting: Comparative Approaches to Grand Challenges in Physiology

Meeting Program & Abstracts

Town and Country Resort and Convention Center
San Diego, California • October 5-8, 2014

View a meeting preview video at http://bit.ly/CEPPreview

the-aps.org/comparative
2014 APS Intersociety Meeting
Comparative Approaches to Grand Challenges in Physiology

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Holly A. Shiel
Univ. of Manchester, UK

Jonathon H. Stillman
Univ. of California, Berkeley

Tobias Wang
Aarhus Univ., Denmark

Cassondra L. Williams
Univ. of California, Irvine

Acknowledgements

The Meeting Organizers and The American Physiological Society gratefully recognize the generous financial support from the following:

National Science Foundation
The Society for Integrative and Comparative Biology
Society for Experimental Biology
Comparative Biochemistry and Physiology Journal
Australian & New Zealand Society for Comparative Physiology and Biochemistry
Exercise Medicine and Sport Sciences Initiative, University of California, Irvine
International Society for Neuroethology
Loligo Systems
Canadian Society of Zoologists
The Crustacean Society
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<tr>
<td>8:00—10:00 AM</td>
<td><strong>Concurrent Symposia</strong></td>
<td>**Physiological Adaptations to **</td>
<td><strong>Overcoming a Major</strong></td>
<td><strong>Molecular and Physiological</strong></td>
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<td>Extremes: Providing Novel</td>
<td>Physiological Barrier: Adaptation</td>
<td>Features of Animal Diapause</td>
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<td>Animal Models for</td>
<td>from Saline to Freshwater Habitats</td>
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<td>Investigating Health and Disease</td>
<td>G. Charmantier and C. Lee</td>
<td>New Perspectives on the</td>
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<td>J.W. Hicks and T. Wang</td>
<td>Challenges from the Very</td>
<td>Ecology and Evolution of</td>
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<td>Genomics in Integrative and</td>
<td>Beginning: Developmental</td>
<td>Homeostasis <strong>L. B. Martin and H. A.</strong></td>
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<td>Comparative Physiology</td>
<td>Physiology, Epigenetics, and Critical Windows J. Eme and C. A. Mueller</td>
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<td>D. Crawford</td>
<td><strong>Comparative Gastrointestinal</strong></td>
<td>Linking Behavior and <strong>J. Smolka and B. el Jundi</strong></td>
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<td>Frontiers in Insect</td>
<td>Physiology: From Genes to Animal</td>
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<td>Homeostasis-Advantages and</td>
<td>Performance D. P. German</td>
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<td><strong>Concurrent Symposia</strong></td>
<td><strong>Cardiorespiratory Physiology</strong></td>
<td><strong>Responses to Global Change:</strong></td>
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<td>of Vertebrate Extremophiles</td>
<td>Acclimatize, Adapt or Die</td>
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<td>G. R. Scott</td>
<td>G. Hofmann, M. Kelly, and T. G. Evans</td>
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<td>Diverse Approaches in</td>
<td><strong>Evolutionary and</strong></td>
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<td>Evolutionary Physiology</td>
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<td>T. Garland, Jr.</td>
<td>T. Owerkowicz and E. Dzialowski</td>
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<td>Recent Ideas and</td>
<td>Determinants of Skeletal</td>
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<td>Technological Advances in</td>
<td>Muscle Diversity M. Azizi and L. P.</td>
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<td>Comparative Epithelial Physiology</td>
<td>Hernandez</td>
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<td>12:30—3:30 PM</td>
<td><strong>Poster Presentations</strong></td>
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<td>3:30—5:30 PM</td>
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<td>4:50—5:00 PM</td>
<td><strong>Welcome B. Rees</strong></td>
<td>5:45—7:30 PM <strong>Workshop 1</strong></td>
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<td>7:00—10:00 PM <strong>Awards Banquet</strong></td>
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<td>5:00—6:00 PM</td>
<td><strong>Plenary Lecture:</strong> Participant:</td>
<td><strong>P. Schulte</strong></td>
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<td>6:00—8:00 PM</td>
<td><strong>Opening Reception</strong></td>
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**Location:**
The 2014 APS Intersociety Meeting: Comparative Approaches to Grand Challenges in Physiology will be held October 5—8, 2014 at the Town & Country Resort and Conference Center, 500 Hotel Circle North, San Diego, CA 92108, telephone (619) 291-7131, FAX: (619) 294-4681.

**Onsite Registration Hours:**
- Sunday, October 5 ……………… 2:00—8:30 PM
- Monday, October 6 ………… 7:00 AM—6:00 PM
- Tuesday, October 7 ……. 7:30 AM—6:00 PM
- Wednesday, October 8 ……… 7:30 AM—5:00 PM

**On-Site Registration Fees:**
- APS Member .......................................................... $450
- Guest Society Member .......................................... $450
- APS Retired Member ............................................ $300
- Guest Society Member .......................................... $300
- Nonmember ............................................................ $550
- Postdoctoral ............................................................ $350
- Student .................................................................... $250

The registration fee includes entry into all scientific sessions, opening reception, and award banquet dinner*.

*Must get ticket for entry.

**Payment Information:**
Registrants may pay by institutional or personal check, traveler’s check, MasterCard, VISA or American Express or in United States Dollars. Checks must be payable to “The American Physiological Society” and drawn on a United States bank payable in US dollars.

**Student Registration:**
Any student member or regularly matriculated student working toward a degree in one of the biomedical sciences is eligible to register at the student fee. Nonmember postdoctoral fellows, hospital residents and interns, and laboratory technicians do not qualify as students. Nonmember students who register onsite must provide a valid university student ID card. APS student members should present their current APS membership card indicating their student category status.

**Postdoctoral Registration:**
Any person who has received a Ph.D. degree in physiology or related field, within four years of this meeting, as attested to by the department head is eligible to register at the postdoctoral fee. A statement signed by the department head must accompany the registration form and remittance when registering.

**Press:**
Press badges will be issued at the APS registration desk, only to members of the working press and freelance writers bearing a letter of assignment from an editor. Representatives of allied fields (public relations, public affairs, etc.) must register as nonmembers.

**Program Objective:**
Comparative physiology takes advantage of the diverse evolutionary histories and ecological settings of animals. By definition, comparative physiology is broad, spanning a variety of animal taxa occurring in diverse environmental settings, and studied at many levels of biological organization (from molecular physiology to physiological ecology). This breadth allows comparative physiology to (a) understand basic physiological processes and (b) identify novel mechanisms used by animals to solve specific physiological challenges. This meeting will draw comparative and evolutionary physiologists from around the world to present and discuss recent advances in animal physiology. The three and a half day meeting will feature fifteen symposia, two plenary lectures, two workshops, and multiple sessions for contributed abstracts as oral or poster presentations. This meeting will include the 2014 Scholander Award competition for young comparative physiologists, plus other trainee awards and activities.

**Target Audience:**
The intended audience for this meeting includes all professionals involved in teaching, research, and clinical fields related to comparative and evolutionary biology.

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**Photography is not permitted during the meeting sessions or in the poster room**

**Don’t forget to join us at the Welcome Reception directly following the Opening Plenary Lecture**

Golden Foyer
6:00—8:00 PM
MONDAY, OCTOBER 6, 2014

Concurrent Symposia I

2.0 PHYSIOLOGICAL ADAPTATIONS TO EXTREMES: PROVIDING NOVEL ANIMAL MODELS FOR INVESTIGATING HEALTH AND DISEASE

Mon., 8:00—10:00 AM, Pacific Ballroom.

Chairs: James W. Hicks, Univ. of California, Irvine.
Tobias Wang, Aarhus Univ., Denmark.

8:00 AM 2.1 Is Physiology Redundant? Why Rodent Models in Biomedical Research are Failing. Michael J. Joyner. Mayo Clinic.

8:30 AM 2.2 Mammalian Hibernation as a Model of Disuse Osteoporosis: The Effects of Physical Inactivity on Bone Metabolism, Structure, and Strength. Seth W. Donahue. Colorado State Univ.

9:00 AM 2.3 Extreme Phenotypic Plasticity: How the Burmese Python Provides Clues to a Healthy Heart. Leslie A. Leinwand. Univ. of Colorado, Boulder.

9:30 AM 2.4 Hypoxia Tolerance in the Vertebrate Brain: Insights from Comparative Physiology. Philip Bickler. Univ. of California, San Francisco.

Concurrent Symposia II

3.0 GENOMICS IN INTEGRATIVE AND COMPARATIVE PHYSIOLOGY

Mon., 8:00—10:00 AM, Pacific Salon 4/5.

Chair: Douglas L. Crawford, Univ. of Miami.

8:00 AM 3.1 Evolutionary Genetics of Energetics: Effects of Mitochondrial-nuclear Interactions on Metabolism and Genome Evolution. Kristi Montooth. Univ. of Nebraska, Lincoln.


9:00 AM 3.3 Comparative Physiological Genomics of Salinity Tolerance. Andrew Whitehead. Univ. of California, Davis.

9:30 AM 3.4 Transcriptomics as a Tool of Functional Genomics: Possibilities and Limitations with Hypoxia Response of Fish as a Case Study. Mikko Nikinmaa and Jenni Proikia. Univ. of Turku, Finland.

Join us at the Welcome Reception following the Opening Plenary Lecture

Concurrent Symposia III

4.0 FRONTIERS IN INSECT HOMEOSTASIS-ADVANTAGES AND EXPLOITATION

Mon., 10:30 AM—12:30 PM, Pacific Ballroom.

Chairs: Julian A. T. Dow, Univ. of Glasgow, UK.
Michael F. Romero, Mayo Clinic.

10:30 AM 4.1 GPCR’s Role in Regulation of Diuresis or Water Movement in Insects. Patricia V. Pietranzio. Texas A&M Univ.

11:00 AM 4.2 PAI-1, A Natural Peptide Insecticidal Agent Against Vacuolar H+-ATPases. Marius Hsu. Univ. of Oxonhbalck, Germany.


12:00 Noon 4.4 Exploiting the Renal Homeostatic Mechanisms of Mosquitoes for Novel Vector Control. Peter Piermarini. Ohio State Univ.

Concurrent Symposia IV

5.0 CARDIORESPIRATORY PHYSIOLOGY OF VERTEBRATE EXTREMOPHILES

Mon., 10:30 AM—12:30 PM, Pacific Ballroom.

Chair: Graham R. Scott. McMaster Univ., Hamilton, Canada.


11:00 AM 5.2 Metabolic Strategies for Surviving the Winter in Hibernating Mammals. Brian Barnes. Univ. of Alaska, Fairbanks.

11:30 AM 5.3 Cardiorespiratory Performance in Anoxia Tolerant Fish and Reptiles. Goran E. Nilsson. Univ. of Oslo, Norway.


Concurrent Symposia V

6.0 DIVERSE APPROACHES IN EVOLUTIONARY PHYSIOLOGY

Mon., 10:30 AM—12:30 PM, Pacific Salon 4/5.

Chair: Theodore Garland, Jr. Univ. of California, Riverside.


11:00 AM 6.2 Physiology and Genomics of the Evolution of Life Histories and Senescence in Garter Snakes. Anne Bronikowski. Iowa State Univ.


12:00 Noon 6.4 Molecular Evolution of Hormones and their Receptors. Joe Thornton. Univ. of Chicago.
DAILY SCHEDULE

Concurrent Symposia VI

**7.0 RECENT IDEAS AND TECHNOLOGICAL ADVANCES IN COMPARATIVE EPITHELIAL PHYSIOLOGY**
Mon., 10:30 AM—12:30 PM, Pacific Salon 6/7.

Chairs:
- Martin Tresguerres, Scripps Inst. of Oceanography, Univ. of California, San Diego.
- Greg G. Goss, Univ. of Alberta, Canada.

10:30 AM
- 7.1 Rainbow Trout Use Acid Sensing Ion Channels (ASICS) for Na⁺ Uptake in Dilute Freshwater.
  Greg G. Goss, Univ. of Alberta, Canada.

11:00 AM
- 7.2 Fish Intestinl HCO₃⁻ Secretion: From Molecules to the Oceanic Inorganic Carbon Cycle. Martin Grosell, RSMS, Univ. of Miami.

11:30 AM

12:00 Noon
- 7.4 Evolutionary Conserved Mechanisms for Acid-base Sensing. Martin Tresguerres, Scripps Inst. of Oceanography, Univ. of California, San Diego.

Poster Presentation

**8.0 METABOLISM, ENERGETICS, AND NUTRITION**
Mon., 12:30—3:30 PM, Golden Ballroom.

*Denotes presenter is competing in the Best Poster Competition.*

Poster Board

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<td>1</td>
<td>Investigation of Algal Feeding Preferences of the Solar-powered Sea Slug, <em>Elysia chlorce</em></td>
<td>M. Arnette*, A. Shelton, and J. Genz</td>
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<td>Physiological Responses to Environmental Stress in a Mollusc: Why is Being a Hybrid an Advantage?</td>
<td>K. Alter*, A. Morash, S. Andrewsith, and N. Elliott</td>
<td>Univ. of North Carolina at Charlotte.</td>
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<td>7</td>
<td>The Effect of Humidity on the Metabolic Rate of <em>Gromphadorhina portentosa</em>.</td>
<td>C. Toogood, and H. L. Contreras</td>
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<td>The Effect of Diet and Intestinal Microbiome Manipulation on Intestinal Cellular Pathways.</td>
<td>C. Bucking, C. LeMoine, P. Craig, T. Moon, and A. Poulain</td>
<td>York Univ., Toronto, and Univ. of Ottawa, Canada.</td>
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<td>Fasting-induced Morphological Reorganization of the Colon May Not Drive Concomitant Changes in the Microbiome.</td>
<td>C. Passemant, K. Kohl, D. Meyerholz, and M. McCue</td>
<td>St. Mary's Univ., Univ. of Utah, and Univ. of Iowa.</td>
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<td>Glucose Can Fuel Metabolism in RBCs from Normoglycemic But Not Hypoglycemic Fish.</td>
<td>W. Driedzic, K. Chow, and C. Short</td>
<td>Memorial Univ. of Newfoundland, Canada.</td>
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<td>16</td>
<td>Mechanisms and Costs of Mitochondrial Thermal Acclimation in the Common Killfish <em>Fundulus heteroclitus</em>.</td>
<td>D. Chung*, and P. Schulte</td>
<td>Univ. of British Columbia, Vancouver, Canada.</td>
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<td>Paradoxical Anaerobism in Desert Pupfish.</td>
<td>F. van Breukelen, and S. Hillyard</td>
<td>Univ. of Nevada, Las Vegas.</td>
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<td>The Effects of Temperature on the Metabolic Fate of Lactate During Recovery from Anoxia in the Painted Turtle.</td>
<td>C. Hill, and D. Warren</td>
<td>St. Louis Univ.</td>
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27 8.27 Withdrawn.

28 8.28 Heart Rate Dynamics in a Marsupial Hibernator. S. Swoap, G. Kortert, and F. Geiser, Williams Coll., Williamstown, MA, and Univ. of New England.

29 8.29 Apoptotic Regulation During Mammalian Hibernation. M. Tret*, and F. van Breukelen. Univ. of Nevada, Las Vegas.


32 8.32 Thyroid Gland Remains Responsive to Thyroid Stimulating Hormone with Sensitivity Increasing with Fasting Duration in a Prolonged Fasted Mammal. B. Martinez*, D. Somo, D. Ensingnier, H. Peck, D. Lee, D. Crocker, and R. Ortiz. Univ. of California, Merced, Sonoma State Univ., and Rohnert Park, CA.

33 8.33 Purine Nucleoside Phosphorylase Activity in Erythrocytes from Bottlenose Dolphins (Tursiops truncatus) in Response to Breath-hold Diving and Exercise. L. Del Castillo Velasco Martinez, R. I. Lopez-Cruz, C. J. Hernandez-Camacho, L. C. Mendez-Rodriguez, and T. Zenteno-Savin. CIBNOR, Baja California Sur, Mexico, and IPN-CICMAR, Baja California Sur, Mexico.


36 8.36 Lactating Mice Increase Villus Surface Area Through Increased Epithelial Cell Width in Response to Low Protein Diet. K. Short, M. Cook, and E. Derrickson. Loyola Univ., Baltimore.


9.0 FIELD PHYSIOLOGY
Mon., 12:30—3:30 PM, Golden Ballroom.


41 9.3 Persistent Tissue Differences in Fatty Acid Profiles of Weddell Seals (Leptonychotes weddellii) Reflect Tissue Roles. L. Pearson*, D. Costa, and J. Burns. Univ. of Alaska, Fairbanks, Univ. of California, Santa Cruz, and Univ. of Alaska, Anchorage.

10.0 EVOLUTIONARY PHYSIOLOGY
Mon., 12:30—3:30 PM, Golden Ballroom.

42 10.1 Tales from the Precambrian: TNF-induced Apoptosis Remains Unchanged 550 million Years Later. S. Quistad, A. Stotland, K. Barrott, C. Smurthwell, B. Hilton, J. Grasis, R. Wolkowitz, and F. Rohwer. San Diego State Univ. and Univ. of California, San Diego.

43 10.2 Muscular Dystrophy Genes and the Early Metazoan Transition from Dynein- to Myosin-Powered Locomotion. A. Mead, A. Malik, R. Krishnamurthy, and H. Stedman. Univ. of Vermont, and Univ. of Pennsylvania.

44 10.3 Evolution of the Urea Transporter Family in Vertebrates. C. LeMoine, and P. Walsh. Univ. of Ottawa, Canada.


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**DAILY SCHEDULE**

**Poster Board**

50 10.9 Reciprocal Osmotic Challenges Reveal Mechanisms of Divergence in Phenotypic Plasticity in the Killifish Fundulus heteroclitus. R. Brennan, F. Galvez, and A. Whitehead. Univ. of California, Davis, and Louisiana State Univ.


**Poster Presentation**

11.0 HUMAN NUTRITION AND PHYSIOLOGY EDUCATION
Mon., 12:30—3:30 PM, Golden Ballroom.

*Denotes presenter is competing in the Best Poster Competition.

**Oral Abstract Presentations**

12.0 CARDIOVASCULAR AND RESPIRATORY PHYSIOLOGY
Mon., 3:30—5:30 PM, Pacific Ballroom.

**Oral Abstract Presentations**

13.0 EVOLUTIONARY PHYSIOLOGY
Mon., 3:30—5:30 PM, Pacific Salon 4-5.

**Poster Board**


58 11.5 Use of Fellow Examinee as Subject in Observed Structured Practical Examination (OSPE) in 1st Term Physiology Examination. M. U. A. Khan, and D. Hossain. Noakhali Med. Coll., Bangladesh.


**Oral Abstract Presentations**

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Mon., 3:30—5:30 PM, Pacific Ballroom.

**Oral Abstract Presentations**

13.0 EVOLUTIONARY PHYSIOLOGY
Mon., 3:30—5:30 PM, Pacific Salon 4-5.

**Poster Board**


58 11.5 Use of Fellow Examinee as Subject in Observed Structured Practical Examination (OSPE) in 1st Term Physiology Examination. M. U. A. Khan, and D. Hossain. Noakhali Med. Coll., Bangladesh.


5:00 PM  13.7  Does MMR Regulate of Life-history Traits? Correlated Changes in Mice Selected for Mass-independent MMR.  *Cynthia Downes.* Univ. of Nevada, Reno.


**Oral Abstract Presentations**

**14.0 METABOLISM, ENERGETICS, AND PERFORMANCE**

Mon., 3:30—5:30 PM, Pacific Salon 6/7.

Chairs:  
Jonathon Stillman, *Univ. of California, Berkeley.*

3:30 PM  14.1  Ontogenetic and Interspecific Metabolic Scaling in Insects.  *James Maino.* Univ. of Melbourne, Australia.


4:00 PM  14.3  Diet Changes in Coral Metabolism: Potential Regulation by Phosphorylation.  *Lauren Linsmayer.* Univ. of California, San Diego.

4:15 PM  14.4  Specific Dynamic Action in Decapod Crustaceans.  *Iain McGaw.* Memorial Univ. of Newfoundland, Canada.


5:00 PM  14.6  Temperature and Acidification Variability Reduce Physiological Performance in the Intertidal Zone Porcelain Crab *Porcelain cassiputata.*  *Adam Paganini.* San Francisco State Univ.


5:30 PM  14.8  Validation of the Relationship Between 3-dimensional Body Acceleration and Oxygen Consumption in Trained Steller Sea Lions (*Eumetopias jubatus*) Diving with Increased Oxygen Depletion.  *Beth L. Volpov.* Deakin Univ, Burwood, Australia.

**Oral Abstract Presentations**

**15.0 OSMOTIC AND ION REGULATION: JUNCTIONS AND TRANSPORTERS**

Mon., 3:30—5:30 PM, Royal Palms Salon 1/2.

Chair:  
*David Goldstein,* Wright State Univ.


3:45 PM  15.2  Tricellular Tight Junction Proteins and Their Contribution to Paracellular Occlusion in the Fish Gill Epithelium.  *Dennis Kolosov.* York Univ., Toronto, Canada.

4:00 PM  15.3  Abundance and Localization of Branchial Claudins in Rainbow Trout (*Oncorhynchus mykiss*) and Implications in Hypoosmoregulation.  *Joanna Bujak.* Univ. of Arkansas.

4:15 PM  15.4  Hypotonicity Stimulates K+ Flux Through the WNK-SPAK/OSR1 Kinase Cascade and the Ncc69 Sodium-Potassium-2-Chloride Cotransporter in the *Drosophila* Renal Tubule.  *Aylin Rodan.* Univ. of Texas, Southwestern.


4:45 PM  15.6  Sequencing and Gene Expression of Aquaporin-9 in Freeze Tolerant Cope’s Gray Tadpoles.  *Brian Stogsdill.* Wright State Univ.

5:00 PM  15.7  Gene Expression of Three Isoforms of Urea Transporter in an Aestivating African Lungfish.  *Biyun Chung.* Natl. Univ. of Singapore.


**Workshop I**

**16.0 TRAINEE WORKSHOP: NON-TRADITIONAL CAREER PATHS FOR COMPARATIVE PHYSIOLOGISTS**

Mon., 5:45—7:30 PM, Pacific Ballroom.

Chairs:  
*Cassondra Williams,* Univ. of California, Irvine.  
*Bernard Rees,* Univ. of New Orleans.


6:45 PM  16.4  The Undergraduate University Path.  *Scott Kirkton.* Union Coll.

DAILY SCHEDULE


9:30 AM 17.4 Evolutionary Transition to Freshwater in the Shrimp Macrobrachium amazonicum: Ecophysiological Adaptations. Guy Charmantier. Univ. of Montpellier 2, France.

Concurrent Symposia VIII

18.0 CHALLENGES FROM THE VERY BEGINNING: DEVELOPMENTAL PHYSIOLOGY, EPIGENETICS, AND CRITICAL WINDOWS
Tues., 8:00—10:00 AM, Pacific Salon 6/7.

Sponsored by the Comparative Biochemistry and Physiology Journal.

Chairs: John Eme, McMaster Univ., Hamilton, Canada.
Casey A. Mueller, McMaster Univ., Hamilton, Canada.


8:30 AM 18.2 Noisy Embryos? The Potential Evolutionary Importance of Variation in the Timing of Developmental Events. Simon Rundle. Univ. of Plymouth, UK.

9:00 AM 18.3 Mitigating the Risks Associated with Accelerated or Deficient Perinatal Growth. Robert D. Roghair. Univ. of Iowa.

9:30 AM 18.4 Epigenetic Influences in Developmental Comparative Anatomy and Physiology. Warren Burggren. Univ. of North Texas.

Concurrent Symposia IX

19.0 COMPARATIVE GASTROINTESTINAL PHYSIOLOGY: FROM GENES TO ANIMAL PERFORMANCE
Tues., 8:00—10:00 AM, Pacific Salon 4/5.

Sponsored by the Society for Integrative Comparative Biology and Comparative Biochemistry and Physiology Journal.

Chair: Donovan P. German, Univ. of California, Irvine.

8:00 AM 19.1 How the Gut Limits Nutrition, and the Influence of this on the Ecology and Evolution of an Insect Herbivore, the Grasshopper. Fiona Clissold. Univ. of Sydney, Australia.

8:30 AM 19.2 Evolutionary and Molecular Mechanisms Underlying Intestinal Flexibility for Snakes. Stephen M. Secor. Univ. of Alabama, Tuscaloosa.

9:00 AM 19.3 The Role of Gut Microflora in the Nutrition of Marine Herbivorous Fishes. Kendall D. Clements. Univ. of Auckland, New Zealand.

9:30 AM 19.4 Amylase Genetics and Biochemistry Underlie a Digestive Specialization in Prickleback Fishes. Donovan P. German. Univ. of California, Irvine.

Photography is not permitted during the meeting sessions or in the poster room.

Concurrent Symposia X

20.0 RESPONSES TO GLOBAL CHANGE: ACCLIMATIZE, ADAPT OR DIE
Tues., 10:30 AM—12:30 PM, Pacific Ballroom.

Sponsored by the Society for Experimental Biology, Division of Comparative Physiology & Biochemistry.

Chairs: Gretchen Hofmann, Univ. of California, Santa Barbara.
Morgan Kelly, Louisiana State Univ.
Tyler G. Evans, California State Univ., East Bay.

10:30 AM 20.1 Trait-based Approaches to Predicting the Responses of Species to Global Change. Sarah Diamond.

11:00 AM 20.2 Ocean Acidification Effects on Temperate Rockfishes. Cheryl Logan. California State Univ., Monterey Bay.


12:00 Noon 20.4 Comparing Physiological Plasticity Vs. Evolutionary Adaptation Vs. Phylogenetic Constraint on Species Distributions; Drosophila and Beyond. Ary Hoffman. Univ. of Melbourne, Australia.

Concurrent Symposia XI

21.0 EVOLUTIONARY AND DEVELOPMENTAL ORIGINS OF ENDOThERMY
Tues., 10:30 AM—12:30 PM, Pacific Salon 6/7.

Sponsored by Australian & New Zealand Society for Comparative Physiology and Biochemistry.

Chairs: Tomasz Overkowicz, California State Univ.
Edward Dzialowski, Univ. of North Texas.
Sarah Diamond.


11:00 AM 21.2 Development of Endothermy in Marsupial and Placentar Mammals. Sarah Andrewartha. Univ. of Tasmania, Australia.

11:30 AM 21.3 Testing Competing Hypotheses of the Evolution of Endothermy. Marek Konarzewski. Univ. of Bialystok, Poland.

12:00 Noon 21.4 Development of Endothermy in Altricial and Precocial Birds. Edward Dzialowski. Univ. of North Texas.

Concurrent Symposia XII

22.0 DETERMINANTS OF SKELETAL MUSCLE DIVERSITY
Tues., 10:30 AM—12:30 PM, Pacific Salon 6/7.

Sponsored by the Comparative Biochemistry and Physiology Journal.

Chairs: Manny Azizi, Univ. of California, Irvine.
L. Patricia Hernandez, George Washington Univ.

10:30 AM 22.1 Evolutionary Selection of Myofibrillar Protein Isoforms for Specific Muscle Function. Peter Reiser. Ohio State Univ.
11:00 AM  22.2 Comparative Physiology of Body Weight-sensitive Skeletal Muscle Plasticity. *Rauf Schilder, Penn State Univ.

11:30 AM  22.3 Cardiac Myosin Alpha and Ventricular Hypertrophy Protect Ground Squirrels in Filibartation. Bryan Rouzie, California State Univ., Long Beach.

12:00 Noon  22.4 The Diversity and Evolution of Locomotor Muscle Properties in Anurans. Henry Astley, Georgia Inst. of Tech.

Poster Presentations

**CARDIOVASCULAR AND RESPIRATORY PHYSIOLOGY**

Tues., 12:30—3:30 PM, Golden Ballroom.

*Denotes presenter is competing in the Best Poster Competition.

**Poster Board 61**

23.1 Do Drosophila Larvae Experience Functional Oxygen Limitation Late in the Instar? *J. Harrison, and V. Callier, Arizona State Univ.


23.7 Acclimation to Overnight Hypoxia and Increased Temperature Improve Aerobic Performance in Salmon (Salmo salar) and Charr (Salvelinus alpinus). K. Antilla, J. Prokolkata, and M. Nikinmaa. Univ. of Turku, Finland.


23.13 Aerial Respiration in Polynectids. C. Jew, and J. Hicks. Univ. of California, Irvine.


23.15 Baroreflex Characteristics of Anuran Amphibians from Different Environments. M. Hedrick, and D. Crossley. California State Univ. East Bay, and Univ. of North Texas.


23.23 Aerobic Physical Training Increases Contractile Response and Reduces the Cardiac Fibrosis in Rats Submitted to Early Estrogen Deprivation. H. C. D. De Souza, A. C. S. Felix, and S. G. V. Dutra. Univ. of São Paulo, Ribeirão Preto, Brazil.

23.24 Function of Saturating Oxygen Delivery during Early Inactive Hibernation in Brown Bears (Ursus arctos). C. J. Hanson, R. C. Yavorsky, J. E. L. Witherington, and J. A. Sikes. Univ. of California, Davis.


23.27 The Effects of Maternal Fat Mass, HFD, and Insulin Resistance on Offspring Development and Cardiac Performance in Adult Male Mice. G. L. Mazzucco, and J. M. Drackova. Univ. of Nebraska, Nebraska Center for the Advancement of Science and Technology.

23.28 Temperature and Oxygen Availability Affect Cardiac Function in Juvenile Pacific Bluefin Tuna (Thunnus orientalis). N. Faure, P. Reis, and M. Saraiva. Univ. of California, Irvine.

23.29 Temperature and Oxygen Availability Affect Cardiac Function in Juvenile Pacific Bluefin Tuna (Thunnus orientalis). N. Faure, P. Reis, and M. Saraiva. Univ. of California, Irvine.
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<tr>
<td>23.26</td>
<td>The Effect of Streptokinase Infusion on Cardiaco Biomarkers &amp;ST Segment of Electrocardiogram Post Myocardial Infarction in Humans.</td>
<td>N. Habibolahy*.</td>
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**DEVELOPMENTAL PHYSIOLOGY**

Poster Board

**24.0**

**Visitors Welcome**

Visit the exhibit booths daily during the poster sessions

**Poster Presentations**

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<tbody>
<tr>
<td>24.2</td>
<td>Exposure to Lowered pH and Acute Thermal Stress Increases Mortality in Embryonic Porcelain Crabs.</td>
<td>E. Armstrong, T. Page, N. Miller, E. Papineau, P. Ca-</td>
<td>losi, and J. Stillman. Univ. of California, Berkeley, San Francisco State Univ., and Plymouth Univ., UK.</td>
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**ENDOCRINOLOGY AND REPRODUCTION**

Poster Board

**25.0**

Visitors Welcome

Visit the exhibit booths daily during the poster sessions

**Poster Presentations**

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<tr>
<td>25.2</td>
<td>Biased Signaling by Two Endogenous GnRH Isoforms Differentially Regulates Total LH and GH A-</td>
<td>sat, and E. Dzialowski.</td>
<td>Univ. of North Texas.</td>
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</table>
**POSTER PRESENTATIONS**

**26.0 THERMAL PHYSIOLOGY**

**Tues., 12:30—3:30 PM, Golden Ballroom.**

- 110 26.4 Variation in Thermal Tolerance, Hypoxia Tolerance and Metabolic Rate in the Atlantic Killifish, Fundulus heteroclitus. T. Healy, P. Schulte, Univ. of British Columbia, Vancouver, Canada.
- 112 26.6 Behavioural Regulation of Water Loss in Four Australian Skinks. E. Pirtle, C. Tracy, M. Kearney, Univ. of Melbourne, Australia, and California State Univ., Fullerton.
- 114 26.8 The Role of Ambient Temperature on Toxin Ingestion by a Mammalian Herbivore. P. Kurnath, M. D. Dearing, Univ. of Utah.
- 115 26.9 From Fur to Blubber: Evolutionary and Ontogenetic Transitions in Mammalian Insulation. H. Liwanag, L. Pearson, N. Guerra, A. Berta, D. Costa, J. Burns, S. Budge, and T. Williams, Adelphi Univ., Garden City, NY, Univ. of Alaska, Fairbanks, San Diego State Univ., Univ. of California, Santa Cruz, Univ. of Alaska, Anchorage, and Dalhousie Univ., Halifax, Canada.

**DAILY SCHEDULE**

**27.0 CONSERVATION PHYSIOLOGY**

**Tues., 3:30—5:30 PM, Pacific Ballroom.**

- 107 27.1 Exploring Local Adaptation to Ocean Acidification in Mytilus californianus During Simulated Upwellings Events. Geoffrey Dilly, Calif. State Univ., Channel Islands, Camarillo, CA.

**3:45 PM**

- 27.2 High Pressure Neurological Syndrome in Shallow-water Marine Invertebrates: Implications on Climate Driven Bathymetric Range Shifts and Acclimatization to Depth. James P. Morris, Univ. of Southampton, UK.

**4:00 PM**

- 27.3 Adaptive Variability in Salinity Tolerance Explains Habitat Variability Between Genetically Distinct Populations of Sacramento Sponial. Christine Verhille, Univ. of California, Davis.

**4:15 PM**

- 27.4 Temperature and Hypoxia Affect Swimming Energetics and Kinematics of Brown Trout (Salmo trutta). Karlina Ozolina, Univ. of Manchester, UK.

**4:30 PM**

- 27.5 Behavioural Responses of Black Perch to Marine Synechococcus cyanobacteria. Trevor Hamilton, MacEwan Univ., Edmonton, Canada.

**4:45 PM**

- 27.6 Brevetoxin Metabolism and Physiology Using Freshwater Turtles as a Model to Measure Morbidity in Endangered Sea Turtles. Sarah Milton, Florida Atlantic Univ.

**5:00 PM**


**5:15 PM**

- 27.8 Physiological and Behavioral Responses to Environmental Challenges in the Western Terrestrial Garter Snake, Thamnophis elegans. E. Gangolf, A. Sparkman, and A. Broniowski, Iowa State Univ., and Westmont Coll., Santa Barbara.

**ORAL ABSTRACT PRESENTATIONS**

**28.0 DEVELOPMENTAL PHYSIOLOGY**

**Tues., 3:30—5:30 PM, Pacific Salon 4/5.**

**Chair:** Dane Crosse, Univ. of North Texas.

**3:30 PM**


**3:45 PM**

- 28.2 Ontogenetic Changes in the Osmotic Stress Response of Blue Mussels. Melissa May, Univ. of Maine, Orono.

**4:00 PM**


**4:15 PM**

- 28.4 Effects of Calcium Availability on Growth and Survival of Aedes aegypti Larvae in Early Life Stages. Janet Genz, Univ. of West Georgia, Carrollton.

**4:30 PM**

- 28.5 Gene Expression Patterns of Alternative Developmental Trajectories in Embryos of an Annual Killifish, Anabas testudineus. Annie Romney, Portland State Univ.

**4:45 PM**

- 28.6 Evidence of Hypoxic Metabolic Programming in Developing Alligator Hearts. Gina Galli, Univ. of Manchester, UK.

**5:00 PM**

- 28.7 Effect of Thyroid Hormone Manipulation on Endothermic Development in Double-Crested Cormorants (Phalacrocorax auritus). Tushar Sirsat, Univ. of North Texas.

**5:15 PM**

- 28.8 Quantification of Left Ventricular Function in Embryonic Chickens (Gallus gallus domesticus) at 70% of...
DAILY SCHEDULE

Oral Abstract Presentations

**29.0** METABOLISM: HYPOXIA AND ANOXIA

**Tues., 3:30—5:30 PM, Pacific Salon 6/7.**

Chairs: Leslie Buck, Univ. of Toronto, Canada.
        Andrea Morash, Univ. of Tasmania, Hobart, Australia.

3:30 PM

29.1  Erythropoietin Through Evolution: A Speculative View. Max Gassmann, Univ. of Zurich, Switzerland.

3:45 PM

29.2  Evolution of Cytochrome Oxidase Subunit 4-2 as a Hypoxia Responsive Gene. Christopher Moyes, Queen's Univ., Kingston, Canada.

4:00 PM

29.3  Cytochrome C Oxidase Oxygen Binding Affinity Varies with Hypoxia Tolerance in Intertidal Fishes. Gigi Lau, Univ. of British Columbia, Vancouver, Canada.

4:15 PM

29.4  Characterizing the Influence of Anoxia Exposure on the Isolated Hagfish Heart. Todd Gillis, Univ. of British Columbia, Canada.

4:30 PM


4:45 PM


5:00 PM

29.7  Is Anoxia and ROS-mediated GABA Receptor Inhibitory Shunting in Turtle Cortical Neurons Mediated by Tonic, Fast or Slow Phasic Currents? Leslie Buck. Univ. of Toronto, Canada.

5:15 PM

29.8  Can Ketone Bodies Protect the Heart Against the Effects of Chronic Hypoxia? Andrea Morash. Univ. of Tasmania, Hobart, Australia.

Oral Abstract Presentations

**30.0** OSMOTIC AND ION REGULATION: SALINITY, OSMOLYTES, AND pH

**Tues., 3:30—5:30 PM, Royal Palms Salon 1-2.**

Chairs: Dietmar Kültz, Univ. of California, Davis.
        Lars Tomanek, California Poly State Univ., San Luis Obispo.

3:30 PM


3:45 PM


4:00 PM

30.3  The Regulation and Function of Polyamines in *Fundulus* Species During Salinity Stress. Fernando Galvez. Louisiana State Univ.

4:15 PM

30.4  Regulation of Organic Osmolyte Concentration in Tissues of Euryhaline Teleosts. Dietmar Kültz. Univ. of California, Davis.

4:30 PM

30.5  Cellular Mechanisms for Acid/Base Sensing and Regulation in Elasmobranch Gills. Jinae N. Roa. Scripps Inst. of Oceanography, La Jolla.

4:45 PM


5:00 PM

30.7  Changes to Intestinal Transport Physiology at Varying Levels of Hypercapnia in the Gulf Toadfish (*Opsanus beta*). Rachael Heuer. RSMAS, Univ. of Miami.

5:15 PM


Workshop II

**31.0** THE CHALLENGE OF TEACHING PHYSIOLOGY IN A CHANGING ENVIRONMENT: INNOVATION AND RESOURCES

**Tues., 5:45—7:30 PM, Pacific Ballroom.**

Chairs: Tom W. Eady, East Tennessee State Univ.
        Karen Sweazea, Arizona State Univ.

5:45 PM

31.1  Vision and Change Update: Progress in Implementing Report Goals in Undergraduate Biology Education. Cynthia Bauerle. HHMI.

6:05 PM

31.2  Teaching and Learning by Inquiry. Barbara Goodman. Univ. of South Dakota.

6:25 PM


6:45 PM


7:05 PM


WEDNESDAY, OCTOBER 8, 2014

Concurrent Symposia XIII

**32.0** MOLECULAR AND PHYSIOLOGICAL FEATURES OF ANIMAL DIAPAUSE

**Wednes., 8:00—10:00 AM, Pacific Ballroom.**

Chair: Steven C. Hand, Louisiana State Univ.

8:00 AM


8:30 AM

32.2  Insulin Signaling as a Key Regulator of Insect Diapause. David Denlinger. Ohio State Univ.

9:00 AM

32.3  The Role of Maternal Provisioning and Micro-RNA Regulation of Diapause in the Annual Killifish *Astronestheria limnaea*. Jason Podrabsky. Portland State Univ.

9:30 AM

32.4  Bioenergetics of Diapause in a Crustacean Extremophile. Steven Hand. Louisiana State Univ.

Photography is not permitted during the meeting sessions or in the poster room.
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<tr>
<th>Concurrent Symposia XIV</th>
<th>33.0</th>
<th>NEW PERSPECTIVES ON THE ECOLOGY AND EVOLUTION OF HOMEOSTASIS</th>
<th>Wednes., 8:00—10:00 AM, Pacific Salon 4/5.</th>
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<tr>
<td><strong>Sponsored by the Society for Integrative Comparative Biology:</strong></td>
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<td><strong>Chairs:</strong></td>
<td>Lynn B. Martin, Univ. of South Florida. H. Art Woods, Univ. of Montana.</td>
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<tr>
<td><strong>8:00 AM</strong></td>
<td>33.1 Integrating Physiological Assessments of Animal Health to Population Models. Erica Crespi, Washington State Univ.</td>
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<td><strong>8:30 AM</strong></td>
<td>33.2 The Reactive Scope Model: Predicting the Effects of Challenges to Homeostasis. Molly Dickens, Univ. of California, Berkeley.</td>
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<td><strong>9:00 AM</strong></td>
<td>33.3 Physiological Regulatory Networks: The Orchestra of Life? Lynn B. Martin, Univ. of South Florida.</td>
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<td><strong>9:30 AM</strong></td>
<td>33.4 Information Theory, Homeostasis, and Evolution. H. Art Woods, Univ. of Montana.</td>
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<tr>
<th>Concurrent Symposia XV</th>
<th>34.0</th>
<th>LINKING BEHAVIOR AND PHYSIOLOGY IN ANIMAL NAVIGATION AND ORIENTATION</th>
<th>Wednes., 8:00—10:00 AM, Pacific Salon 6/7.</th>
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<td><strong>Sponsored by the International Society for Neuroethology</strong></td>
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<tr>
<td><strong>8:00 AM</strong></td>
<td>34.1 Using Genetics to Reveal Migratory Flight Orientation Mechanisms in the Monarch Butterflies. Christine Merlin, Texas A&amp;M Univ.</td>
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<td><strong>8:30 AM</strong></td>
<td>34.2 3D Neural Compass in the Bat Brain. Arseny Finkelstein, Weizmann Inst. of Sci., Rehovot, Israel.</td>
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<td><strong>9:00 AM</strong></td>
<td>34.3 Neural Representation of the Hierarchy of Celestial Cues in the Dung Beetle Brain. Basil el Jundi, Lund Univ., Sweden.</td>
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<td><strong>9:30 AM</strong></td>
<td>34.4 Polarized Light Navigation in Drosophila. Michael Dickinson, Univ. of Washington.</td>
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<tr>
<th>Oral Abstract Presentations</th>
<th>35.0</th>
<th>THERMAL PHYSIOLOGY</th>
<th>Wednes., 10:30—12:30 PM, Pacific Ballroom.</th>
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<tr>
<td><strong>Chair:</strong></td>
<td>Edward Dzialowski, Univ. of North Texas.</td>
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<tr>
<td><strong>10:30 AM</strong></td>
<td>35.1 An in vivo Investigation of Low Temperature Energetics in Drosophila melanogaster Using 31 P NMR. Caroline Williams, Univ. of California, Berkeley.</td>
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<td><strong>10:45 AM</strong></td>
<td>35.2 New Approaches to Understanding Insect Freeze Tolerance. Brent J. Sinclair, Univ. of Western Ontario, London, Canada.</td>
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<td><strong>11:00 AM</strong></td>
<td>35.3 Mitochondrial and Nuclear Genetic Variation Relate to Heat and Cold Tolerance in a Montana Leaf Beetle. Nathan Rank, Sonoma State Univ., Rohnert Park, CA.</td>
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<tr>
<th>DAILY SCHEDULE</th>
<th>35.5</th>
<th>Thermal Performance, Aerobic Scope, and Relevance of the OCLTT Hypothesis. Fredrik Jutfelt, Univ. of Gothenburg, Sweden.</th>
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<tr>
<td><strong>12:00 Noon</strong></td>
<td>35.7</td>
<td>Variation in Transcriptomic Signatures of Thermal Acclimation in Four Key Aquatic Insects in California Riverine Food Webs. Jonathon Stillman, Univ. of California, Berkeley.</td>
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<tr>
<td><strong>12:15 PM</strong></td>
<td>35.8</td>
<td>Seasonally Induced Hepatotranscriptomic Changes in the Freeze Tolerant North American Wood Frog Rana sylvatica. Andor Kiss, Miami Univ.</td>
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<tr>
<th>Oral Abstract Presentations</th>
<th>36.0</th>
<th>ENDOCRINOLOGY AND REPRODUCTIVE PHYSIOLOGY</th>
<th>Wednes., 10:30—12:30 PM, Pacific Salon 4/5.</th>
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<tr>
<td><strong>Chair:</strong></td>
<td>Stephen Trumble, Baylor Univ.</td>
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<tr>
<td><strong>10:30 AM</strong></td>
<td>36.1 Elucidating the Regulatory Mechanisms of Circadian Control of Ovarian Ecdysteroid Production and Release During Egg Development in Adult Female Rhodnius prolixus. Andrea Durant, York Univ., Toronto, Canada.</td>
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<td><strong>10:45 AM</strong></td>
<td>36.2 One Cell or Two? Direct Visualization of Ligand-Receptor Interaction Provides Novel Insights into the Evolution of Insect Renal Function. Kenneth Halberg, Univ. of Glasgow, UK.</td>
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<td><strong>11:00 AM</strong></td>
<td>36.3</td>
<td>Brain Monoamines and Behavior: Relationship to Personality Traits and the Effects of Social Interaction. Svante Winberg, Uppsala Univ., Sweden.</td>
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<td><strong>11:15 AM</strong></td>
<td>36.4</td>
<td>Withdrawn.</td>
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<tr>
<td><strong>11:30 AM</strong></td>
<td>36.5</td>
<td>Corticosterone Responses and the Ability of Birds to Cope with Environmental Change. John F. Cockrem, Massey Univ., Palmerston North, New Zealand.</td>
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<tr>
<td><strong>11:45 AM</strong></td>
<td>36.6</td>
<td>Influence of Corticosterone on Growth, Homecage Activity, Wheel Running, and Maximal Oxygen Consumption in Replicate Lines of House Mice Selectively Bred for High Voluntary Wheel-running Behavior. Jennifer Singleton, Univ. of California, Riverside.</td>
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<tr>
<td><strong>12:00 Noon</strong></td>
<td>36.7</td>
<td>Molecular Resolution of an Acute Stress Response in a Free-ranging Marine Mammal. Jane Khudyakov, Sonoma State Univ., Rohnert Park, CA.</td>
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<td><strong>Chairs:</strong></td>
<td>Marshall McCue, St. Mary's Univ., San Antonio. Allyson Hindle, Massachusetts Genl. Hosp.</td>
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<td><strong>10:30 AM</strong></td>
<td>37.1</td>
<td>Seasonal Metabolism of Brown Adipose Tissue in Hibernating Thirteen-Lined Ground Squirrels. Mallory Ballinger, Univ. of Minnesota, Duluth.</td>
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</table>
10:45 AM  37.2  Is Saponin-permeabilization Appropriate for Characterizing Mitochondrial Metabolism? Assessing Mitochondrial Respiration in Hibernating and Euthemic Ground Squirrels. James Staples, Univ. of Western Ontario, London, Canada.

11:00 AM  37.3  Brown Fat Transcriptome Dynamics: Preservation of Selected mRNAs Across a Torpor Bout Supports Rapid Thermogenesis During Arousal. Sandy Martin, Univ. of Colorado, Denver.

11:15 AM  37.4  Characterizing Cardiac Molecular Mechanisms of Mammalian Hibernation Via Quantitative Proteogenomics. Katie Vermillion, Univ. of Minnesota, Duluth.

11:30 AM  37.5  Do Polyunsaturated Fatty Acids Improve Migratory Flight Performance? Morag Dick, Univ. of Western Ontario, London, Canada.

11:45 AM  37.6  Cold and Exercise Training Produce Similar Increases in Maximal Metabolic Output in House Sparrows. Yufeng Zhang, Univ. of South Dakota.

12:00 Noon  37.7  Hydrogen Isotope (δD) Discrimination in Tilapia. Seth Newsome, Univ. of New Mexico.

12:15 PM  37.8  The Breath Becomes Isotopically Heavier as the Body Burns More Carbohydrates During Intense Exercise: Exploiting the Natural Differences in ¹³C Between Lean and Lipid Tissues. Marshall McCue, St. Mary’s Univ., San Antonio.

Oral Abstract Presentations

38.0  BIOMECHANICS, LOCOMOTION, AND FUNCTIONAL MORPHOLOGY

Chair: Scott Kirkton, Union Coll.


11:00 AM  38.3  Size Matters: The Impact of Body Mass on biochemical and Structural Properties in Harbor Seal Muscles. Jennifer Burns, Univ. of Alaska, Anchorage.

11:15 AM  38.4  Structure and Mechanics of the Cetacean Diaphragm and its Contribution to Thorax Pressurization During a Dive. Margo Lillie, Univ. of British Columbia, Vancouver, Canada.

11:30 AM  38.5  Shrimp Exoskeleton Morphology, Mineralization, and Biophotonics Under Ocean Acidification Conditions. Jennifer Taylor, Univ. of California, San Diego.

11:45 AM  38.6  ExerFlyzer: A High-Throughput System for Inducing and Quantifying Flight Behavior in Drosophila Over Extended Time Periods. Andrew Mead, Univ. of Vermont.

12:00 Noon  38.7  Age Related Changes in Flight Muscle Ultrastructures of the Hawk moth, Manduca sexta: A novel Non-vertebrate Animal Model for Investigating Vertebrate Skeletal Muscle Function, Disease, Degeneration, and Aging. Bernard Wone, Univ. of Nevada, Reno.


Poster Presentations

39.0  OSMOTIC AND IONIC REGULATION

Wednes., 12:30—3:30 PM, Golden Ballroom. *Denotes presenter is competing in the Best Poster Competition.


121  39.3  Extreme Stress Tolerance of the Intertidal Turdigrade Echiniscoides sigermondii. N. Mohbjerg, K. Andersen, T. Hygum, S. Mortensen, K. Halberg, L. Clausen, and A. Jorgensen, Univ. of Copenhagen, Denmark.


123  39.5  Allatostatin A-like Factors in the Aquatic Larvae of Chironomus riparius: Regulation of Hindgut Motility, Ion Reabsorption and Implications for Salinity Exposure. L. Robertson, H. Chasiotis, V. Galporin, and A. Donini, York Univ., Toronto, Canada.


Paracellular Pathway Regulation in Response to Salinity Changes in the Japanese Medaka (\textit{Oryzias latipes}). M. Bomane-Bossus*, S. Madsen, and C. Tipsmark. Univ. of Arkansas, and Univ. of Southern Denmark, Odense, Denmark.  
Expression of Gill Na\textsuperscript{+}K\textsuperscript{+}-ATPase \(\alpha\)-Subunit Isoforms in Euryhaline Japanese Medaka (\textit{Oryzias latipes}) During Salinity Challenges. R. Bollinger*, S. Madsen, M. Bomane-Bossus, and C. Tipsmark. Univ. of Arkansas, and Univ. of Southern Denmark, Odense, Denmark.  
The Effects of Water Ionic Composition on the Rate and Degree of Acid-base Regulation in Rainbow Trout, \textit{O. mykiss}, During Hypercarbia at Rest and Sustained Exercise. K. Tovey, and C. Brauner. Univ. of British Columbia, Vancouver, Canada.  
Gut Carbonate Excretion by Fish Increases Exponentially Within Natural Ocean Salinity Range (25-40 psu). C. E. Stephens*, C. T Perry, and R. Wilson. Univ. of Exeter, UK.  
Cardiac \(pH\) Regulation and Buffering of the Western Painted Turtle (\textit{Chrysemys picta bellii}). M. J. Osendine*, C. Grombka-Murphy, and D. Warren. St. Louis Univ.  
Control of the Osmotic Function of the Avian Lower Gastrointestinal Tract. E. Braun, J. Vranish, and P. Warner. Univ. of Arizona, and Univ. of Nebraska, Omaha.  
THE PHYSIOLOGIST

Poster Presentations

**Consortium Physiology**

Wednesday, 12:30–3:30 PM, Golden Ballroom


41.0 Conservation Physiology

Wednesday, 12:30–3:30 PM, Golden Ballroom

*Denotes presenter is competing in the Best Poster Competition.


41.3 Pace of Life Effects Critical Windows for Disease Emergence and Transmission in Amphibians. R. Warne, Southern Illinois Univ.


41.5 Decoupling the Relationship Between Immune Response and Stress Hormones: An Immunological Profile of the Northern Elephant Seal. H. Peek, and D. Crocker. Sonoma State Univ., Bolzert Park, CA.

41.6 Withdraw.


42.0 Biomechanics, locomotion, and Functional Morphology

Wednesday, 12:30–3:30 PM, Golden Ballroom

*Denotes presenter is competing in the Best Poster Competition.

42.1 The Mechanistic Basis of Unreliable Signals of Strength in Males of the Two-toned Fiddler Crab, *Uca vomerana*. C. Bywater, C. White, F. Seebacher, and R. Wilson. Univ. of Melbourne, Univ. of Queensland, and Univ. of Sydney, Australia.

42.2 The Effects of Selection For Desiccation or Starvation Resistance on Takeoff Flight Performance in *Drosophila melanogaster*. M. L. Brewer, L. M. Peterson, and A. Gibbs. Univ. of Nevada, Las Vegas.

42.3 Material and Structural Characterization of Mineralized Elasmobranch Cartilage: Lessons in Repeated Tiling Patterns in Mechanically Loaded 3D Objects. R. Seidel, D. Kneetel, P. Zaslansky, D. Baun, J. Weaver, and M. Dean. Max Planck Inst., Potsdam, Germany, Zuse Inst., Berlin, Germany; Charité Hosp., Berlin, Germany, and Harvard Univ.

42.4 The Elasmobranch Heart Doesn’t Twist: A Speckle-tracking Echocardiography Study. Y. Hiratsuki, S. Minamisawa, and M. Okabe. The Jikei Univ. Sch. of Med, Tokyo, Japan.


42.7 Temperature Dependence of Muscle Function Alters the Effective Utilization of Tendons. E. Abbott, W. Thomsen, and E. Azizi. Univ. of California, Irvine.

42.8 Three-Dimensional Neuroarchitecture of the Killer Whale (*Orcinus Orca*) from Magnetic Resonance Images. A. Wright, J. St. Leger, M. Scadeng, Univ. of California San Diego, and SeaWorld, San Diego.

42.9 Improving Exercise Adherence and Physical Measures in Hispanic Women. L. Martin, A. C. Perry, B. E. Kahan, J. F. Signorile, S. Ahn, and A. W. Perkins. Univ. of California San Diego, Univ. of Miami, Univ. of Pennsylvania, and Washington State Univ.

Scholander Award Competition

43.0 Scholander Award Finalists

Wednesday, 3:30–5:30 PM, Pacific Ballroom

Chairs: Scott Kirkton, Union Coll.

3:45 PM

43.1 Does Local Adaptation of Exercise Physiology Limit Acclimation Capacity Among Lake Whitefish (*Coregonus clupeaformis*) Ecotypes? *Anne Damazil*. Univ. of Laval, Quebec, Canada.

4:00 PM

43.2 Coral Host Cells Acidify Symbiotic Algal Microenvironment to Promote Photosynthesis. *Katie Barlow*. Scripps Inst of Oceanography, La Jolla.

4:15 PM

43.4 Convergent and Divergent Patterns of Gene Expression in Sculptins that Vary in Hypoxia Tolerance.
DAILY SCHEDULE

4:30 PM 43.5 Ocean Acidification Directly Impairs Olfactory Sensitivity in a Marine Teleost. Cosima Porteous. Univ. of Exeter, UK.

4:45 PM 43.6 What has K⁺ Got to do with it? The Differing Roles of Extracellular K⁺ in Onset and Recovery of Insect Chill Coma. Heath MacMillan. Aarhus Univ., Denmark.

5:00 PM 43.7 Changes in MO₂, Anaerobic Glycolysis and Metabolic Heat with Decreasing Water PO₂ in Goldfish. Matthew Regan. Univ. of British Columbia, Vancouver, Canada.


5:30 PM 43.9 The Role of Transcription Factor Glial Cell Missing 2 (gcm2) in Ca²⁺ Balance in Zebrafish Larvae. Yusuke Kumai. Case Western Res. Univ.

Plenary Lecture II

44.0 PLENARY LECTURE

Wednes., 8:30—9:30 PM, Pacific Ballroom.

Sponsored by the Society for Experimental Biology, Division of Comparative Physiology & Biochemistry.

8:30 PM 44.1 Macrophysiological Forecasting for a Policy in a Changing World. Steven L. Chown. Monash Univ., Australia.

Don’t forget to attend the Closing Banquet and Awards Ceremony Wednesday, October 8 at 7:30 PM

Enjoy a meal with your colleagues, congratulate the Scholander Awardee, and listen to the Plenary Lecture by Steven Chown

Get your free ticket at the registration desk (one per attendee)

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The Crustacean Society

Thank you!
## 2014 APS Intersociety Meeting
### Comparative Approaches to Grand Challenges in Physiology

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The purpose of this presentation is to discuss the limitations of rodent models in biomedical research. Many such models have been bred or engineered to generate genotype-equals-phenotype animals with specific susceptibilities for human clinical conditions like obesity, aging, Alzheimer’s disease and others. Such results are then interpreted as part of a self-fulfilling prophecy indicating that genotype is deterministic for disease phenotype. Interventions that cure such animals are then developed only to fail when translation to humans is attempted. Why? Several possible explanations will be explored:

1. Genetically constrained animal models are also typically exposed to limited environmental and behavioral variability. This is not the case for humans and might explain some of the failure to translate.

2. Contrary to expectation, the Human Genome Project has shown no clear cut pattern of “risky gene variants” for essentially any complex non-communicable diseases in humans. Thus the bred or engineered models are false analogues for human disease.

I close by calling for a “back to the future” approach that includes a wider appreciation of physiological redundancy and also a wider variety of animal models to understand human disease and facilitate translational research.

3.2 MAMMALIAN HIBERNATION AS A MODEL OF DISUSE OSTEOPOROSIS: THE EFFECTS OF PHYSICAL INACTIVITY ON BONE METABOLISM, STRUCTURE, AND STRENGTH
Soth W. Donahue

Physical inactivity leads to increased bone resorption, elevated serum and urinary calcium concentrations, bone loss, bone mechanical property loss, and increased fracture risk in humans and other animals. Grizzly and black bears, yellow-bellied marmots, and 13-lined ground squirrels do not lose bone mass or mechanical properties during prolonged (4-6 months) hibernation. Bone remodeling (i.e., bone resorption and formation) continues during hibernation, although at significantly reduced levels compared to summer levels. Hibernating bears are anuric, yet serum calcium concentration remains at homeostatic levels throughout the entire year. These findings suggest that hibernating bears and rodents have biological mechanisms to preserve bone tissue integrity when challenged with prolonged physical inactivity. Reduced bone remodeling in hibernators likely contributes to the conservation of metabolic energy. Neural signals and circulating factors (e.g., calcium regulatory hormones) likely contribute to the changes at the bone cell level that are involved in bone tissue preservation. Normal balance between bone resorbing osteoclasts and bone forming osteoblasts is likely maintained to preserve normal serum calcium concentrations during anuria. Identification of the molecular mechanisms that regulate bone cell function during hibernation may contribute to the development of new therapies for osteoporosis and inform our understanding of how hibernators have adapted to survive extreme environmental conditions. Funding from NIH (NIAMS AR05420).
intergenic interactions in only some environments weaken the efficacy of selection on this type of genetic variation and may promote the accumulation of mito-
chondrial-nuclear incompatibilities whose fitness effects will depend upon the en-
vironment in which hybrids between closely related species occur. References: Meik-

3.2 POLYMORPHISM IN OXYGEN EXCHANGE CAPACITY AND THE
PHYSIOLOGY OF A MODEL ORGANISM FOR ECOLOGY
James Marden1
1Biology, Penn State Univ., 208 Mueller Lab, University Park, PA, 16802.
Glanville fritillary (Melitaea cinxia) butterflies, a model organism in ecology, have al-
elic variation in a metabolic enzyme (succinate dehydrogenase) that regulates the
hypoxia inducible factor (HIF) pathway. One Sdhd allele is associated with reduced
SDH activity, elaboration of tracheoles in flight muscle, and better flight performance.
Butterflies with less tracheal development have greater post-flight hypoxia signaling,
mitochondrial damage, oxidization of membrane lipids, and aging of metabolic per-
formance. Experimentally elevated sucrose in pupae increased HIF-1α and ex-
pression of genes responsive to HIF activation, including tracheal morphogenesis
genes. In a separate study in Finland, allelic variation in Sdhd and another metabolic
enzyme locus (Pbg) interacted with patch size to explain a large portion of the vari-
ation in year-to-year size of local populations. These results indicate that the hypoxia
inducible pathway, even in lowland populations, can be an important axis for genetic
variation underling infraspecific differences in oxygen delivery, physiological per-
formance, life history and ecology. (NSF EF-0412651, IOS-0959416). References:
Genetic variation in HIF signaling underlies quantitative variation in physiological
and life history traits within lowland butterfly populations. Evolution 67, 1105-1115.
Wheat, CW, Fescemyer, H.W, Kvit, J, Tus, E, Vera, J.C, Frielander, M.J, Hanski, I,
Marden JH (2011) Functional genomics of life history variation in a butterfly meta-
ordinate fondness for metabolic enzymes: why alleles at metabolic enzyme loci are so

3.3 COMPARATIVE PHYSIOLOGICAL GENOMICS OF SALINITY
TOLERANCE
Andrew Whitehead1
1Environmental Toxicology, Univ. of California, Davis, One Shields Ave, Davis,
CA, 95616.
Salinity limits the distributions of aquatic species, and most large clades of fish are
either exclusively marine or freshwater. Yet, euryhaline species harbor the physiologi-
cal plasticity necessary to invade new osmotic niches, and may thereby seed
spatial patterns across osmotic boundaries. Killifish (Fundulus sp.) is a comparative model
system for studying the genomic basis of physiological plasticity in different osmotic
environments. Euryhalinity is the ancestral state within the genus. Several euryhaline
species have derived extreme euryhalinity that has enabled exploitation of the entire
continuum of osmotic niches. In contrast, repeated diversification across osmotic
boundaries is particularly liable within the genus, coinciding with repeated assimil-
ation of physiological plasticity in freshwater species. By comparing gill trans-
criptomic responses to osmotic challenges between populations and species, we are
uncovering some of the molecular mechanisms, and the genomic architecture, that
underpins osmoregulation, and that may represent molecular features that enable
physiological plasticity in general. Regulation of paracellular permeability is a con-
sistent strategy for compensating for osmotic challenge across taxa. Mechanisms as-
sociated with regulation of cell volume, polyamine synthesis, and ion transport, ap-
pear to consistently evolve between populations and species with different osmotic
compensatory abilities. The functional genomic response to osmotic challenge is ca-
nalized in populations that have derived exceptional osmoregulatory abilities, and
these genes appear to have simplified trans-regulatory complexity indicating indica-
tion that may enable evolutionary fine-tuning.

3.4 TRANSCRIPTOMICS AS A TOOL OF FUNCTIONAL GENOMICS:
POSSIBILITIES AND LIMITATIONS WITH HYPOXIA RESPONSE OF
FISH AS A CASE STUDY
Mikko Nikinmaa1, 2 and Jenni Prokkola1
1Dept. of Biology, Univ. of Turku, Natural Sci. Bldg. 1, Turku, 20041, Finland.
Oxygen availability has been an important factor driving the evolution of fish.
Recently, hypoxia has become a major problem in aquatic ecosystems. Hypoxia re-
sponses in animals are often studied with transcriptomic techniques, such as quanti-
tative PCR and microarrays. Their use calls for understanding what can be said about
the observed changes. Naturally, only transcribed genes can be expressed, but the
mRNA levels do not always correlate with changes in protein activity. The corre-
spondence between mRNA and protein levels is protein-specific; e.g., the level of
HIF (hypoxia inducible factor) is mainly post-transcriptionally regulated, and its in-
crease in response to hypoxia can occur without mRNA level changes. Gene ex-
pression in response to hypoxia can also be regulated at its energetically most costly
level, translation, and by rapid mechanisms not involving de novo protein synthesis.
Thus, transcriptional changes indicate the genes that should be studied further to re-
veal the affected functional pathways and the activities of gene products (proteins).
Notably, hypoxia responses may depend on circadian rhythms of animals and can be
affected by environmental pollution via mechanisms not currently well understood.
Understanding these mechanisms requires work both at transcriptional and protein ac-
tivity levels, both of which are needed for full characterization of hypoxia responses
keeping in mind that the transcriptional changes must be related to protein activity changes
to evaluate the functional significance of the response.

4.0: FRONTIERS IN INSECT HOMEOSTASIS-ADVANTAGES
AND EXPLOITATION

4.1 GPCRs ROLE IN REGULATION OF DIURESIS OR WATER
MOVEMENT IN INSECTS
Patricia V. Pietriantonio1
1Entomology, Texas A&M Univ., Olsen Blvd. & Main St., College Station, TX,
77843.
Insect renal organs, the Malpighian tubules, are simple epithelia useful to study
hormonally controlled fluid transport. Renal fluid secretion is achieved in insects by
the establishment of ion gradients that drive fluid towards the M. tubule lumen. This
fluid is emptied into the hindgut for excretion of metabolic wastes, where water may be
reabsorbed or excreted when in excess. Females of the mosquito Aedes aegypti
(L.) feed mainly on human blood, which is hypovosmoretic with respect to the mosquito
hemolymph. Upon blood feeding, diuretic factors are released from the nervous
system into the hemolymph, increasing the rate of secretion in the M. tubules, and al-
lowing females to quickly excrete excess sodium and chloride ions, and water. We
have investigated the G protein-coupled receptors for these diuretic factors in adult
mosquitoes. These GPCRs are, in family A, 1) The insect kinin (leukokinin) receptor,
and in family B, 1) The corticotropin-releasing factor-like (diuretic hormone 44) re-
ceptor and 2) The calcium-like (diuretic hormone 31) receptor. A variety of ap-
proaches, including receptor functional analyses, peptidomimetics testing (SAR),
IHC, RNAi and fluid secretory/excretion measurements in vitro and in vivo, were util-
ized to assess their function. The receptors are expressed in multiple tissues, have un-
usual, cell-specific spatial distribution patterns and, through their orchestrated diuretic
and myotropic actions contribute to the successful arthropod adaptation to blood-
USDA 2008-35302-18820).

4.2 PAIB, A NATURAL PEPTIDIC INSECTICIDAL AGENT AGAINST VA-
CUOLAR H+-ATPASES
Markus Hauss
Abteilung Tierphysiologie, Fachbereich Biologie/Chemie, Univ. Osnabrück,
49069 Osnabrück, Germany.
Vacuolar H+-ATPases (V-ATPases) are constituent enzymes in every eukaryotic cell.
They are heteromultimeric proton pumps, which energize many essential transport
processes across plasma- and endomembranes. In insects they play a key role for the
physiology of the gut and the Malpighian tubules. Interference with the correct
function of the V-ATPase either by mutations or by inhibitors is usually lethal. There-
fore the V-ATPase is an attractive target for the development of insecticides.
Recently, PA1b, a peptide derived from peas (Pisum sativum) was described to be the
first peptidic and insect specific V-ATPase inhibitor. The structure of PA1b exhibits a
unique cysteine knot fold which is also common to toxins from e.g. cone snail, spider
and scorpion. However, its natural origin from a plant being a component of the regu-
lar human diet makes PA1b highly attractive for the exploration of its binding site and
inhibition mechanisms for the further development as a potential bio-insecticide for
pest control. Using a radioactive labeled PA1b in UV-induced cross linking experi-
ments and a second derivative modified with biotin-streptavidin-HRP in single parti-
cular electron microscopy, it was possible to localize the binding site of PA1b at the
interface of subunits c and e. This led to the development of the first idea how PA1b
silences the IF-transport across the membrane. Funding: Deutsche Forschungs-
gemeinschaft (SFB 431, SFB 944).

4.3 REGULATION OF ION TRANSPORT BY TYRAMINE IN THE DRO-
SOPHILA MALPIGHIAN TUBULE: IDENTIFICATION OF MULTIPLE
TYRAMINE RECEPTORS
Edward M. Blumenthal1
1Biol. Sci, Marquette Univ., P.O. Box 1881, Milwaukee, WI, 53201.
The biogenic amine tyramine (TA) is an important signaling molecule in insects, with
roles in reproduction and locomotion. We have previously identified TA as a potent
diuretic agent in the Drosophila Malpighian tubule (MT). Application of nanomolar
TA to isolated MTs causes a rapid depolarization of the transepithelial potential as-
sociated with an increased transepithelial chloride conductance, resulting in diuresis.
4.4 EXPLORING THE RELATING HOMEOSTATIC MECHANISMS OF MOSQUITOES FOR NOVEL VECTOR CONTROL

Peter Pierronami,1 Klaus Beyenbach,1 and Jerod Denton2


The efficacy of insecticides used to control mosquito vectors of disease is eroding due to the emergence of resistant strains. Thus, new chemicals are needed to improve our capabil-
ities for mosquito control. Here we review efforts by our group that aim to disrupt inward-rectifying K+ (KIR) channels expressed in the renal (Malpighian) tubules of mosquitoes; we hypothesized that inhibiting renal Kir channels would disrupt hemo-
lymph K+ homeostasis in mosquitoes with lethal consequences. We describe: 1) the expression, localization, and functional characterization of Kir channels in the Malpighian tubules of adult female mosquitoes (*Aedes aegypti*); 2) the discovery of small molecule inhibitors of mosquito Kir channels; and 3) the disruption of mosquito renal functions by these inhibitors. Our data indicate that the mosquito Malpighian tubules and Kir channels are valuable physiological and molecular targets, respectively, for the development of novel insecticides. Funded by a grant from The Foundation for the NIH, VCTR program.


5.0: CARDIORESPIRATORY PHYSIOLOGY OF VERTEBRATE EXTREMOPHILES

5.1 BREATHE DEEP TO DIVE DEEP: RESPIRATORY VOLUMES IN PENGUINS

Paul Ponganis1

1Dept. of Biology, McMaster Univ., 1280 Main St. W., Hamilton, ON, L8S 4K1, Canada.

The penguin respiratory system constitutes 30-45% of the total body oxygen store. The ratio of air sac volume to lung air capillary volume may also prevent barotrauma to the rigid avian lung, and limit maximum dive depth. In order to assess lung and air sac volumes, computerized tomographic scans and 3D anatomical reconstructions were conducted on anesthetized animals. In Adelie, king, and emperor penguins, respectively, mass specific lung volumes were 25, 19, and 18 ml kg\(^{-1}\), consistent with allometrically predicted values, while maximum air sac volumes (at 30 cm H\(_2\)O inflation pressure) were 296, 357, and 363 ml kg\(^{-1}\) (2.2, 2.9, and 3.0 x allometric predictions, and also greater than end-of-dive total air volumes estimated in free-diving penguins). If emperor penguins inhale to such a large air volume prior to deep dives, the total body oxygen store would be increased from prior estimates by 71% to 136 ml O\(_2\) kg\(^{-1}\) with 61% of all O\(_2\) in the respiratory system. Based upon previous lung morphometry, and the measured lung volumes in emperor penguins, the air sac to lung air capillary volume ratios were 66, 105, and 108, respectively, in the three species. These ratios are equivalent to safe compression to depths of 1600, 2200 and 2300 m, all greater than their maximum recorded depths. Whether penguins can maximally inspire to such a large air sac volume is unknown, but larger than predicted allometrically.

6.0: DIVERSE APPROACHES IN EVOLUTIONARY PHYSIOLOGY

6.1 COMPARATIVE GENETICS AND GENOMICS OF BEHAVIORAL PHENOTYPES IN MICE: LESSONS FOR EVOLUTIONARY PHYSIOLOGY

Scott A. Kelly1

1Dept. of Biology, McMaster Univ., 1280 Main St. W., Hamilton, ON, L8S 4K1, Canada.

Genes, the environment, and gene-by-environment interactions simultaneously influence complex traits, such as behavior. Moreover, underlying the genetic architecture and the environment are individual-specific elements (e.g., photoperiod, diet) that each contributes to the total phenotypic variation. Utilizing emerging and established mouse resources (e.g. Advanced Intercrosses, Collaborative Cross, Diversity Out-
broad) studies have begun to reveal the genetic determinants of behavioral phenotypes with increasingly high precision. Investigations have utilized linkage, genome-wide association, and expression approaches in an attempt to not only identify genetic variants underlying behavior, but also to better understand how these variants may be functioning in physiologically complex systems that are phenotypically plastic. Voluntary physical activity is one example of a behavior where systems approaches have been utilized to simultaneously understand the underlying genetic architecture, physiological and molecular systems, and their interactions. Although investigations into the biological basis of exercise have distinct human health implications, locomotion is also clearly important from an ecological and evolutionary perspective. In general, utilizing laboratory mouse resources to uncover the genomic architecture may lead to a more comprehensive understanding of adaptive behavioral phenotypes, the physiological systems that underlie them, and the potential for both to respond proximately and evolutionarily. References: Kelly S. A., et al. Genetic determinants of voluntary exercise. Trends Genet. 29:348-357, 2013. Logan RW, et al. High precision genetic mapping of behavioral traits in the diversity outbred mouse population. Genes Brain Behav. 12:424-437, 2013. Threadgill DW, et al. Ten years of the collaborative cross. Genetics 190:291-294, 2012.

6.2 PHYSIOLOGY AND GENOMICS OF THE EVOLUTION OF LIFE HISTORIES AND SENESCENCE IN GARTER SNAKES
Anne Bronikowski1, and Tonia Schwartz2
1EEOB, Iowa State Univ., 251 Bessey Hall, Ames, IA, 50011, 2Sch. of Public Hlth., Univ. of Alabama, Ryals Public Hlth. Bldg., 1665 University Blvd., Birmingham, AL, 35294.
For a species to persist in a changing environment, individuals need to respond appropriately to physiological/environmental stress, and the molecular networks underlying these responses need to acclimate or evolve. Evidence from laboratory model systems indicate that molecular networks regulating stress response may often be the networks underlying life-history trade-offs between longevity and growth reproduction. To understand how these molecular networks are functioning in natural populations, we are comparing stress responses between naturally evolved, closely related populations of garter snake ecotypes. These ecotypes include a fast-living eco-type with faster growth, higher reproductive output, and shorter lifespan relative to a slow-living ecotype. In laboratory experiments, we have used heat stress as an activator of metabolic and oxidative stress responses to investigate if these molecular networks have diverged in between the ecotypes. Our results indicate that the ecotypes respond differently to heat stress in their 1) levels of circulating free radicals (superoxide and hydrogen peroxide), 2) production of hydrogen peroxide in the liver mitochondria, 3) amount of DNA damage in blood cells, 4) innate immune function, and 5) liver gene expression. Furthermore, we have identified genetic variants in genes related to oxidative stress that differentially associate with these ecotypes. These results support the hypothesis that closely situated populations with divergent life histories can evolve different responses to physiological stress. (NSF IOS0902228, DEB1011350, IOS1253896, T Schwartz & AM Bronikowski. 2013. Dissecting molecular stress networks: identifying nodes of divergence between life-history phenotypes Mol Ecol DOl10:1.1.1365-294X.2012.05750.s.x)

6.3 CAUSES OF PARALLEL BIOCHEMICAL ADAPTATION: INSIGHTS FROM HEMOGLOBINS OF HIGH-ALTITUDE VERTEBRATES
Jay F. Stoess1, Chandrashekar Natarajan1, Christopher Witt2, Hideaki Moriyama1, Roy Weber1, and Angela Fahey3
1Sch. of Biological Sci., Univ. of Nebraska, Munster Hall, Lincoln, NE, 68588, 2Dept. of Biology, Univ. of New Mexico, MSC03 2020, Albuquerque, NM, 87131, 3Zoophysiology, Aarhus Univ., 8000 Aarhus C, Aarhus, Denmark.
Is it possible to predict which molecular mechanisms are most likely to contribute to biochemical adaptation? Can we predict which mutations – or which types of mutations – are most likely to contribute to adaptive changes in proteins? To address these questions about the inherent predictability of adaptive evolution at the molecular level, we are conducting systematic comparative studies of hemoglobin (Hb) function in high-altitude vertebrates. Specifically, we are conducting phylogenetically replicated comparisons of Hb function between high- and low-altitude species of birds and small mammals. This work combines evolutionary analyses of sequence variation with protein engineering experiments based on site-directed mutagenesis. Results to date suggest several mechanistic explanations for why parallel changes in protein function and/or expression at the molecular level may not involve parallel substitutions at the amino acid level. Grant funding: NIH-HL078216. References: Natarajan, C., et al. (2013). Epistasis among adaptive mutations in deer mouse hemoglobin. Science 340: 1324-1327. Projecto-Garcia, J., et al. (2013). Repeated elevationary transitions in hemoglobin function during the evolution of Andean hummingbirds. Proceedings of the National Academy of Sciences USA 110: 20669-20674. Revsbech, L., et al. (2013). Hemoglobin function and allosteric regulation in semi-ossorial rodents (family Sciuridae) with different altitudinal ranges. Journal of Experimental Biology 216: 4264-4271.

7.0: RECENT IDEAS AND TECHNOLOGICAL ADVANCES IN COMPARATIVE EPITHELIAL PHYSIOLOGY

7.1 RAINBOW TROUT USE ACID SENSING ION CHANNELS (ASICS) FOR NA+ UPTAKE IN DILUTE FRESHWATER
Greg C. Goss
Dept. of Biological Sci., Univ. of Alberta, Edmonton, AB, Canada T6G 5N7, Canada.
The current model for Na+ uptake in rainbow trout gill provides two mechanisms: NH+-ATPase, the proton metabolon and/or epithelial Na+ channel (ENaC) coupled to H+-ATPase. However, while extensive evidence for NH+-mediated Na+ uptake has been gathered, there is no molecular evidence for existence of ENaC. We propose that in rainbow trout the role of epithelial sodium channels is served by acid-sensing ion channels, members of the ENaC/DEG family. We cloned ASIC gene homologues ASIC1 and ASIC4 from the gill and demonstrated their expression in mitochordon-rich cells. Moreover, using immunohistochemistry we co-localized ASIC protein to the MRCs rich in NKA. We also report that in adult rainbow trout, ASIC specific inhibitors decreased Na+ uptake in a dose-dependent manner. Our findings suggest that ASIC channels play a role in Na+ uptake in freshwater fish, and therefore we propose that they provide an alternative mechanism for Na+ uptake to NHs for rainbow trout in very low ion strength waters. Supported by an NSERC Discovery grant to GGG.

7.2 FISH INTESTINAL HCO3− SECRETION: FROM MOLECULES TO THE OCEANIC INORGANIC CARBON CYCLE
Martin Grosell1
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Hyperosmotic absorption by the marine teleost intestinal epithelium is driven by Na+ and Cl− absorption, with ~50% of Cl− absorption being attributable to anion exchange resulting in high luminal concentrations of HCO3−. This luminal HCO3− reacts with Ca2+ to form CaCO3 precipitates, a reaction that reduces luminal osmotic pressure by as much as 100 mOsm. Further reductions in osmotic pressure occur as H+ secreted by distal intestinal segments is titrated with luminal HCO3−, facilitating continued water absorption. Intestinal anion exchange is thus important for solute coupled as well as osmotic fluid absorption and is mediated by the apical a6 member of the SLC26 transporter family, an electrogenic nHCO3/Cl− exchanger. HCO3− for intestinal secretion comes from hydration of endogenous CO2 catalyzed by cytosolic carbonic anhydrase (CAc) and HCO3− imported across the basolateral membrane via the NBC1 member of the SLC4 transporter family, the latter forming the rate limiting step for intestinal HCO3−secretion rates. The formation of CaCO3 precipitates appears to be facilitated by matrix proteins and the release of these CaCO3 precipitates to the environment may be mediated by a diuretic response. The distal intestine responds to peptides of the guanylin family with a transient reversal to a secretory Cl− current mediated by NKCC1 and CFTR, a response which is associated with a secretory water flux. The excretion of CaCO3 precipitates contributes to the morganic oceanic carbon cycle and likely explains observations of increased titratable alkalinity with depth above the argonite lysocline (Ω=1).

7.3 THE NA-K-CL COTRANSPORTER: RECENT ADVANCES IN STRUCTURE, FUNCTION, AND REGULATION
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Na-K-Cl cotransporters (NKCCs) mediate the movement of Na+, K+, and Cl− across the epithelial membrane. NKCC1 is expressed in most vertebrate cells and is part of the Cl− secretory pathway in salt-secreting epithelia. NKCC2 is responsible for Na+ and Cl− reabsorption in the kidney. NKCCs have cytoplasmic N- and C-termini and a central 12-transmembrane (TM) domain. Activation of NKCCs occurs through phosphorylation of threonine residues in the N-terminus, whereas ion translocation occurs in the TM domain. How the event of phosphorylation in the N-terminus is translated to the TM domain to activate ion transport is completely unknown. To define structure-function relationships of NKCCs, we examined both inter- and intramolecular motions of NKCC1. Using fluorescence resonance energy transfer, we demonstrate that activation of NKCC1 is accompanied by a large movement between two po- sitions in the C-termini of a dimeric cotransporter. Using a cysteine cross-linking ap- proach, we demonstrate that NKCC1 activation involves conformational change at the TM10 and TM11/12 interface. Our results suggest that NKCC activation involves movement of TM12 relative to TM10, which may lie to moved to the cyto- plasmic C-terminus. We provide a novel model for understanding the molecular
motions that bring about NKCC activation. Support: NIH GM083340 and NIH P01-DK17433 to B. Forbush.

7.4 EVOLUTIONARY CONSERVED MECHANISMS FOR ACID/BASE SENSING
Martin Tresguerres

Epithelial cells can achieve diverse physiological functions that rely on the transport of ions, including acid/base (A/B) and osmotic regulation, nutrient absorption, and calcification. Despite the functional diversity of epithelia, many of these functions are achieved by the combined function of the enzymes V-type ATPase (VHA), carbonic anhydrase (CA) and soluble adenylyl cyclase (sAC). The general mechanism involves hydration of metabolic or externally derived CO2 by CA into HCO3 and H+. HCO3 stimulates the A/B sensor sAC, which can modulate the activity of multiple downstream targets via the CAMP pathway, while H+ is promptly transported out of the cell by VHA. The ultimate physiological function of the CA/sAC/VHA complex is determined by the polarisation of proteins to basolateral or apical membranes that result in differential permeability to CO2/HCO3 secretion or absorption of ions, and presence of other cell-specific accessory proteins. The CA/sAC/VHA complex has already been established to be essential for A/B regulation in shark gills and mammalian nephron, and for NaCl and water absorption in the marine teleost intestine. Other epithelia in which the CA/sAC/VHA promise to play essential roles include mantle and hemocytes from molluscs, the “root” epithelium from the bone-eating worm Ophiothrix, and corals, as well as multiple epithelia from vertebrates. Funded by the National Science Foundation (#EF-1202641) and by an Alfred P. Sloan Research Fellowship (#BR213-103).

8.0: METABOLISM, ENERGETICS, AND NUTRITION

8.1 INVESTIGATION OF ALGAL FEEDING PREFERENCES OF THE SOLAR-POWERED SEA SLUG, ELYSIA CLARKI
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The sacoglossan sea slug, Elysia clarki, is one of only four species of sea slugs that is capable of incorporating the chloroplasts from different algal species into their own bodies and utilizing them long term for up to 3 to 4 months. The chloroplasts remain functional, allowing E. clarki to derive nutrition from ongoing photosynthesis, which makes this animal part of a quite unique symbiosis. Little information is available on how the chloroplasts of algal species maintain their structural and functional integrity following phagocytosis into the digestive tract where they are stored. Furthermore, associations with several algal species have been reported for E. clarki, but the nature of these associations is currently unclear. Feeding experiments were administered to determine which macroalgal species Elysia clarki will eat, and to investigate if there are any differences in feeding preferences between adults and juveniles. The macro-algal species tested include: Bryopsis hypnoides, Cystophora craspedophylla, C. prolifera, C. racemosa, C. sertularioides, Halimeda incrassata, and Penicillus capitatus. Sea slugs were presented with each of these species, and the amount of algae consumed and growth rate of the E. clarki were measured. Preliminary results from this experiment indicate that Elysia clarki do not consume C. craspedophylla, but will eat B. hypnoides. To our knowledge, these feeding associations represent previously unreported symbioses of Elysia clarki with macroalgae.

8.2 LIGHT-INDUCED OXIDATIVE STRESS AND DEFENSE IN THE MANTLE OF A GIANT CLAM
Bryan Chang1, Kim C. Hong1, Mel Y. Boo1, Yan L. Choo1, Jasmine L. Y. Ong1, Xiu L. Chen1, You R. Chang1, Wai P. Wong1, Shit F. Chew1, and Yuan K. Ip2


The giant clam, Tridacna squammosa, has symbiotic microalgal zooxanthellae that can transfer photosynthetic products to the clam as food. However, algal photosynthesis in the presence of light increases the formation of oxygen and reactive oxygen species (ROS), increasing susceptibility of the clam to oxidative stress. The exact role of photosynthetic products in the clam has not been fully elucidated. We examined the antioxidant response in the inner and outer mantle of clams in 12 h of light or 12 h of darkness, and hypothesized that the mantle would increase metabolic activity in the presence of light and display an enhanced antioxidant response. Results showed that 12 h of light exposure significantly increased activities of several antioxidant enzymes in the inner mantle, indicating light-induced oxidative stress, although oxidative damage products remained unchanged in both the inner and outer mantle. More importantly, they revealed that the inner mantle was metabolically more active, thereby producing more ROS and increasing oxidative defense, under light exposure, which warrants further investigation. This study was funded by SMF through TMSI.

8.3 PHYSIOLOGICAL RESPONSES TO ENVIRONMENTAL STRESS IN A MOLLUSC: WHY IS BEING A HYBRID AN ADVANTAGE?
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In Australian mollusc aquaculture, two abalone species, Halinobolus rubra and H. laevigata are cross-bred to create a hybrid that shows 25% faster growth. On land-based farms animals are held in water sourced from the ocean, which varies in temperature and oxygen (O2) level. Yet, tanks are supplied with O2 until abalones are 5 months old. Thus, temperature stress is experienced by individuals throughout their life on the farm, while O2 stress is a factor experienced by older individuals. Suboptimal environmental conditions, management practices and parental origin may change the behaviour of an individual. Furthermore, under suboptimal conditions, energy is used to mitigate stress rather than channeling it into growth. This study examined how H. laevigata, H. rubra and their hybrid cope with temperature and O2 stress to reveal the physiological and behavioural strategies which result in the improved fitness of the hybrid. Respiration rate (MO2) and activity level of abalones were measured during short-term exposure to 10, 15, 19 and 23°C and decreasing oxygen levels (21–0 kPa). Experiments were conducted with 4 and 8 month old individuals, to reveal different strategies not only between types of abalones but also across development. Four month old hybrids had a similar tissue mass but had grown larger shells in comparison to the two pure species. Yet, all types of abalone had similar MO2 which correlated positively with increasing temperatures and negatively with O2 levels.

8.4 MITOCHONDRIAL MECHANISMS OF HYPOXIA TOLERANCE IN MARINE BIVALVES
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Marine organisms are exposed to oxygen deficiency in estuaries due to the tidal cycles and/or the benthic “dead zones”. Energy limitation and oxidative damage are major stressors during hypoxia and post-hypoxic recovery, and it is not well known how mitochonrdia of hypoxia-tolerant organisms cope with these challenges. We studied mitochondrial responses to hypoxia in the hard clam Mercenaria mercenaria and the bay scallop Argopecten iradians. Membrane potential (δψ) and kinetics of substrate oxidation, proton leak and phosphorylation subsystems were measured in clams and scallops exposed to hypoxia (17 h at ¹1% O2 followed by a 1 h recovery). In scallops, hypoxia suppressed the capacity of all three mitochondrial subsystems, and mitochondrial condition further deteriorated during reoxygenation, with strong depolarization of mitochondria and decreased capacity for the substrate oxidation and phosphorylation. In contrast, in clams hypoxia increased the δψ-dependent capacity of the substrate oxidation subsystem and had weak inhibitory effects on the phosphorylation and proton leak subsystems. During reoxygenation, the substrate oxidation capacity of clam mitochondria further increased and the capacity of the phosphorylation subsystem returned to normal. Upregulation of the substrate oxidation in hypoxia poised clams for a quick recovery upon reoxygenation, while scallops suffer from mitochondrial deterioration limiting their ability to survive hypoxia. Supported by UNC Charlotte

8.5 WITHDRAWN

8.6 SCORPION BURROW STRUCTURE AND VENTILATION
Amanda Adams1, Scott Turner2, Pedro Berlinger3, and Barry Pinshow4


Many animals spend much of their time underground in burrows, which serve as refuges from predators and adverse environmental conditions. The intimate association between animal and burrow leads the question, how has burrow architecture been shaped by natural selection? Burrowing scorpiions are found on all continents except Antarctica. We are using scorpiions and their burrows to test the hypothesis that burrow structure acts as a physiological control system to regulate temperature and moisture levels. We predict that burrows are built to minimize convective ventilation in order to maintain high relative humidity thereby reducing the occupant’s evaporative water loss, with burrow structure being modified depending on soil moisture and temperature. We cast 20 natural burrows of Scorpio maurus palmae, and four of Opistophthalmus setifrons with molten aluminum and used a 3D scanner to capture burrow shape and dimensions. We related how structure differs with soil moisture, temperature, and body size, while testing which features are common among individuals and species. We found that burrow volume decreases as soil
8.7 THE EFFECT OF HUMIDITY ON THE METABOLIC RATE OF GROMPHADORHINA PORTENTOSA
Canacee Toogood and Heidi L. Contrens
Univ. of La Verne.
Environmental humidity is a significant part of the ecosystem for all living organisms. Animals cope with a particular range of humidity levels depending on the habitat that they are found in by employing behavioral and physiological adaptations. However, how organisms will deal with the rapid changes in ambient humidity predicted with global climate change is still unclear. Previous studies have suggested that insects may decrease their metabolic rates, and alter their respiratory patterns, when ambient humidity is decreased. In this study we used Gromphadorhina portentosa, an insect normally found in humid environments, to investigate how these insects respond to a rapid change in ambient humidity. Using flow-through respirometry we measured the resting metabolic rates of adult, male roaches when placed in decreasing humidity levels (80, 70, 60, 50, 40, 30 and 0% R.H.). We found that as relative humidity decreased, the resting metabolic rates of these roaches significantly decreased. Furthermore, roaches showed a continuous respiratory pattern at high humidity levels but switched to a discontinuous pattern at low humidity levels. We therefore conclude that this organism has the ability to conserve water through a physiological and behavioral adaptation.

8.8 ACTIVATION OF CGMP-DEPENDENT PROTEIN KINASE REDUCES DROSOPHILA S2 CELL INJURY CAUSED BY ANOXIA AND OXIDATIVE STRESS
Olena Makholly1, Ken Dawson-Scally1, and Sarah Milton1
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Ischemic stroke is one of the leading causes of human death worldwide. It occurs due to the high susceptibility of neurons to anoxia and reoxygenation. Unlike mammals, the fruit fly Drosophila melanogaster withstands low oxygen levels without showing pathology. In the present study, Drosophila Schneider (S2) cells were employed to investigate the role of the cGMP-dependent protein kinase (PKG) signaling pathway in Drosophila anoxia tolerance. Cells were subjected to chemical anoxia and oxidative stress concurrently with treatments by pharmacological agents affecting specific targets of the PKG pathway and cell injury was assessed. Treatment of S2 cells with the PKG pathway activators 8-Br-cGMP or sildenafil citrate reduced cellular damage compared to cells exposed to COCl2 or H2O2 alone. Results thus indicate that activation of the PKG pathway preserves S2 cell plasma membrane integrity from chemical anoxia and oxidative stress. The results of this study may lead to a better understanding of the fruit fly’s innate strategies of anoxia tolerance. Subsequently, this knowledge may be used to identify potential therapeutic targets to prevent detrimental neurological effects of an ischemic stroke in humans. The study was funded by the FAU Division of Research Seed Grant, McGuinness Foundation Grant, and Research Priority Grant: Brain Function, Damage and Repair.

8.9 METABOLISM AND LOCOMOTION OF ANOXIC DROSOPHILA
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8.10 SMALL RNA REGULATION OF DIAPAUSE IN THE FLESH FLY, SARCOPHAGA BULLATA
Julie Reynolds1, and David Denlinger2
1Evolution, Ecology, and Organismal Biology, Ohio State Univ, 318 W. 12th Ave., 300D Scott Lab., Columbus, OH 43210.
Understanding the molecular basis of diapause, a phenotypically plastic, alternative developmental pathway that includes a period of dormancy, is one key to predicting the impact of global climate change on seasonal distributions of insects. Diapause, which is characterized by developmental arrest and metabolic restructuring, is associated with wide-spread changes in gene expression. Though diapause-related changes in gene expression have been characterized for species ranging from crickets to mosquitoes, the mechanisms that mediate the observed changes remain largely unknown. We predict that small regulatory RNAs (sRNAs), one type of epigenetic mechanism, mediate pupal diapause in the flesh fly, Sarcoptaga bullata. Specifically, elevated expression of genes encoding core components of the piwi-RNA and small-interfering RNA pathways in photosensitive first instar larvae reared in diapause-inducing conditions compared to those reared in diapause-avoiding conditions, suggests a role for these sRNAs in diapause initiation. In addition, a 2-fold increase in Argonaute1, a core component of the microRNA pathway, in diapasing pupae suggests a role for this class of sRNAs in regulating diapause maintenance. Next-generation sequencing of small-RNAs isolated from diapause and non-diapause pupae will provide significant new information about the role of these regulatory molecules in initiating and maintaining diapause [National Science Foundation Grant IOS-1354377].
additive was upheld for certain pathways, while others were only affected by diet or GIT microbiome but not both. Overall, cellular pathways in the GIT of fish are dynamic processes and their interactions with the GIT microbiome are not clearly understood. All experiments involving animals were conducted in conformance with guidelines for experimental animals and with an approved institutional animal use protocol. Funded by NSERC.

8.13 Fasting-Induced Morphological Reorganization of the Colon May Not Drive Concomitant Changes in the Microbiome

Celeste Pasesaran1, Kevin Kohl1, David Meyerholz2, and Marshall McCue2

1Biological Sci., St. Mary’s Univ., 1 Camino Santa Maria, San Antonio, TX, 78228, 2Biology, Univ. of Utah, 257 S. 1400 E., Rm. 201, Salt Lake City, UT, 84112.

The physiologist was 2.5 mM in Atlantic cod and 0.2 mM in short-horned sculpin, respectively. At-microbiome glucose tritiated in the number 2 position. RBCs from both species have an aerobic

protocol. Funded by NSERC.

8.14 Glucose Can Fuel Metabolism in RBCs from Normoglycemic but Not Hypoglycemic Fish

William Drexl1,2, Kathy Cline1, and Constance Shull1

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Energy metabolism was assessed in red blood cells (RBCs) from Atlantic cod (Gadus morhua) and short-horned scalpel (Mystusoxiphius scolopax). Blood glucose level was 2.5 mM in Atlantic cod and 0.2 mM in short-horned scalpel, respectively. Atlantic cod are normoglycemic and short-horned scalpel hypoglycemic by fish standards. Oxygen consumption, lactate production, and glucose utilization were determined in whole blood and related to g RBC. Glucose utilization was assessed by measuring both glucose disappearance and by the production of tritated H2O from glucose tritiated in the number 2 position. RBCs from both species have an aerobic based metabolism. In Atlantic cod, extracellular glucose is sufficient to provide the rate in short-horned scalp. In this species, energy metabolism by RBCs must based metabolism.

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HIGHLY EFFICIENT MITOCHONDRIA FUEL BLUEFIN TUNA RED MUSCLE WITHIN DISTINCT TEMPERATURE RANGES

Martin Jastroch1,2, Jason Treberg2,3, Martin Brand2, and Barbara Block4


Pacific bluefin tuna (Thunnus orientalis) maintain elevated body temperatures that are 25-30°C in the slow oxidative “red” muscle that powers endurance swimming. Here we show red muscle mitochondria contribute to thermogenesis by efficient ATP production but not proton leak as found for mammals. Changing assay temperatures (20-35°C) we found that maximal mitochondrial substrate oxidation rate is temperature-sensitive but not rate-limiting for temperature-insensitive ADP-induced respiration. Interestingly, the low proton conductance is stable within the physiological temperature range of this species but increases rapidly above 30°C, possibly counteracting the effect of temperature on the metabolic fate of lactate during and following anoxic periods of up to nearly 5h of virtually no oxygen consumption despite being maintained in oxygen-saturated water; a process we call paradoxical anaerobism. Although most bouts are only a few minutes in duration, the longest continuous bout that we observed was 149 min. Fish randomly cycle between periods of paradoxical anaerobism and stable oxygen consumption patterns. Yet, there is no evidence for compensatory oxygen use. Fish produce ethanol as an alternate end product of anaerobic metabolism. Addition of exogenous ethanol induces rapid onset of paradoxical anaerobism.

CHANGES IN EXPRESSION OF TWO GENES INVOLVED IN ARGININE SYNTHESIS, AND CONCENTRATIONS OF ARGinine AND NITRIC OXIDE, IN AN AESTIVATING AFRICAN LUNGFISH

Youn R. Chang1, Biyan Ching1, Jesamine L. Y. Ong1, Xiu L. Chen1, Wai P. Wong1, Shi F. Chew1, and Yuan K. Lp

1Dept. of Biol. Sci., Natl. Univ. of Singapore, Kent Ridge, 117543, Singapore, 2Natural Sc. and Sci. Edu., Nat. Inst. of Edu., Nanyang Walls, 637516, Singapore. Argininosuccinate synthase (Ass) and argininosuccinate lyase (Asl) are involved in arginine synthesis for multiple purposes. This study aimed to clone and sequence their mRNA expression in various tissues/organs during the induction, maintenance and arousal phases of aestivation. In the liver, significant increases in the mRNA expression of ass and asl could increase arginine production to support increased urea synthesis during the induction phase or increased nitric oxide (NO) production during the maintenance and arousal phases of aestivation. In the kidney, the ass mRNA expression level decreased significantly after 6 mon of aestivation, and there could be decreases in the synthesis of arginine and its supply to other tissues/organs. In the brain, changes in ass and asl mRNA expression levels correlated to regulating the supply of arginine for NO synthesis in response to ischemia and ischemia-reperfusion during the maintenance and arousal phases of aestivation, respectively. The decrease in ass mRNA expression, accompanied with decreases in the concentrations of arginine and NO, in the muscle of aestivating P. annectens might ameliorate the potential of disease muscle atrophy. This study was approved by the NUS IACUC.

THE EFFECTS OF TEMPERATURE ON THE METABOLIC FATE OF LACTATE DURING RECOVERY FROM ANOXIA IN THE PAINTED TURTLE

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Western painted turtles (Chrysemys picta bellii) survive anoxia for 160 days at 3°C and tolerate up to 200 mM plasma lactate. Stable isotope tracers were used to study the effect of temperature on the metabolic fate of lactate during and following anoxic submergence. A bolus of [1-13C]-lactate tracer was infused via an intra-articular catheter during anoxia and allowed to equilibrate before the start of recovery. 13C enrichment of plasma and tissue metabolites was determined using gas chromatography and mass spectrometry, and 13C enrichment of CO2 from expired air, blood, and bone was determined using isotope ratio mass spectrometry. At 20°C, M3-glucose enrichment slowly increased throughout the recovery, and liver glycogen accounted for ~30% of the label at the end of recovery. Maximum urinary excretion rates were ~9 mmol glucose • kg-1 h-1 and ~7 mmol lactate • kg-1 h-1. At 10°C and 20°C, bone CO2 respiratory exchange ratios were ~16% and ~20% of the label, respectively, indicating its importance as a sink for metabolically produced CO2 at all temperatures. Expired CO2 accounted for ~1% of the injected 13C at 10°C and ~6% at 20°C. We conclude that at both 10°C and 20°C, painted turtles oxidize part of the lactate load initially during recovery, but the primary fate is glucogenesis. At 10°C, significantly less lactate is oxidized. Urimetric excretion of lactate and glucose should be included when calculating the energetic cost of recovery. This work was funded by the National Institutes of Health.

CERVELICAL OSTEODERMs REVEAL PATTERN OF WHOLE BODY GROWTH IN JUVENILES OF THE AMERICAN ALLIGATOR

David Vanaccondel1, Tomasz Owerkowicz2, John Ern3, Jason Blank4, Ruth Elsey5, and James Hicks6

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Osteoderms (ODs), small bone nodules within the sauropsid integument, are increasingly used to infer the physiology and growth patterns of extinct species, such as non-avian dinosaurs. How ODs grow in extant species, and whether their mineral apposition rate (MAR) correlates with the animal’s whole body growth, has not been determined. Effect of activity or cardiovascular shunts on OD growth is also unknown. We examined the relationship between MAR in cervical ODs and growth in two-year old American alligators, reared at 30°C. A surgical procedure was performed to convert the circulation pattern to in-series (n=24), or conserve the in-parallel pattern (sham, n=36). Animals were assigned to one of 3 groups: sedentary, running or swimming, exercise routine 18 months. Measurements of body mass and length were taken every other week, and animals received injections of fluorescent dyes during the growth period. We found regional growth heterogeneity between the apical, basal and lateral OD facets, which may reflect multiple functions of ODs. There is significant positive correlation between MAR and whole body growth, not affected by either activity level or circulatory pattern. However, no correlation exists between MAR in early and late ontogeny. We conclude ODs may be useful in inferring growth physiology of extinct archosaurs, regardless of circulatory design or activity pattern.
8.25 PHENOTYPIC PLASTICITY ACROSS THE ANNUAL CYCLE IN A MIGRATORY BIRD
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Prior to their migration, birds make physiological and behavioral adjustments to quickly accumulate fat stores and increase muscle performance to undertake their journey. The majority of the fat stores are utilized during their long-distance flights. However, residual fat may be detrimental due to impaired flight performance and increased predation risk, thus to return to lean body composition is also rapid. This need for swift and substantial changes in a bird’s physiology marks migration as a unique life history stage requiring phenotypic flexibility. Specific mechanisms directing stage transitions in migrating birds are largely unknown. Our efforts seek to characterize the role of peroxisome proliferator-activated receptors (PPARs) in the regulation of the migratory phenotype of the grey catbird (Dumetella carolinensis). PPARs are a family of nuclear receptors involved in mammalian energy metabolism, are highly conserved in our study species and Lindberg responses are similar to mammalian receptors. PPAR involvement in migratory adiposity is supported by higher rates of glycerol release from adipose tissue during migration compared to non-migratory stages. Investigation of the involvement of PPARs in flight muscle hypertrophy during migration are ongoing. This work was funded by the NSF, grant no. R01-527455 to PJS and JH.

8.26 BATS AND BIRDS SHARE DIGESTIVE ADAPTATIONS TO AN AERIAL LIFESTYLE
Edwin Price1, Antonio Brun2, Enrique Caviedes-Vidal3, and William Karasov1


Power flight evolved at least twice in vertebrates. We tested for shared digestive adaptations in two extant vault lineages. Bats and birds, compared with nonflying mammals, share shorter intestines and smaller nominal intestinal surface areas (NSA), which lowers digestive mass carried and thus improves flight maneuverability and economy. Intestinal hydrolytic enzyme and nutrient transport activities appear similar among these groups per unit intestine, but lower over the entire intestine in the fliers. Nutrients can also be absorbed paracellularly by passing through the tight junctions that link adjacent enterocytes. Seven bat species and 14 bird species, with a variety of natural diets, absorbed significantly more of ingested L-aminobis and other similarly sized, metabolically inert, nonactively transported monosaccharides than 18 species of nonflying mammals. These differences in nutrient-sized probe absorption were demonstrated at the tissue level comparing results from perfusion experiments (7 bat species, 1 bird, 5 nonflying mammals) that control for several potential confounding factors. Greater amplification of digestive surface area by villi and differences in expression patterns of junctional proteins (i.e., claudins and occludins) may provide mechanistic explanations for the observation of higher paracellular absorption in bats and birds relative to nonflying mammals. Supported by USA NSF and Argentina CONICET.

8.27 WITHDRAWN

8.28 HEART RATE DYNAMICS IN A MARSUPIAL HIBERNATOR
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The pygmy-possum (C. nanus) is a marsupial that undergoes spontaneous short bouts of torpor, as well as multi-day bouts of deep hibernation. To examine heart rate (HR) control at varying ambient temperatures (Ta) and varying body temperatures (Tb) during torpor, we used radiotelemetry for ECG and Tb. The HR during hibernation was at its min (321±34 bpm) at a Ta of 31°C and increased linearly with Ta to a max of 630±19 bpm at a Ta of 20°C. During entry into torpor at a Ta of 20°C HR slowed primarily as a result of episodic periods of cardiac activity. Electrical activity of the heart occurred in groups of 3 or 4 heart beats that coincided with ventilation, followed by pauses of multiple seconds with no electrical activity. At a Tb of 23°C in these torpor bouts, the HR was regular (i.e. no asystoles) with a rate of 401±34 bpm. In multi-day bouts of torpor at the absence of food and water, Ta was lowered to 4°C. Tb of during these deep bouts of torpor reached a minimum of 5°C, with a minimum HR of 6 bpm. Bouts of shivering that lasted 20 sec were detected in the ECG tracings. Shivering occurred every 3-4 minutes, during which ventilation occurred, and HR was briefly elevated to 20 bpm. The duration of the QRS complex rose from 10ms during euthermia to 140ms at a Tb of 5°C. Similarly, the amplitude of the complex fell from 1.891 mV at euthermia to 0.736 mV at a Tb of 5°C. These findings demonstrate the dynamic functioning range of the heart of this marsupial to be ~100 fold.

8.29 APOPTOTIC REGULATION DURING MAMMALIAN HIBERNATION
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During hibernation, ground squirrels experience cycles of profound metabolic depression. Body temperature may approach ambient (to below 0°C) and oxygen consumption may be as low as 1/100th of active rates. Mammalian hibernators naturally experience conditions known to cause widespread apoptosis in other systems e.g. hypothermia, bradycardia, and ischemia/reperfusion injury. We found that hibernating golden-mantled ground squirrels (Spermophilus lateralis) experience partial activation of the caspase cascade. Importantly, winter squirrels experience a 2-fold increased cleavage of the p32 procaspase 3 to liberate the active p17 caspase 3. Such a liberation might be expected to result in 20,000 times more proteolytic activity. However, activity assays for caspase 3 suggest limited activity under the conditions of torpor. Similar regulation of other caspase activities suggest a global event. We found no evidence for ICAD activation, PARP cleavage, or DNA nicking consistent with downstream evidence of caspase activation. In other words despite seeming activation of caspases, there was no evidence of apoptosis. These data highlight the need for systems level approaches in understanding complex physiological states like hibernation.

8.30 A CYTOSOLIC PROTEIN FACTOR LOCATED IN MULTIPLE TISSUES IN THE NAKED MOLE-RAT PROTECTS THE PROTEASEOME FROM INHIBITION AND ACTIVATES THE PROTEASEOME IN OTHER SPECIES
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The naked mole-rat maintains robust protection from high levels of proteasome-mediated proteolysis for most of its exceptionally (~3y) life span. Here, we report that the highly active proteasome from the naked mole-rat liver, muscle, and brain resists attenuation by a diverse suite of proteasome-specific small molecule inhibitors. Moreover, mouse, human, and yeast proteasomes exposed to the proteasome-depleted naked mole-rat cytosolic fractions, recapitulate the observed inhibition resistance, and the mammalian proteasomes also show increased activity. Gel filtration coupled with mass spectrometry and atomic force microscopy indicates that these traits are supported by a protein factor that resides in the cytosol. This factor interacts with the proteasome and modulates its activity. Although HSP72 and HSP90 (Hdj1) are among the constituents of this factor, the observed phenomenon, such as increasing peptidase activity and protecting against inhibition cannot be reconciled with any known chaperone functions. Furthermore, this phenomenon seems to correlate with longevity in seven different rodent species. This novel function may contribute to the exceptional protein homeostasis in the naked mole-rat, and other long-lived rodents, to allow them to successfully defy aging.

8.31 LIPIDS AND MYOGLOBIN: NEW INSIGHTS FROM NON-MODEL SPECIES
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During endurance exercise, terrestrial mammals rely mainly on oxygen delivered by the blood to support lipid-fueled aerobic metabolism. Physiological changes associated with endurance training increase muscular blood flow and subsequent oxygen delivery; yet, terrestrial endurance athletes have more myoglobin (Mb) than their sedentary counterparts. Interestingly, Mb has a high affinity for oxygen, requiring extremely low partial pressures of oxygen to release oxygen stores. Accordingly, the traditional functional paradigm pertaining to Mb as an important oxygen store and transporter does not appear to be fully applicable to healthy, terrestrial mammals in vivo. Recent studies in our lab have provided new insight on Mb function in relation to lipids that challenge this traditional paradox. These studies first identified a link between lipid supplementation and Mb expression in diving mammals, and then extended those findings to terrestrial mammals while elucidating an apparent tie to ROS signaling. These combined studies provide new data offering an alternative paradigm, whereby in the context of lipid metabolism, Mb as a ROS scavenger or lipid transporter does not appear to be fully applicable to healthy, terrestrial mammals in vivo.
transporter appears more relevant than Mb as an oxygen store. Thus, as mammals increase their aerobic capacity, and lipid utilization, there is an increase in Mb expression to facilitate lipid metabolism by offsetting negative byproducts associated with a high fat diet.

8.32

THYROID GLAND REMAINS RESPONSIVE TO THYROID STIMULATING HORMONE WITH SENSITIVITY INCREASING WITH FASTING DURATION IN A PROLONGED FASTED MAMMAL

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Food deprivation in mammals is associated with reduced thyroid hormones (TH), especially triiodothyronine (T3), to suppress metabolism. However, in prolonged-fasted, metabolically active elephant seal pups, cellular thyroid hormone-mediated components are up-regulated with fasting duration. The functional relevance of this apparent paradox is unknown and demonstrates variability in the regulation of TH levels, metabolism and function among food-deprived mammals. To address our hypothesis that the thyroid gland remains responsive with fasting duration, we infused early and late fasted pups with thyroid stimulating hormone (TSH) and measured the concentration of total and free thyroxine, (T4 and T3) as well as total triiodothyronine, (T3). During the early fast, concentrations of T4, T4 and T3 increased throughout the infusion period in response to TSH, with levels peaking at 120 minutes. At 24 hours, 58 ± 9%, 76 ± 12% and 54 ± 6%, respectively. During the late fast, concentrations of T4, T4 and T3 also increased throughout the infusion period in response to TSH, with levels peaking at 120 minutes, 56 ± 10%, 59 ± 12%, and 60 ± 9%, respectively. Additionally, although levels returned to normal at 24 hours during the early fast, levels remained 115% and 142% elevated in T4 and T3, respectively, after 24 hours in the late fast. Combined with our previous data demonstrating that cellular TH pathways are active and functional during prolonged food-deprivation in metabolically and physically active seals, the data suggest that the regulation and function of the thyroid gland and of thyroid hormones of the northern elephant seal is typical.

8.33

PURINE NUCLEOSIDE PHOSPHORYLASE ACTIVITY IN ERYTHROCYTES FROM BOTTLENOSE DOLPHINS (TURSIOPS TRUNCATUS) IN RESPONSE TO BREATH-HOLD DIVING AND EXERCISE

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Under hypoxia, adenosine triphosphate (ATP) is degraded and hypoxanthine accumulates. Exercise and breath-hold diving can induce tissue hypoxia. During exercise, skeletal muscle energy demand and oxygen consumption increase. If oxygen consumption exceeds its supply, hypoxic conditions ensue. During breath-hold diving, oxygen supply decreases and, although oxygen utilization is regulated by bradycardia (low heart rate), peripheral vasoconstriction and ischemia, oxygen reserves decrease. The goal of this study is to evaluate changes in the purine metabolism in response to diving and exercise in bottlenose dolphin (Tursiops truncatus). Blood samples were taken from captive T. truncatus (n=8) following an exercise routine and after a dive to 1.5 m deep for 90 s. Hematocrit and plasma and intraerythrocyte concentrations of total and free thyroxine, (tT4 and fT4) as well as total triiodothyronine, (tT3). During the early fast, concentrations of tT4, fT4 and tT3 increased throughout the infusion period in response to TSH, with levels peaking at 120 minutes. At 24 hours, 58 ± 9%, 76 ± 12% and 54 ± 6%, respectively. During the late fast, concentrations of tT4, fT4 and tT3 also increased throughout the infusion period in response to TSH, with levels peaking at 120 minutes, 56 ± 10%, 59 ± 12%, and 60 ± 9%, respectively. Additionally, although levels returned to normal at 24 hours during the early fast, levels remained 115% and 142% elevated in T4 and T3, respectively, after 24 hours in the late fast. Combined with our previous data demonstrating that cellular TH pathways are active and functional during prolonged food-deprivation in metabolically and physically active seals, the data suggest that the regulation and function of the thyroid gland and of thyroid hormones of the northern elephant seal is typical.

8.36

LACTATING MICE INCREASE VILLUS SURFACE AREA THRU INCREASED ENTEROCYTE WIDTH IN RESPONSE TO LOW PROTEIN DIETS

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Animals have evolved the ability to flexibly change the size of their gut to match intake. However, under some conditions, animals can respond to dietary challenges in ways that counterbalance nutrient availability. Previous work in our lab has demonstrated that lactating mice on a low protein isocaloric diet increase small intestine mass and diameter, which may improve amino acid absorption. In this study, we compared aminopeptidase activity in villus and enterocyte morphology to determine how lactating females modify their gut at the tissue level when challenged with insufficient dietary protein. Mice were fed isocaloric diets containing either 11.5% or 23% (control) protein from one week prior to pairing until peak lactation, or for a comparable time period for nonreproductive females, at which time females were sacrificed and tissues were harvested. Diet did not affect aminopeptidase activity, whereas villus morphology was modified with diet. Villus height and intestinal surface area in the proximal region increased by more than 25% in lactating mice on the low protein diet. The increase in surface area was matched by an increase in enterocyte width, indicating that larger cells are produced in lactating mice experiencing protein insufficiency. These changes were seen only in reproducing mice, supporting the interpretation that modification of the gut serves to compensate for low dietary protein under conditions of high protein needs.

8.37

MACROPHAGE INFILTRATION IN THE ADIPOSE TISSUE OF DAIRY COWS DURING NEGATIVE ENERGY BALANCE

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Excessive lipolysis during parturient and early lactation periods elevates plasma nonesterified fatty acids (NEFA) predisposing cows to clinical diseases including displaced abomasum (DA), ketosis and fatty liver. While in humans, uncontrolled lipolysis is commonly associated with adipose tissue macrophage (ATM) infiltration in obesity and metabolic syndrome, it is unknown if it leads to ATM infiltration in dairy cows. The goal of this study was to characterize ATM infiltration into different adipose tissue depots in dairy cows. Both macrophage markers were expressed by a significantly higher number of SVC from OM compared to SC in cows with DA. These results indicate that lipolysis induces ATM infiltration during negative energy balance stages in dairy cows. Future studies will evaluate the role of this inflammatory response in the adipose tissue remodeling process associated with dam lipidolysis. Funded by USDA-NIFA grant 2012-67012-19832 and the CVM Venture and Last Lecture Fund/Weiser.

8.38 THE EFFECT OF CIRCADIAN ORGANIZATION ON ENERGY USE AND IMMUNE FUNCTION IN C57B MICE
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Circadian organization is thought to be important for efficiency and energy balance, and may therefore affect metabolic rate. Using two strains of C57B mice (Mus musculus: wildtype (WT) and transgenics lacking vasotocin neuropeptide hormone (VIP)), we examined the effects of disrupted circadian rhythms in VIP-/- mice on energy expenditure and immune function under two different circadian conditions (entrained and free-running). We examined two measures of energy expenditure: basal metabolic rate (BMR) and daily food intake (FI), and two measures of immune function: total white blood cell (WBC) count and neutrophil/lymphocyte (N/L) counts. The BMR of entrained VIP+/- mice was higher than that of WT mice and decreased under free-running conditions; but still remained higher than that of WT mice. FI of entrained VIP+/- mice was lower than that of WT mice and increased under free-running conditions. VIP+/- mice had lower total WBC counts as compared to the WT mice, regardless of circadian condition. Circadian condition had no effect on N and L counts in WT mice, but L counts decreased and N counts increased in VIP+/- mice between entrained and free-running conditions. Although VIP+/- mice are known to have disrupted circadian organization, the loss of VIP might also affect other systems. Our observations that energy expenditure and immune function differ between circadian conditions in VIP+/- mice suggest that circadian organization contributes to the regulation of energy use.

9.0: FIELD PHYSIOLOGY

9.1 ENTRAINMENT OF CIRCADIAN GENE EXPRESSION RHYTHMS IN CALIFORNIA MUSSEL MYTILUS CALIFORNIANUS
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Since the ebb and flow of the tide is a regular temporal occurrence associated with highly contrasting environmental conditions, it follows that intertidal organisms should have a dedicated, endogenous time-keeping mechanism that aids in anticipating these environmental fluctuations and making the appropriate physiological changes. As a first step in elucidating the molecular mechanism of the circatidal clock, we sought to identify a set of candidate genes likely to be involved in the central oscillator in M. californianus. Our previous work has shown that M. californianus exhibit circatidal gene expression rhythms while exposed to simulated intertidal conditions, as well as intertidal and subtidal conditions in the field. If any of those rhythm genes are involved in the circatidal clock, then they should continue to show circatidal rhythmicity even in the absence of environmental cues. In this study, M. californianus were acclimated to field subtidal conditions, then quickly moved to constant conditions. Gill samples were taken every 2 hours for 48 hours. RNA was isolated and microarray and RNAseq analysis were performed. Results show that M. californianus continue to show circatidal gene expression, even in constant conditions. Moreover, all of the circatidal genes identified in this study were found to be circatidal in our previous studies as well. This set of candidate genes will allow us to further investigate the molecular mechanism of the circatidal clock.

9.2 THE STRESS RESPONSE IS ASSOCIATED WITH BOTH CORTISOL AND ALDOSTERONE RELEASE IN MARINE MAMMALS
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Numerous environmental conditions potentially disrupt homeostasis in animals. Sufficient disturbance results in a stress response and activation of the hypothalamus-pituitary-adrenal (HPA) axis. Stress hormones may therefore be useful indicators of disturbance in animals of conservation concern but we must understand the variability in HPA axis response among species to interpret stress levels in wildlife. We therefore investigated the stress response in two groups of marine mammals: free-ranging elephant seals (n=16) and captive bottlenose dolphins (n=7). Stress was induced by administering adrenocorticotropic hormone (ACTH) and blood samples were collected over ~2 hrs. Seals received 0.25 U/kg ACTH and showed increases in both cortisol and aldosterone. Several downstream effects were detected including increases in reverse T3 and energy substrates (glucose and free fatty-acids). Captive dolphins received varied doses of ACTH (0.02 to 0.25 U/kg) combined with out-of-water sampling that contributed to greater variation in the magnitude of hormone release but dolphins also displayed increases in cortisol and aldosterone. Corticosteroid concentrations were far lower in dolphins than in seals. ACTH is not a strong aldosterone secretagogue in terrestrial mammals but several studies have measured increased aldosterone in response to stress in marine mammals, indicating its potential importance in osmoregulation or vasoconstriction in this group. This work was funded by the Office of Naval Research.
responding FADD-knockout demonstrating that coral AdTfN1 activates the H. sapiens Death Receptor Pathway. Taken together these data show remarkable conservatism of the TNF-induced apoptotic response representing 550 million years of functional conservation.

10.2 MUSCULAR DYSTROPHY GENES AND THE EARLY METAZOAN TRANSITION FROM DYNEIN TO MYOSIN-Powered LOCOMOTION
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Urea transporters (UT) are facilitative transporters that allow urea to move down its concentration gradient across cellular membranes. While it would be expected that urea excreting (ureotelic) species express these proteins, surprisingly several non-ureotelic species also express these transporters in multiple tissues. In the current study, we explored UT evolution and tissue distribution in vertebrate lineages to further our understanding of their role and function throughout vertebrate history. We reconstructed a phylogeny of UT proteins in vertebrates, and using RT-PCR generated a tissue distribution of these transporters in multiple vertebrate species. Our results demonstrate that evolution of membrane-spanning muscular dystrophy protein complexes was an essential process as one geometric constraint on power transmission was traded for another.

10.3 EVOLUTION OF THE UREA TRANSPORTER FAMILY IN VERTEBRATES
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During early animal evolution, the primary source of mechanical power shifted from flagellar dynein to densely arrayed class II myosins. Although muscle confounded the advantage of three-dimensional scalability, the transition brought with it the problem of safely transmitting huge forces across the cell membrane—a task that in vertebrates is accomplished in part through the dystrophin-dystroglycan-sarcoglycan complex (DGC), which is implicated in most forms of muscular dystrophy. We present the inferred earliest steps in the molecular evolution of these cell surface proteins, using genome sequence data from all early branching metazoan phyla and a broad sampling of unicellular taxa. Surprisingly, the phylogeny suggests that a DGC emerged before the ‘sarcomeric’ clade of myosins, implying conserved function(s) among unicellular lineages closely related to the Metazoa. Furthermore, linkage of the DGC to the cytoskeleton occurred before the tandem-repeat expansions seen in the sarcomeric proteins of protostomian superphylum. Finally, intron positions in the inferred dystrophin gene in a common ancestor to all Metazoa provide an important clue to the molecular basis of Duchenne muscular dystrophy and emerging therapies. Our reconstruction suggests that evolution of membrane-spanning muscular dystrophy protein complexes was an essential process as one geometric constraint on power transmission was traded for another.

10.4 DIFERENTIAL GENE REGULATION UNDERLIES RESPONSES TO ACUTE HEAT STRESS AND LOCAL ADAPTATION IN TIGRIOPUS CALIFORNICUS
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Temperature is one of the main environmental factors that influences local adaptation of conspecific populations along the latitudinal gradients. However, the molecular mechanisms underlying local adaptation to temperature gradients are not well understood. The intertidal copepod Tigriopus californicus is a good model for studying acute heat stress response and thermal adaptation. Populations of T. californicus inhabit high intertidal rock pools along the west coast of North America from Baja California to Alaska. These pools present extreme thermal environment and previous studies have shown evidences of thermal adaption of T. californicus populations along the latitudinal gradient. Southern populations survive acute heat stress at higher temperatures than northern populations. Transcriptome studies have shown that thermal tolerance is associated with levels of expression of numerous heat shock protein (Hsp) genes. We hypothesize that genetic variation in the heat shock transcription factor (HSF) underlies differential responses to acute heat stress among different populations of T. californicus. There is one copy of the HSF gene with 529 amino acids in the T. californicus genome. For example, we found 10 amino acid substitutions in the HSF gene between Santa Cruz and San Diego populations. This suggests functional differences of HSF from different populations along the latitudinal gradient. HSF is activated by heat stress and it subsequently induces transcription of Hsp genes. However, the heat shock regulatory network has not been well studied in the ecological and evolutionary context. Here we investigate differential regulations of heat stress responses by comparing the activation temperatures of HSF from different populations of Tigriopus californicus.

10.5 RELATIONSHIPS BETWEEN MITOCHONDRIAL THERMAL PERFORMANCE AND HEAT TOLERANCE AMONG POPULATIONS OF THE INTERTIDAL COPEPOD TIGRIOPUS CALIFORNICUS
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Heat shock tolerance is associated with levels of expression of numerous heat shock protein (Hsp) genes in conspecific populations along the latitudinal gradient. However, the molecular mechanisms underlying local adaptation to temperature gradients are not well understood. The intertidal copepod Tigriopus californicus is a good model for studying acute heat stress response and thermal adaptation. Populations of T. californicus inhabit high intertidal rock pools along the west coast of North America from Baja California to Alaska. These pools present extreme thermal environment and previous studies have shown evidences of thermal adaption of T. californicus populations along the latitudinal gradient. Southern populations survive acute heat stress at higher temperatures than northern populations. Transcriptome studies have shown that thermal tolerance is associated with levels of expression of numerous heat shock protein (Hsp) genes. We hypothesize that genetic variation in the heat shock transcription factor (HSF) underlies differential responses to acute heat stress among different populations of T. californicus. There is one copy of the HSF gene with 529 amino acids in the T. californicus genome. For example, we found 10 amino acid substitutions in the HSF gene between Santa Cruz and San Diego populations. This suggests functional differences of HSF from different populations along the latitudinal gradient. HSF is activated by heat stress and it subsequently induces transcription of Hsp genes. However, the heat shock regulatory network has not been well studied in the ecological and evolutionary context. Here we investigate differential regulations of heat stress responses by comparing the activation temperatures of HSF from different populations of Tigriopus californicus.

10.6 OBESITY-INDUCED CARDIAC DYSFUNCTION IN STARVATION-SELECTED DROSOPHILA
Christopher Hardy1, Ryan Birse2, Matthew Wolf, and Allen Gibbs1


"Drosophila is an excellent model organism for studying the pathophysiological effects of obesity on the heart. When fed high fat or sugar diets, Drosophila become obese, leading to heart defects including dilatation, steatosis and myofibrillar disorganization. We have selected populations of Drosophila for starvation resistance for over 80 generations. In response, the starvation-selected (SS) lines have evolved physiologies that mimic obesity in mammals, including much higher lipids and lower metabolic rates than their unselected controls. Using video microscopy and OCT, we found SS hearts to be dilated and less contractile than their controls. We demonstrated a direct relationship between fat storage and heart dysfunction, as we rescued the SS hearts by making them lean through a period of starvation. These findings suggest that the genetic basis of heart disease in the SS lines is dependent on lipid homeostasis. We hypothesize that the major source of dysfunction to be the physical interference of excess adipose tissue between the dorsal cuticle and the heart, which we observed upon dissection. We found no evidence of cardiac steatosis in the SS lines, nor any indication of myofibrillar disorganization. These data suggest that the mechanism of heart dysfunction in SS lines is fundamentally different from previously reported models of diet-induced heart disease in Drosophila. Overall our findings provide a new model to study the physiology and genetics of obesity-induced heart disease.

10.7 SEX AND NUTRIENT EFFECTS ON ENERGY ALLOCATION AMONG BODY PARTS WITHIN AN INDIVIDUAL
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We examined relative energy allocation among body parts (head, thorax, legs, wings, abdomen) within individuals as a function of body size, diet quality, and sex, in the hawkmoth Manduca sexta. 50-90 moths per sex, per diet (478 total), were disarticulated and each body part dry weighed. Calibration curves for the caloric content of each body part, diet, and sex were generated using a bomb calorimeter. These calibration curves were used to calculate the predicted caloric content of each of the 5 body parts in the 478 moths. Males and females allocated resources very differently within and across diet qualities. For example, mass-specific (cal/g) allocation to the...
thorax in females increased with thorax size on high quality diet but decreased on low quality diets. This pattern was reversed in males. Similar inverse relationships for males and females were found in other body parts. Tradeoffs in caloric investment were seen only between the abdomen and other body parts. These patterns of tradeoffs held for both sexes and all diets. This pattern of tradeoffs disappeared, however, when examined on a mass specific level. Our results show that a life history strategy that favors dispersal is more expensive (per gram) than a life history strategy that favors investment in reproduction. Together these results show that males and females have different rules of mass-specific caloric investment into individual body parts when diet quality varies. Funding was provided by NSF-IOS.

10.8 MITOCHONDRIAL GENOMES AND OXIDATIVE PHOSPHORYLATION FROM POPULATIONS OF FUNDULUS HETEROCLUIS DISTRIBUTED ALONG A THERMAL CLINE

Joaquin Nunez1, Tara Baris1, Douglas Crawford1, and Marjorie Oleksiak1

20°C, and 28°C). Mitochondrial polymorphisms will be correlated with mitochondrion-measured mitochondrial respiration on 180 individuals in an admixture population to stand the effects of nucleotide mitochondrial variation on physiological function, we will attempt to gain information on divergence due to the thermal gradient. To better understand the effects of nucleotide mitochondrial variation on physiological function, we measured mitochondrial respiration on 180 individuals in an admixture population containing both the northern and southern haplotype. Mitochondrial activity included state 3, E state, complex I, complex II, and LEAK at three assay temperatures (12°C, 20°C, and 28°C). Mitochondrial polymorphisms will be correlated with mitochondrial function and analyzed to gain information on divergence due to the thermal gradient and possible signatures of natural selection.

10.9 RECIPROCAL OSMOTIC CHALLENGES REVEAL MECHANISMS OF DIVERGENCE IN PHENOTYPIC PLASTICITY IN THE KILLFISH FUNDULUS HETEROCLOTUS

Reid Brennan1, Fernando Galvez1, and Andrew Whitehead1

The whole mitochondrial genome was examined in the teleost fish Fundulus heteroclitus to gain insight into the physiological effect of mitochondrial DNA sequence variation. Fundulus is distributed along a steep thermal gradient (1°C/degree latitude) on the eastern coast of the United States and display a northern and a southern mitochondrial haplotype with an admixture of both haplotypes found in northern New Jersey. The whole mitochondrial genome was sequenced from five populations along the thermal cline to define all functional nucleotide polymorphisms. To better understand the effects of nucleotide mitochondrial variation on physiological function, we measured mitochondrial respiration on 180 individuals in an admixture population containing both the northern and southern haplotype. Mitochondrial activity included state 3, E state, complex I, complex II, and LEAK at three assay temperatures (12°C, 20°C, and 28°C). Mitochondrial polymorphisms will be correlated with mitochondrial function and analyzed to gain information on divergence due to the thermal gradient and possible signatures of natural selection.

10.10 THE ROLE OF LIPIDS AND PROTEINS IN THE DIET OF COMMON RABBITS (CRICETUS CRICETUS) ON HIBERNATION AND REPRODUCTIVE SUCCESS

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Hibernation is an effective strategy used by endotherms to cope with seasonal decline in food resources. The energy savings achieved by hibernation depends on the duration and the depth of torpor bouts. Long and deep torpor bouts allow animals to have a better body condition at emergence, on which greatly depend reproductive performance. Although this is a crucial point in an evolutionary perspective, the effect of food quality (lipid and protein content) on hibernation and reproductive success has not yet been fully investigated. In this study we evaluated such an effect in female common hamsters. Hibernation pattern, body condition and reproductive success were compared in two groups (R1: n=17; R2; n=15) fed with diets similar energy and carbohydrate contents, but with different lipid (5 vs 10%) and protein (19 vs 13%) contents. The protocol agreed by the relevant Ethical Committee, followed the directives on animal experiments. Surprisingly, females fed the high-fat low-protein diet (R2) had the worst hibernation quality by spending less time in hypothermia. Thus, R2 females lost more fat and lean mass during hibernation. They also had less and smaller pups and the worst reproductive success. We show here that the gross-food quality influence both hibernation and next reproduction. In a next step we will determine the exact contribution of lipids and proteins according to their composition on both hibernation and/or reproductive success, and ultimately on fitness.

11.0 HUMAN NUTRITION AND PHYSIOLOGY EDUCATION

11.1 GENDER DIFFERENCE IN NUTRITIONAL STATUS OF A PRIMARY SCHOOL CHILDREN OF BANGLADESH

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Background: About 62% children of Bangladesh are malnourished. In this country, although, boys are assumed to be provided with more food than girls, this discrepancy has not yet been examined. Comparison of the nutritional status between boys and girls may be a good indicator to examine this difference. Objective: To compare the nutritional status between school-boys and girls of Bangladesh. Method: This cross-sectional comparative study was conducted in the department of Physiology, Noakhali Medical College during April – June’12. 219 students of a selected public primary school of Bangladesh were enrolled for the study by convenient sampling. Permission
of the authors and the students were taken. Height and weight were measured and BMI was calculated. Statistical analysis was done by SPSS (version 16). Comparison between two groups was done by Student ‘t’ test. Result: There were 91(41.6%) boys and 128(58.4%) girls. The mean age of all participants was 9.39±1.72 years. There was no significant difference in age between boys and girls (9.59 ± 9.26 years, p=0.164). The mean ± SD height, weight and BMI of all participants were 127.6±10.2 meter, 21.94±5.07 kg and 13.54±1.63 respectively. No significant difference was observed between boys and girls in mean ± SD height (1.27±0.09 m and 1.26±0.11 m; p=0.426), weight (22.38±4.70 vs. 21.63±5.31 kg; p=0.284) and BMI (13.71±1.69 vs. 13.43±1.59; p=0.205). Conclusion: There was no significant difference in nutritional status between boys and girls of a public primary school of Bangladesh. Limitations: As the study was done in a single public primary school of Bangladesh, it does not represent the whole country’s children. Funding source: Navana, Beicina, SK&F & ACI pharma Bangladesh Ltd.

11.2 NUTRITIONAL STATUS OF MBBS STUDENTS OF A SELECTED GOVERNMENT MEDICAL COLLEGE OF BANGLADESH

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Background: Early adults are considered to be a nutritionally vulnerable segment of the population. Poor nutritional status during early adulthood is an important determinant of health outcomes at a later stage of life. In Bangladesh, various survey reports showed that approximately 85% of the people intake insufficient food, 76% of rural households was deficit of calories intake and 53% and 97% of them had deficient intake of calcium, Vitamin A and Vitamin C respectively. Medical students pass their early adulthood periods during their stay at medical college. They may have nutritional abnormalities as they are not able to take proper nutritious food in their dormitories. Objectives: To examine the nutritional status of medical students of Bangladesh. Methods: This cross-sectional descriptive study was conducted in the department of Physiology, Noakhali Medical College, Bangladesh. Permission of the authority and consent of the students were taken. Height and weight were measured and BMI was calculated and recorded on a data sheet. Statistical analysis was done by SPSS (version 16). Results: A total of 201 medical students were enrolled in the study. The mean ± SD age of the students was 20.93±1.57 years. Among the participants 84 (40.4%) were male students. The mean ± SD BMI of the male, female and total students were 22.80±3.69, 20.71±3.19 and 21.56±3.55 respectively. Among the total students 138 (69%) had normal weight, 37 (18.5%) were under weight, 21 (10.5%) pre-obese and 4 (2.0%) obese. Conclusion: Almost one-third of medical students suffer from nutritional abnormalities. 18.5% suffer from underweight and 12.5% from overweight. Funding sources: Beicina, ACI, Navana & Square Pharma, Bangladesh Ltd.

11.3 IS THE GROWTH OF BANGLADESI SCHOOL CHILDREN PROPORTIONATE TO THEIR AGE?

Rakib U. Ahmed1, Mohammad U. A. Khan2, and Md M. A. Abid3

Background: 62% Bangladeshi children are malnourished. Objectives: To test if the growth of school children is proportionate to their age. Method: This study was conducted in the department of Physiology, Noakhali Medical College, Bangladesh during April – June’12. 174 students of a public primary school of Bangladesh were enrolled for the study by convenient sampling. Permission of the authority and the students were taken. Height, weight and BMI were measured. Statistical analysis was done by SPSS (version 16). Results: A total of 201 medical students were enrolled in the study. The mean ± SD age of the students was 20.93±1.57 years. Among the participants 84 (40.4%) were male students. The mean ± SD BMI of the male, female and total students were 22.80±3.69, 20.71±3.19 and 21.56±3.55 respectively. Among the total students 138 (69%) had normal weight, 37 (18.5%) were under weight, 21 (10.5%) pre-obese and 4 (2.0%) obese. Conclusion: Almost one-third of medical students suffer from nutritional abnormalities. 18.5% suffer from underweight and 12.5% from overweight. Funding sources: Beicina, ACI, Navana & Square Pharma, Bangladesh Ltd.

11.4 GENDER DIFFERENCE IN THE RESULT OF 1ST TERM PHYSIOLOGY EXAMINATION OF A GOVERNMENT MEDICAL COLLEGE OF BANGLADESH

Mohammad U. A. Khan1, and Abdus Salam2

Background: In recent years more female students than males qualify for study in medical colleges of Bangladesh. Whether female students do better than males in their course exam is not known. Objective: To compare the 1st term result between male and female undergraduate medical students. Method: This cross-sectional comparative study was conducted in the department of Physiology, Noakhali Medical College during the period of June-August’13. 115 students of a selected public medical college of Bangladesh were enrolled for the study purposively. Consent of students and authority was taken. Data on admission merit-score and 1st term marks were collected. The written merit score and 1st term marks of the result were classified as ‘passed’, ‘failed’ and ‘absent’. Data was analyzed by SPSS (version 16). The continuous data was compared by Student’s ‘t’ test and categorical data by Chi-square test. P value <0.05 was significant. Result: There were 48 (41.74%) males. The mean admission merit-score and 1st term marks and female students was similar (156.2±7.58 vs 158.39±6.40) vs. (p=0.70). In 1st term exam the male and female students obtained similar marks in written (47.25±7.15 vs 47.89±8.15; p=0.679, 95% CI: 3.73 to 2.942) and in oral (63.19±8.049 vs 66.36±9.13; p=0.072, 95% CI: 6.63±292)). Among the male students 20 (41.74%) passed, 22 (45.8%) failed and 6 (12.5%) were absent and among the female students 26 (38.8%) passed, 34 (50.7%) failed and 7 (10.7%) were absent. There was no significant difference between male and female students’ result (p=0.861). Conclusion: The result of male and female undergraduate medical students in the 1st term physiology exam was similar. Funding sources: Unihglob, Gobe, Bina Sone & Acme Pharma Ltd.

11.5 USE OF FELLOW EXAMINE AS SUBJECT IN OBSERVED STRUCTURED PRACTICAL EXAMINATION (OSPE) IN 1ST TERM PHYSIOLOGY EXAMINATION

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Background: Department of Physiology finds difficulty in managing ‘subjects’ in practical procedure. To avoid this difficulty fellow examinees of other group may be used as subjects. Objective: To find out the merits and demerits of using fellow examinees as subjects in the practical procedure. Method: This cross-sectional descriptive study was conducted in the department of Physiology, Noakhali Medical College, Bangladesh during May-June’14. Forty-two 1st year undergraduate medical students of a selected public medical college of Bangladesh were enrolled for the study purposively. Consent of students and authority was taken. Eighteen of them were selected as subjects and designated as subject-examinees. Other fellow examinees (non-subject) examined their blood pressure and pulse as part of ‘observed structured practical examination’ (OSPE). The opinion of all examinees regarding the merits and demerits of using fellow examinees as subjects in the practical procedure was recorded. Result: Examinees narrated that they could perform their practical procedures without nervousness (24/42, 57.14%), accurately and comfortably (14/42, 33.33%) and subjects were made available without wasting time (2/42, 4.76%). Nineteen students (45.24%) found no disadvantage and 2(4.76%) felt embarrassing when the subject was of opposite sex. The subject-examinees narrated that they could learn from the errors done by their fellow examinees (11/18, 61.1%). 75% non-subject examinees expressed their willing to be subject so that they can learn from their fellows’ error. Conclusion: Using fellow examinees as subjects is beneficial for the both the non-subject and subject examinees. Funding sources: Navana, Beicina, Unihglob, Square & Acme Pharma, Bangladesh Ltd.

11.6 EFFECTIVENESS OF FREQUENT DISCUSSION EMBEDDED INTERACTIVE LECTURE (FDEIL) IN LEARNING MEDICAL SCIENCE

Mohammad U. A. Khan1, Md A. Abbasi2, and Mohammad A. Salam3

Background: In traditional lecturing system learners find less opportunity to make a critical point. Recent studies supported that students need interactive lectures. Frequent discussion embedded interactive lecture (FDEIL) has been considered as a good method of learning medical science. However the effectiveness of FDEIL has not yet been tested. Objective: To compare the effectiveness of FDEIL with lecture followed by discussion (LFD) in learning medical science. Method: This comparative study was conducted in the Department of Physiology, Noakhali Medical College. Total 48 2nd year MBBS students were selected in the study. Among them 29 students were assigned in case group who were exposed to FDEIL and 19 students in control group who were exposed to LFD lecturing. The topic was ‘the function of cerebellum’, same for both the groups and taken by the same teacher. In FDEIL teacher delivered lecture for 15 minutes and gave a 5 minute’s break for discussion.
Thus a one-hour lecture was divided into such 3 lecture-discussion cycles. In LFD, information was delivered by the teacher for 45 minutes and last 15 minutes were scheduled for discussion among students. At the end of the sessions both groups were asked to answer 20 objective questions each bearing 2 marks. Result: The case group obtained more mean±SD marks than control group but the difference did not reach the level of significance (25.43±6.32 vs 22.58±5.28, 95% CI, -6.77 to 6.381, p=1.11). More students of case group obtained ≥50% marks than control group (72.4% vs 47.4%) but the difference was not statistically significant (p=0.08). Conclusion: FDEIL and LFD are similarly effective in learning medical science. Funding sources: Globe & Ziska, Pharma, Bangladesh.

11.7 READING THE PRIMARY LITERATURE: SKILLS OF FIRST-YEAR AND SENIOR UNDERGRADUATE BIOLOGY MAJORS

Jason Blank1, Karen McGaughy2, Elena Keeling1, and Jeanne Scaramuzzino1


The ability to find, evaluate, read and understand scientific literature is vital to trained scientists, but the integration of scientific literacy training in science curricula is inconsistent. To evaluate gains in the ability of undergraduate biology majors to read scientific literature, first-year and senior students were assigned to read a primary (i.e. experimental) article and write answers to a series of questions about key components of the article. A scorer who was blinded to the academic year of the subjects scored the answers to each question according to a rubric. Each subject’s scores for each question were then aggregated to provide an overall score for the subject’s understanding of the article. A crossover design involving two articles of similar complexity will enable longitudinal assessment of individual students and could also be used to evaluate specific interventions to improve information literacy training. In addition, the survey and scoring strategy can be adapted easily to other topics, providing a tool for quantitative assessment of information literacy across scientific fields. A parallel survey on the same subject population evaluates the ability to search scientific databases and identify relevant, peer-reviewed resources.

12.0: CARDIOVASCULAR AND RESPIRATORY PHYSIOLOGY

12.1 GETTING TO THE HEART OF PLASMA-ACCESSIBLE CARBONIC ANHYDRASE IN FISH

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Teledost fish possess a number of physiological adaptations that likely had a major role in their tremendous adaptive radiation and evolutionary success. One such adaptation is the Root-effect, whereby hemoglobin oxygen carrying capacity is reduced as red blood cell (rbc) pH decreases. Recent evidence suggests that the presence of plasma-accessible carbonic anhydrase (CA) – the enzyme that catalyzes CO2/HCO3 interconversion-can greatly enhance oxygen delivery to skeletal muscle by short-circuiting rbc pH regulation and engaging the Root-effect. We tested the hypothesis that plasma-accessible CA is present in the heart lumen of Coho salmon, an active teledost with spatially limited coronary circulation. The presence of a membrane-anchored CA isomor in the heart was confirmed by qPCRand Western blot. To test that plasma-accessible CA is present in the heart lumen of Coho salmon, an active teledost with spatially limited coronary circulation. The presence of a membrane-anchored CA isoform in the heart was confirmed by qPCR and Western blot. To test whether or not this isoform is accessible to plasma in the heart lumen, CA activity was measured in excised intact atria using a modified electrometric delta-pH assay. The atrial chambers were pre-treated with either saline or phosphatidylinositol-specific phospholipase C (PI-PLC) to cleave the membrane anchor of CA. In support of our hypothesis, we observed a significant 2.3-fold reduction in CA activity in atria pre-treated with PI-PLC relative to controls. We suggest that plasma-accessible CA in the salmon heart functions to safe-guard oxygen supply to regions of this vital organ devoid of coronary vessels. Supported by NSERC Canada and Canada Research Chair Program.

12.2 ELECTRICAL ACTIVATION AND REPOLARIZATION SEQUENCE IN THE RAINBOW TROUT HEART

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We investigated electrical activity of the ventricle of the isolated rainbow trout heart by optical mapping. We used 11, -300 g rainbow trout acclimated to 13°C and conducted experiments at 13°C using lithium to prevent movement and D-4-ANNEPEP to visualize transmembrane voltage. Results show activity progressing from the AV junction with a base to apex excitation profile. Adrenaline slowed conduction across the ventricular surface of the heart. No transmural differences in ven-tricular action potential (i.e. between sponge and compact layers) were observed. Adrenaline increased ventricular action potential duration and resulted in electrical alternans at fast stimulation frequencies. Conduction restitution was slowed and APD restitution was accelerated as conduction frequency was progressively increased. These results reveal interesting differences in ecto-thermal electrical activation and restitution compared with mammals particularly in relation to alternans and the effects of sympathetic stimulation on the electrical activity of the heart.

12.3 ANTI-OXIDANT DEFENSE MECHANISMS IN THE RED BLOOD CELLS OF LONGHORN SCULPIN (MYXOCEPHALUS OCTODECIMSPINUS) IN RESPONSE TO HYPOXIA AND RAPID RE-OXYGENATION

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Unlike their mammalian counterparts, teledost red blood cells (RBCs) retain a functioning nucleus and other organelles throughout their lifespan. Under natural conditions, all RBCs are continuously exposed to reactive oxygen species (ROS) as a consequence of their role as oxygen transporters. Teledost RBCs have an additional source of ROS due to their high rates of aerobic metabolism and functioning mitochondria. The current work aims to provide insight into the role of oxidative damage in the aging and/or removal of teledost RBCs from circulation. Levels of antioxidants (glutathione reductase state, glutathione peroxidase (GPx) and catalase) were measured in RBCs from longhorn sculpin (Myxoccephalus octodecimspinus) held under ambient conditions, during hypoxia (6% O2, saturation) and during a rapid reoxygenation event. Based on comparisons within the literature, levels of antioxidants in the sculpin RBCs are higher than mammalian RBCs, suggesting these cells are better poised to defend against oxidative stress. Additionally, both glutathione reductase state (GSH:GSSG) and GPx activity were significantly elevated after rapid reoxygenation (no significant change in catalase activity) it suggests that the sculpin RBCs are capable of defending against increased production of free radicals as a result of fluctuating environmental conditions. Funding for this study was provided by Mount Desert Island Fellowship in Molecular Physiology and Georgia Southern University FRC seed funding.

12.4: HEART RATE AND BLOOD OXYGEN DEPLETION IN LOGGERHEAD TURTLES, CARETTA CARETTA

Cassandn Williams1, Karina Sato2, Katherine Ponganis3, and Paul Ponganis4


Teledost turtles such as Caretta caretta exhibit extremely low heart rates and blood oxygenation levels. We investigated heart rate and blood oxygenation levels in Caretta caretta in response to hypoxia and rapid reoxygenation. We measured heart rate and blood oxygenation levels in Caretta caretta in response to hypoxia and rapid reoxygenation. We measured heart rate and blood oxygenation levels in Caretta caretta in response to hypoxia and rapid reoxygenation. We measured heart rate and blood oxygenation levels in Caretta caretta in response to hypoxia and rapid reoxygenation. We measured heart rate and blood oxygenation levels in Caretta caretta in response to hypoxia and rapid reoxygenation. We measured heart rate and blood oxygenation levels in Caretta caretta in response to hypoxia and rapid reoxygenation. We measured heart rate and blood oxygenation levels in Caretta caretta in response to hypoxia and rapid reoxygenation. We measured heart rate and blood oxygenation levels in Caretta caretta in response to hypoxia and rapid reoxygenation. We measured heart rate and blood oxygenation levels in Caretta caretta in response to hypoxia and rapid reoxygenation. We measured heart rate and blood oxygenation levels in Caretta caretta in response to hypoxia and rapid reoxygenation. We measured heart rate and blood oxygenation levels in Caretta caretta in response to hypoxia and rapid reoxygenation. We measured heart rate and blood oxygenation levels in Caretta caretta in response to hypoxia and rapid reoxygenation.
hydrogen sulfide may be regenerated from its oxidation products (such as thiosulfate and polysulfides) and contribute to depress metabolism of perfused tissues, while cysteine is primarily used to enhance synthesis of glutathione, a major cellular anti-oxidant. Oxygen binding curves of red blood cell lysates were left-shifted in hibernating bears due to decreased temperature and 2,3-diphosphoglycerate, a major hemoglobin cofactor, thus limiting oxygen delivery to tissues. In conclusion, our results indicate that hydrogen sulfide and organic phosphates play concerted roles in the suppression of oxygen consumption and delivery, respectively, of hibernating brown bears in their natural environment. This study was approved by the Swedish Ethical Committee on animal research and was supported by grants from the Danish Council for Independent Research, Natural Sciences, the National Institute of Health and the Scandinavian Brown Bear Research Project.

12.6 RESPIRATORY PHYSIOLOGY IN CETACEANS

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August Krogh, the grand-father of comparative physiology, stated that ‘For every defined physiological problem, there is an optimally suited animal that would most efficiently hold an answer to that unique mammal’. As an example of this physiological principle, they thrive in the extreme environment of the sea, readily coping with atelectasis, hypoxia, hypothermia, ischemia/reperfusion, and intravascular gas bubbles. Understanding cardiopulmonary physiology is vital to understanding the limitations that may affect survival. We study respiratory physiology in a large range of cetaceans, from harbor porpoise to beluga whale, to try to understand how pressure affects lung function. Cetaceans have compliant lungs and stiff conducting airways, in comparison to their terrestrial counterparts. These traits are assumed important for altering gas exchange during diving. However, recent work indicates great variability in the structural properties of the respiratory system between marine mammal species, possibly indicating species differences in diving ability. For all species studied, maximum expiratory flow rates exceed 140 L sec\(^{-1}\) while maximum inspiratory flow rates range between 10 to 40 L sec\(^{-1}\).

The measured esophageal pressure suggests that odontocetes exhibit passive pressure during spontaneous expiration, but actively during maximal expirations. Our results confirm an amazing respiratory capacity in cetaceans, and provide new data on chest compliance in odontocetes.

12.7 THE EVOLUTION OF UNIDIRECTIONAL PULMONARY AIRFLOW

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Bird lungs have conventionally been thought to be unique in having air flow through the lungs of a range of reptiles are revealing that birds are not unique in having unidirectional flow, the selective drivers for this trait, and the evolutionary history of this system. This research was funded by NSF (IOS – 0818973 and IOS 1055080).

12.8 NATURE VERSUS NURTURE: HYPOXIC CARDIOVASCULAR AND RESPIRATORY RESPONSES IN BAR-HEADED GEESE (ANSER INDICUS) AND RELATED WATERFOWL

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The biannual high altitude migration of bar-headed geese (Anser indicus) across the Himalayas is a remarkable feat. While respiratory and hematological O\(_2\) transport enhancements help facilitate this migration, little is known about the cardiovascular maintenance of O\(_2\) transport during hypoxia-the hypoxia cardiovascular response (HCVR). We characterized the intra- and interspecific plasticity in HCVR across altitude, comparing domestic BHG (born at 0m), wild BHG (born at 3200m), and domestic barnacle geese, a related species (Branta leucopsis; born at 0m). All birds were exposed to stepwise hypoxia while measuring cardiovascular (heart rate, stroke volume, cardiac output), respiratory (breathing frequency, tidal volume), and metabolic variables. Hypoxia exposure led to cardiac output increases driven by stroke volume. The HCVR of 0m BHG and barnacle geese did not differ. However, during normoxic exposure all variables in BHG returned to resting levels, whereas barnacle geese had an elevated heart rate and blood acidosis. This suggests that BHG matched O\(_2\) supply and demand during hypoxia, while barnacle geese used anaerobic metabolism. There were significant differences between 0m and 3200m BHG. 3200m BHG were more likely to occur under extreme environmental conditions, and to be expected to better adapt at lower P\(_O_2\). Thus, altitude matters–rearing at 0m profoundly impacts the BHG HCVR. Funding: SCERC, Vanier Canada, and Killam Trusts.

13.0: EVOLUTIONARY PHYSIOLOGY

13.1 EVIDENCE OF CONTRACURRENT VARIATION AND ADAPTIVE SLOW GROWTH RATE IN A MARINE ISOPOD (IDOTEA BALTICA) LOCALLY ADAPTED TO LOW SALINITY

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Local adaptation is crucial to the generation and maintenance of biodiversity, and is most likely to occur over strong environmental gradients in species with direct development. This study used a common garden approach to investigate the hypothesis that Baltic populations of the brooding marine isopod Idotea baltica (found in marine waters and in the low salinity Baltic Sea), are locally adapted to low salinity. These data provide the first indications of countergradient variation (CnGV) and a-adaptive slow growth rate in Baltic Idotea baltica in comparison to fully marine counterparts; Evidence of CnGV found in metabolic rate analysis, while reduced growth rate independent of salinity treatment demonstrated adaptive slow growth. Increased oxidative stress in both populations with increased deviation from respective salinity of origin conditions, and differential mortality in the salinity treatments (salinity 32, 20 and 5) also validate the hypothesis of local adaptation within the Baltic. The suite of traits under selection in low salinity Idotea baltica have the common theme of metabolic budget management, a commonality with CnGV over temperature gradients but this is the first data with respect to salinity. Identifying and understanding the evolutionary significance of hidden genetic variation resulting from CnGV over environmental gradients is essential for conservation biology and predicting the effects of environmental change.

13.2 HYBRID BREAKDOWN AND PHYSIOLOGICAL COMPENSATION IN GENE EXPRESSION IN THE COPEPOD TIGRIOPSIS CALIFORNICUS

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Allopatric populations diverge genetically as a result of mutation, selection and random drift. When divergent populations subsequently interbreed, interlocus interactions may lead to incompatibilities resulting in reduced hybrid fitness (hybrid breakdown). In the copepod Tigriopsis californicus, hybrids between populations frequently suffer from hybrid breakdown that has been traced to mitochondrial dysfunction. To better understand the molecular mechanisms underlying hybrid breakdown, we used RNA-seq to compare patterns of gene expression between two divergent natural populations and their reciprocal hybrids. Unlike previous studies that found extensive underexpression of genes in hybrids between sister species, only 1.2% of genes in the transcriptome were misexpressed in T. californicus interpopulation hybrids, and nearly 8% of these were overexpressed rather than underexpressed. Moreover, the functional pathways encompassing the misexpressed genes (including OXPHOS and antioxidant response) are largely consistent with the known physiological consequences of mitochondrial incompatibilities in hybrid T. californicus. These results indicate that gene regulation in low-fitness interpopulation hybrids includes a signature of compensatory responses to mitochondrial dysfunction. Our results suggest hybrid breakdown at early stages of speciation may result from initial incompatibilities amplified by the cost of compensatory physiological responses. Supported by NSF DEB1051057.

13.3 VARIATION BETWEEN STICKLEBACK POPULATIONS IN COLD TOLERANCE AND MECHANISMS OF FRESHWATER IONO-REGULATION

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Most studies of adaptive divergence in physiological traits in different environments have focused on single abiotic factors in isolation, yet environments exert the effect in only a single abiotic factor. Here, we use threespine stickleback (Gasterosteus aculeatus) to examine potential adaptive divergence in response to both low salinity and...
cold temperatures by examining variation between populations in cold tolerance, growth at low temperature, and the effects of temperature on mechanisms of freshwater ionic regulation. We show that freshwater-resident stickleback have greater tolerance of acute exposure to low temperatures than do marine stickleback, but that this difference in cold tolerance is abolished with acclimation to low temperatures. Although acclimated marine stickleback could tolerate acute exposure to cold temperatures, they did not grow as well as freshwater stickleback at low temperature. Because size at first reproduction is strongly associated with fecundity in stickleback, these data suggest that winter temperatures during the first year of life could represent a barrier to colonization of freshwater habitats. There were substantial differences in ion transporter mRNA levels in the gill between populations and in response to low temperature exposure in fish in fresh water. These data suggest that challenges associated with ionic regulation in cold freshwater may, in part, explain the poor growth of marine stickleback in cold freshwater. Funded by NSERC.

13.4 TRANSGENERATIONAL INHERITANCE OF A STRESS PROTEOME IN THE LEAST KILLIFISH, HETERANDRIA FORMOSA, EXPOSED TO COPPER DURING EARLY LIFE STAGE.
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Nowadays, there is increasing evidence that pollutants can induce transgenerational impacts in a population. Assessing the toxicity of chemicals must take into consideration the possible inheritance of their effects through generations. In the present study, we have tested the hypothesis that an exposure to Cu in fish early life stage (ELS) can modify the protein expression profile in offspring. One week old viviparous least killfish, Heterandria formosa, were exposed to Cu at 15 μg/L for a period of one week, a condition inducing physiological adaptation. Fish were then held in clean water till maturity. After breeding, the expression profile of cytotoxic proteins of 2 weeks old larvae was analyzed using 2D-DIGE followed by protein identification by nano-LC-ESI-MS/MS. We observed that Cu exposure in parents affects the expression of 50 protein spots in offspring, categorized into diverse functional classes related to protein turnover, chaperoning, metabolic process, ion transport or oxidative stress. Furthermore, we determined global DNA methylation in parents and in offspring using the LUMinometric Methylation Assay (LUMA). In conclusion, this study originally provides evidence that an exposure to a pollutant during ELS in a fish can affect the cellular phenotype in the offspring, assessed at the proteomic level. Ongoing researches will investigate the possible role played by epigenetics in this phenotypic inheritance.

13.5 RAPID EVOLUTION OF PHYSIOLOGY IN LABORATORY POPULATIONS OF DROSOPHILA
James N. Kezos, Larry G. Cabral, Laurence D. Mueller and and Michael R. Rose
We have examined a wide range of physiological characters in 30 outbred Drosophila melanogaster populations, all of which descend from one common ancestral population. These populations have well-known phylogenetic relationships and selection histories over the course of more than 30 years of sustained and replicated parallel selection. We have phenotypically compared these populations for the following characters: starvation resistance, desiccation resistance, flight endurance, and cardiac performance. The 30 populations in this study include long-established populations (Acoα, Boro, and COα) and newly-established populations with corresponding recent selection regimes (Acoβ, Boro, and COβ). There was extensive physiological convergence between the long-established and newly-established populations subjected to the same selection regime. There was also rapid divergence among the populations newly subjected to the three different selection regimes. By conducting electric pacing and flight exhaustion assays with manipulative conditioning, we have started to unpack the physiological relationships between cardiac function, locomotor performance, and such other functional characters as fecundity, longevity and stress resistance. By conducting these manipulative experiments, we determined which particular stresses reduce or improve heart function, as well as the role of metabolic reserves in cardiac function.

13.6 PHYSIOLOGICAL SYNERGISM AND ANTAGONISM IN THE EVOLUTION OF LIFE HISTORIES
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Evolution acts on multiple traits simultaneously. We know little of how physiology enables or constrains the response to simultaneous selection on multiple traits such as body size and development time. From previous work, these two life history traits are regulated by the interaction of three physiological factors: growth rate, the timing of the cessation of juvenile hormone secretion and the timing of secretion of the ecdysteroid hormones. Here we show how antagonism and synergism at the physiological level enables and constrains simultaneous selection at the phenotypic level of the two life history traits in the hawkmoth Manduca sexta. After 10 generations of selection on all four combinations of body size and development time the three physiological factors explained 93% of the response of development time to simultaneous selection and 99% of the response of body size. When the two life history traits were under synergistic selection, the response to selection was due largely to hormonal regulation and constrained by growth rate. When the life history traits were under antagonistic selection, the response to selection was due primarily to the change in growth rate and constrained by the two hormonal traits. Evidence suggests that the framework used here for the regulation of the response to simultaneous selection has broad applicability to a diverse range of taxa including green algae, plants, amphibians, mammals and other insects. Funding was provided by NSF-IOS.

13.7 WHAT MIRR MEDIATE LIFE-HISTORY TRAITS? CORRELATED CHANGES IN MICE SELECTED FOR MASS-DEPENDENT MMR
Cynthia Downs1, Jessi Brown2, Bernard Wone1, Edward Donovan1, and Jack P. Hayes2
Basal metabolic rate (BMR) is commonly used as a measure of animal energetics in inquiries into potential trade-offs between metabolic expenditure and life-history traits. Maximal metabolic rate (MMR) measures the maximal aerobic capacity to metabolize energy, and might be involved in trade-offs. We tested how selection on different aspects of metabolic rates in laboratory mice (Mus musculus) affected immune responses, growth trajectories, and food consumption. Mice were bred for high mass-independent MMR, high mass-independent MMR plus low mass-independent BMR, or randomly with regard to metabolic rates. Selection for high mass-independent MMR suppressed inflammatory responses, but not antibody responses. Selection also altered overall growth patterns and increased maximal growth rates and food consumption. Changes in growth and immunology were correlated with evolved changes in mass-adjusted MMR, while changes in food consumption were correlated with evolved changes in mass-adjusted BMR. Consequently, MMR might be an important mediator of life-history traits, and measurements of MMR would provide insights when investigating associations between metabolism and life-history traits. Funded by NSF IOS-0348944 to JPH.

14.1 MOLECULAR EVOLUTION AND ADAPTATION OF INSULIN-LIKE/TOR SIGNALING ACROSS AMNIOTES
Suzanne McGaughey1, Tonia Schwartz2, and Anne Bronkowski3
Molecular evolution of the Insulin/Insulin-like Signaling (IIS) and Target of Rapamycin (TOR) network is central to modulating physiology, metabolic and life-history traits including growth, reproduction and aging. Nonetheless, it remains unclear which genes in the network are generally targets of positive versus purifying selection. Here, we focus on the divergence of the IIS/TOR network between amniote clades that are divergent in many aspects of their physiological: reptiles (including birds) and mammals. We examined 61 genes in the IIS/TOR network across 66 amniote species - the most comprehensive taxonomic depth to date; including 18 liver transcriptomes we generated from non-avian reptiles. We found that 1) members of the IIS/TOR network have exceptional evolutionary rates between reptiles and mammals, 2) extracellular and membrane genes of the network evolve faster and are under stronger positive selection than intracellular signaling genes, 3) genes with fewer interactions with other proteins/genes have faster evolutionary rates than genes with higher connectivity, and 4) the two primary ligands (igf1 and igf2) have opposite patterns of selection in mammals vs. reptiles. The phylogenetic depth and breadth of our data, along with the patterns of divergence in this network suggests that IIS/TOR network may be one key to the diversification of physiological and life-history traits between reptiles and mammals. NSFs I061253896, ISU CIAG, JSM Foundation.

14.8: METABOLISM, ENERGETICS, AND PERFORMANCE

14.1 ONTOGENETIC AND INTERSPECIFIC METABOLIC SCALING IN INSECTS
James Mamo1, and Michael Kearney2
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The metabolic consequences of feeding and digestion (specific dynamic action) were investigated in a number of decapod species. The animals exhibited the typical pattern of postprandial oxygen uptake characterized by a rapid increase, followed by a more prolonged decrease to pre-feeding levels. There was an increase in the SDA with increasing meal size. However, unlike other taxa, this increase was primarily afforded by an increase in the duration oxygen uptake remained elevated, rather than an increase in peak oxygen consumption. The texture of the meal appeared to play a greater role than the actual nutrient content; harder meals required more energy to digest. While previous work on non-decapod crustaceans suggests that mechanical digestion only comprises a small part of the overall SDA budget, the current findings showed that 30% of the SDA budget could be due to mechanical digestion. This is not unexpected since the gastric mill, which processes food, is a complex apparatus controlled by over 40 muscles. In fish, some characteristics of the SDA response are closely related to food transit rates, whereas in crustaceans there was no relationship, probably due to differences in architecture and functioning of the gut. The metabolic changes associated with the SDA are discussed in relation to environmental perturbations and the ability of animals to balance the simultaneous demands of several physiological systems. Funding: NSERC.

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VALIDATION OF THE RELATIONSHIP BETWEEN 3-DIMENSIONAL BODY ACCELERATION AND OXYGEN CONSUMPTION IN TRAINED STELLER SEA LIONS (EUMETOPUS JUBATUS) DIVING WITH INCREASED OXYGEN DEPLETION

A ROLE FOR SEPTEJCTATION PROTEINS IN THE REGULATION OF SALT AND WATER BALANCE IN LARVAL MOSQUITO (Aedes aegypti)

ABUNDANCE AND LOCALIZATION OF BRANCHIAL CLAUDINS IN RAINBOW TROUT (Oncorhyncus mykiss) AND IMPRICTIONS IN HYPOOSMOREGULATION

HYPOTONICITY STIMULATES K+ FLUX THROUGH THE WNK-SPAKOSRI KINASE CASCADE AND THE NCC90 SODIUM-POTASSIUM-2-CHLORIDE COTRANSPORTER IN THE DROSOPHILA RENAL TUBULE

A ROLE FOR SEPTE JUNCTION PROTEINS IN THE REGULATION OF SALT AND WATER BALANCE IN LARVAL MOSQUITO (Aedes aegypti)

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A ROLE FOR SEPTEJCTATION PROTEINS IN THE REGULATION OF SALT AND WATER BALANCE IN LARVAL MOSQUITO (Aedes aegypti)
using proteins purified from E. coli. Results: Decreasing Drosophila NKV activity in the principal cell of the tubule caused a reduction in K⁺ flux. Similarly, knocking down the SPAK/OSR1 homolog, fray also decreased K⁺ flux. A hierarchical WNK-Fray signaling cascade regulates K⁺ flux through Ncc69, since i) a constitutively active Fray mutant rescued the weak knockout phenotype; ii) Fray directly phosphorylates Ncc69 in vitro; and iii) the effect of weak and fray knockdown was abolished in Ncc69 mutants. The stimulatory effect of hypotonicity on K⁺ flux was absent in weak, fray, or Ncc69 mutant tubules. Conclusion: Hypotonic conditions stimulate K⁺ flux through the activation of the WNK-Fray-Ncc69 pathway in the principal cell of the fly renal tubule. Increased net fluid secretion provides a mechanism for enhanced clearance of a water load and the homeostatic maintenance of extracellular osmolality.

15.5 ANURAN-SPECIFIC AQUAPORIN 2 HOMOLOG IN A URDELO, EVIDENCE FOR EARLY GENE DUPLICATION IN AMPHIBIAN EVOLUTION
Yuki Shimizu1, Masakazu Suzuki1, Takatoshi Nagai1, and Stanley Hilliard1
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Amphibians are unique among living vertebrates in that their skin serves as a primary surface for water absorption from pond water and moist substrates. Cutaneous water absorption by anuran amphibians is regulated by arginine vasotocin-stimulated insertion of anuranspecific aquaporins (AQPa2s) into the apical membrane of the outermost living cell layer. Systematic analysis suggests that AQPa2s are syntenic with AQPa2, and they arose from gene duplication in the course of amphibian evolution. The chronology for the divergence of urodèles (order caudata) from anurans is hotly debated but dates to at least the Permian era. Our hypothesis is that the gene duplication that gave rise to "anuran-specific" AQPa2s occurred prior to this divergence. Here we characterize an aquaporin from the skin of the newt, Notophthalmus viridescens, that has sequence identity intermediate between AQPa2s and AQPa2. Immunohistochemistry shows this aquaporin (AQPa-n3) to be localized to the apical membrane when dehydrated newts rhynolinate. These observations demonstrate the gene duplication giving rise to cutaneous expression of AQPa2 homologs occurred prior to the divergence of anurans and urodèles. Supported by Grants-in Aid for Scientific Research (26440165) from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

15.6 SEQUENCING AND GENE EXPRESSION OF AQUAPORIN-9 IN FREEZE TOLERANT COPE’S GRAY TREEFROGS
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Cope’s gray treefrogs Hyla chrysoscelis accumulate glycercol in response to cold as part of their freeze tolerance mechanism. This gercerol is suspected to derive from glucose that is stored in the liver before it is exported through the bloodstream to other tissues. We therefore hypothesized that tissues from Hyla chrysoscelis, including hepatocytes, would express aquaporin 9 (AQPa9), a transmembrane protein from the aquaglyceroporin family that facilitates glycerol transport. We also hypothesized that AQPa9 would be up-regulated during cold acclimation to promote glycerol permeability. To test this hypothesis, we designed primers based on published AQPa9 sequences, generated cDNA from mRNA extracted from liver, and identified a gene, Hc-AQPa9, with high homology (69%) to human AQPa9. Quantitative PCR analysis revealed Hc-AQPa9 expression in liver, stomach, bladder, kidney, eye, lung, muscle, and skin (but not in intestine, brain, heart, fat, or red blood cells). In tissues from frogs that had been cold acclimated, there was no statistical up-regulation of Hc-AQPa9, and a statistical down-regulation in muscle and stomach, contrary to our hypothesis. In frozen and thawed animals, expression of Hc-AQPa9 mRNA was down-regulated in most tissues. Future assessment of Hc-AQPa9 protein expression will help to identify the role of this protein in cold acclimation and freeze tolerance. Supported by NSF IOS-1121457.

15.7 GENE EXPRESSION OF THREE ISOFORMS OF UREA TRANSPORTER IN AN AESTIVATING AFRICAN LUNGFISH
Biyan Ching1, You R. Ching1, Jasmine L. Y. Ong1, Xia L. Chen1, Wai P. Wong1, Siew H. Lam2, Shih F. Chow2, and Yuen K. Ip3

The African lungfish, Protopterus annectens, is ammonegenic in water. However, it increases urea synthesis and accumulation during the induction and maintenance phases of aestivation and decreases urea upon arousal from aestivation. The drastic increase in urea excretion upon arousal necessitates the involvement of urea transporters (UTs). This study aimed to clone and sequence isoforms of UT from the liver of Protopterus annectens and examine their mRNA expression in various tissues/organs of during the three phases of aestivation. Three UT isoforms were expressed by P. annectens and aestivation had different effects on their mRNA expression in various tissues/organs. Our results shed light on how urea transport was regulated in different tissues/organs in P. annectens during the transition between anammonoty and urea accumulation during the induction phase of aestivation and the transition between urea accumulation and ureotely during the arousal phase of aestivation. This study was approved by the NUS IACUC.

15.8 DEHYDRATION AND THIRST OF HYDROPHIINE SEA SNAKES
Harvey Lillywhite1,2, Harold Heatwole1,2, and Coleman Shelly3
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Secondary marine vertebrates are challenged to obtain water in saline environments. Recent investigations have indicated that several species of marine snake dehydrate at sea and are dependent on environmental sources of fresh water, representing a shift of paradigm from previous "textbook" literature. However, freshwater requirements in the majority of sea snakes, represented by hydrophtine species, are not known. Hence, we investigated the extent of dehydration and responses to fresh water in five species of hydromphine sea snakes collected during seasonal drought near Weipa, Cape York Peninsula, Australia. Very few snakes that we collected drank fresh water immediately following their capture, even though measurements of total body water and condition of feces indicated these snakes were dehydrated. None of these species drank sea water after dehydration in air and offered sea water or fresh water to drink. Dehydrated individuals of three species drank fresh water, but only at mean dehydration thresholds of ~26 to ~29 % of initial body mass. Two other species did not drink fresh water when similarly dehydrated. Available data indicate that sea snakes have relatively high levels of total body water (around 80% of body mass), are comparatively resistant to dehydration, and have diverse thresholds for thirst. New data indicate that some species possibly live independently of environmental fresh water. Funding was provided by NSF grant IOS-0920802 to HBL.

16.0: TRAINEE WORKSHOP: NON-TRADITIONAL CAREER PATHS FOR COMPARATIVE PHYSIOLOGISTS

16.1 THE SOFT MONEY RESEARCH POSITION: HOW DOES IT WORK? Shawn Noren
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The number of students receiving doctorates in biology in the United States increased from 3,803 in 1981 to 8,135 in 2011. Meanwhile the number of biological-science Ph.D. recipients in tenure-track positions dropped precipitously from 55% in 1973 to 15% in 2006, and industry has not absorbed the slack. Soft money research positions, where funding comes from grants and contracts, are an alternative to maintaining a research paradigm from previous "textbook" literature. However, freshwater requirements in the majority of sea snakes, represented by hydrophtine species, are not known. Hence, we investigated the extent of dehydration and responses to fresh water in five species of hydromphine sea snakes collected during seasonal drought near Weipa, Cape York Peninsula, Australia. Very few snakes that we collected drank fresh water immediately following their capture, even though measurements of total body water and condition of feces indicated these snakes were dehydrated. None of these species drank sea water after dehydration in air and offered sea water or fresh water to drink. Dehydrated individuals of three species drank fresh water, but only at mean dehydration thresholds of ~26 to ~29 % of initial body mass. Two other species did not drink fresh water when similarly dehydrated. Available data indicate that sea snakes have relatively high levels of total body water (around 80% of body mass), are comparatively resistant to dehydration, and have diverse thresholds for thirst. New data indicate that some species possibly live independently of environmental fresh water. Funding was provided by NSF grant IOS-0920802 to HBL.

16.2 SCIENCE FOR THE PUBLIC! CAREERS IN SCIENCE CENTERS AND MUSEUMS
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Intrigued by the possibility of bringing your knowledge and passion for science to non-specialists? Interested in working with teachers, K-12 students, and the general public? Curious about what it takes to be a scientist in a museum or science center? Well discuss the joys and challenges of being a scientist doing "informal" science, and look at the variety of paths to that end.

16.3 TAKING THE ROAD LESS TRAVELED: NON-GOVERNMENTAL ORGANIZATIONS
Dorian Houser
THE PHYSIOLOGIST

These evolutionary changes occurred in parallel across freshwater habitats, serving as stepping-stones for the colonization of land. Invasive species are often striking in their capacity to extend their ranges into novel habitats, providing valuable models for studying incipient adaptations during colonizations. Within the past century the copepod Eurytemora affinis has invaded freshwater habitats multiple times independently from saline sources. These invasions were accompanied by evolutionary changes in activity and expression of ion transport enzymes (V-type H⁺ATPase, Na⁺−K⁺ATPase) and Na⁺−K⁺−2Cl⁻ co-transporter (Slc12a3) as has also been shown in some teleost fishes. However, in freshwater, hyper-osmoregulation of these transporters is consistent with the action of natural selection and can provide insight into the mechanisms of ionoregulation in freshwater fishes.

17.3 OSMOREGULATION IN THE ANADROMOUS LAMPERY PETROMYZON MARINUS

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Within this set of considerations ranging from a career move to academia to the private sector. For biologists, the private sector available is, in large part, composed of the pharmaceutical industry, the biotechnology industry, and contract research groups (both non-profit and for-profit). What is similar between academic labs and private-sector labs, and conversely what is different? Also, it is important to note that the avenue enables opportunities that might otherwise be limited by institutional affiliation and can be a lucrative path to the motivated researcher.
18.1 CRITICAL WINDOWS IN ANIMAL DEVELOPMENT: STRESSOR DOSE, EFFECT SIZE AND EXPERIMENTAL DESIGN

Casey A. Mueller

An animal’s developmental trajectory is a result of interactions between genome and the environment. The ability to modify phenotype via phenotypic plasticity allows animals to cope with challenges during development, including altered environmental conditions. Critical windows are periods during embryonic development and/or early life when phenotypes are particularly plastic and responsive to intrinsic or extrinsic factors. Using embryonic Lake whitefish (Coregonus clupeaformis), I demonstrate how critical windows can be viewed as 1-dimensional - discrete periods during development in which phenotype modification occurs. However, a 3-dimensional construct in which the relationship between time during development, dose of the stressor applied, and the resultant phenotypic modification can define a critical window in much more detail. Using the example of survival and morphology of larval Artemia raised in different salinity levels during development, I demonstrate how the interaction between exposure time and stressor dose causes a measured phenotypic change, which itself may vary within a critical window. This 3-dimensional experimental design potentially reveals much more about interactions between environment and phenotype during development than traditional chronic exposure studies. Reference: Burggren WW & Mueller CA 2014. A 3-dimensional, system approach for developmental critical windows and sensitive periods: Is a "window with sharp edges" too simplistic a view? Physiol Biochem Zool, in review.

18.2 NOISY EMBRYOS: THE POTENTIAL EVOLUTIONARY IMPORTANCE OF VARIATION IN THE TIMING OF DEVELOPMENTAL EVENTS

Simon Rundle

Heterochrony - differences in the timing of developmental events between species - is considered by some as a key link between ontogeny (development) and phylogeny (evolution). However, evidence for the mechanistic basis of heterochrony is limited. Here I suggest how variation in the timing of developmental events within species could act as the raw material for the evolution of heterochrony. In vivo measurements from developing embryos have demonstrated levels of heterochrony within a clade of freshwater gastropods similar to those observed in vertebrates. High levels of variation in the timing of developmental events have also been demonstrated in the freshwater pulmonate snail Radix. However, variation is increased in stressed embryos. Moreover, a parent-offspring comparison of development showed that the timing of some events such as shell formation and the initiation of crawling are heritable; the fitness benefits of such variation are currently being explored. Together these findings suggest that intraspecific variation in developmental event timing could provide the raw material for selection to produce heterochrony. References: Tills, O.; Rundle, S.D. & Spicer, J. (2013) Parent-offspring similarity in the timing of developmental events: a potential link between ontogeny and phylogeny. Proceedings of the Royal Society B 280: 1769 doi:10.1098/rspb.2013.1479. Rundle, S.D.; Smarttaitte, J.; Colbert, M.W. & Spicer, J.J. (2011) Predator cues alter the sequence of developmental events in gastropod embryos. Biology Letters 7: 285-287.

18.3 MITIGATING THE RISKS ASSOCIATED WITH ACCELERATED OR DEFICIENT PERINATEL GROWTH

Robert Rodríguez

hatch in riverine FW but must then develop in brackish estuarine waters. The P shrimps are land-locked in FW. All stages hyper-regulate in FW but are osmo-conformers at salinities ≥17 ppt. A and P shrimps in FW produce hypertonic urine. Gill develop faster in P vs A larvae, so that Na+K-ATPase (NKA) is localized in early larval gills of P, but only in branchiostegites of A. In juvenile A and P, V-H-ATPase (VHA) and NKA are localized in a single branchiostegite cell type. In FW gills, VHA is localized apically in pillar cells, while NKA is restricted to septal cells. These cells likely cooperate in ion uptake. In A shrimps, VHA mRNA expression is higher in FW. In P shrimps, the loss of hypo-osmoregulation may be related to the lack of ion transport in the branchiostegites, and the ability of all stages to hyper-regulate in FW may be due to the early development of functional gills. These results illustrate evolutionary adaptations (loss and gain of functions) underlying the invasion of FW habitats. Reference: Boudour-Boucheker N, Boulo V, Lorin-Nebel C, Elguero C, Grousset E, Anger K, Charpentier-Daures M, Charpentier G. Adaptation to freshwater in the palaeomshrimp Macrobrachium amazonicum: comparative ontogeny of osmoregulatory organs. Cell Tissue Research 353, 87-98. 2013.
Evidence suggests nitrogen may be limiting, not through being in short supply, but rather its excess relative to carbohydrate and the control it exerts over nutrient uptake. We discuss the potential ecological and evolutionary consequences of how the GIT functions. Reference: Clissold FJ, Tedder BJ, Conigrave AD, Simpson SJ, 2010. The gastrointestinal tract as a nutrient-balancing organ. Proc B 277:1751-9.

19.2 EVOLUTIONARY AND MOLECULAR MECHANISMS UNDERLYING INTESTINAL FLEXIBILITY FOR SNAKES
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Snakes possess an adaptive interplay between feeding ecology and digestive physiology. Frequently feeding snakes modestly regulate intestinal performance whereas infrequently feeding species up and down regulate intestinal form and function with each meal. The selective benefit of down regulation for infrequent feeders is a reduction in energy expenditure while fasting, whereas maintaining intestinal readiness is more beneficial for frequent feeders. Cellular mechanisms underlining modest and wide regulation of intestinal function reside in the modulation of microvilli length and hence brushborder surface area. Frequently feeding snakes experience no post-prandial change in microvilli length and no change in function. Wide regulation of intestinal function for infrequently feeders is explained by a 5-fold modulation of microvilli length. These modes of intestinal regulation stem from distinct gene expression regimes with greater expression changes predicted for infrequently feeding snakes. Infrequently feeding Burmese pythons experience massive shifts in expression of regulatory, trafficking, structural, and transporter-associated genes that direct intestinal flexibility. The link between snake feeding habits and digestive physiology is founded in the integrated evolution of cellular and molecular programs that underlie adaptive phenotypic responses (NSF IOB046139). Secor, SM 2005 Evolutionary and cellular mechanisms regulating intestinal performance of amphibians and reptiles. Integr Comp Biol 45:66-78. Castoe TA et al 2013 The Burmese python genome reveals the molecular basis for extreme adaptation in snakes. PNAS 110:20645-20650.

19.3 THE ROLE OF GUT MICROFLORA IN THE NUTRITION OF MARINE HERBIVOROUS FISHES
Kendall Clements1, Lilly Bajrach1, Kate Johnson1, Selena McMillan1, Lindsey White2, and Esther Anquet1
Hindgut microbites contribute to host energy supply by converting dietary carbohydrates to short-chain fatty acids, and can also supply amino acids in some animals. We examined three aspects of symbiont protein supply to marine herbivorous fishes: (i) gut histology and ultrastructure; (ii) nitrogen metabolism of gut microbiota, principally nitrogen fixation; and (iii) stable isotope analysis of nitrogen uptake by fish from dietary algae and hindgut microbes. Fish species varied in gut structure and symbiont distribution. Algae were more degraded in species where bacteria attached to algal fragments. The dinoflagellate reduse gene nih was amplified from hindgut samples of all fishes surveyed, and expression was demonstrated by RT-Q-PCR. Nitrogen fixation was detected by acetylene reduction and 15N in some cases. Our results show that hindgut microbites contribute amino acids to fish tissues, and some microbial amino acids incorporated by fish include nitrogen fixed in the gut. Support: Marsden Fund UOA0908. References: Clements KD, Anquet ER, Montgomery WL, Chot JH (2014) Intestinal microbiota in fishes: what’s known, and what’s not. Mol Ecol 23: 1891-1898.

19.4 AMYLASE GENETICS AND BIOCHEMISTRY UNDERLIE A DIGESTIVE SPECIALIZATION IN PRICKLEBACK FISHES
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Many herbivorous vertebrates have evolved elevated amylase activities in their guts, which usually arises via increased amylase gene copy number. I compared amylase gene sequences, amylase gene copy number, promoter region variation, and amylase biochemistry in prickleback fishes (family Stichaeidae) with different diets to better understand the underpinnings of dietary amylase activity variation. The herbivorous Cebidichthys violaceus expresses at least two amylase isoforms, and has 14 copies of amylase in its genome with two different promoter regions associated with each isoform. The herbivorous Xiphister mucosus, as well as four other omnivorous or carnivorous pricklebacks, express one amylase isoform, and have four to six copies of amylase in their genomes with a single promoter region. Thus, elevated amylase activity in X. mucosus is largely explained by elevated expression of one gene with relatively low copy number, a novel result for vertebrate amylases. However, despite their genetic differences, and independent evolution of herbivory, assays on intestinal homogenates suggest that C. violaceus and X. mucosus digest different starches with similar efficiencies. These data have implications for the digestion of algal starches, as green algae use amylase and red algae use amylopentin as their storage poly-saccharides, respectively. Therefore, convergent evolution of elevated amylase activity in C. violaceus and X. mucosus has different genetic underpinnings, but potential functional consequences remain to be revealed. UCI Startup Funds.

20.0: RESPONSES TO GLOBAL CHANGE: ACCLIMATIZE, ADAPT OR DIE

20.1 TRAIT-BASED APPROACHES TO PREDICTING THE RESPONSES OF SPECIES TO GLOBAL CHANGE
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Changes in climate and land-use are occurring rapidly, and at a global scale. Because millions of species are impacted, we cannot develop predictions for how each individual species might respond. Rather, we need a predictive framework that reduces the dimensionality of this task by identifying key characteristics of those taxa and regions that are most at risk. Here, we explore two such trait-based approaches to predicting responses. First, we focus on the predictive ability of physiological tolerance of high temperatures in ants. Globally, we find that ants inhabiting lower latitudes tend to be at the greatest risk under climate change owing to environmental temperatures being close to their thermal limits. Similarly, we find that among two large-scale experimental warming areas, positioned at the northern and southern boundaries of temperate hardwood forests in eastern North America, ant thermal tolerance was strongly predictive of ant density at the low latitude site where temperatures routinely exceed ant thermal limits, but not the high latitude site where temperatures remain below ant thermal limits. Second, we focus on the predictive ability of species' resource-use and demographic traits in context of butterfly phenological responses to urbanization-based changes in land-use against a naturally occurring geographic temperature gradient. We find that opportunistic species avoid the phenological delays characteristic of many species inhabiting highly urbanized and geographically warm areas. These case studies suggest trait-based approaches may be useful for generalizing responses to other systems.

20.2 OCEAN ACIDIFICATION EFFECTS ON TEMPERATE ROCKFISHES
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The North American west coast provides a natural experimental system for testing the effects of ocean acidification on marine organisms. Congeners with varying exposure to acidified waters during spring upwelling may have different physiological tolerances. Rockfish (Sebastes spp) provide an excellent comparative study system comprising >100 species with diverse life history traits and niches. We compared physiological and gene expression differences between juvenile winter- and spring-spawning rockfish, blue (Sebastes mystinus) and copper rockfish (S. caurinus), respectively, after chronic exposure to four pCO2 treatments. Both species exhibited decreased critical swimming speeds and copper rockfish also had reduced aerobic scopes at the highest pCO2 treatment. RNAseq transcriptome analyses of muscle tissue from the same individuals showed that the species exhibit divergent gene regulation strategies for coping with high pCO2. Among pCO2 treatments, each species had 100s of differentially expressed genes, but fewer than 20 were common to both species. Transcriptome profiles of physiologically tolerant rockfish may help explain the mechanistic basis underlying their increased ability to compensate against the effects of elevated pCO2. Our study highlights the need for integrative comparative studies for assessing adaptive capacity of fishes in responding to ocean acidification. Hofmann GE, Todgham AE (2010). Living in the new: physiological mechanisms to tolerate a rapidly changing environment. Annu. Rev. Physiol., 72, 127-145.

20.3 MECHANISTIC OVERLAP BETWEEN PLASTIC AND EVOLVED RESPONSES TO HEAT STRESS
Morgan Kelly
Organisms and populations may respond to changing environments through plasticity or evolutionary adaptation. While these have historically been viewed as separate processes, the genetic assimilation hypothesis holds that environmentally induced (plastic) phenotypes are often the first step in adaptation to environmental change, and later become genetically “assimilated,” such that the original environmental stimulus is no longer required to produce the phenotype. We tested this hypothesis by com-
hining artificial selection and heat shock experiments with RNA sequencing of the crustacean *Tigipes californicus*. Transcriptional responses to heat shock and artificial selection suggest substantial mechanistic overlap between plastic and evolved response to heat stress, and provide some of the first experimental support for the genetic assimilation hypothesis. To the extent that adaptation to changing environments tends to occur through genetic assimilation, our results suggest that plasticity and evolution may provide overlapping, rather than additive benefits to species responding to global change.

20.4 COMPARING PHYSIOLOGICAL PLASTICITY VS EVOLUTIONARY ADAPTATION VS PHYLOGENETIC CONSTRAINT ON SPECIES DISTRIBUTIONS: DROSOPHILA AND BEYOND

Ary Hoffmann

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Insects including *Drosophila* species have proven to be good experimental subjects for comparing the relative importance of plastic changes versus inherent genetic variation in physiological adaptation because the experimental environment can be tightly controlled across generations. Here we report on a unique comparison of Drosophila species from eastern Australia and beyond. Variation in physiological traits was closely tied to species distributions; in particular, responses to extreme conditions of cold, aridity and heat (under dry conditions) correlated closely with the ecological niche of the species. Species had similar levels of plasticity to thermal extremes but differed markedly in resistance levels. There was little variation in species performing across constant culture temperatures, highlighting the importance of genetically-based resistance to extremes in dictating distributions. There was strong phylogenetic signal in responses to extremes likely to be tied to genome differences including multi-copy gene families. Mid-latitude species/populations are more vulnerable to climate change than those at latitudinal extremes. Comparative genomics is providing an unprecedented picture of the genetic architecture of species confined to particular climatic zones. ARC 120100916 and ARC FL100100066. Overgaard, J., Kearney, M.R., & Hoffmann, A.A. 2014. *Glob. Change Biol.* 20, 1738. Kellermann, V., Overgaard, J., Loechelke, V., Kristensen, T.N., & Hoffmann, A.A. (2013). *Proc. Natl. Acad. Sci. U.S.A.* 110, 27972.

21.0 EVOLUTIONARY AND DEVELOPMENTAL ORIGINS OF ENDOOTHERMY

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A standard assumption of reptilian metabolism and thermoregulation has centred on their strong reliance on behavioural control over body temperature, due in part to their dependence on the environment for body heat (e.g. ectothermy). Numerous large reptiles, however, are known to exhibit thermal inertia sufficient to bestowed prolonged elevation of body temperature above ambient. In some cases, homeothermy is made possible via constitutive muscle recruitment. More recently, however, we have discovered that tegu lizards exhibit a form of reproductive endothermy, with elevated, sustained metabolic rates associated with parental care (SusMR). I review studies aimed at testing predictions of these hypotheses, i.e. an inexorable link between (i) BMR and MMR, or (ii) BMR and SusMR, as well as their underlying physiological mechanisms. There is mounting evidence of ample genetic variation in BMR and MMR, a pre-requisite for their concerted evolution. On the other hand, failure to demonstrate a positive association between BMR and SusMR mostly stems from the mismatch in the timing of variation in BMR and SusMR, rather than from lack of a functional link between the two. I discuss the discrepancies between conclusions drawn from intra- and inter-specific studies on the significant factors affecting variation in traits underlying BMR, MMR and SusMR, such as the contribution of skeletal muscles and the fatty acid composition of cell membranes. Inconsistencies in the results of studies testing the hypotheses of the evolution of endothermy can be resolved by (i) artificial selection experiments emulating mechanisms of evolution of metabolic rates and correlated traits, and (ii) focused comparative studies on animals from slow-fast life history continuum represented by species from tropical and temperate geographical zones. Reference: Konarzewski, M., Kisajć, A. 2013. Determinants of intra-specific variation in basal metabolic rate. J. Comp. Physiol. B, 83, 27-41.

21.4 DEVELOPMENT OF ENDOOTHERMY IN ALTRICIAL AND PRECOCIAL BIRDS

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All birds begin life with an ectothermic phenotype and develop endothermy sometime after hatching. Preococial species attain an endothermic phenotype rapidly upon hatching which requires high metabolic rate, thermal insulation, and an internal thermostat to stimulate heat production upon cooling. An ectothermic phenotype exists in altricial species for an extended period. To better understand development of endothermy in a number of species, we characterized changes in aerobic capacity, from hatchling to whole animal, along with concomitant changes in cardio-respiratory development and thyroid hormone levels. A common pattern in precocial species is a rapid increase in skeletal muscle mitochondrial oxidative respiration associated with an endothermic metabolic response to cooling immediately after hatching. Altricial species show greater variability in the timing of peak skeletal mitochondrial respiration compared with their endothermic metabolic response. Thyroid hormone levels rise rapidly upon hatching in precocial species and gradually in altricial species. Manipulation of thyroid hormones in altricial species influences development of resting metabolism, organ growth, insulation, and timing of obtaining endothermy. Future focus of research into development of endothermy should include development of muscle metabolism and cardio-respiratory function and their association with hormonal regulation of endothermic metamorphosis. Supported by NSF Grant IOS-1146758.

22.0 DETERMINANTS OF SKELETAL MUSCLE DIVERSITY

22.1 EVOLUTIONARY SELECTION OF MYOFIBRILLAR PROTEIN ISOFORMS FOR SPECIFIC MUSCLE FUNCTIONS

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Understanding the role of myofibrillar protein isoforms in the regulation of muscle contractile properties has greatly advanced over the past three decades. This is due, in large part, to the ability to study contractile properties of single muscle fibers and the determination of their myofibrillar protein isoform composition. This approach has been applied to multiple vertebrate species with natural variations in isoform expression patterns and to transgenic models in which the expression of a specific isoform was altered. The results from many laboratories have led to several well-accepted generalizations, including the pivotal role of myosin heavy chains in determining shortening velocity and power generation, the fundamental role of myosin light chains in modulating the same properties, and the key roles of isoforms of tropomyosin and of troponin subunits in regulating calcium activation of contraction. With this solid knowledge base, we can now ask which specific patterns of contractile
protein isoform expression have been selected to serve the incredibly diverse range of motor functions in different muscles, especially among vertebrate craniofacial muscles, where isoform expression patterns are highly divergent among different species. Addressing this will provide an understanding of how myofibrillar protein isoforms contribute to the fascinating and elaborate comparative physiology of muscle contraction. I will provide illustrations of specific patterns of contractile protein isoform expression and their association with contractile properties across a broad range of vertebrate motor functions.

22.2 COMPARATIVE PHYSIOLOGY OF BODY WEIGHT-SENSITIVE SKELETAL MUSCLE PLASTICITY

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Efficient animal locomotion requires skeletal muscle function to be finely tuned to variation in body weight. We know very little about how animals sense their weight quantitatively nor do we know how such fine tuning occurs exactly. The main focus of research addressing such questions has been on skeletal muscle mass plasticity with less attention being paid to plasticity and body weight-dependency of skeletal muscle properties that occurs in parallel. Here I will present a growing body of work that demonstrates the existence of an evolutionarily conserved body weight sensing mechanism that controls plasticity in skeletal muscle properties at the level of the somatotopic gene expression patterns. In addition, I will discuss how dietary quality and disease can affect this mechanism in both insect and mammalian study systems.

22.3 CARDIAC MYOSIN ALPHA AND VENTRICULAR HYPERTROPHY PROTECT GROUND SQUIRRELS IN HIBERNATION.

Bryan Rothermel1,2, and Lynne Nelson3

As deep hibernators, golden-mantled ground squirrels (Callospermophilus lateralis) have multiple challenges to cardiac function during torpor and subsequent arousals. At dramatically slower heart rates, chamber dilation and reduced cardiac output could lead to congestive myopathy. We performed echocardiography on squirrels before and after hibernation. The left ventricle hypertrophied 30% during hibernation. Left atrial ejection generally was absent during hibernation, but returned weakly with a-rousals. We analyzed cardiac myosin heavy-chain (MyHC) isoforms in squirrels before and during hibernation, and prior to emergence. Relative abundance of cardiac MyHC alpha increased during hibernation, at hibernacula temperatures of 20 and 4 °C. An increase in contractile speed and power from MyHC alpha may aid force generation at low temperature and low heart rates. Unlike cardiomyopathies where MuHC alpha is replaced by MyHC beta to reduce oxygen consumption, squirrels demonstrate a cardioprotective mechanism to maintain cardiac output during torpor. Squirrels thus prevent cardiac dilation and reduced cardiac output at body temperatures and heart rates far below what non-hibernators can tolerate. (NIH M05 S06 GM063119 (BCR) Reference: Nelson, O.L and Rourke, B.C. B.C. Increase in cardiac myosin heavy-chain (MyHC) alpha protein isoform in hibernating ground squirrels, with echocardiographic visualization of ventricular wall hypertrophy and prolonged contraction, J Exp Biol 216, 4678-4690.

22.4 THE DIVERSITY AND EVOLUTION OF LOCOMOTOR MUSCLE PROPERTIES IN ANURANS

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Anuran jumping is a model system for linking muscle physiology to organismal performance. Additionally, anuran species display substantial diversity in their locomotion and morphology, reflecting their habitats (including aquatic, terrestrial, arboreal, and fossorial environments) as well as other anatomical or life history traits (such as protective toxins). Some anurans are renowned for performing powerful leaps from riverbanks or tree branches, but other species move predominantly via burrowing, swimming, short hops, or even diagonal-sequence jumps. Many anurans with similar locomotion and morphology are actually convergent, (e.g. multiple independent evolutions of “tree frogs”), while closely related species may differ drastically, as with the bullfrog-like river toad compared to other Bufonid toads. These multiple independent evolutionary changes in locomotion allow us to test the hypothesis that evolutionary increases in locomotor performance will be linked to the evolution of faster, high-power muscles. We tested the jumping, swimming and running performance of fourteen species of anurans and one salamander, followed by measurement of the contractile properties of the semimembranosus and plantaris muscles and anatomical measurements, then analyzed these data using both traditional statistics and phylogenetic independent contrasts. Contrary to our hypothesis, we found that locomotor performance showed little correlation to muscle properties, but was tightly linked to anatomical properties. This suggests that locomotor performance is more dependent upon anatomy than upon muscle contractile properties, and the evolution of increased locomotor performance is not linked to the evolution of faster, higher-power muscles.

23.0: CARDIOVASCULAR AND RESPIRATORY PHYSIOLOGY

23.1 DO DROSOPHILA LARVAE EXPERIENCE FUNCTIONAL OXYGEN LIMITATION LATE IN THE INSTAR?

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Anurans gain considerable mass between molts, increasing oxygen demand with some aspects of the respiratory system fixed in size until the next molt. We hypothesized that internal hypoxia could be one of several cues that could trigger molting in insects and other arthropods. In support of this hypothesis, hypoxic rearing (10 kPa) triggered an ecdysone peak and molting to adulthood at a smaller body size in Drosophila (JEB 216:4334). Here we tested for evidence that late-third instar Drosophila larvae show signs of functional oxygen-limitation. Lactate levels did not increase later in the third instar, and were unaffected by rearing in hypoxia. However late-instar larvae had stronger capacities to generate lactate in response anoxia, suggesting past selection for coping with late-instar oxygen limitation. Metabolic rates rose linearly with mass early in the instar, but showed no further increase with mass later in the instar. Hypoxia (30 kPa) increased late-third instar larval metabolic rates, though hypoxia (10 kPa) did not lower them. Together these data support the conclusions that Drosophila, and likely other insects, can experience functional oxygen limitations later in the instar, that internal hypoxia is one of several factors capable of inducing molting, and that molting occurs before internal PO2 decreases sufficiently to trigger anarchoesis. This research was supported by NSF IOS 1256745.

23.2 A STUDY ON EMBRYONIC DEVELOPMENT RATE AND CARDIORESPIRATORY PERFORMANCE OF THE NORWAY LOBSTER, Nephrops norvegicus, DURING ACUTE EXPOSURE TO ELEVATED PCO2, MANGANESE, AND HYPOXIA

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As a consequence of the anthropogenic carbon dioxide (CO2) emissions since mid-18th century, our oceans have gradually become more acidic, a process named ocean acidification (OA). Aquatic organisms experience a multitude of simultaneous environmental factors and combined stressors have the potential to be synergistic. Thus, experiments should combine OA with other ecologically relevant stressors, in order to fully understand future impact on species, populations and ecosystems. The marine deepclad crustacean Nephrops norvegicus is of immense importance to both ecology and economy. It lives in sedimentary burrows and is seasonally exposed to periods of prolonged hypoxia (hypoxia, <30% O2 saturation), as thermoclines build up and oxygen is consumed. These events are exacerbated by anthropogenic eutrophication and are predicted to increase both spatially and temporally, as well as in severity, due to thermal elevation caused by climate change. Hypoxia causes reduction of MnSO4 in sediments and release manganese into the overlying water, where it becomes bioavailable to benthic organisms. In this study, berried Nephrops norvegicus were exposed to ocean acidification (1600 ppm) for a period of seven weeks and an additional stressor the final week, i.e. hypoxia (~25% O2) or manganese (8 mg/l). The aim was to investigate development rate (yolk consumption), cardiorespiratory performance (heart rate and rate of oxygen consumption) as well as wet dry weight during embryonic development.

23.3 REPEATABILITY AND MORPHOLOGICAL CORRELATES OF FISH BEHAVIOR IN HYPOXIA

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The habitat of the gulf killifish, Fundulus grandis, is characterized by variable oxygen levels. In this study, we evaluated the repeatability and morphological correlates of two behaviors of fish in hypoxia: aquatic surface respiration (ASR), where fish ventilate their gills with oxygen-rich surface waters, and loss of equilibrium (LOE), a non-lethal measure of tolerance to extreme hypoxia. We exposed fish to gradually lowered oxygen levels and recorded the time and oxygen levels when fish conducted ASR and LOE. We also measured total filament length, total filament number, and average filament length of gills from these fish. Results from 14 fish used in 2 to 4 trials, each spaced by approximately 2 weeks, show that the time when fish conduct ASR and the oxygen levels at ASR, are significantly dependent upon the individual (P < 0.05) and have a repeatability of about 50%. The time to LOE approached statistical significance (P = 0.09) with a repeatability of 22%. Mass-adjusted average filament length was significantly correlated with time at ASR (r = 0.53, P < 0.05) and showed
weak negative relationship with the oxygen levels at ASR (r = -0.44, P = 0.11), suggesting that fish with longer gill filaments do not conduct ASR until oxygen levels reach lower values. Thus, fish behaviors during hypoxia, ASR in particular, vary in a repeatable fashion among fish, which may be explained, in part, by intraspecific variation in gill morphology. Funded by NSF (DB 104996).

23.4 EFFECTS OF HYPERCAPNIA ON GILL VENTILATION, STANDARD METABOLIC RATE, OXYGEN SUPPLY CAPACITY AND SWIMMING PERFORMANCE IN RED DRUM (SCIAENOPS OCELLATUS) Rasheeda Enli1, and Andrew Eschau1

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Oceanic CO2 levels are predicted to reach 1000 ppm by the end of this century. In fish, exposure to elevated water CO2 levels (hypercapnia) reduces the CO2 gradient across the gills, causing CO2 accumulation in the blood and respiratory acidosis. During metabolic acidosis fish partially restore blood pH by increasing CO2 excretion via hyperventilation. A hyperventilatory response is also seen during hypercapnia exposure and is assumed to alleviate acid-base disturbances; however, this likely comes at an osmoregulatory cost owing to the osmorespiratory compromise of fish. As such, hypercapnia induced hyperventilation could potentially carry energetic trade-offs tied to an increase in cost of osmoregulation. We show that gill ventilation in red drum (Sciaenops ocellatus) increases after acute exposure to 1000 and 4000 ppm hypercapnia, respectively. Standard metabolic rate, however, was unchanged indicating that the hyperventilatory response to ocean acidification places, at most, only a minor energetic load on this species. Furthermore, we examine the effects of acute and chronic hypercapnia exposure on critical oxygen tension, aerobic scope, critical swimming speed and cost of transport. Our results show that ocean acidification has little effect on swim performance or oxygen supply capacity. All experiments were conducted in accordance with the University of Texas at Austin Institutional Animal Care and Use Committee. This work was funded by NSF (EF 1315290).

23.5 OPTIMIZATION OF IN VITRO INCUBATION OF GILLS FROM FUNDULUS GRANDIS Kristina Farrugia1, Joseph Diaz1, and Bernard Rees1

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Low dissolved oxygen, hypoxia, is a prevalent feature of many aquatic habitats, and fish that occur in these habitats employ a suite of behavioral, morphological, and physiological mechanisms to deal with low oxygen. As the site of gas exchange, fish gills are directly subject to variation in ambient oxygen levels. The purpose of this study was to optimize in vitro culture conditions for intact gills from the Gulf killifish, Fundulus grandis, with the ultimate goal of using these preparations to study the cellular and molecular responses of this tissue to hypoxia. Gills were dissected and incubated in two media, using two apparatus, and assayed for viability by trypan blue staining and lactate dehydrogenase (LDH) leakage at 24 h incubation compared to 2 h, neither was significant, partly due to high variability in both measures of tissue viability. Current work continues to optimize culture conditions. Funded by the Louisiana Board of Regents.

23.6 PURIFICATION AND CHARACTERIZATION OF ANTIBODIES AGAINST KILLIFFISH HIF-1α Janet Gonzalez-Rosario1, and Bernard Rees1

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The hypoxia-inducible factor (HIF) is a transcription factor that regulates gene expression in animals exposed to low oxygen concentrations, or hypoxia. Previously, we generated polyclonal antibodies in chickens against HIF-1α from the killifish Fundulus heteroclitus. The objectives of the current project were to clone, express, and purify a fragment of HIF-1α and used it to affinity purify these antibodies. A 300 bp region of F. heteroclitus HIF-1α cDNA was cloned and used to express a recombinant HIF-1α protein fragment of approximately 13 kDa. The protein fragment was purified by affinity, ion-exchange, and size-exclusion chromatography. The purified protein was coupled to AminoLink Plus resin and used to affinity purify the chicken anti-killifish HIF-1α antibodies. In western blots, the affinity purified antibodies specifically recognized full length killifish HIF-1α (ca. 95 kDa) made by in vitro transcription and translation (IVTT). Next, we used these antibodies to immunoprecipitate IVTT HIF-1α, the buffer composition; amounts of primary antibody and the immununoprecipitating resin; incubation times; and the recovery of input HIF-1α.

The next phase of this project is to immunoprecipitate and measure endogenous HIF-1α from multiple tissues harvested from killifish exposed to various durations and levels of hypoxia. Funded by the Louisiana Board of Regents and the National Science Foundation (DB 104996).

23.7 ACCLIMATION TO OVERNIGHT HYPOXIA AND INCREASED TEMPERATURE IMPROVE AEROBIC PERFORMANCE IN SALMON (SALMO SALAR) AND CHAR (SALVELinus ALPINES) Katja Annila, Jenni Prokopkova, and Mikko Nikinmaa

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Fish are increasingly influenced by rising temperatures and decreasing oxygen levels in aquatic ecosystems. However, the capacity of fish to tolerate the combination of these stressors is not well understood. The purpose of the current study was to analyze the effects of warming and overnight hypoxia on capacity of fish to tolerate hypoxia. One-year-old salmon and charr were acclimated to cold (8 °C) and warm (14 °C) temperatures in either normoxic conditions or with an overnight hypoxia for one month, after which their heart tissue was collected and hypoxia tolerance measured. Both warm acclimation and overnight hypoxia more than doubled the hypoxia tolerance of salmon and also increased the tolerance of charr by 25%. Charr were significantly more tolerant to hypoxia than salmon. Charr also had larger hearts and thicker compact layers than salmon. The relative ventricle mass was higher in cold acclimated fish, but the thickness of the compact layer of the ventricle increased with both warm and hypoxia acclimation. This may contribute to improved hypoxia tolerance. The results show significant species differences in the tolerance of multiple environmental stressors in salmonid fish. The experiments were done following APS guiding principles for the care and use of animals (animal testing permit nr. EAV140684/10.07/2013). The study was funded by Kone Foundation, BIONT Doctoral program and the Academy of Finland.

23.8 LONG-TERM ACCLIMATION TO HYPOXIA DOES NOT CONFER CROSS-TOLERANCE TO HIGH TEMPERATURE IN STEELHEAD TROUT (Oncorhynchus mykiss) Tommy Norin1, Romar Mowda1, and A. Kurt Gumpel1

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It has been suggested that acclimation to high temperatures or hypoxia confers tolerance against the other oxygen-limited stressor. Thus, we investigated if acclimation to hypoxia (>3 months at 40% air sat.) improved the trout’s critical thermal maximum (CTM), or affected primary physiological variables that determine upper thermal tolerance [oxygen consumption rate (M0,1), hematocrit (Hct), and cardiovascular function and morphology] M0,1 (resting and max.), CTM (24.7 vs. 25.3°C), and Hct did not differ between hypoxic- and normoxia-acclimated trout when measured in normoxia. However, cardiac output (Q) plateaued in hypoxic trout >20°C, despite similar increases in heart rate as compared to normoxic fish, and this resulted in a lower Qmax. This limited pumping capacity was not associated with changes in cardiovascular morphology or in vitro maximum stroke volume, suggesting that alterations in ventricular filling dynamics or myocardial contractility constrain cardiac function in hypoxia-acclimated fish at high temperatures. Our finding that hypoxia-acclimated trout consumed more oxygen for a given increase in Q is consistent with data on Atlantic cod, and suggests that long-term hypoxia improves tissue oxygen extraction or utilisation, and that this compensates for diminished heart performance. In summary, our data do not support the concept of ‘cross-tolerance’ with respect to these two environmental variables, but offer additional insights into fish physiological plasticity.

23.9 HYPOXIA-INDUCED APOPTOSIS IN THE HEARTS OF HYPOXIA-TOLERANT TILAPIA (Oreochromis hybrid sp.) AND HYPOXIA-SENSITIVE STRIPED BASS (Morone saxatilis) Amanda Reynolds1, and Johanne M. Lewis1

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Oxygen deprivation (hypoxia) is a common stressor affecting cardiomyocytes when blood flow is reduced or cut off from the heart. In response, cardiomyocytes have been shown to undergo apoptosis which is suggested to have a role in the pathogenesis of cardiovascular diseases, the leading cause of mortality in developed nations. Since many species of fish can survive low oxygen levels that would be fatal to mammals, fish are an ideal model system to study changes at the cellular and molecular level that prevent or repair hypoxia-induced damage in cardiomyocytes. Evidence of apoptosis at the cellular level (caspase-3/7 activity) will be measured in a hypoxia-sensitive species, striped bass (Morone saxatilis) and a hypoxia-tolerant species, tilapia (Oreochromis hybrid sp.) at four key time points: 1) prior to hypoxia exposure (normoxic control), 2) after four hours at the species’ specific PaO2 (hypoxia), 3) immediately upon return to normoxia (reperfusion), and 4) after four hours at normoxia (recovery). Additionally, RT-qPCR will be used to quantify changes in the tran-
scriptome of cardiomyocytes of pro-apoptotic (BAX and FAS), anti-apoptotic (BCL2 and FASL) and repair (Hsp70) genes. We hypothesize pro-apoptotic genes will have higher gene expression and caspase activity will be higher in stressed bass while anti-apoptotic and repair genes will have higher expression in tilapia. Funding was provided by Georgia Southern University via a GSO grant (AR) and FRC seed funding (JML).

23.10 ACUTE THERMAL CHALLENGES OF CARDIAC FUNCTION IN PACIFIC BLUEFIN TUNA
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Understanding the physiology of vertebrate thermal tolerance is critical for predicting how animals respond to climate change. Pacific bluefin tuna experience a wide range of ambient temperatures and occupy the largest geographic niche of all tunas. Their capacity to endure thermal change is due in part to cardiac specialisations that improve performance across a range of temperatures. To better understand the cellular mechanisms that enable bluefin hearts to maintain function across thermal gradients we combined confocal microscopy and electrophysiology to define interactions between temperature, adrenergic stimulation and contraction frequency on the electrical activity and Ca dynamics of Pacific bluefin ventricular myocytes. We demonstrate that acute cooling and acute warming modulate the excitability of the cardiomyocyte by altering ion fluxes during the action potential. With constraining constant sympathetic stimulation (500 nM adrenaline) we found that compensatory changes in the action potential and ion influx resulted in fairly constant Ca cycling across a 20 °C acute temperature gradient. The results indicate the tuna heart maintains consistent contraction and relaxation cycles during acute temperature changes. We hypothesize that the capacity to operate across larger thermal gradients plays a key role in the bluefin's capacity for broad thermal niche utilization. Study supported by NOAA, Monterey Bay Aquarium Foundation and The University of Manchester.

23.11 TEMPERATURE DEPENDENCE OF BLOOD-OXYGENATION IN JUVENILE SANDBARRIER SHARKS (CARCHARHINUS PLUMBEUS)
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Juvenile sandbar sharks (Carcharhinus plumbeus) use shallow inshore areas on the east coast of the United States (e.g., Chesapeake Bay) as nursery grounds before emigrating to southern coastal waters or into deep adjacent waters as adults, and thus experience a great range of environmental temperatures. In the nursery grounds water temperatures typically range from 20-26°C, but sharks are known to experience water temperatures between 15-30°C throughout their lives. Sandbar sharks are ectothermic and thus their blood temperature equilibrates with that of the ambient water. Consequently, water temperature could have a profound influence on blood-oxygenation due to the generally exothermic nature of oxygen (O2) binding to the hemoglobin groups of hemoglobin. We hypothesized that the temperature dependence of blood-O2 binding would be minimal in juvenile sandbar sharks, permitting sufficient O2 uptake in the warm waters of their nursery grounds. To test the effects of temperature changes on blood-O2 binding in the sandbar shark we constructed O2 equilibrium curves (OECs) on whole blood at a range of temperatures that juvenile sandbar sharks typically encounter throughout the year. Representative OECs and blood O2 tensions at 50% of Hb-O2 saturation (Po2) are reported for two carbon dioxide tensions over the experimental temperature range, and interpreted with respect to the thermal niche of juvenile sandbar sharks. Funding provided by NSERC.

23.12 EFFECT OF TEMPERATURE ACCCLIMATION ON HEMOGLOBIN-OXYGEN BINDING PROPERTIES IN PACIFIC BLUEFIN TUNA (THUNNUS ORIENTALIS) AND YELLOWFIN TUNA (THUNNUS ALBACARES)
Laura Lilly1, Joe Bonaventura2, Richard Lipnick3, and Barbara Block1
Current climate change trends may threaten the survival of many marine species, so understanding responses to increasing ocean temperatures and CO2 is critical for predicting future impacts. This study investigates thermal acclimation effects on hemoglobin-oxygen (Hb-O2) binding properties in Pacific bluefin tuna (Thunnus orientalis) and yellowfin tuna (Thunnus albacares) maintained in captive tanks at 17, 20 and 24°C acclimation temperatures. Oxygen binding properties of acclimated hemoglobin were examined under experimental temperatures (15-35°C) and CO2 levels (0%, 0.5% and 1.5%). Bluefin tuna demonstrated a reverse temperature-dependent trend between 15-30°C at each acclimation and all CO2 levels. In contrast, yellowfin tuna produced a normal temperature-dependent effect at each acclimation at 0% CO2, but temperature-independent and reverse temperature-dependent effects at 0.5% and 1.5% CO2. Both species demonstrated a normal Bohr Effect. Thermal acclimation in bluefin increased production of O2-affinitly at 17°C-acclimation, and a significantly steeper oxygen equilibrium curve slope at 24°C-acclimation compared to the other acclimations. These findings indicate that Pacific bluefin tuna possess a reverse temperature-dependent effect, consistent with other bluefin species, while yellowfin tuna utilize a mix of effects. While temperature acclimation produced changes in Hb-O2 binding properties, more work is needed to clarify the functional significance of such changes.

23.13 AERIAL RESPIRATION IN POLYPTERIDS
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Polypterus are a basal family of actinopterygian fishes (ray-finned) that share similarities with extant sarcopterygians (lungfishes and tetrapods) including the ability to breathe air using paired ventral lungs. Two polypterid genera, Erpetoichthys and Polypterus, are able to completely satisfy O2 demand breathing air, however, only Erpetoichthys is known to be amphibious. To investigate whether gas exchange capacity and ventilatory mechanisms have evolved in response to environmental and ecological factors, we measured O2 consumption, CO2 production, and respiratory partitioning between pulmonary and cutaneous gas exchange in Erpetoichthys and Polypterus over a 3-hour period in air. Pulmonary gas exchange accounted for 80% and 57% of total VO2 and VCO2 in Erpetoichthys and 82% and 60% in Polypterus. Differences in respiratory partitioning of O2 and CO2 resulted in reduced respiratory exchange ratios (RER) at the lungs and elevated RERs at the skin in Erpetoichthys (0.6-lungs and 1.7-skin) and Polypterus (0.6-lungs and 1.9-skin). Other amphibious fishes when removed from water typically show low air-breathing organ RERs (<0.7) indicating an inability to fully excrete CO2 into the air with this organ. Our data show similar findings in Polypterus where pulmonary gas exchange participates in CO2 excretion, yet cutaneous gas exchange continues to play a significant role. Erpetoichthys, on the other hand, shows an elevated ability for pulmonary CO2 excretion. Regardless, the total RERs of both genera were 0.7-0.9 indicating that Polypterus did not differ in its overall aerial respiratory capacity to process CO2 from its amphibious relative Erpetoichthys. Our data suggest that in polypters, gas exchange ability was not the limiting constraint in the evolution of amphibious behavior. Instead, factors such as, lung ventilation mechanics, locomotive ability, and ecological factors may have been important influences.

23.14 THE EFFECTS OF UMBELLULARIA CALIFORNICA ESSENTIAL OIL ON THE CUTANEOUS VASCULATURE OF FROGS
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Most plant species produce chemical compounds called secondary metabolites that enhance fitness in a variety of ways. Many of these compounds are also physiologically active in vertebrates and have widespread medical uses. The most ubiquitous secondary metabolite are the terpenoids, many of which cause vasodilation of the aorta and mesenteric arteries. In this study, we examined the vasoactive effects of the essential oil of Umbellularia californica, which contains the terpenoid umbellalone. Oil obtained via steam distillation using aerial portions of U. californica was applied directly to cutaneous arterioles of frogs. Arteriole diameter was monitored before and after oil application by video microscopy. Within seconds of application, the oil caused significant vasoconstriction that persisted until the oil was washed off. Our control, medical grade sesame oil, caused no observable effects when applied using the same protocols. These results are opposite to the vasoconstrictor effects of terpenoids on aortic rings and mesenteric arteries. This suggests that the vasoactive effects of umbellalone are different from other terpenoids, that the vasoactive effects of terpenoids differ depending on blood vessel type, or that application of the complete essential oil affects vasculature differently than application of the isolated terpenoid. This research was approved by SUU-IACUC and funded by a grant from SUU Undergraduate Research and Scholarship Program.

23.15 BAROREFLUX CHARACTERISTICS OF ANURAN AMPHIBIANS FROM DIFFERENT ENVIRONMENTS
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Anurans from terrestrial environments have an enhanced ability to maintain arterial blood pressure through lymph mobilization in response to desiccation or hemorrhage.
We compared baroreflex function in three species of anurans that span a range of environments, dehydration tolerance and an ability to maintain arterial blood pressure with dehydration and hemorrhage. The cardiac limb of the baroreflex loop was studied using pharmacological manipulation of blood pressure and the resulting changes in heart rate were quantitatively analyzed using a four-parameter sigmoidal logistic function. Resting mean arterial blood pressure (Pm) in the aquatic species, Xenopus laevis, was 3.6±0.3 kPa and was less (P<0.005) than the semiaquatic species, Rhinella marina (4.7±0.2 kPa). The maximal baroreflex gain was not different among the three species and ranged from 12.1 to 14.3 beats min⁻¹ kPa⁻¹ and occurred at Pm ranging from 3.0 to 3.8 kPa, which were slightly below the resting Pm for each species. Resting Pm in the three species were near the upper saturation point of the baroreflex curve which would enable animals to respond primarily to hypertensive, rather than hypotensive, changes in blood pressure. This is consistent with the hypothesis that the baroreflex is a key sensory component that allows anurans to maintain arterial blood pressure by mobilization of sympathetic return in response to hypotension.

23.16
does the right-to-left shunt affect assimilation efficiency, digesta transit, and postprandial metabolic changes in alligators? Christopher Slay1, John Erve2, and James Hicks1

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Multiple hypotheses exist regarding the functional significance of the right-to-left (“pulmonary bypass”) shunt utilized by non-avian reptiles. The unique cardiovascular arrangement of crocodilians, including the complete anatomic separation of right and left ventricles and presence of right and left aortae, provides a useful model for testing these hypotheses, as the shunt can be ablated by surgical occlusion of the left aorta. This study aims to clarify the role of the right-to-left shunt in digestion. The shunt is hypothesized to aid digestion by delivering hypercapnic blood to the oesophageal cells of the stomach, increasing the rate of acid secretion and, consequently, digestion of bone contained therein. Additional experiments, however, demonstrated that animals with chronic occlusion of the left aorta were not remarkably smaller than sham-operated controls and would have likely reached sexual maturity during the same breeding season. In this study, we use flow through respirometry to determine postprandial metabolic rate (specific dynamic action, SDA), and total fecal collection to measure transit time of digesta along with apparent assimilation efficiency. The results may clarify whether there is a functional or adaptive role of the reptilian right-to-left shunt.

23.17
cyphemic ventilatory response in lizards (Tropidurus torquatus) acclimated to different temperatures Lays de Souza Porto1, Kátia C. Bicego1, Wilfried Klein2, and Luciane H. Gargaglione1

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Temperature is a factor that influences physiological processes and may cause changes in breathing patterns of some species. We evaluated the ventilatory responses to CO2 of the lizard population, Tropidurus torquatus, from the South of Brazil (biome Parana), acclimated to three different temperatures. To this study, animals (0.040 ± 0.005 kg, males) were collected in Alegre, (Rio Grande do Sul, Brazil) during the summer season and were acclimated to 20 (n=2), 25 (n=2) or 30°C (n=2) during 30 days. Pulmonary ventilation was measured by pneumotachographic method at 25°C during normocapnia, hypercapnia (5%CO2) and post-hypercapnia. Lizards acclimated to 25°C showed a higher ventilatory response to CO2 compared to animals acclimated at 20 and 30°C, due to an increase in tidal volume. Regarding post hypercapnia response, no difference was observed between groups. Therefore, our preliminary data suggest that animals acclimated to 25°C appear to be more sensitive to CO2. Financial Support: FAPESP and CNPq.

23.18
the avian paradox: avian resistance to protein glycation Karen Sweezey1, Chad Borges2, and Sean Rayle3

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Avian plasma glycine (Pm) concentrations are naturally 1.5-2 times those of mammals of similar body mass. Avian tolerance of exceptionally high Pm is enigmatic considering that mammals with similar values would suffer severe pathologies stemming from hyperglycemia-induced protein glycation. What is not currently known is how birds protect circulating proteins and the vasculature from high Pm. The purpose of this study was to explore this “avian paradox.” Because protein glycation is an exothermic chemical process, we hypothesized that the unique physiology of birds (higher body temperatures than mammals) would help protect them from protein glycation. Since protein glycation is an exothermic chemical process, we hypothesized that the unique physiology of birds (higher body temperatures than mammals) would help protect them from protein glycation.
Decompression sickness (DCS) is a collection of symptoms caused by bubbles forming due to gas phase separation in the body. Anatomical, physiological, and behavioral traits are thought to protect breath-hold diving marine vertebrates against DCS. However, increasing evidence suggests that under certain circumstances marine mammals and sea turtles appear to experience gas bubbles following diving. The a-bundance and gas composition of bubbles found after severe decompression versus decompression was experimentally established in New Zealand White Rabbits. Using our methods we found that 85% of stranded marine mammals, 100% of bycaught marine mammals, and 43% of bycaught sea turtles, presented with gas bubbles. Deep divers presented with larger amounts of gas bubbles than non-deep divers. The gas composition of bubbles found in cetaceans, pinnipeds, and sea turtles was similar to the gas composition of bubbles in compressed and decompressed rabbits. The results suggest that diving air-breathing vertebrates deal daily with gas management and potential gas bubble formation. In extreme cases, animals seem to exhibit DCS lesions and symptoms similar to human divers.

23.22
ADAPTATION TO HIGH ALTITUDE IS LINKED TO GROWTH OF THE LUNGS AND HIGHER ALVEOLAR SURFACE IN MICE BUT NOT IN RATS
Alexandra Jochmans-Lemoine1, Gabriella Villalpando2, Marcelino Conzales2, Ibana Valverde2, Rudy Sorti,1 and Vincent Joseph1

Introduction: Decompression sickness (DCS) is a collection of symptoms caused by bubbles forming due to gas phase separation in the body. Anatomical, physiological, and behavioral traits are thought to protect breath-hold diving marine vertebrates against DCS. However, increasing evidence suggests that under certain circumstances marine mammals and sea turtles appear to experience gas bubbles following diving. The abundance and gas composition of bubbles found after severe decompression versus decompression was experimentally established in New Zealand White Rabbits. Using our methods we found that 85% of stranded marine mammals, 100% of bycaught marine mammals, and 43% of bycaught sea turtles, presented with gas bubbles. Deep divers presented with larger amounts of gas bubbles than non-deep divers. The gas composition of bubbles found in cetaceans, pinnipeds, and sea turtles was similar to the gas composition of bubbles in compressed and decompressed rabbits. The results suggest that diving air-breathing vertebrates deal daily with gas management and potential gas bubble formation. In extreme cases, animals seem to exhibit DCS lesions and symptoms similar to human divers.

23.23
AEROBIC PHYSICAL TRAINING INCREASES CONTRACTILE RESPONSE AND REDUCES THE CARDIAC FIBROSIS IN RATS SUBMITTED TO EARLY ESTROGEN DEPRIVATION

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Introduction: We investigated the effects of early estrogen deprivation on the heart and the role of physical training in this condition using different approaches: contractility, morphology and function, and cardiac fibrosis. Methods: Female Wistar rats (N=48) were assigned into two groups: ovarioctomized (OVX; 22 weeks-old) and control rats (SHAM; 22 weeks-old). Each group was subdivided into two subgroups, sedentary and trained (aerobic physical training by swimming for 10 weeks). Results: The contractile responses to β-agonists were similar, including an increased response to a β-agonist (dobutamine) observed after physical training. The OVX sedentary group presented changes in cardiac morphology, which resulted in a decreased ejection fraction, fractional shortening and cardiac index in relation to the SHAM sedentary group. Physical training did little to alter these findings. Moreover, the histology analysis showed a significant increase in cardiac fibrosis in the sedentary OVX group, which was not observed in the trained OVX group. Conclusions: Early estrogen deprivation impairs cardiac morphology and cardiac function. This condition also increases cardiac fibrosis; however, it does not affect contractility induced by dobutamine and salbutamol (β-agonists). Furthermore, this model of physical training prevented increased fibrosis and promoted an increased cardiac contractile response but had little effect on the morphological and functional parameters. Funded: FAPESP (2014/1937-9); CNPQ (135531/2011-0).

23.24
DYNAMICS OF OXYGEN UTILIZATION DURING PASSIVE AND ACTIVE CYCLING EXERCISE

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Physiology, Univ. of Sao Paulo, Av. Bandeirantes, 3900, Bairro Monte Alegre, Ribeirão Preto, 14045-905, Brazil.

Introduction: We investigated the effects of early estrogen deprivation on the heart and the role of physical training in this condition using different approaches: contractility, morphology and function, and cardiac fibrosis. Methods: Female Wistar rats (N=48) were assigned into two groups: ovarioctomized (OVX; 22 weeks-old) and control rats (SHAM; 22 weeks-old). Each group was subdivided into two subgroups, sedentary and trained (aerobic physical training by swimming for 10 weeks). Results: The contractile responses to β-agonists were similar, including an increased response to a β-agonist (dobutamine) observed after physical training. The OVX sedentary group presented changes in cardiac morphology, which resulted in a decreased ejection fraction, fractional shortening and cardiac index in relation to the SHAM sedentary group. Physical training did little to alter these findings. Moreover, the histology analysis showed a significant increase in cardiac fibrosis in the sedentary OVX group, which was not observed in the trained OVX group. Conclusions: Early estrogen deprivation impairs cardiac morphology and cardiac function. This condition also increases cardiac fibrosis; however, it does not affect contractility induced by dobutamine and salbutamol (β-agonists). Furthermore, this model of physical training prevented increased fibrosis and promoted an increased cardiac contractile response but had little effect on the morphological and functional parameters. Funded: FAPESP (2014/1937-9); CNPQ (135531/2011-0).

23.25
THE EFFECTS OF MODULATING ENDOTHELIAL NITRIC OXIDE SYNTHASE (ENOS) ACTIVITY AND COUPLING IN CORONARY, HINDLimb, RENAL, AND MESENTERIC VASCULAR INFLAMMATION MODELS

Alexandra Lopez1, Kerry-Anne Perkins1, Amber Koon1, Armelie Botten1, Qian Zhou1, Robert Barstow1, and Linda Young1


Introduction: Atherosclerotic vascular disease is linked to inflammation in the vascular wall. The NO-cGMP pathway is an important target for treatment of cardiovascular disease (CVD). cGMP can be degraded by phosphodiesterase (PDE), which reduces bioavailability of NO. The aim of this study was to investigate the effects of modulating NO bioavailability by inhibiting PDE in vivo. We employed a rat model of acute inflammation treated with an NO donor and assessed the effects of an NO synthase (NOS) inhibitor and an NO donor on PDE activity and inflammation.

Methods: Adult male Sprague-Dawley rats (n=12) were randomized into three groups: control, acute inflammation treated with an NO donor (20 mg/kg, intraperitoneal injection), and acute inflammation treated with an NO donor and a PDE inhibitor (1 mg/kg, intraperitoneal injection). The inflammation model was established by injecting a solution of lipopolysaccharide (LPS) and bradykinin into the right carotid artery. The rats were sacrificed 24 hours after the injection, and the thoracic aorta was collected for histological analysis. The thoracic aorta was stained with hematoxylin and eosin (H&E) and analyzed using a light microscope. The inflammatory response was quantified by counting the number of inflammatory cells in the atherosclerotic lesions.

Results: The number of inflammatory cells in the atherosclerotic lesions was significantly lower in the group treated with the NO donor and PDE inhibitor compared to the group treated with the NO donor alone. The PDE inhibitor had no effect on the number of inflammatory cells in the atherosclerotic lesions.

Discussion: These results suggest that modulating NO bioavailability by inhibiting PDE can reduce inflammation in the vascular wall. Future studies will investigate the effects of modulating NO bioavailability on other vascular diseases, such as CVD and atherosclerosis.

23.26
THE EFFECT OF STREPTOKINASE INFUSION ON CARDIAC BIO-MARKERS & ST SEGMENT OF ELECTROCARDIOGRAM POST MYOCARDIAL INFARCTION IN HUMANS

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Introduction: Myocardial infarction (MI) is a common and serious cardiovascular disease that results in the death of cardiac muscle tissue. The management of MI is crucial to reduce mortality and morbidity. Thrombolytic therapy is the most common reperfusion strategy for MI, but the success rate is limited by the time delay from symptom onset to hospital arrival. Early recognition and timely intervention are essential for the successful treatment of MI. The aim of this study was to investigate the effects of streptokinase infusion on cardiac biomarkers and ST segment of electrocardiogram in patients with acute MI.

Methods: In this randomized, double-blind, placebo-controlled trial, 100 patients with acute MI were enrolled. The patients were randomized into two groups: streptokinase infusion group and placebo group. The group received an intravenous infusion of streptokinase at a dose of 1.5 million units over 30 minutes, while the placebo group received an intravenous infusion of saline. The primary outcome measure was the change in cardiac biomarkers (troponin I and troponin T) at 6 hours after the onset of symptoms. The secondary outcome measure was the change in ST segment of electrocardiogram at 6 hours after the onset of symptoms.

Results: The results showed that the streptokinase infusion group had a significantly lower troponin I level at 6 hours compared to the placebo group. There was no significant difference in the troponin T level between the two groups. The ST segment of electrocardiogram showed a significant improvement in the streptokinase infusion group compared to the placebo group.

Discussion: These results suggest that streptokinase infusion may be an effective strategy for reducing myocardial damage and improving cardiac function in patients with acute MI. Further studies are needed to confirm these findings and to evaluate the long-term outcomes of streptokinase infusion.
24.1 GENETICALLY DETERMINED VARIATION IN METABOLIC ALLOCATION—POTENTIAL FOR ADAPTATION TO ENVIRONMENTAL CHANGE

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Defining the genetic basis of physiological variance is key for predicting adaptive potential of populations to climate change. For most organisms of interest to comparative physiologists – in particular, for marine animals – this goal has been elusive, owing to a lack of genetically enabled models. We crossed pedigreed lines of the Pacific oyster Crassostrea gigas to produce >20 families that were screened for physiological contrasts. Larvae were assayed for variation in physiology of Na+, K+–CATION–POTENTIAL for adaptation to environmental change, others may be resilient. Supported by NSF grant EF1220587.

Angeles, CA, 90089-0371.


Pupal development is particularly sensitive to environmental perturbations, because the insect is immobile while physiological systems essential for the adult are constructed from larval and imaginal tissues. Severe environmental fluctuations during the pupal period could disrupt development, resulting in a negative impact on adult performance. In the alfalfa leaf-cutting bee, Megascolia rotundata, exposure to cold temperatures during pupal development causes sub-lethal effects on adult performance, including flight deficiencies, altered metabolic rates, behavioral changes, and decreased longevity; however, the mechanisms underlying these effects are not known. Disruption of one or more developing systems may be responsible for the deleterious effects seen in adults after pupae were exposed to cold temperatures; however, the internal changes associated with pupation are a “black box.” We used μCT to investigate the development of flight structures, metabolic reserves, and the digestive tract during M. rotundata pupal development. Pupal development occurs over three weeks, during which time we were able to clearly see development of these structures. Gut and flight structures appear to change continuously during the pupal period. However dense metabolic reserves may decrease only slightly throughout pupal development. These data will allow us to better understand what happens during metamorphosis, and to make comparisons about how these systems are altered after cold exposure.

24.5 SHIFTCUTTING TEMPERATURES ALTER HEART RATE AND OXYGEN CONSUMPTION OF LAKE WHITEFISH EMBRYOS AND HATCHLINGS

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Critical windows are periods of developmental susceptibility when an animal’s phenotype may be vulnerable to environmental fluctuations. We incubated whitefish embryos at control temperatures of 2°C, 5°C, or 8°C, and shifted embryos into another of those same temperatures at the end of gastrulation or organogenesis to determine the effects of temperature on heart rate and oxygen consumption. This study was supported by the Center for Chronic Disorders of Aging at PCOM.

Seawater acidification had minimal effect on size, growth and metabolic rate. However, substantial biochemical rate compensation occurred to sustain this resilience at the organismal level. Larvae of similar size and metabolic rate responded to acidification with ~50% increases in protein synthesis rate and ion transport by Na+, K+–ATPase. Up to 86% of the ATP pool was allocated to these two processes. In terms of the hierarchy of ATP allocation, protein synthesis is more significant than the cost of supporting changes in ion transport. We are currently manipulating pCO2 and pH independently to determine which component of the seawater carbonate chemistry is responsible for the observed biochemical changes. We conclude that the apparent minimal responses to acidification at the organismal level actually involve major changes at the cellular level, with substantial impacts on energy allocation within fixed ATP pools. Supported by NSF EF1220587.
termite if gusulation or organogenesis represent critical windows. Heart rate (fH) and oxygen consumption (VO2) were measured across embryonic development, and VO2 was measured in 1d larvae. Thermal shifts up or down from initial incubation temperatures caused fH and VO2 to differ from control embryos measured at the same temperature (2°C, 5°C, 8°C). Most prominently, when embryos were measured at organogenesis compared to controls, exposure to 2°C or 5°C through gasuturation resulted in lower VO2 and fH at 8°C, exposure to 2°C resulted in lower VO2 and fH at 5°C, and exposure to 5°C resulted in lower VO2 at 2°C. Through the latter half of development, VO2 and fH trended toward recovery to control values for thermally shifted treatments. However, in hatching larvae measured at 2°C, VO2 was higher in groups exposed to 8°C or 5°C through organogenesis, compared to 2°C controls (43-65% increase), which further indicates that development through organogenesis represents a critical window of physiological plasticity. This study presents a unique experimental design that identified periods of thermal sensitivity in fish embryonic development.

### 24.6 PHYSIOLOGICAL CONSEQUENCES OF COMPENSATORY GROWTH IN THE CHECKERED GARTER SNAKE, THAMNOPHIS MARCIANUS

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Variation in physical and developmental environments can influence life-history traits through phenotypic plasticity. Poor natal nutrition can lead to subsequent energy intake being diverted between compensatory growth or delayed maturation. If an organism does allocate energy to compensatory growth vs “on-time” maturation, there may be consequences across physiological axes. For example, immune defense is an energetically expensive biological process, which can trade-off with traits such as growth. Here we test how poor natal nutrition impacts growth, immune function and glucocorticoid production both during a natal phase of poor nutrition and a subsequent phase of rich nutrition in the checkered garter snake, Thamnophis marciusus. We found accelerated growth rates after the switch to high-quality food, with no impact on innate immune function and glucocorticoid production. However, we found negative impacts on subsequent reproduction, with those individuals experiencing poor natal nutrition producing smaller offspring. This suggests that innate immune function is not compromised when excess energy is allocated towards growth compensation. Thus immune function may be maintained during periods of stress at the expense of other attributes.

### 24.7 HYPOXIA DURING CRITICAL WINDOWS OF ONTOGENY ALTERS ORGAN MASS AND CARDIOVASCULAR FUNCTION IN THE AMERICAN ALLIGATOR (ALLIGATOR MISSISSIPPIENSIS)

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Hypoxic incubation represents an important and relevant challenge to the maturation of embryonic reptiles. We have previously reported that chronic hypoxic (10% O2; H) incubation of American alligator eggs results in a decrease in embryonic mass, increase in relative heart mass, decrease in heart rate and decrease in arterial pressure. Further investigation has identified a “critical window” for hypoxic application is within the 20-70% of incubation period. Our goal was to further isolate the critical period by narrowing the windows of hypoxic incubation as well as reciprocal shifts between normoxic (N) conditions and 10% O2 conditions at 50 and 70% with measurements taken at 90% of incubation. Shifting of hypoxic embryos from 10% O2 to normoxia (H to N) at 50% or 70% of incubation resulted in a relative enlargement of the heart compared to control values. Further while embryonic mass was decreased in eggs moved back to normoxia at 70%, embryonic mass of eggs that were returned to normoxia at 50% did not significantly differ from the control groups. Functional cardiovascular phenotypes produced as a result of hypoxia occur within a critical window spanning a 20% segment of incubation. Currently we are investigating changes in gene expression that may provide insight into the basis for the morphology and functional difference between these groups. NSF CAREER IBN IOS-0845741 to DAC.

### 24.8 TEMPERATURE EFFECTS ON HEART RATE AND BAROREFLEX FUNCTION OF EMBRYONIC AMERICAN ALLIGATOR (ALLIGATOR MISSISSIPPIENSIS)

Derek Nelson1, Kevin Tate1, Ruth Eves1, and Dane Crossley1

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Nest temperatures of American alligators are known to fluctuate on a diurnal cycle. While these fluctuations have been previously documented, the impact on embryonic cardiovascular physiology is poorly understood. Our prior studies have demonstrated that cardiovascular regulatory capacity in alligator embryos is limited compared to adults and the embryo relies heavily on endocrine control of function. Further, embryonic alligators possess a hypertensive baroreflex response only when studied at 30°C. A recent study suggested that cardiovascular homeostatic mechanisms are temperature- sensitive in embryonic alligators, with vagal tone activated both above and below 30°C. Based on these findings we hypothesized that a hypertensive baroreflex would be functional when embryos are studied at temperatures above and below the 30°C threshold. Further, the baroreflex gain would increase in response to the elevation in resting heart rate. We assessed heart rate and arterial pressure responses of embryonic alligators at 90% of incubation to increasing and decreasing temperatures. In addition the Oxford method was used to assess the cardiac limit of the baroreflex. The project is ongoing and the findings of these investigations will be presented. Project funded by NSF CAREER IBN IOS-0845741 to DAC.

### 24.9 THE EFFECTS OF CHRONIC AND ACUTE HYPOXIA ON CARDIAC FUNCTION IN EMBRYONIC CHICKENS

Dane Crossley1, Michale Espinoza1, Erica Davis2, George Ginaud3, and Sonnet Jonker4


Chronic hypoxia is a known developmental insult, and studied extensively for its hemodynamic effects in early chicken development. However the effects of chronic and acute hypoxia on cardiac function and arterial coupling in the late-stage embryonic chickens. Our goal was to quantify how chronic and acute hypoxia affects cardiac function. Eggs incubated at 21% or 15% O2. At 19 days, prior to lung ventilation, embryos were anesthetized and surgically instrumented to study left ventricular (LV) pressure-volume loops. Hearts were collected for mRNA analysis. Hypoxia-incubation led to growth restriction (-20%) increased heart/body ratio (+17%) and LV/body ratio (+40%). The maximal rate of LV pressure generation, dP/dTmax was lower in hypoxia-incubation (-20%), as were end systolic LV elastance (Ees; +30%), arterial elastance (Ea; +120%), and LV output (-46%). Both hypoxia-incubation and acute hypoxia (10% O2) lengthened tau, the half-time of relaxation (+20%). Acute hypoxia reduced heart rate (-8%) and increased end diastolic pressure (+20%) without changing dP/dTmax. These were accompanied by reduced mRNA for intracellular calcium handling genes. Hypoxia-incubation reduces LV function slowing pressure generation and relaxation, possibly driven by altered intracellular excitation-contraction coupling. The ratio of Ees/Ea is much higher with hypoxia-incubation, indicating decreased cardiac efficiency.

### 24.10 DEVELOPMENTAL PHYSIOLOGY OF THE PEKIN DUCK (ANAS PEKIN) DUCTUS ARTERIOSUS

Fancine Mascanerhas1, and Edward Dziakowski1

1Biological Sci., Univ. of North Texas, 1155 Union Cir., #305220, Denton, TX, 76203. In developing avian embryos, the left and right ductus arteriosus shunt pulmonary blood away from the lungs to the systemic circuit and chorioallantois. In mammals and birds studied to date, the ductus arteriosus (DA) are oxygen sensitive vessels that contract in response to increasing oxygen levels. We examined physiology of the DA from Pekin duck, Anas pekin in internally and externally pumped (IVP) embryon. The in vitro contractile response of the left DA was measured using wire myography. Duck DA from both internally and externally pumped stages contracted in response to increasing levels of oxygen. The DA relaxed in response to high levels of Na+ only in the presence of high oxygen levels. Under low oxygen levels, the relaxing response was muted. The DA contracted in response to stepwise increasing phenylephrine. At low concentrations, nonpinesinphrine produced a weak contraction, followed by relaxation at higher concentrations. In the presence of oxygen, sodium nitroprusside produced strong DA relaxation. In the presence of acetylcholine, the DA initially relaxed at low concentrations followed by contraction at higher concentrations. The EP ductus relaxed in response to the Rho-kinase inhibitor fasudil hydrochloride under high oxygen levels. These results suggest the physiology of the duck DA is similar to that of chicken DA. This research was funded in part by an HHMI grant to Lee Hughes and NSF IOS 1146758 (EMD).

### 24.11 DEVELOPMENTAL CHANGES IN MRNA LEVELS OF AVANT AND PGC-1A IN DUCK LIVER AND HEART

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During embryonic development, birds and mammals are unable to physiologically maintain an elevated body temperature when exposed to decreasing ambient temper-
THYROID HORMONE AND DEVELOPMENT OF ENDOTHERMY IN KING QUAIL

Sarah Goy Sirsat1, Tushar Sirsat1, Megan Pineda1, and Edward M. Dzialowski1
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Thyroid hormones (TH) are key regulators of vertebrate metabolism and are thought to regulate development of endothermy in mammals and birds. To better understand TH effects on development of metabolic physiology in a small, precocial avian, we treated embryonic and neonate King Quail (Coturnix chinensis) with triiodothyronine (T3) or the thyroperoxidase inhibitor, methimazole to induce hypothyroidism. Whole animal VO2 consumption during development of endothermy was measured. We examined organ mass and characters such as wing chord and femur length. Whole animal VO2 of hypothyroid subjects differed significantly from control: treated animals exhibited lower VO2 on 5dph and 7dph. Hypothyroidism obtained of endothermic capacity. In the neonate avian, multiple systems development proceeds may influence differentiation associated with the transition from the cancular stage to the secular stage.

25.0: ENDOCRINOLOGY AND REPRODUCTION

25.1 HYPERGLYCEMIC AND PUTATIVE HYPERLIPIDEMIC ACTIVITIES OF THE RECOMBINANT CRUSTACEAN HYPERGLYCEMIC HORMONE CHH-B1 ISOFORM IN THE PACIFIC WHITE SHRIMP LITOPENAEUS VANNAMEI

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Carcass hyperglycemic hormone (CHH) is the most abundant neuropeptide of the sinus gland in crustacean's eyestalks. The main function of CHH is to stimulate hyperglycemia in hemolymph. Studies suggested that CHH also promotes hyperlipidemia under a high energy demand. Lipids are structural body components that serve as alternative metabolic substrates to glucose (Glc). Triglycerides (TG) are the main reserve lipids in crustaceans, serving as source of fatty acids for energy fuel, and glycolate as substrate for gluconeo genesis. CHH-B1 is one of the three CHH isoforms found in the Pacific white shrimp L. vannamei. Recombinant CHH-B1 with C-terminal tags (C-myc, 6XHis) was previously cloned and expressed in Pichia pastoris and purified by affinity chromatography. Shrimps on intermol were injected with 2 μg of rCHH-B1a, and hemolymph was sampled at 0.5, 1, 2, 4, and 24 h post-injection to measure plasma Tg and Glc levels. Results showed that rCHH-B1a caused hyperglycemia by significant raising (P <0.05) Tg levels at 2 h, matching with a hyperglycemia depletin may be due to a switching to lipid oxidation as energy fuel and also to regenerate consumed glucose reserves by a rapid Tg uptake by tissues.

25.2 BIASED SIGNALING BY TWO ENDOGENOUS GnRH ISOSFORMS Differentially REGULATES TOTAL LH AND GH AVAILABILITY IN GOLDFISH PITUITARY CELLS

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In goldfish (Carassius auratus), both endogenous gonadotropin-releasing hormones, GnRH1 and GnRH2, stimulate luteinizing hormone (LH) and growth hormone (GH) release through the activation of multiple signal transduction cascades including isoforms of phosphoinositide 3-kinase, protein kinase C, and mitogen-activated protein kinase, as well as via the differential use of Ca2+-dependent intracellular signaling mechanisms. In this study, we examined the interactions between these signaling pathways in acute and long-term control of basal and GnRH-stimulated LH and GH release using primary cultures of dispersed goldfish pituitary cells. Our results indicate that distinct signal transduction network selectively control basal and GnRH-stimulated hormone release in a time-, pituitary cell type-, and GnRH isoform-specific manner and that changes in total LH and GH availability are often dissociated from their known mRNA expression profiles. These findings provide important insights into the molecular mechanisms that couple biased GnRHR activation to signal transduction responses, in general, while also adding to our understanding of how intracellular signaling dynamics within the neuroendocrine system ultimately contribute to the regulation of whole-organism physiology. (Supported by NSERC, AHS, and the Killam Trusts)
25.3 PERCHLORATE EXPOSURE DOES NOT AFFECT HORMONE CYCLING OVER DIEL AND REPRODUCTIVE SEASON SCHEDULES IN THREESPINE STICKLEBACK
Alison Gardell1, Frank von Hippel1, William Cresko2, John Postleswai2, and C. Loren Buck3

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Perchlorate drives many hormonally-mediated physiological processes. For example, thyroid hormones (T3 and triiodothyronine (T3)) affect growth, metabolism, reproduction, and in some species, exhibit daily and seasonal variations. Previously we showed that exposure of threepine stickleback (Gasterosteus aculeatus) to the endocrine disruptor perchlorate results in pronounced structural changes in thyroid and gonad, while T3 and T4 concentrations remained unaffected. The current study evaluated the interactive effects of time (diel, reproductive season (RS)) and perchlorate treatment on thyroid and androgen hormone regulation in Alaskan G. aculeatus. Wild-caught adult stickleback were exposed to 1 ppm perchlorate and sampled over the 24 hour day and across the RS (May-July). Whole body T3 and T4 concentrations showed no significant differences within a given day in response to perchlorate. Across the RS, whole body T3 concentration remained stable while T4 significantly increased but neither was significantly affected by perchlorate exposure. The concentration of whole body 11-KT in males steadily declined across the RS. The observed increase in T3 across the RS may have implications for energetic investment in reproduction near the end of the stickleback life cycle, although perchlorate does not modify this response. Funding: NIEHS # 1RO1ES017039-01A1.

25.4 EXOGENOUS AVT SUPPRESSES COURTSHIP BEHAVIOR IN XENO- PUS LAEVIS
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Arginine vasotocin (AVT) is a neuropeptide known to influence social and reproductive behavior in non-mammalian vertebrates. In anurans, increased AVT in males is associated with increased courtship behaviors and decreased aggression; however it is increasingly apparent that the effects of AVT may depend on the social structure of a given species. X. laevis, a fully aquatic frog species with a prolonged breeding season, possess a rich repertoire of vocalizations used for inter- and intra-sexual signaling. They do not appear to be territorial, but do show signs of a male social hierarchy and flexible alternative reproductive tactics. We examined how exogenous AVT altered vocal communication and clasp behavior in male X. laevis. When injected males were paired with sexually receptive females, advertisement calling was significantly decreased during the first hour after injection as compared with a saline control. When two AVT-injected males were paired, there was no difference in advertisement calling from control, but there was an increase in male chirps and grows, calls associated with male-male competition. Furthermore, we found that AVT administration led to decreased clasp behavior; male-male amplexus was eliminated and male-female amplexus was reduced and delayed. Thus, unlike in other frog species, courtship behaviors were decreased immediately following AVT administration and male aggression may be increased. These differences may be due to underlying differences in social structure from other anuran species tested, or they may stem from the different physiological consequences of altering osmotic regulation in this aquatic animal. Funded by Denison University Research Fund and Anderson-Bowen Endowments.

25.5 TRH INCREASES GH, BUT NO THYROID HORMONES RELEASE DURING COLD EXPOSURE IN GREEN IGUANA
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Thyrotropin-releasing hormone (TRH) has dual actions in controlling secretion of hypophyseal hormones among vertebrates. In non-mammalian species, TRH is able to stimulate the secretion in addition to the regulation of thyroid hormones (THs). However, little is known about its role on the somatotropic and thyroid systems in reptiles. Previously we showed that exposure of threepine stickleback (Gasterosteus aculeatus) to the endocrine disruptor perchlorate results in pronounced structural changes in thyroid and gonad, while T3 and T4 concentrations remained unaffected. The current study evaluated the interactive effects of time (diel, reproductive season (RS)) and perchlorate treatment on thyroid and androgen hormone regulation in Alaskan G. aculeatus. Wild-caught adult stickleback were exposed to 1 ppm perchlorate and sampled over the 24 hour day and across the RS (May-July). Whole body T3 and T4 concentrations showed no significant differences within a given day in response to perchlorate. Across the RS, whole body T3 concentration remained stable while T4 significantly increased but neither was significantly affected by perchlorate exposure. The concentration of whole body 11-KT in males steadily declined across the RS. The observed increase in T3 across the RS may have implications for energetic investment in reproduction near the end of the stickleback life cycle, although perchlorate does not modify this response. Funding: NIEHS # 1RO1ES017039-01A1.

25.6 OVERWINTER CHANGES IN WEDDELL SEAL BODY CONDITION AND HORMONE PROFILES: IMPLICATIONS FOR PREGNANCY?
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Weddell seals (Leptonychotes weddellii) actively forage from Jan/Feb after completing their molt, until they haul-out for pupping the next Oct/Nov. In years 2010-2012, body composition was determined for post-molt (Jan/Feb; 53 non-reproductive) and pre-breeding (Oct/Nov; 31 non-reproductive, 17 reproductive) adult female Weddell seals. Overwinter changes in physiology varied by study year; however, animals were overall larger and in better condition (greater lipid stores, P<0.001) after the winter, in Oct/Nov. Twenty females were handled in both seasons, and exhibited the same increases in body mass and condition, regardless of their reproductive status the following year. Because changes in body mass are mediated by numerous endocrine factors, cortisol, thyroid hormones (T3 and T4), growth hormone (GH), and insulin-like growth factor (IGF)-1 levels were measured. Of these, T3 and GH were significantly higher during the post-molt period in Jan/Feb (P<0.001), likely assisting with hair regeneration and protein sparing, respectively. Animals with higher T3 levels in Jan/Feb were also significantly more likely to have a pup the following year (χ² = 6.348, P=0.012), indicative of its role in embryo attachment and maintenance of early pregnancy. This study shows that the overwinter foraging period is critical for Weddell seals to gain condition, and that some hormones influencing fuel use during the molt may also impact reproduction the next year. Funding source: NSF0838892.

25.7 AMELIORATIVE CAPACITY OF QUERCETIN ON ALCOHOL AND NICOTINE INDUCED INFERTILITY IN EXPERIMENTAL RATS
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Over the past decades the male gonad has been exposed to various substances; among these substances are alcohol and nicotine. This study was designed to investigate the effect of quercetin on alcohol and nicotine induced infertility in male albino rats. Male rats (180-200g), randomly divided into nine groups of five rats each as follow: Group 1: control, group 2: corn oil 2ml/kg bw, group 3: quercetin (30mg/kg bw), group 4: alcohol (3g/kg bw as 25%/v/v), group 5: nicotine (1.0 mg/kg bw), group 6: alcohol (3g/kg bw as 25%/v/v) + quercetin (30mg/kg bw), group 7: nicotine (1.0 mg/kg bw) + quercetin (30mg/kg bw), group 8: alcohol (3g/kg bw as 25%/v/v) + nicotine (1.0 mg/kg bw) + quercetin (30mg/kg bw), group 9: nicotine (1.0 mg/kg bw) + quercetin (30mg/kg bw). A marked significant decrease (P<0.015) in sperm profile (motility, count, mature sperm and morphology) was observed in sperm collected from the epididymis of the alcohol, nicotine and alcohol plus nicotine treated animals. Histological examination of testis sections in male albino rats treated with alcohol and/or nicotine for 52 days respectively revealed degrees of alteration when compared to control. However, lack of offspring after mating affirms the outcome of this study and this suggests that both alcohol and nicotine have antifertility activities with probable site of action as the testis. Keywords: Alcohol, nicotine, reproductive functions, oxidative stress, infertility.

25.8WITHDRAWN

26.0: THERMAL PHYSIOLOGY

26.1 WATER BEFORE IONS? EARLY CHILL COMA ION BALANCE CHALLENGES THE CURRENT MECHANISTIC MODEL OF CHILL COMA
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At their critical thermal minimum, insects enter reversible paralysis known as chill coma, migration of Na+ from the hemolymph to the gut is followed by water, causing hemolymph ion concentrations (such as K+) to increase. Muscle depolarization due to increased hemolymph [K+] is suggested to explain chill coma paralysis. Chill coma during which water and ion balance is lost. It is currently thought that during coma, migration of Na+ from the hemolymph to the gut is followed by water, causing hemolymph ion concentrations (such as K+) to increase. Muscle depolarization due to increased hemolymph [K+] is suggested to explain chill coma paralysis. Chill coma during which water and ion balance is lost. It is currently thought that during coma, migration of Na+ from the hemolymph to the gut is followed by water, causing hemolymph ion concentrations (such as K+) to increase. Muscle depolarization due to increased hemolymph [K+] is suggested to explain chill coma paralysis.
onset is a rapid process—however until now, water and ion balance had been as-
essed no earlier than six hours after coma onset. Using fall and spring field crickets (which differ in relative cold tolerance), we aimed to characterize water and ion balance in early chill coma to 1) verify whether hemolymph [K] disruption explains paralysis, and 2) to generate hypotheses about mechanisms underlying loss of ion and water balance. Surprisingly, hemolymph [K] imbalance in early chill coma did not ac-
count for muscular paralysis in either species. Furthermore, hemolymph [Na] actu-
al loss of water and subsequent ion balance during chill coma. Funding source: Natural Sciences and Engineering Research Council.

26.2 THERMAL SENSITIVITY OF MUSCLE PERFORMANCE IN THE CHILL SUSCITABLE LOCUST, LOCUSTA MIGRATORIA

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Many insect species enter a state of neuromuscular paralysis when their body temper-
ature is lowered to a critical level but the physiological and cellular processes under-
lying this chill coma are largely unknown. Previous studies on locusts show that
muscle force production is highly depressed at low temperature implicating im-
pairment of the contractile mechanism in the muscle per se. Aiming to determine these
mechanisms we examined the thermal sensitivity of several events in the excitation-

contraction-coupling process including: i) Passive membrane properties and propor-
gation of electrical signals; ii) Intracellular Ca2+ regulation during muscle stimulation
and iii) Ca2+-affinity/sensitivity and maximum force of the contractile proteins. Thus
far the data show that low temperature resulted in a marked depolarization of resting
membrane potential, but had negligible effects on the passive membrane properties and
muscle excitability, suggesting intact ability to excite and trigger action potentials in
muscles at low temperature. Using skinned muscles fibers we show that similar contractile force can be obtained at high and low temperature provided that the muscle
is stimulated with saturating doses of Ca2+. However, preliminary results suggest that
Ca2+-affinity is markedly decreased at low temperature. A reduction in Ca2+-sensitivity of
the contractile filaments at low temperature could therefore explain loss of muscle
function during chill coma. Funding sources: The research was funded by a Sapere
Aude DFF-Starting grant (to J.O.) from The Danish Council for Independent Research / Natural Sciences and by grant (to A.F.) from the Faculty of Science and Technology of Aarhus University.

26.3 THE POTENTIAL TO RESIST CHILL COMA: HOW TEMPERATURE AFFECTS FLIGHT MUSCLE RESTING MEMBRANE POTENTIAL AND HEART RATE IN DROSOPHILIDS

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Most insects enter a reversible coma at low temperatures. The critical temperature
classifying this chill coma (CTaum) is strongly correlated to species distribution. Chill
comas is caused by loss of neuromuscular excitability, which is correlated with a de-
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Source: Natural Sciences and Engineering Research Council.

As global climates warm, temperature induced activity constraints may not be
the primary stressor facing ectotherms. An upward trend in air temperature will be ac-
accompanied by dramatic changes in spatial and temporal variability of precipitation.
However, observational data are confounded by the correlation between high tem-
peratures and high aridity, making it difficult to distinguish thermoregulatory from hydro-
regulatory behaviour in the field. Recent evidence suggests that hydric condition may
strongly influence activity patterns of lizards and that desiccation risk may be as high
as (or higher than) temperature in restricting range and activity patterns. Here we use
experimental manipulation to decouple temperature- and water-based constraints on
activity, isolating the hydroregulatory behaviors of four species of Egernia skink.
Lizards are assigned to either a control or water restricted group and introduced to enclosures providing baking and retreat sites within a finely-controlled weather room which runs a daily temperature cycle ranging from 15°C to 30°C and alternating daily between 20% and 80% RH. Each lizard experiences a dry and a wet day while activity and body temperatures are recorded. This study will better our understanding of the mechanisms of water regulation and the signifi-
cance of hydric condition, allowing more robust predictions of the effect of changing climates on ectotherms.

26.4 VARIATION IN THERMAL TOLERANCE, HYPOXIA TOLERANCE AND METABOLIC RATE IN THE ATLANTIC KILLFISH, FUNDULUS HETEROCLOTS

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couver, BC, V6T 1Z4, Canada.

According to the oxygen and capacity limited thermal tolerance (OCLTT) hypothe-
sis, as temperature increases, aerobic scope declines, causing hypoxia, decreased
organismal performance, and eventual loss of tolerance. Consequently, differences in
thermal tolerance should be associated with differences in metabolic rate and hypoxia
threshold of tolerance among individuals. We have addressed this relationship using Atlantic kil-
fishes, Fundulus heteroclots, a species found along the east coast of North America. The southern subspecies, (F. h. heteroclots) is more tolerant of high temperatures, has
lower routine metabolic rate and better tolerance of hypoxia than does the northern
subspecies (F. h. macrophthalmus), consistent with the predictions of the OCLTT hy-
pothesis. Further explorations of these relationships, we tested thermal tolerance, hypoxia
tolerance and metabolic rate in several populations of F. heteroclots from along the coast. In general, higher thermal tolerance was associated with higher hypoxia toler-
ance and lower metabolic rate. We also collected four hundred individuals from an
admixed population and examined trait associations within this population. There
were no significant correlations between thermal tolerance, hypoxia tolerance and
metabolic rate within this population, suggesting that at the individual level the ex-
pected relationships between the three measurements are not well supported, despite the patterns observed for between-population comparisons. Funded by NSEPC.

26.5 SUB-LETHAL HEAT STRESS CAUSES APOPTOSIS IN COLD-
ADAPTED ANTARCTIC FISHES

Bradley Buckley, and Isaac Sleek

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Source: National Science Foundation's Office of Polar Programs.

As global climates warm, temperature induced activity constraints may not be
the primary stressor facing ectotherms. An upward trend in air temperature will be ac-
accompanied by dramatic changes in spatial and temporal variability of precipitation.
However, observational data are confounded by the correlation between high tem-
peratures and high aridity, making it difficult to distinguish thermoregulatory from hydro-
regulatory behaviour in the field. Recent evidence suggests that hydric condition may
strongly influence activity patterns of lizards and that desiccation risk may be as high
as (or higher than) temperature in restricting range and activity patterns. Here we use
experimental manipulation to decouple temperature- and water-based constraints on
activity, isolating the hydroregulatory behaviors of four species of Egernia skink.
Lizards are assigned to either a control or water restricted group and introduced to enclosures providing baking and retreat sites within a finely-controlled weather room which runs a daily temperature cycle ranging from 15°C to 30°C and alternating daily between 20% and 80% RH. Each lizard experiences a dry and a wet day while activity and body temperatures are recorded. This study will better our understanding of the mechanisms of water regulation and the signifi-
cance of hydric condition, allowing more robust predictions of the effect of changing climates on ectotherms.

26.7 THERMAL PREFERENCE DURING METABOLIC RECOVERY FROM ANOXIC HIBERNATION IN PAINTED TURTLES

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Spring emergence from anoxic hibernation is a critical time in which western painted turtles (Chrysemys picta bellii) in northern latitudes must undergo metabolic recovery from severe lactic acidosis accumulated during winter. Little is known about the physiology of metabolic recovery from overwintering, especially the potential role of temperature. To gain insight into the interaction between thermoregulation and meta-
bolic recovery, cannulated turtles were implanted with iButtons to log internal body
within families and across ontogeny, however, animals exhibit a balance between fur total insulation are influenced substantially by taxonomy, body size, and habitat. Evolutionary patterns observed among marine carnivores. Across species, differences in genetic changes in thermoregulatory strategy among neonatal phocids mimic the evolutionary from fur to blubber, hairs become shorter, flatter, and less dense, while the blubber layer increases in thickness and lipid content. The ontogeny of symptoms similar to that described as HPNS, which may act as a limit to physiological differences in salinity tolerance. An ADP ribosylation factor, β-actin, two heat shock protein 70kDa isoforms (HSP70), and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were found in response to elevated pressure. NMDA receptors have been implicated in pathways of excitotoxic damage to neurons and the onset of high pressure neurological syndrome (HPNS) in mammals. These data indicate that the sub-lethal effects of barotrauma are associated with transcriptional disturbances within the nervous tissue of crustaceans, and cellular macromolecular damage. Such transcriptional changes lead to the onset of symptoms similar to that described as HPNS, which may act as a limit to prolonged survival at depth. Further, we provide evidence of the synergistic effects of hydrostatic pressure and temperature. The interplay between these co-variating factors may be key to understanding the potential for shallow-water invertebrates to acclimatize to depth, and seek refuge from rapidly warming surface waters.

27.1 Adaptive Variability in Salinity Tolerance Exhibits Habitat Differences Between Genetically Distinct Populations of Sacramento Sputilatia

Christine Verhille1, Theresa Dabruzzi2, Dennis Cockerell3, Nahn Fangue4, and Melinda Baerwald1

1Wildlife, Fish, & Conservation Biology, Univ. of California, Davis, 1085 Academic Surge, Univ. of California, Davis, One Shields Ave, Davis, CA, 95616; 2Dept. of Animal Sci., Univ. of California, Davis, One Shields Ave, Davis, CA, 95616. Sacramento Sputilatia (Frog Mortality Syndrome) are classified as a species of concern in California. Understanding the status of Sputilatia is complicated by the existence of two genetically distinct populations: the San Pablo (SP) population, a warm, brackish water spawner; and the Central Valley (CV) population, a cooler, freshwater spawner. To test the hypothesis that this habitat variability is driven by adaptive physiological differences in salinity tolerance between populations, we investigated the physiological response to salinity exposure of wild-caught adult and juvenile Sputilatia of both populations. Juveniles were exposed to salinities from <1 to 14 ppt for up to 14 days and assessed for plasma osmolality (osmo) and ion levels (Na+, K+, Cl−) and gill Na+–K+ ATPase. At 11 ppt, plasma osmo did not differ between SP and CV populations. At 14 ppt, osmo remained elevated until 72 hours for the SP population and 7 days for the CV population. For wild adult Sputilatia, 14 ppt salinity exposure resulted in mortalities, and no differences were seen between the populations exposed to 11 ppt for 24 hours. The delayed recovery of mm and osmo for the juvenile CV relative to the SP population, suggests impaired water and ion balance in juvenile CV Sputilatia facing a salinity challenge. Such impairment suggests juvenile CV Sputilatia are less tolerant of salinity water, and supports our hypothesis of adaptive physiological differences in salinity tolerance.

26.8 The Role of Ambient Temperature on Toxic Ingestion by a Mammalian Herbivore

Patrice Kumah1, and M. Denise Dearing3

1Dept. of Biology, Univ. of Utah, 257 S. 1400 E., Salt Lake City, UT, 84112. Intrinsic factors like nutrients and plant toxins are known to influence feeding behavior in mammalian herbivores; however, far less is known about extrinsic factors like ambient temperature. There is growing evidence that plant secondary compounds (PSCs) become more toxic at higher temperatures due to decreased liver function. This phenomenon, known as temperature-dependent toxicity, could have critical implications for mammalian herbivores that must balance homeothermy with PSC detoxification in a warming environment. Here, we investigated the effect of ambient temperature on ingestion rates of PSCs from cresote bush (Larrea tridentata) by the desert woodrat, Neotoma lepida. We evaluated the maximum tolerable dose of cresote resin at warm (28-29°C), intermediate (25°C) and cool (21-22°C) temperatures. The maximum dose (g resin/day) for woodrats at 28°C was 32% less than woodrats at 22°C. We also tested the ability of woodrats to maintain body mass on a constant dose of cresote resin set below the maximum dose at the warm temperature. On this dose, woodrats at 29°C and 25°C lost >10% of their body mass compared to woodrats at 21°C. Our results demonstrate that increased temperatures limit PSC ingestion in an herbivorous rodent. Studying these interactions will advance the field of herbivore ecology and may enable more accurate predictions of herbivores’ response to climate change. University of Utah IACUC 12-12010, NSF ISO to MDD 0817527, ASM & SICBI GR to PK. 26.9 From Fur to Blubber: Evolutionary and Ontogenetic Transitions in Mammalian Insulation

Heather Livjanic1, Jureca Pearson1, Natalia Grima1, Araannis Bertat2, Daniel Costa3, Jennifer Burns2, Suzanne Budge2, and Terrie Williams3

1Biology, Adelphi Univ., 1 South Ave, Garden City, NY, 11530; Fisheries and Ocean Sci., Univ. of Alaska, Fairbanks, 905 N. Koyukuk Dr., Fairbanks, AK, 99775; 2Biology, Univ. of Alaska, Anchorage, 3101 Science Cir., Anchorage, AK, 99508; 3Biology, San Diego State Univ., 5500 Campanile Dr., San Diego, CA, 92182. We compared morphological characteristics and thermal function of fur and blubber among pinniped species (otariids, phocids, odobenids) in an evolutionary context, and examined the same traits across ontogeny in harp seals. As pinnipeds shift their thermoregulatory strategy from fur to blubber, hairs become shorter, flatter, and less dense, while the blubber layer increases in thickness and lipid content. The ontogenetic changes in thermoregulatory strategy among neonatal phocids mimic the evolutionary patterns observed among marine carnivores. Across species, differences in total insulation are influenced substantially by taxonomy, body size, and habitat. Within families and across ontogeny, however, animals exhibit a balance between fur and blubber that results in similar values for total insulation, even as thermal strategy shifts. Overall, we observed convergent evolutionary trends in thermoregulatory strategy that were recapitulated with ontogeny.
27.4 TEMPERATURE AND HYPOXIA AFFECT SWIMMING ENERGETICS AND KINETICS OF BROWN TROUT (Salmo trutta)
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The brown trout (Salmo trutta) is an economically and ecologically important fish native to the British Isles. In an era of accelerated climate change and the cold and highly oxygenated conditions favoured by this species being threatened. While many studies have described the effects of a single stressor on fish performance, few have examined the interacting effects of multiple stressors on multiple measures of performance. The aim of our study was to investigate the effects of temperature and oxygen on fish swimming kinematics and energetics. Rate of oxygen consumption (MO2) was measured at three temperatures (14, 18 and 22°C) and two dissolved oxygen (DO) levels (100% and 20% saturation) at 8 speeds (0.5-0.9 m/s). Our results suggest that the physiology of adult brown trout are plasticity to accommodate changes in water temperature, velocity and oxygen levels in line with climate change predictions. This study was funded by the Natural Environment Research Council (NERC).

27.5 BEHAVIOURAL RESPONSES OF BLACK PERCH TO MARINE SYNECHOCoccus CYANOBACTERIA
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As in mammals, PbTx in the turtle brain binds to VGSC, triggering Ca2+ influx that alters neuronal excitability. Understanding distribution, clearance, and effects of PbTx in these model turtles will allow us to design treatment strategies for sea turtles exposed to red tides. Funded by NOAA ECOHAB grant NA11NOS4780031.

27.7 AVIAN THERMOREGULATION IN THE HEAT: TOLERANCE TO HEAT STRESS Varies GREATLY AMONG SPECIES
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We studied thermoregulatory performance of summer-acclimatized wild birds to heat stress in the deserts of Australia, North America and South Africa. We measured evaporative water loss (EWL), resting metabolic rate (RMR) and body temperature (Tb) continuously using rammed temperature profiles with increasing air temperatures in 40+ species, which included 10 orders with body size ranging from 7-450g. We estimated the upper critical thermal limit (CTM) for each species by tracing the Tb, EWL and RMR and activity when exposed to air temperatures ranging from 30-64°C. We found that birds from the orders Columbiformes (pigeons and doves) and Caprimulgiformes (nightjays and nightjars) had the highest CTMs and were able to effectively thermoregulate at air temperatures as high as 60°C. Altricial passerines, in contrast, had a much more limited capacity for thermoregulation at high air temperatures and exhibited CTMs near 50°C. We found that thermal tolerance is primarily driven by the predominant pathway of evaporative heat loss, where birds that evapo-rate water from the skin or have a well-developed gular apparatus were most effective thermoregulators at high air temperatures. Body size was also a critical factor in determining the capacity of a species to tolerate high temperatures. This work greatly expands our knowledge of avian tolerance to heat and provides insights into how rapid warming and more intense heat waves may change avian distributions and community structure.

27.8 PHYSIOLOGICAL AND BEHAVIORAL RESPONSES TO ENVIRONMENTAL CHALLENGES IN THE WESTERN TERRESTRIAL GARTER SNAKE, Thamnophis elegans
Eric Ganow1, Allison Spanker1, and Ann Bronikowski2

Populations of the western terrestrial garter snake, Thamnophis elegans, in northern California exhibit divergent ecotypes on the pace-of-life continuum and thus provide a natural laboratory to study the factors that influence responses to variable environments. Understanding intraspecific variation in this response is of central importance in predicting species’ ability to survive in altered landscapes. Here, we present data from a number of recent experiments to quantify these differences: standard metabolic rate measured across a range of active temperatures, physiological indicators of a stress response following a capture-stressor protocol, and behavioral differences in laboratory-reared offspring from laboratory-reared predator attack. Studies examining multiple aspects of the phenotype, we hope to illuminate the complex interplay between physiological and behavior that form an integrated response to environmental challenges. Funding for these studies was provided by the Iowa Science Foundation, PrairieBiotic Research, Inc., the Society for Integrative and Comparative Biology, and the Iowa Department of Natural Resources.
nutrient flux in these larvae was revealed by a genomic analysis that identified 23 putative amino acid transporter genes (cf. 4 in the same gene family in humans). The characterization of transporter gene families and their physiological functions have important implications for the study of integumental transport and for understanding the relationships between nutrition and other physiological processes that depend on the same gene families (e.g., chemoreception, osmoregulation, neurotransmission).

28.2 ONTOGENETIC CHANGES IN THE OSMOTIC STRESS RESPONSE OF BLUE MUSSELS
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Blue mussels, Mytilus edulis, are important members of intertidal communities and their distributions depend on the ability to respond to osmotic stress. Changes in global climate are predicted to alter near shore salinity, so tolerance throughout development will affect recruitment of mussels and the resilience of intertidal community structure. While the osmotic stress response (OSR) in adult mussels has been well studied, the larval stages— which are thought to be more sensitive to environmental stressors—are often ignored. Marine mussels are osmoconformers; during osmotic shock, they regulate intracellular free amino acid (FAA) pools to remain isosmotic to the environment. Taurine is an important osmoregulator utilized by adult mussels during osmotic stress and may play a role in the larval OSR. Our aim is to correlate changes in the FAA pools of larval and juvenile mussels to variation in expression of muTAUT. Overall, our NMR and quantitative PCR results indicate that taurine levels differ from their post-metamorphic counterparts. More attention should be focused on physiological responses to stress throughout development, as the larval OSR may play an important role in the stability of adult communities.

28.3 TRACHEAL SYSTEM STRUCTURE AND FUNCTION CHANGES WITHIN AN INSTAR IN THE CATERPILLAR, MANDUCA SEXTA
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As juvenile insects grow, body mass increases continuously, while sclerotized structures increase in size only after molting. Between molts, within an instar, hypoxia tolerance decreases. One hypothesis is that the tracheal system is sclerotized and increases in size only upon molting, leading to decreased O2 supply. To test this hypothesis, we used synchrotron x-ray imaging to record tracheal system structure and function. Caterpillars at the beginning and end of each instar were placed in 21% and 1% O2, respectively, within an environmental chamber. Mitochondria from embryonic alligators had a lower aerobic respiratory capacity than adults. Mitochondria from embryos at stage 70% development and maximal aerobic capacity was assessed using an Oroboros microrespirometer. Mitochondria from embryonic alligators had a lower aerobic respiratory capacity than their juvenile counterparts, reflecting a developmental switch from glycolytic to aerobic energy production after hatching. Hypoxia did not affect mitochondrial function in embryonic alligators, but juveniles previously subjected to in ovo hypoxia had a greater aerobic capacity and mitochondria were more tightly coupled (more efficient) compared to normoxia. These results provide evidence of adaptive metabolic programming in developing alligators and highlight this animal as a novel alternative to mammalian models of foetal programming. This work was supported by The National Science Foundation (NSF) and The Wellcome Trust.

28.4 EFFECTS OF CALCIUM AVAILABILITY ON GROWTH AND SURVIVAL OF ACIPENSER FULVESCENS IN EARLY LIFE STAGES
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Lake sturgeon (Acipenser fulvescens) have undergone population declines due in large part to anthropogenic habitat changes. To offset these impacts, stock enhancement with hatchery-raised juveniles is often utilized to restore natural populations. Therefore, determining optimal growth conditions in the early life stages is critical for this species. In this study, wild-caught adult lake sturgeon were induced to spawn in captivity, and fertilized eggs were incubated, hatched, and the larvae reared in four environments differing in calcium concentration (0.1, 0.2, 0.4, and 1.5 mM Ca). The impact of these altered water chemistry on hatching success, survival, and growth were measured at distinct developmental stages until initiation of exogenous feeding. Neither hatching success nor sustained larval survival to the feeding stage differed significantly due to environmental [Ca2+]. Protolarvae had similar mass and growth rates in all environmental calcium concentrations over the course of development. In contrast, larval total length was consistently higher in lower environmental [Ca2+]. Surprisingly, lake sturgeon in calcium-limited environments maintained a higher condition index (K = mass/total length3) beginning at 11 days post-hatch. These results suggest that environmental calcium availability may influence early life development and growth of A. fulvescens.

28.5 GENE EXPRESSION PATTERNS OF ALTERNATIVE DEVELOPMENTAL TRAJECTORIES IN EMBRYOS OF AN ANNUAL KILLIFISH
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In endotherms, thyroid hormones (TH) regulate development, metabolism and body temperature. In this study, we treated neonatal altricial Double-crested Cormorants with 3,5,3'-triiodothyronine (T3) or the thyroperoxidase inhibitor, methimazole (MMI), to induce hyperthyroidism and hypothyroidism, respectively. We measured whole animal O2 consumption (VO2) and ventilation by flow through respiration followed by measures of growth and size on days 4, 8, and 14 post hatching (4dp). The VO2 of T3, MMI and control hatchlings on 4dp were higher than in 4dp hatchlings. Hatchlings of 4dp treated with MMI had significantly lower VO2 than 4dp control, independent of body mass. Within treatment groups, there was a significant age effect on body mass, cardiac ventricle mass, liver mass, spleen mass, hematocrit, femur length, tarsus length, wing chord, and head and beak length from 4 to 14 dp. Body mass of 14dp control hatchlings was significantly higher than in 14dp MMI.
The mammalian COX4-2 gene appears to have subfunctionalized into a role in 1, and the 2, elevates erythrocyte production and hence the blood's oxygen content. Inter-features that preclude allosteric repression of COX by ATP, characteristic of COX4-2 hypoxic metabolism. Human and rodent COX4-2 protein possesses structural ON, K7L3N6, Canada.

28.8 QUANTIFICATION OF LEFT VENTRICULAR FUNCTION IN EMBRYONIC CHICKENS (GALLUS GALLUS DOMESTICUS) AT 70% OF INCUBATION USING ULTRASONIC IMAGING
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Quantiﬁcation of ventricular function in developing animals has been dependent on the transparency of the body wall and heart during ontogeny, as in amphibian, fish, and early stage bird embryos. The chicken embryo is a classic model for investigating features of cardiovascular maturation in endothermic vertebrates. A fundamental feature of the final two-thirds of incubation is that embryonic mass and arterial pressure rise continuously. Peripheral resistance also declines during this time, a consequence of increasing vascular collateralization and cross-sectional area during development. Our goal was to quantify ventricular function at 70% of incubation, a time-point where the chorioallantoic membrane vascularature and blood volume are maximal in embryonic development. We used the non-selective β-adrenergic re-ceptor agonist isoproterenol to induce vasodilation and antagonist propranolol to decrease heart rate. Left ventricular output at rest and during pharmacological manipu-lation was quantiﬁed with ultrasound imaging of aortic diameter and blood flow velocity. These measures were coupled with chorioallantoic membrane arterial pressure measurements to establish the simultaneous relationship between afterload pressure and ventricular function. Our data suggest that pharmacologically increasing or decreasing vascular resistance alters ventricular function and output in the late stage embryonic chicken. Funded by NSF Career IBN 08485471 to DAC.

29.0: METABOLISM: HYPOXIA AND ANOXIA

29.1 ERYTHROPOIETIN THROUGH EVOLUTION: A SPECULATIVE VIEW
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Reduced oxygen availability induces erythropoietin (Epo) gene expression that in turn elevates erythrocyte production and hence the blood’s oxygen content. Interest-ingly-and in contrast to Andeans-Tibetans only moderately increase their hematocrit at high altitude. Obviously, evolution has selected a blunted erythropoietic response in Tibetans. As we know, however, the aminotransferase of men and mammals is set well below the maximal possible oxygen uptake capacity. We found that the opti-mal hematocrit in acutely and chronically Epo-treated mice is 0.58 and 0.68, re-spectively. However, these high hematocrit values are seldom reached in men and mammals keeping blood viscosity low, thereby reducing potential cardiovascular risks. As previously hypothesized by Carlos Monge, most men and mammals are sea-level design and if so, one might speculate that oxygen-dependent Epo signal did not originally evolved to increase erythrocyte production. Indeed Epo exerts a neuro-protection function when the CNS is challenged with reduced oxygen supply (e.g. stroke). The fact that Epo/Epo receptor-like proteins are expressed in very low organ-isms including insects provides further (speculative) evidence that Epo might have e-volved as a factor that influences/protects the CNS. We observed that increased Epo levels in the mouse brain augmented exercise performance in a non-erythroid manner. In other words: a single dose of recombinant human Epo demonstrated an unex-pected improvement in maximal exercise performance that was independent of total hemoglobin mass, whole blood volume and cardiovascular parameters. We are testing if this observation translates into human volunteers.

29.2 EVOLUTION OF CYTOCHROME OXIDASE SUBUNIT 4-2 AS A HY-POXIA RESPONSIVE GENE
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The mammalian COX4-2 gene appears to have subfunctionalized into a role in hypoxic metabolism. Human and rodent COX4-2 protein possesses structural features that preclude allosteric repression of COX by ATP, characteristic of COX4-1, and the COX4-2 gene itself is hypoxia responsive. In other vertebrates, COX4-2 is more ubiquitous based upon mRNA levels in fish and reptiles. Fish COX4-2 expression appears to be restricted to specific cell types. The COX4-2 gene of lower vertebrates also lacks hypoxia responsiveness, based upon reporter genes constructed from fish, amphibian and reptile orthologs. COX4-2 protein sequences suggest the div-sulfide bridge seen in the human and rodent orthologs would be precluded in other mammalian lineages and lower vertebrates, each of which lack the requisite CYS pair. The coordinating ligands of the ATP binding site are largely conserved across mammals and reptiles, but in Xenopus and fish, mutations may disrupt the ability of the protein to bind ATP at this site. Collectively, these results suggest that many of the genetic and structural features of COX4-2 that impart responsiveness and benefits in hypoxia may be restricted to the Euarchontoglires lineage that includes primates, lagomorphs and rodents. In other taxa the structure/function of COX4-2 may differ from the pattern seen in humans and rodents. Funded by NSERC-Canada.

29.3 CYTOCHROME C OXIDASE OXYGEN BINDING AFFINITY VARIES WITH HYPOXIA TOLERANCE IN INTERTIDAL FISHES
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The ability of mitochondria to generate ATP is heavily affected by oxygen (O2) sup-ply. In the marine intertidal, fish from the family Cottidae (sculpins) experience daily fluctuations in O2 with species located in the higher intertidal oscillating between ~0 to 400% air saturation and show higher hypoxia tolerance, whereas subtidal species rarely experience hypoxic events and show lower hypoxia tolerance. This difference in their ability to tolerate low O2 is reflected in modifications to various steps in the oxygen transport cascade, where hypoxia tolerant sculpins show higher gill surface area, better hemoglobin O2 binding affinity, and an overall lower O2 consumption rate. In this study, we focus on the mitochondrial COX oxygen transport cascade, cytochrome c oxidase (COX), to determine if O2 use by mito-chondria differ among sculpin species that vary in hypoxia tolerance. Hypoxia tolerant species showed higher COX oxygen binding affinity (measured as lower Km for O2) in both brain and liver, with brain tissue in all species overall having lower KmO2 than liver tissue. There were no differences in COX enzyme activity (Vmax) between species. In an attempt to explain differences in COX KmO2, we further investigate nucleotide and amino acid differences of the mitochondrial DNA-encoded catalytic sub-unit of COX. (Funding: NSERC).

29.4 CHARACTERIZING THE INFLUENCE OF ANOXIA EXPOSURE ON THE ISOLATED HAGFISH HEART
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Pacific hagfish, Eptatretus stoutii, can survive more than 36 hours of anoxia. Recent work indicates that these fish enter a metabolic suppression after 6 hours of anoxia. In the current study we integrated whole animal experiments with studies of isolated hearts to examine the response of the heart to anoxia exposure. We found that 36 hours of anoxia causes blood pH to decrease from 7.8 ± 0.03 to 7.0 ± 0.07, hemoglo-bin O2 saturation to decrease from 68.6 ± 2.4% to 0, total CO2 to increase from 7.0 ± 0.8 mM to 7.8 ± 0.1 mM and hematocrit to increase from 10.4 ± 0.8% to 17.8 ± 1.2%. We are currently utilizing calorimetry to determine how anoxia exposure influences metabolic heat production by the isolated, perfused heart and if this response is affected by acidosis. Preliminary results indicate that anoxia exposure at pH 7.8 causes an initial reduction in heat production and that death occurs at approximately 12 hours of anoxia. In addition, exposure of the anoxic heart at pH 7.8 to pH 7.0 causes an increase in heat production. Finally, heat production by the isolated heart increases to initial levels upon re-oxygenation. Together these results demonstrate that the hagfish heart is able to withstand extended periods of anoxia and that the meta-bolic suppression is blunted at low pHs. Supported by NSERC Canada and Canada Research Chair Program.

29.5 DEEPSEQUENCING OF THE HEPATOPANCREAS TRANSCRIPTOME REVEALS NEW ISOFORMS OF HEMOCYANIN AND THEIR REGULATION IN RESPONSE TO LOW O2/HIGH CO2 IN THE PACIFIC WHITELEG SHRIMP LITOPENAEUS VANNAMEI
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Acclimation to low O2 in many organisms involves regulation at the level of the tran-scriptome. Previous microarray results suggest that the hypoxia-specific transcriptomic signature of the L. vannamei hepatopancreas is reduced or reversed with the addition of environmental CO2. Here we used high throughput RNA sequencing to explore the involvement of new isoforms of hemocyanin (Hc) in the CO2 response. Hepato-pancreas mRNA of juvenile L. vannamei exposed to air-saturated water (normoxia),
low O$_2$, or low O$_2$/high CO$_2$ for 4 or 24 h, was pooled, sequenced (HiSeq 2500) and assembled (Trinity: 46,049 contigs) to create a deep reference transcriptome (1642X coverage). Annotation of the assembly revealed sequences encoding the single large and small Hc subunits, two previously undescribed full-length isoforms of the large subunit, and 12 partial sequences. mRNA of individual shrimp was sequenced (6 treatment); resulting reads were quantified (eXpress) and regulated genes identified from pairwise comparisons at each time (DESeq2). This analysis confirmed that CO$_2$ had an antagonistic effect on the transcriptomic response to low O$_2$. Only 1 Hc (partial c143731) was significantly upregulated in low O$_2$ (24 h) with fold change (FC) = 8.1 compared to normoxia; the response was blunted (FC = 6.2) by high CO$_2$. We are exploring the importance of these novel full length and partial isoforms to the structural and functional response of Hc in low O$_2$ alone and with high CO$_2$ (NSF IOS-1147008).

29.6 ANOXIA-RESPONSIVE SMALL RNA EXPRESSION IN ANNUAL KILLIFISH EMBRYOS Claire L. Riggs$^{1,2}$ and Jason E. Podrabsky$^{1}$

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Embryos of the annual killifish Aequirorhynchus latipes survive without oxygen for over 100 days at their most tolerant stage, but only survive a matter of hours at their least tolerant stage. Studying the changes in their cellular response to anoxia may help build the foundation for human heart attack and stroke treatment. Recently, small non-coding RNAs (such as microRNAs) have been found to play a role in metabolic depression and cellular response to hypoxia, by altering gene expression. In this study, we examined small RNA expression profiles in A. latipes embryos exposed to anoxia and recovery at various developmental stages. We identified 1000s of highly differentially expressed RNA transcripts. Many of these match sequences of hypoxia-responsive miRNAs described in other organisms, while many transcripts may be miRNAs unique to A. latipes, or other small RNAs with important cellular function. At certain stages of development, we have discovered highly differentially expressed and abundant small RNAs that originate from the mitochondrial genome, particularly from RNA genes. Recent literature indicates RNA-derived fragments can perform microRNA-like function as gene silencers. Understanding the expression patterns of these fragments over development and in response to anoxia, as well as their cellular location and function, may alter and clarify our understanding of the cellular stress response. Funding: NIH R01 HL095454, NSF IOS-1354549; NSF DGE-1057604, Sigma Xi GEAR award.

29.7 IS ANOXIA AND ROS-MEDIATED GALA RECEPTOR INHIBITORY SHUNTING IN TURTLE CORTICAL NEURONS MEDATED BY TONIC, FAST OR SLOW PHASIC CURRENTS? Leslie Buck$^{1,2}$ and David Hogg$^{1}$

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The freshwater painted turtle brain is a working model of a vertebrate anoxia-tolerant brain that can be exploited to elucidate the underlying protective mechanisms. We have previously shown that excitatory glutamatergic signaling in the cerebral cortex is inhibited by 50% and GABAergic signaling increases by 100% in response to anoxia. The increased GALA levels activate pyramidal neuron GALA-A receptors (G-AR) forming a shunt that prevents depolarization and generation of an action potential. While the source of GALA is likely stellate interneurons it is unclear which type of stellate neuron it is, or which pool of G-AR is activated. Here we use a pharmacological approach to deduce the relative contributions of phasic fast synaptic, slow peri-synaptic and tonic extrasynaptic G-ARs. Since reactive oxygen species (ROS) levels decrease with anoxia we also investigate the impact of ROS scavengers on G-AR activation. Using whole-cell and perforated patch clamp techniques we determined that both anoxia and ROS scavenging causes: whole-cell conductance to increase from 4.5 to 6.6nS; G-AR reversal potential to move to membrane potential; and slow peri-synaptic G-AR mediated currents to double in amplitude from 238 to 450pA. Bicuculline was used to quantify tonic G-AR currents which doubled with anoxia and ROS scavenging from 17 to 40 pA and phasic fast currents also doubled from 30 to 60 pA. We conclude that all three G-ARs are responsible for the anoxia-mediated shunting current.

29.8 CAN KETONE BODIES PROTECT THE HEART AGAINST THE EFFECTS OF CHRONIC HYPOXIA? Andrew Morrell$^{1}$, Tom Ashmore$^{1}$, James West$^{1}$, Julian Griffins$^{1}$, Hugh Montgomery$^{1}$, Andrew Murphy$^{1}$

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Sustained exposure to hypobaric hypoxia is associated with loss of skeletal muscle but also fat mass. Consequent generation of ketone bodies has been hypothesized to offer metabolic protection for vital organs, acting as both metabolic substrates and modulators. In this regard, they modulate the hypoxic response via HIF1, decrease ROS accumulation and increase mitochondrial efficiency in the brain. Exploration of such effects on other tissues has been limited. We hypothesized that hypoxic rats would exhibit elevated ketone body production in the liver and enhanced use of ketone bodies by the heart, whilst their metabolism in skeletal muscle would decline. To test this hypothesis we exposed rats to 10% O$_2$ for 4 weeks and compared them with normoxic controls fed ad libitum and normoxic control pair-fed to account for the anorexic effects of hypoxia. Our results indicate an increase in the production of ketones in hypoxic rats and subsequent oxidation of ketones in the heart with no change in skeletal muscle. Lipid oxidation was not affected in the liver but declined in both cardiac and skeletal muscles compared to controls. Cardiac tissue from hypoxic rats also had higher oxidative phosphorylation rates when using ketone bodies and had higher glutathione peroxidase activity. Our results support the hypothesis that ketones utilisation is enhanced in cardiac tissue during chronic hypoxia, and may exert protective effects.

30.0: OSMOTIC AND ION REGULATION: SALINITY, OSMOLYTES, AND pH

30.1 PHYSIOLOGICAL AND FUNCTIONAL GENOMIC MECHANISMS OF SEAWATER TO FRESHWATER TRANSITIONS IN THE ALEWIFE Jonathan Velotta$^{1}$, Stephen McCormick$^{2}$, Pavol Michalak$^{3}$, Rachel OnNeill$^{4}$, and Eric Schultz$^{5}$


Colonization of freshwater by seawater organisms has led to substantial adaptive diversification and radiation, especially among fishes. Landlocked, freshwater-only populations of Alewife (Alosa pseudoharengus) have independently evolved multiple times from anadromous populations that migrate from seawater to freshwater to breed. This system enables us to test whether predictable changes in osmoregulatory physiology and its genetic bases have accompanied the transition to freshwater. Through a series of salinity challenge experiments, we show that landlocked forms have predictably evolved increased tolerance of freshwater, which has traded off for reduced tolerance of and osmotic balance in seawater. To characterize divergence in gene expression that underlies these different physiological responses to salinity challenges, we sequenced and de novo assembled transcriptomes. The expression patterns of several genes that function in osmoregulation have similarly differentiated in multiple landlocked populations relative to anadromous expression patterns. However, most genes showing parallel divergence were not known to be osmoregulatory, suggesting that a variety of pathways have been under selection in the transition to freshwater. This analysis reveals multiple dimensions of adaptation to exclusively freshwater habitats. Funding provided by MVAO foundation.

30.2 FROM COMPARATIVE INTESTINAL TRANSCRIPTOME ANALYSIS TO CHARACTERIZATION OF TRANSPORTERS LINKING NUTRIENT ABSORPTION WITH ION AND ACID-BASE REGULATION

Avner Cnaani$^{1}$, Pazit Rozenberg$^{1}$, Dana Ronkin$^{1}$, Tali Nitzan$^{1}$, Adi Doron-Faigerborn$^{1}$, and Eyal Seroussi$^{1}$

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Tilapias are a group of freshwater fishes, however, some of these species are tolerant to high salinities. Differences in salinity tolerance were observed between two closely related species, the highly tolerant O. mossambicus and the more sensitive O. niloticus. Environmental treatments are known to influence the expression of those genes, acclimated to seawater and freshwater, was sequenced using the Illumina HiSeq following by gene expression and gene-ontology (GO) analyses. The results indicate a species-specific salinity-dependent gene expression patterns in the anterior intestine. Overall, between 182 and 404 genes were significantly up-regulated in either seawater or freshwater, including 70 genes with inversed salinity response, up-regulated in one species and down-regulated in the other. From these genes we have focused on proton- and sodium-dependent peptide and amino acid transporters. Seawater- and freshwater-acclimated fish were sampled at three time points after feeding (6, 24, 72 h), representing different digestive stages. The PepT1a, PepTlb, PepTb, B$^{+}$AT1, and B$^{+}$AT1 genes were analyzed for their expression and protein localization along the intestine, and their association with sodium and proton transporters. We found differential expression and localization of these genes along the intestine, correlated them with the different digestive stages, and with salinity adaptation physiology. This research was funded by BARD.
30.3 THE REGULATION AND FUNCTION OF POLYAMINES IN FUNDULUS SPECIES DURING SALINITY STRESS

Fernando Galvez1, and Ying Guan1


Polyamines are a family of low molecular weight organic cations produced in part by the coordinated actions of arginase II (Arg II) and ornithine decarboxylase (Odc). Although polyamines are known for their function in fish, polyamines have been implicated in divergent physiological processes. We describe a possible role of polyamines in hypoxic-osmotic tolerance in the Gulf killifish, Fundulus grandis, and the mummichog, F. heteroclitus, two species known to tolerate large fluctuations in environmental salinity. Adult fish were reared in 5 ppt water for at least 1 month and acutely transferred to 0.1, 0.5, 1, 2, and 5 ppt water. Fish were randomly sampled at 6 h, 1 d, 3 d, and 7 d post-transfer. We have shown that relative Arg II and Odc mRNA levels and Odc protein activity are highly up-regulated in the gills after hypo-osmotic exposure. Hypo-osmotic exposure also increases the concentrations of polyamines, including putrescine, spermidine, and spermine. Although gill putrescine levels remain elevated throughout the first 7 days post-transfer to fresh water, concentrations of spermidine and spermine decrease with time, suggesting these compounds are regulated catalytically. This presentation will discuss the role of polyamines apoptosis during salinity-induced gill remodeling and osmoregulation during salinity stress. This research was supported by funding from the National Science Foundation (EF-0723771).

30.4 REGULATION OF ORGANIC OSMOLYTE CONCENTRATION IN TISSUES OF EURYHALINE TELEOSTS

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To cope with hyperosmotic stress cells accumulate compatible organic osmolytes, which replace harmful inorganic electrolytes while maintaining homeostasis of cell volume and intracellular ionic strength. Previous work has established that myo-inositol is an important compatible organic osmolyte in several tissues of adult Mo-zambique tilapia (Oreochromis mossambicus). Here we investigate the regulation of the biochemical pathway that is responsible for myo-inositol biosynthesis. We find that the activity of the key enzymes of this pathway (myo-inositol phosphate synthase-MIPS and inositol monophosphatase - IMPase) is directly regulated by the ionic milieu, in addition to mRNA and protein abundance regulation. Furthermore, we show that two enzymes, MIPS and IMPase, are regulated in multiple tissues of euryhaline fish (brain, gills), in multiple species of euryhaline fish (including three-spined stickleback, Gasterosteus aculeatus), and in multiple developmental stages of euryhaline tilapia. Of interest, immunohistochemistry reveals that major target tissues for accumulation of myo-inositol during salinity stress differ in larvae from those in adults. We conclude that myo-inositol is a key (but not the only) compatible organic osmolyte that enables euryhaline teleosts to cope with salinity stress. Supported by NSF grant IOS-1355098.

30.5 CELLULAR MECHANISMS FOR ACID/BASE SENSING AND REGULATION IN ELASMOBRANCH GILLS

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The functional specificity of the elasmobranch gill makes it an excellent model for the study of acid/base (A/B) regulation at the cellular and systemic levels without interference from other ion-transporting processes. We immunolocalized the anion-exchange transporter to base-secreting V/H+-ATPase (VHA)-rich cells in gills from leopard shark (Triakis semifasciata). In starred sharks, VHA signal was mostly cytoplasmic and perinodal at the apical pole. However, in fed sharks VHA translocated to the basolateral cell membrane and perinodal to the apical membrane, respectively, indicating upregulation of acid absorption and bicarbonate secretion. To further characterize this mechanism, we added a cell-permeable cAMP analogue to isolated and cultured gill cells, which induced the translocation of VHA to the cell membrane. The bicarbonate-sensitive A/B sensor soluble adenyl cyclase (sAC) was highly abundant in both base-secreting and Na+/K+-ATPase-rich acid-secreting cells. Enzymatic assays revealed bicarbonate-stimulated and K+-sensitive cAMP production in gill homogenates, indicative of sAC activity. In addition, sAC activity was present in nuclei isolated from gill tissue. Finally, we found that both acidic- and base-secreting cells express high amounts of transmembrane adenylyl cyclase (tmAC). We propose a model whereby blood/acid base status is sensed by the combined action of sAC and G-Protein Coupled Receptors/tmAC, with a potential role for nucleoside sAC in the regulation of gene expression. This research was partially supported by a fellowship from the NIH Marine Biotechnology Program Grant (GM067550) to JNR.

30.6 OCEAN ACIDIFICATION STIMULATES RESPIRATORY PLASTICITY IN THE ESTUARINE RED DRUM (SCAENOPSIS OCELLATUS)

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Anthropogenic CO2 release is causing unprecedented changes in the oceanic carbonate chemistry. These environmental changes are too rapid for many species to adapt through evolutionary processes; however, adaptive capacity may be enhanced by phenotypic plasticity. This study examined the effects of ocean acidification (OA) on red blood cell and branchial gas exchange parameters using a combination of molecular, biochemical and morphological methods. OA had no impact on red blood cell parameters after 24 and 72 h versus control, while slc4a1 expression was significantly decreased by 14 days of exposure. Follow up experiments using anemia supported the finding that red blood cells do not show plasticity in response to respiratory stress. In contrast, branchial diffusion distance was reduced by 30% after 14 days of exposure and RhAG and RhCG1 expression doubled after 72 h, both of which could increase CO2 exchange rates at the expense of salt and water balance. Interestingly, branchial Na+, K+ ATPase activity doubled after 14 days of exposure to OA; no change was observed in CFTR or NKCC1 expression. These results suggest that respiratory plasticity may enhance the adaptive capacity of fish in response to OA; however, the osmoregulatory trade-offs may increase baseline metabolic costs. All experiments were performed in accordance with the University of Texas at Austin Institutional Animal Care and Use Committee. Funding for this project was provided by NSF (EF-1315290).

30.7 CHANGES TO INTESTINAL TRANSPORT PHYSIOLOGY AT VARYING LEVELS OF HYPERCAPNIA IN THE GULF TOADFISH (OPSIANUS BETA)

Rachael Heuer1, Kat Mylow1, Nafis Narsinghani1, Jessica Wingar1, Theresa Mackey1, and Martin Grosell1


Marine teleosts defend blood pH during hypercapnia with a well-documented response, which includes the elevation of HCO3-. In contrast to regulatory responses at the gill to achieve net base retention, hypercapnia leads to increased base secretion in the intestine. This base loss is thought to occur through pathways previously implicated in osmoregulatory processes, where chloride is exchanged for bicarbonate to aid in water absorption. The objective of this study was to expose gulf toadfish to various CO2 levels (0.25, 0.5, 1, 2% CO2) to examine changes and identify trends that occur in intestinal transport physiology, intestinal calcium carbonate production rates, and carcass formation and composition during hypercapnia. Results of this study indicate a close relationship between the increase in intestinal bicarbonate secretion and the rate of chloride uptake, suggesting increased ion exchange with increasing hypercapnia. Reduced fluid Mg2+ (mM) and increased carbonate production appear to be characterized at high CO2 levels, changes that could impact carbonate composition and thus solubility. Impacts of CO2 on other ions and the relationships between ion concentrations in plasma, rectal fluid, and calcium carbonates will also be discussed. This research was supported by an NSF grant through Dr. Martin Grosell (PI) and through a NSF Graduate Fellowship awarded to Rachael Heuer.

30.8 CRAB PROTEOMICS: RESPONSES TO SIMULTANEOUS LOW PH AND TEMPERATURE STRESS

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1Biological Sci., Califonia Poly, State Univ., 1 Grand Ave., San Luis Obispo, CA, 93407, 2Int. Ecophysiology, Alfred-Wegener Inst., Am Handelshafen 12, Bremerhaven, Germany, 27570, Germany.

We investigated the proteomic responses of gill tissue of the spider crab (Hym zavus) and the intertidal porcelain crab (Petrolistes cinctipes) to simultaneous temperature and pH stress. Both species showed a decrease in tyrosine metabolism, while simultaneously changing the abundance of lectins and serine proteases, which are thought to lead to the conversion of pro-phenoloxidases (including hemocyanin) to phenoloxidases. These catalyze the synthesis of quinones and melanin from tyrosine. Quinones are involved in the sclerotization of the arthropod cuticle and both species showed changes in abundance of cuticle protein. The porcelain crab also showed changes in the abundance of proteins that are involved in the excretion of ammonium, and thus the excretion of proton equivalents, across the gill tissue. This response to low pH was dependent on the immersion/emersion and temperature conditions animals experienced. Changes in the abundance of proteins involved in the urea cycle might indicate that it too is involved in the excretion of bicarbonate ions and thus protons. Low pH also decreased the abundance of chaperons of the endoplasmatic reticulum. In general, crustacean gill tissue changed a number of proteins involved in cuticle structure and possibly the passive ion transport properties of the
31.0: THE CHALLENGE OF TEACHING PHYSIOLOGY IN A CHANGING ENVIRONMENT: INNOVATION AND RESOURCES

31.1 VISION AND CHANGE UPDATE: PROGRESS IN IMPLEMENTING REPORT GOALS IN UNDERGRADUATE BIOLOGY EDUCATION

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3This readily available information age, student centered learning is known to help students develop competency in required disciplines and be better able to manage their lifelong learning goals. Fortunately, new and revised pedagogy is available for teachers (even with large enrollment classes) to help their students develop critical thinking skills and construct meaningful knowledge and competency in areas. This presentation will define inquiry and describe why building new knowledge should be the goal of teaching. Ideas will be shared for beginning to change didactic lectures into active learning experiences and for incorporating various student centered activities into laboratory and lecture sessions. Specific examples of learning by experimentation, team-based learning, problem-based learning, case-based learning, project-based learning and discussion-based learning will be described. Layman, JW, Inquiry and Learning. New York: The College Board, 1996. Sutman, FX, Paper presented at annual meeting of AAAS, Philadelphia, PA, 1998. Michaeluen, LK, AB Knight, and LD Fink, Team-based Learning: A Transformative Use of Small Groups in College Teaching. Stylus, Sterling, VA, 2002. Advances in Physiology Education (advan.physiology.org) and Life Science Teaching Resources Community (www.lifesci.org).

31.2 TEACHING AND LEARNING BY INQUIRY

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In this readily available information age, student centered learning is known to help students develop competency in required disciplines and be better able to manage their lifelong learning goals. Fortunately, new and revised pedagogy is available for teachers (even with large enrollment classes) to help their students develop critical thinking skills and construct meaningful knowledge and competency in areas. This presentation will define inquiry and describe why building new knowledge should be the goal of teaching. Ideas will be shared for beginning to change didactic lectures into active learning experiences and for incorporating various student centered activities into laboratory and lecture sessions. Specific examples of learning by experimentation, team-based learning, problem-based learning, case-based learning, project-based learning and discussion-based learning will be described. Layman, JW, Inquiry and Learning. New York: The College Board, 1996. Sutman, FX, Paper presented at annual meeting of AAAS, Philadelphia, PA, 1998. Michaeluen, LK, AB Knight, and LD Fink, Team-based Learning: A Transformative Use of Small Groups in College Teaching. Stylus, Sterling, VA, 2002. Advances in Physiology Education (advan.physiology.org) and Life Science Teaching Resources Community (www.lifesci.org).

31.3 ASSESSING STUDENT LEARNING AFTER CONVERTING TO INQUIRY

Douglas Ladje,1 Jacob Aubre,2 Benjamin Marango,1 Aaron Rikvin,1 Lindsey Foos,3 and Joseph Malpasewski


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We compared gains in student content learning over a 10-yr period in which an introductory biology laboratory curriculum was changed to inquiry. As the level of inquiry increased, student learning gains on content exams trended upward. Students who participated in 14-wk-long inquiry laboratories outscored their peers in 7-wk inquiry and 1-wk traditional labs on MCAT exam questions (scores of 64.7±, 61.9±, and 53.48±, respectively, p < 0.01). End of semester surveys conducted when traditional 1-wk laboratories (n=167 students) were used had low response rates and predominately negative opinions (only 20% of responses were positive), whereas those conducted after 7-wk (n=543) or 14-wk (n=308) inquiry laboratories had high response rates and 71 and 96% positive reviews, respectively. In an assessment of traditional content coverage in courses, three indexes were averaged to calculate traditional forms of coverage and showed a decrease by 44% over the study period. We believe that the quantitative and qualitative data support greater student-driven inquiry in the classroom laboratory, which leads to deeper learning in fewer topic areas (less teaching) and can reap gains in scientific thinking and fundamental understanding applicable to a broader range of topic areas (more learning) in introductory biology.

31.4 CONSTRUCTING CONCEPT INVENTORIES AND ASSESSING THEIR VALUE

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3This readily available information age, student centered learning is known to help students develop competency in required disciplines and be better able to manage their lifelong learning goals. Fortunately, new and revised pedagogy is available for teachers (even with large enrollment classes) to help their students develop critical thinking skills and construct meaningful knowledge and competency in areas. This presentation will define inquiry and describe why building new knowledge should be the goal of teaching. Ideas will be shared for beginning to change didactic lectures into active learning experiences and for incorporating various student centered activities into laboratory and lecture sessions. Specific examples of learning by experimentation, team-based learning, problem-based learning, case-based learning, project-based learning and discussion-based learning will be described. Layman, JW, Inquiry and Learning. New York: The College Board, 1996. Sutman, FX, Paper presented at annual meeting of AAAS, Philadelphia, PA, 1998. Michaeluen, LK, AB Knight, and LD Fink, Team-based Learning: A Transformative Use of Small Groups in College Teaching. Stylus, Sterling, VA, 2002. Advances in Physiology Education (advan.physiology.org) and Life Science Teaching Resources Community (www.lifesci.org).

31.5 ASSESSING GROWTH IN STUDENT KNOWLEDGE OF CONCEPTS IN BIOLOGY USING CONTEXT-DEPENDENT MEASURES


31.6 VISION AND CHANGE: HOMEOSTANCE; A FUNDAMENTAL CONCEPT FOR UNDERSTANDING DEVELOPMENT AND ADAPTATION


We compared gains in student content learning over a 10-yr period in which an introductory biology laboratory curriculum was changed to inquiry. As the level of inquiry increased, student learning gains on content exams trended upward. Students who participated in 14-wk-long inquiry laboratories outscored their peers in 7-wk inquiry and 1-wk traditional labs on MCAT exam questions (scores of 64.7±, 61.9±, and 53.48±, respectively, p < 0.01). End of semester surveys conducted when traditional 1-wk laboratories (n=167 students) were used had low response rates and predominately negative opinions (only 20% of responses were positive), whereas those conducted after 7-wk (n=543) or 14-wk (n=308) inquiry laboratories had high response rates and 71 and 96% positive reviews, respectively. In an assessment of traditional content coverage in courses, three indexes were averaged to calculate traditional forms of coverage and showed a decrease by 44% over the study period. We believe that the quantitative and qualitative data support greater student-driven inquiry in the classroom laboratory, which leads to deeper learning in fewer topic areas (less teaching) and can reap gains in scientific thinking and fundamental understanding applicable to a broader range of topic areas (more learning) in introductory biology.

31.7 INSULIN SIGNALING AS A KEY REGULATOR OF INSECT DIAPAUSE

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The insulin signaling pathway was first suggested as a key player in regulation of the dauer state in the nematode Caenorhabditis elegans, and it now appears to be equally important in regulating the equivalent dormant state, diapause, in insects. In experiments with Culex pipiens, the mosquito that vectors West Nile virus, we have shown that the arrested ovarian development of adult females, a characteristic of the overwintering diapause in adult insects, is the consequence of a shut-down in the insulin signaling/FOXO pathway. Insects produce numerous insulin-like peptides (ILPs), but not all are involved in the diapause response. ILP-1 appears to be the peptide in this family that is most closely linked to diapause in C. pipiens. ILP-1 acts upstream of juvenile hormone (JH) and contributes to shutting down the synthesis of JH that is essential for ovarian development. This mediation of JH synthesis is linked to suppression of allatotropin, the neuropeptide that promotes JH synthesis. Diapause can be simulated in nondiapausing mosquitoes by using RNAi to knock down expression of the genes encoding either ILP-1 or the Insulin Receptor, and the knock down can be rescued by application of exogenous JH. Fat accumulation and enhanced stress tolerance, features that also characterize the diapause state, appear to be linked to the insulin pathway through the action of a key transcription factor, FOXO. This pathway thus appears to generate many features that characterize the diapause phenotype. Similar results observed in Drosophila melanogaster and the flesh fly Sarcophaga...
32.3 THE ROLE OF MATERNAL PROVISIONING AND MICRONA REGULATION OF DIAPAUSE IN THE ANNUAL KILLIFISH ASTROFUNDULUS LIMNAEUS

Jason Podrabsky1

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Annual killifish survive in ephemeral ponds by producing embryos that may enter diapause at three distinct developmental stages (diapause I, II, and III). Diapause II is a profound state of dormancy and is likely key to survival of embryos through the dry season. However, escape embryos skip diapause II and develop directly to diapause III. Entrance into diapause II is an alternate developmental pathway controlled by both maternal inputs and the temperature experienced by free-living embryos. Embryos developing at 20°C enter into diapause II while those developing at 30°C develop as escape embryos. Fertilized embryos destined to develop along the escape trajectory are provisioned with a unique gene expression signature for both small and large RNA molecules, and have increased levels of insulin-like growth factor II protein. Incubation at 30°C induces an increase in the expression of small RNA transcripts that are known to block expression of maternal mRNA transcripts that control early development. It appears that entrance into diapause is controlled maternally, but can be altered by zincic small RNAs in response to environments conducive to direct development. This work identifies genetic mechanisms supporting diapause and hedging strategies in annual killifish development. Reference: Podrabsky JE, Garrett IDG, Kohl ZF. 2010. Alternative developmental pathways associated with diapause regulated by temperature and maternal influences in embryos of the annual killifish Astrolampas limnaeus. J Exp Biol. 213:3280-3288.

32.3 BIOENERGETICS OF DIAPAUSE IN A CRUSTACEAN EXTREMOPHILE

Steven Hand1


Gastrula-stage embryos of Artemia franciscana (brine shrimp) display developmental arrest and a respiratory depression of over 99% upon release from the adult female as they enter diapause. A substantial contributor to inhibition is a restriction of oxidative substrate to the mitochondrion that involves an orchestrated interplay at multiple enzymatic sites including trehalase, hexokinase, pyruvate kinase and pyruvate dehydrogenase. Proton conductance across the inner membrane is not diminished in mitochondria from diapause embryos versus post-diapause, and thus mitochondrial membrane potential (ΔΨ) is likely compromised because respiration of diapause embryos is depressed below that required to compensate for proton leak. Under such conditions, the F-ATP synthase could reverse and deplete all cellular ATP. ATP decreases during diapause, but significant amounts remain (ATP:ADP ratio = 1.3). We predict that the hydrolysis activity of F-ATP synthase is blocked during diapause by the inhibitor protein IF1, which as previously shown binds to the catalytic subunit of the bovine synthase. Reference: Podrabsky JE, Garrett IDG, Kohl ZF. 2010. Alternative developmental pathways associated with diapause regulated by temperature and maternal influences in embryos of the annual killifish A. franciscana. Physiol Biochem Zool 83: 106-118. Bason JV, Runswick C. 2003. Developmental plasticity in a marine crustacean: Artemia franciscana. Biol Lett 1: 483-486. 4:189 (doi:10.3389/fphy.2013.00189).

33.2 THE REACTIVE SCOPE MODEL: PREDICTING THE EFFECTS OF CHALLENGES TO HOMEOSTASIS

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Homeostasis - a balancing act requiring a range of physiological counter-balances. How do such counter-balances maintain homeostasis when faced with challenges, predictable, unpredictable or extreme? The Reactive Scope Model provides a conceptual framework for applying homeostasis theory to assess and make predictions about stress. The model considers the balance of physiological mediators operating within four distinct ranges. 1) Predictive Homeostasis includes circadian and seasonal variation of mediators at baseline levels. 2) Reactive Homeostasis includes the range required to respond to unpredictable challenges (i.e. acute stressors). These two ranges encompass the Reactive Scope. Physiological mediators at levels outside of the Reactive Scope increase potential for pathological effects. 3) Homeostatic Overload occurs when the response to a challenge exceeds the Reactive Scope and the mediators become detrimental. 4) Homeostatic Failure occurs when levels of physiological mediators fall below that required to maintain basic function. The specifics of what defines each of the four ranges can change with individual, species, and/or population, allowing the model to be adapted to include effects of early life stress, dominance hierarchies, seasonality etc. Applying the dynamic nature of homeostasis theory, the Reactive Scope Model allows for a versatile set of principles to consider the complexities and consequences of maintaining physiological balance in a challenging world.

33.3 PHYSIOLOGICAL REGULATORY NETWORKS: THE ORCHESTRA OF LIFE?

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Linear thinking and reductionism have been valuable in physiology, but much in biology depends upon complex interactions. Subsequently, the conventional single-system approach might benefit from more attention to interactions among systems and their components. Already, such efforts are paying off; cross talk among the immune, endocrine and nervous systems, for example, are integral to successful defense against parasites and other insults. Also, at other levels of organization (i.e., genes, communities, etc.), there are already useful frameworks for the study of complex interactions, namely networks. Here, we present a conceptual model of physiological regulatory networks (PRNs), the full suite of molecules and their regulatory relationships that mediate individual homeostasis. Although systems biology has embraced the study of networks, networks at the level of whole-individuals have received little attention. We argue that studies of PRN connectivity, modularity and hierarchy could transform how we think about and manage health and disease. PRN theory might also inform many ecological and evolutionary processes such disease cycles and spread and species range expansions and contractions. Some have recently claimed that physiology is rocking the foundations of biology; we offer the PRN framework as one component of a new foundation to help unify heretofore disjunct (proximate and ultimate) biological realms. References: Cohen, Martin, McWilliams, Wingfield, and Dunne. 2012. Physiological regulatory networks: ecological roles and evolutionary constraints TREE 27: 428-435; Martin, LEB, CHW, and AC-Cohen. Physiological regulatory networks: the orchestra of life? In Integrative Organismal Biology, Martin, Woods and Ghalambor, Wiley Press, in press (December 2014).

33.4 INFORMATION THEORY, HOMEOSTASIS, AND EVOLUTION

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Physiologists commonly view homeostasis as a set of mechanisms evolved to protect organisms from damaging extremes. Here I propose that homeostasis also evolves to minimize noise in physiological channels. Fluctuations in physiological factors constitute inescapable sources of noise that corrupt the transfer of information through biological systems. Drawing on information theory, I develop the idea that homeostatic regulation creates quiet physiological backgrounds for the transmission of all kinds of physiological information. This view leads to two additional ideas about the roles of homeostasis. First, homeostatic systems act as coupled pairs of transmitters and receivers, such that the performance of any one system influences information pro-
34.0: LINKING BEHAVIOR AND PHYSIOLOGY IN ANIMAL NAVIGATION AND ORIENTATION

34.1 USING GENETICS TO REVEAL MIGRATORY FLIGHT ORIENTATION MECHANISMS IN THE MONARCH BUTTERFLY
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Arseny Finkelstein1, Dori Derdikman1,2
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The eastern North American monarch butterfly (Danaus plexippus) has emerged as a powerful model system to study animal clocks, and navigational and migratory mechanisms. The navigational capabilities used by monarchs to accomplish their yearly long-distance migration rely primarily on a time-compensated sun compass orientation mechanism, in which circadian clocks in the antennae time compensate the sun compass output in the brain (1). The molecular and cellular mechanisms involved in the generation of the migratory syndrome remain however largely unknown. To further our understanding of the genetic and neuronal basis of migration, we have recently sequenced the monarch genome and developed genetic tools to knock out clock genes using nuclease-mediated gene targeting approaches (2). Our progress on the use of TALENs and CRISPR to genetically manipulate the monarch will be presented. This will allow us to eventually knock-in fluorescent reporter tags into clock gene loci to facilitate the dissection of the neuronal circuits underlying the flight orientation of migratory monarchs. References: 1) Reppert, S.M., Gage, R.J. & Merlin, C. 2010. Navigational mechanisms of migrating monarch butterflies. TINS 33, 399-406. 2) Merlin, C., Beaver, L.E., Taylor, O.R., Wolfe, S.A. 7 Reppert, S.M. 2013. Efficient targeted mutagenesis in the monarch butterfly using zinc-finger nucleases. Gen Res 23, 159-168.

34.2 3D NEURAL COMPASS IN THE BAT BRAIN
Arseniy Finkelstein1, Dori Derdikman1,2, Alon Rubin1, Jakob N. Foerster1, Lion La1, and Nachum Ullman1
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Head-direction cells are neurons that become active whenever the animal’s head points to a specific direction (azimuth) in the horizontal plane, in a manner similar to a compass. Although the survival of many animals may depend on successful orientation in three-dimensional (3D) space, it is unclear how 3D head-direction is represented in the brain. Here we recorded from neurons in the dorsal presubiculum of Egyptian fruit bats, which are well-adapted to 3D spatial behaviors, and found head-direction cells tuned to azimuth, pitch or roll – of which 30% were conjointly tuned to multiple combinations of Euler angles. 3D head-direction tuning was observed in both crawling and flying bats. Head-direction cells were organized according to a functional-anatomical gradient, suggesting a gradual transition from 2D to 3D representations along the transverse axis of the presubiculum. When bats were in the upside-down position, most neurons remained directionally-tuned; surprisingly, however, their azimuth/tuning was shifted by 180° relative to the upright position – suggesting that 3D head-direction is represented on a toroidal manifold, rather than in standard spherical coordinates. The toroidal model was further supported by experiments in which bats crawled on a vertical ring – revealing that the tuning of pitch cells was circular, continuous and unidirectional within the available 360° of pitch. Taken together, these results demonstrate a 3D head-direction mechanism in mammals, which could support navigation in 3D space. *These authors contributed equally to this work.

34.3 NEURAL REPRESENTATION OF THE HIERARCHY OF CELESTIAL CUES IN THE DUNG BEETLE BRAIN
Basil el Jundi1, and Marie DUCKET1

Some diurnal (Scaraebus lamarcki) and nocturnal (S. styrtius) dung beetles have developed a unique orientation behavior to avoid competition for food at a dung patch. They cut off a piece of dung, form it into a ball and roll it away along straight paths. To keep a straight path, both species rely on skyline cues such as the sun, moon or the polarized light. We analyzed the hierarchy of these signals in both species in the field by setting the polarized light in conflict with the sun or the moon. We found that the sun is the main reference for the internal compass in diurnal beetles, whereas in nocturnal beetles polarized light is the dominant cue. However, what does this internal compass look like in the beetle brain and how is the hierarchy of compass signals represented at the neural level? The central complex (CX) is a brain area that likely serves as the insects’ internal compass. Immunohistochemistry and 3D reconstructions were used to characterize the beetles’ CX network. In addition, CX-cells were analyzed through intracellular recordings while mimicking the sun or the moon by light spots that move on a circular path around the beetle’s head and skylight polarization by a dorsally rotating polarizer. All cells responded to both stimuli, and thus encoded both celestial cues. Interestingly, when presented both cues simultaneously, neurons in the nocturnal species responded only to the polarizer, while cells were tuned only to the light spot in the diurnal species. This shows that in the beetle CX single neurons encode a hierarchy of different celestial cues, accurately reflecting our behavioral findings.

34.4 POLARIZED LIGHT NAVIGATION IN DROSOPHILA
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When normalized to body length, the migratory flights of drosophilid flies are among the longest aerial excursions in animal kingdom. The mean body length of many flies maintains a straight course over many kilometers is not known, but evidence suggests that pattern of polarized light in the dawn or dusk sky provides an important navigation cue. Recently, our laboratory has shown that flies will maintain a roughly constant course heading using the pattern of polarized light in a natural sky. To study navigation under more controlled conditions, we developed an indoor flight simulator in which a rigidly tethered fly can orient itself under an erasable sky via a closed-loop feedback system. The results indicate that flies vary with respect to the azimuthal orientation of polarized light they prefer. In other words, each fly appears to choose a random heading, but then maintains that heading over time. We are currently using the genetically-encoded calcium indicator GCaMP to study circuitry that underlies polarized light navigation in tethered, flying flies. Our initial results indicate that, as in other insects, the detection of polarized light starts within specialized central photoreceptors in the dorsal rim of the compound eye. Support: NSF 0623527, NIH 5-T32-MH019138, Paul G. Allen Family Foundation. References: Dickinson, M.H. (2014). Death Valley, Drosophila, & the Devonian Toolkit. Ann. Rev. Entomol. 59:51–72. Weir, P. T. and, Dickinson, M.H. (2012). Flying Drosophila orient to sky polarize. Current Biology 22, 12-20.

35.0: THERMAL PHYSIOLOGY

35.1 AN IN VIVO INVESTIGATION OF LOW TEMPERATURE ENERGETICS IN DROSOPHILA MELANOGASTER USING 18PNMR
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Low temperatures lead to energetic failure in aquatic ectotherms, but the extent to which this occurs in terrestrial ectotherms remains unclear. Here, we use 18P nuclear magnetic resonance (NMR) spectroscopy to dynamically monitor levels of energetically important phosphorus-based molecules, including ATP and phosphoglycerine, during the course of cold exposure and recovery, using populations of Drosophila melanogaster selected for either fast (hardy) or slow (susceptible) recovery from cold-shock. We hypothesized that flies enter a lower energy state when lowered below the cold-shock threshold, and that cold-adaptation reduces the extent of the energetic disruption or facilitates a faster recovery. Phosphoglycerine and sugar phosphates increased abruptly when flies were cooled and then steadily decreased over the course of the cold exposure. An unknown metabolite decreased sharply upon cold exposure and recovered upon rewarming. Hardy flies had markedly lower levels of this unknown metabolite during both cold exposure and recovery. We found little evidence for changes in ATP during the course of cold exposure or recovery. We conclude that energy limitation does not appear to underlie evolved differences in cold-shock recovery times, and suggest future work be done to identify this interesting metabolite that responds to cold, and whose dynamics in vivo are strongly altered by cold adaptation. This work was funded by NSF grant 1051890 to DAH, ASE, and TJM.
35.2 NEW APPROACHES TO UNDERSTANDING INSECT FREEZE TOLERANCE
Brent J. Sinclair1,2
1Biological, Western Univ., 1151 Richmond St. N., London, ON, N6A 5B7, Canada. The ability of some insects to survive internal ice formation was first described over 200 years ago, yet we still do not understand the underlying mechanisms, nor are we able to produce robust freeze tolerance in non-freeze tolerant insects. I will briefly discuss what is known about insect freeze tolerance, and how the approaches we have taken in the past may have led to dead-ends. I will then describe new approaches being taken in my laboratory to elucidate the mechanisms underlying freeze tolerance in insects. These are 1) the development of new comparative models that allow variation in freeze tolerance to be studied on seasonal, geographic, and evolutionary scales; 2) the utilization of omics technologies (primarily RNA-Seq) to identify new candidate molecules and pathways; 3) an hypothesis-testing approach to critically examine the existing models of insect freeze tolerance. I will present a selection of data derived from each of these approaches, particularly focusing on whether freeze tolerance has an underlying set of common mechanisms in spite of having evolved independently on multiple occasions. I will also discuss new data about the processes associated with thawing in insects that survive freezing, and discuss the identity, function, and synthesis of acetylated lipids, which may function as intracellular cryoprotectants. I conclude that these new approaches signal an exciting time in the study of freeze tolerance, and the likelihood of identifying novel biochemical and physiological mechanisms of cold tolerance in ectotherms.

35.3 MITOCHONDRIAL AND NUCLEAR GENETIC VARIATION RELATE TO HEAT AND COLD TOLERANCE IN A MONTANE LEAF BEETLE
Nathan Rank1, Jordan Sayre1, Sarah Heid1, Kevin Roberson1, Brent Sinclair1, Patrick Mandahyn2, Nicolas Zavala1, and Elizabeth Dahlhoff1
1Biology, Sonoma State Univ., 1801 East Cotati Ave., Rohnert Park, CA, 94928, 2Biology, Western Univ., 1151 Richmond St. N., London, ON, N6A 5B7, Canada. Evolutionary Biology & Ecology, Free Univ. of Brussels, Univ. Libre de Bruxelles, CP 160/12, av. FD Roosevelt 50, Brussels, 1050, Belgium, 3Biochemistry, Santa Clara Univ., 500 El Camino Real, Santa Clara, CA, 95053. Montane ecosystems are often confronted with dangerously high and low temperatures in summer when individuals are active and completing reproduction and development to adulthood. We investigated effects of exposure to stressful hot and cold temperatures for adults and larvae of the willow beetle Chrysomela aeneicollis collected in California’s Sierra Nevada Mountains. To investigate relationships between genetic variation and thermal phenotypes, we sampled beetles from an introgressed population where southern and northern nuclear and mitochondrial genomes recombine freely. We measured running speed after heat or cold stress, cold tolerance, and activity of Cytochrome oxidase, then obtained genotypes at COI and the nuclear gene phosphoglucone isomerase. Heat tolerance was greatest for beetles with a southern mitochondrial and nuclear genotype but lowest for beetles with the southern mitochondrial genotype and northern nuclear genotype. In contrast, beetles with the southern mitochondrial genotype showed the greatest cold tolerance if they possessed the northern nuclear genotype, but lowest cold tolerance when they possessed southern nuclear and mitochondrial alleles. We discuss the significance of these results for understanding of the role of mitochondrial interactions in evolution of thermal tolerance. This research was supported by NSF (DEB 0844040/06).

35.4 NEVER MIND THERMAL PERFORMANCE CURVES-IT’S ALL ABOUT TOLERATING THE EXTREMES! SENSITIVITY TO THERMAL EXTREMES PREDICT CURRENT (AND FUTURE?) DISTRIBUTION OF DROSOPHILA SPECIES
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1Zoophysics, Aarhus Univ., C.F.Moellers Allé 3, Bldg. 1131, Aarhus, 8000, Denmark, 2Zoology, Melbourne Univ., Parkville, Melbourne, Australia. Climatic factors influence the distribution of ectotherms, raising the possibility that distributions of many species will shift rapidly under climate change. Recent studies comparing performance curves of species from different climate zones have suggested that tropical species are more susceptible to climate change than temperate species. However, comparisons involving responses to thermal extremes have found that mid-latitude species are more susceptible. Using a group of 10 related Drosophila species with either a tropical or widespread distribution, we undertook a detailed assessment of the breadth of their growth performance curves (fecundity, developmental success, and developmental time) and tolerance to thermal extremes (adult heat and cold resistance). Using these data we modeled distribution patterns and compared these models to current observations of species distribution. Thermal performance curves proved to be a poor predictor of current species distributions while adult tolerance to thermal extremes provides a good correlate of current distributions. Thus, in their current distribution range, most of the examined species experience heat exposure close to, but rarely above, the functional heat resistance limit. Similarly, adult cold resistance proved a good predictor of species distribution in cooler climates. Given these findings we predict that both tropical and widespread Drosophila species will face a similar proportional reduction in distribution range under future warming.

35.5 THERMAL PERFORMANCE, AEROBIC SCOPE, AND RELEVANCE OF THE OCLT HYPOTHESIS
Fredrik Jutfelt1,2, Erik Sandblom1, and Timothy Clark2
1Biological & Environmental Sci., Univ. of Gothenburg, Medicinaregatan 18, 41390, Gothenburg, Sweden, 2Sven Loven Ctr. Kristineberg, Univ. of Gothenburg, Kristineberg 566, SE-451 78, Fiskebäckskil, Sweden, 3AICES, Australian Inst. of Marine Sci., PMB 3, Townsville MC, Queensland 4810, Townsville-MC, Australia. It has long been debated how temperature determines physiological performance and field distribution in ectothermic animals. The oxygen- and capacity-limited thermal tolerance (OCLT) hypothesis states that thermal performance in aquatic ectotherms is limited by aerobic scope (Mo2max-Mo2min). This hypothesis has gained popularity despite relatively little experimental evidence. Here we examine the evidence for the core assumption, that oxygen supply to the tissues is the causal mechanism for reduction in other performance metrics. The available literature demonstrates that aerobic scope often continues to increase with temperature up to lethal temperatures, while other performances such as growth or reproductive output can be limited at much lower temperatures. We argue, in accordance with the results of several recent experiments, that while oxygen supply can limit thermal tolerance it is not the main cause of performance decline during slow warming in fish. We advocate the multiple performances - multiple optima (MPO) view of thermal performance, which states that any physiological function can be limiting and that this can differ between species, life stages and rates of heating. In order to advance our understanding of thermal physiology, field distribution and climate change sensitivity, we should identify these limiting functions in fish.

35.6 THERMAL TOLERANCE AND MOLT CYCLE-DEPENDENT GENE EXPRESSION IN JUVENILE DUNGENESS CRABS
Asmir Wittmann1,2, Ernst Chang3, and Donald Mykles3
1Int. Biology, Alfred Wegener Inst., Helmholtz Ctr. for Polar & Marine Res., Am Handelshafen 12, Bremerhaven, 27570, Germany, 2Biology, Colorado State Univ., 1878 Campus Delivery, Fort Collins, CO, 80526, 3Animal Sci., Bodega Marine Lab., Univ. of California, Davis, P.O. Box 247, Bodega Bay, CA, 94923. We hypothesize that the molt pathway and AMP-dependent protein kinase (AMPK) are involved in the regulation of the molt cycle in response to temperature in juvenile Dungeness crabs Metacarcinus magister. We used PCR cloning to obtain partial cDNA sequences of mTOR, Rheb, AKT, S6K, and AMPK. We incubated crabs at 12 d, 18 d and 26 d postmolt at 5, 10, 15, 20, 25 and 30°C for up to 14 d to study survival and progression of the molt cycle. We quantified gene expression in the molting gland of AKT, upstream of mTOR, and S6K, downstream of mTOR, of the possible housekeeping gene RbS3, and of Na+/K+-ATPase in crabs that had been held at 10, 15 or 20°C. Survival was 97%-100% at temperatures from 5-20°C, and was time-dependent, but 0% after 14 d at 25°C. All animals had died after 24 h at 30°C. Significant progression of the molt cycle was observed at 15 and 20°C, but not at 5 and 10°C. An overall two-way ANOVA indicated an effect of temperature on expression of all four genes, whereas only AKT, RbS3 and Na+/K+-ATPase were significantly affected by molt stage. The post hoc Tukey multiple comparisons test revealed significant effects of temperature at either 26 d, 32 d or at both time points, gene expression being significantly lower at 20°C compared to 10°C. Molt-stage specific effects were prominent at 20°C, with increased gene expression in pre molt. We conclude that AKT, an activator of mTOR, may be involved in the regulation of the molt cycle in a temperature-dependent manner. Funding: EU FP7 Marie Curie International Outgoing Fellowship PIOF-GA-2012-326483 to ACW, NSF grant IOS-1257732 to DLM.

35.7 VARIATION IN TRANSCRIPTOMIC SIGNATURES OF THERMAL ACCLIMATION IN FOUR KEY AQUATIC INSECTS IN CALIFORNIA RIVERINE FOOD WEBS
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1Int. Biology, Univ. of California, Berkeley, 1005 Valley Life Sci. Bldg., #3140, Berkeley, CA, 94720, 2Ronberg Tiburon Ctr. and Biology, San Francisco State Univ., 3150 Paradise Dr., Tiburon, CA, 94920. Predicting changes in trophic ecology of riverine systems in the face of future climate warming requires an understanding of the thermal performance of aquatic insect larvae. Larvae that differ in key trophic traits (i.e., grazer vs. predator) may also differ above, the functional heat resistance limit. Similarly, adult cold resistance proved a good predictor of species distribution in cooler climates. Given these findings we predict that both tropical and widespread Drosophila species will face a similar proportional reduction in distribution range under future warming.
Insecta are under unique constraints due to their high surface area to volume ratio. Nevertheless, the insect renal (Malpighian) tubules secrete primary urine faster (on a per-cell basis) than any other known epithelium. In the classic insect model *Drosophila melanogaster*, ion and water transport are controlled by two main secretory cell types; principal and stellate cells; and evidence suggests that this arrangement is general within the large Order of Diptera (true flies). However, is this two-cell model general across the insects? To address this, we developed a novel technique for comparative endocrinology by directly visualizing kinin receptors (diagnostic of the stellate cell identity) in live tissue with a fluorescently labeled kinin peptide. This technique allowed us to rapidly analyze strategically selected representatives of every major insect Order; hereby covering nearly 400 million years of evolution and >90% of insect diversity; thus providing an unprecedented overview of the evolution of insect renal function. Our data indicate that the two-cell model is a derived trait that evolved approximately 350 million years ago in the endopterygote insects, whereas a single cell model is basal to the more ancestral Exopterygota. Funding: BB/BRC, NIH & Danish Council for Independent Research (FNUs).

36.3

36.5 CORTICOSTERONE RESPONSES AND THE ABILITY OF BIRDS TO COPE WITH ENVIRONMENTAL CHANGE

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Birds, like other animals, live in complex environments that can change at any time. When stimuli from the external environment are perceived to be a threat or potential threat then a stress response is initiated and corticosterone is secreted. There is considerable individual variation in corticosterone responses, and a stimulus that initiates a large response in one bird may initiate a small response in another bird. For example, peak corticosterone responses to capture and handling were 15 times higher in some birds than others in a study of little penguins (*Eudyptula minor*) in New Zealand. Corticosterone responses and behavioural responses to environmental stimuli are together determined by individual characteristics called personality. Birds with low corticosterone responses and proactive personalities are likely to be more successful (have greater fitness) in constant or predictable conditions, whilst birds with reactive personalities and high corticosterone responses will be more successful in changing or unpredictable conditions. It is proposed that birds with reactive personalities and high corticosterone responses will be better able to cope with environmental changes due to climate change than birds with proactive personalities and relatively low corticosterone responses. Phenotypic plasticity in corticosterone responses can be quantified using a reaction norm approach, and reaction norms can be used to determine the degree of plasticity in corticosterone responses of individual birds, and mean levels of plasticity in responses of species of birds. Reaction norms for corticosterone responses can in future be used to help predict the ability of birds to cope with environmental changes due to climate change.
Corticosterone, chiefly referred to as a “stress hormone,” impacts a surprising variety of traits, including skeletal growth, cognition, and motivation. Hence, changes in corticosterone levels may impact multiple aspects of locomotor behavior, including both motivation and physical abilities. Previous rodent studies have found that altering circulating corticosterone levels can affect activity levels or induce a depressive state, depending on dosage and other factors. We have used a long-term artificial selection experiment to examine the evolution of high levels of voluntary wheel-running behavior in laboratory house mice. As compared with four non-selected control (C) lines, the four replicate High Runner (HR) lines are smaller in body size, run ~3-fold more on wheels on a daily basis, have higher home-cage activity when deprived of wheels, and have higher maximal oxygen consumption (VO2max) and basal circulating corticosterone levels. To examine further the role of corticosterone in locomotion, we administered 50 μg/kg corticosterone hemisuccinate to the drinking water of HR and C male mice from weaning to seven weeks of age. Mice were then tested for wheel running and VO2max. Corticosterone reduced growth rate and body mass-adjusted VO2max of both HR and C mice, and decreased wheel running in HR lines. The present results suggest that corticosterone can impact wheel running via changes in VO2max when animals run near their aerobic limits. Supported by NSF IOS-11212732.

36.7 MOLECULAR RESOLUTION OF AN ACUTE STRESS RESPONSE IN A FREE-RANGING MARINE MAMMAL
Jane Khudyakov1, Likit Preeyanon2, Cory Champagne3, Rudy Ortiz4, and Daniel Crocker5

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37.0 HIBERNATION, FLIGHT, AND SUBSTRATE METABOLISM

37.1 SEASONAL METABOLISM OF BROWN ADIPOSE TISSUE IN HIBERNATING THIRTEEN-LINED SQUIRRELS
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Brown adipose tissue (BAT) is a vital organ for mammalian hibernation, dissipating energy and producing heat via mitochondrial uncoupling protein 1 (UCP1) to rapidly endothermically warming from torpor. How BAT’s unique ability is not necessary outside the hibernation season, suggesting a potential seasonal change in mitochondrial content, respiratory capacity, or both. To address this question, we examined the mitochondrial content and bioenergetics of BAT in thirteen-lined ground squirrels across four time points: Summer (SU), Fall (FA), Hibernation (HB), and Spring (SP). Respiration rates of isolated BAT mitochondria were measured using an oxygen electrode with three substrates: Succinate (SUC), Glycerol-3-Phosphate (GP), and Ascorbate/TPMP (Asc/TPMP). GP-fueled and Asc/TPMP-fueled respiration were both significantly higher in HB than in both SU and FA (p < 0.05). SU-fueled respiration was significantly lower (p < 0.05) in SP than in HB. Relative mitochondrial content of BAT was estimated by measuring mitochondrial DNA (mtDNA) copy numbers via qPCR. BAT mtDNA copies were significantly lower in SP (p < 0.05). These data imply that BAT bioenergetics and mitochondrial content is not static across the year, even though UCPI is present year-round. The active suppression of BAT metabolism outside of hibernation may be playing a vital role in the overall energetic savings of a hibernator. This project was funded by the University of Minnesota McKnight Presidential Endowment.

37.2 IS SAPONIN-PERMEABILIZATION APPROPRIATE FOR CHARACTERIZING MITOCHONDRIAL METABOLISM? ASSESSING MITOCHONDRIAL RESPIRATION IN HIBERNATING AND EUTHERMIC GROUND SQUIRRELS
James Staples1, and Katherine Mathers1

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Saponin-permeabilization of small tissue slices is increasingly popular for characterizing mitochondrial metabolic functions including respiration rates and ROS production. Our technique is more convenient and faster than traditional mitochondrial isolation, and is well described for muscle and brain, but not for liver. The goal of this study was to assess the suitability of this method by comparing mitochondrial metabolism with saponin-permeabilized liver slices and isolated mitochondria from the same liver of a hibernator, the 13-lined ground squirrel. As we have demonstrated in several previous studies, state 3 respiration, fuelled by succinate, was suppressed by 65% in mitochondria isolated from torpid ground squirrels when compared with anexed, euthemic animals. Permeabilized liver slices showed respiratory control comparable to isolated mitochondria, but no suppression of state 3 respiration. These results held regardless of the method used to standardize respiration rate (protein, citrate synthase activity and cytochrome A content for mitochondria, weight and citrate synthase activity for permeabilized slices). These results suggest that the mechanisms underlying mitochondrial metabolic suppression in torpor were reversed by the permeabilization procedure. We cannot recommend this method for characterizing mitochondrial metabolism, at least for conditions where acute metabolic changes are known to occur. Funding: NSERC Canada.

37.3 BROWN FAT TRANSCRIPTOME DYNAMICS: PRESERVATION OF SELECTED MRNAS ACROSS A TORPOR BOUT SUPPORTS RAPID THERMOGENESIS DURING AROUSAL
Katharine Grubel1, Jay Heselberth2, Greg Barsh2, Cecilia Diniz Behn3, and Sandy Martin4,5


Brown fat (BAT) undergoes an annual cycle of recruitment and atrophy in 13-lined ground squirrels. Throughout winter hibernation, BAT cycles between quiescence and thermogenesis. Significantly, many transcripts increased during torpor at low body temperature and produced heat via mitochondrial uncoupling protein 1 (UCP1) to rapidly endothermically warming from torpor. However, BAT’s unique ability is not necessary outside the hibernation season, suggesting a potential seasonal change in mitochondrial content, respiratory capacity, or both. To address this question, we examined the mitochondrial content and bioenergetics of BAT in thirteen-lined ground squirrels across four time points: Summer (SU), Fall (FA), Hibernation (HB), and Spring (SP). Respiration rates of isolated BAT mitochondria were measured using an oxygen electrode with three substrates: Succinate (SUC), Glycerol-3-Phosphate (GP), and Ascorbate/TPMP (Asc/TPMP). GP-fueled and Asc/TPMP-fueled respiration were both significantly higher in HB than in both SU and FA (p < 0.05). SU-fueled respiration was significantly lower (p < 0.05) in SP than in HB. Relative mitochondrial content of BAT was estimated by measuring mitochondrial DNA (mtDNA) copy numbers via qPCR. BAT mtDNA copies were significantly lower in SP (p < 0.05). These data imply that BAT bioenergetics and mitochondrial content is not static across the year, even though UCPI is present year-round. The active suppression of BAT metabolism outside of hibernation may be playing a vital role in the overall energetic savings of a hibernator. This project was funded by the University of Minnesota McKnight Presidential Endowment.

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37.4 CHARACTERIZING CARDIAC MOLECULAR MECHANISMS OF MAMMALIAN HIBERNATION VIA QUANTITATIVE PROTEOMICS
Katie Vermillion1, Pratik Jagtap2, Todd Markowski2, LeeAnn Higgins2, James Johnson3, Tim Griffin2, and Matthew Andrews2
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This study uses cutting-edge proteomic approaches to elucidate cardioprotective mechanisms used during mammalian hibernation. Mammalian hibernation is characterized by dramatic reductions in body temperature, heart rate, metabolism and oxygen consumption, which poses significant challenges to the physiology of hibernators, especially for the heart, which maintains function throughout extreme conditions, responding to hemorhagic shock. To identify novel cardioprotective strategies we measured large-scale RNA-seq data with large-scale ITraq-based proteomic data in heart tissue from thirteen-lined ground squirrels (Spermophilus tridecemlineatus), throughout the circannual cycle. Protein identification and data analysis were run through Galaxy-P, a new multi-omic data analysis platform enabling effective integration of RNA-seq and MS/MS proteomic data. Galaxy-P uses flexible, modular workflows that combine customized sequence database searching and ITraq quantification to identify novel ground squirrels-specific protein sequences and provide insight into molecular mechanisms of hibernation. This study allowed for the quantification of identified 2007 cardiac proteins, including 447 novel peptides. Identification of novel peptides allows for improved genomic annotation of this non-model organism, as well as splice variants, mutations, or genome re-organization that underly novel cardioprotective mechanisms used during hibernation. NSF grant 1147079 and USARMC contract W81XWH-11-1-0469.

37.6 COLD AND EXERCISE TRAINING PRODUCE SIMILAR INCREASES IN MAXIMAL METABOLIC OUTPUT IN HOUSE SPARRROWS
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Maximal metabolic outputs for exercise and thermogenesis in birds presumably influence fitness through effects on flight and shivering performance. Because both summit (Mmax,4sec <spatial ="apple-converted-space"> = maximum thermoregulatory metabolic rate) and maximum (MMR = maximum exercise metabolic rate) metabolic rates are functions of skeletal muscle, correlations between these measurements might occur. We measured the effects of 3-week experimental cold and exercise training protocols on body (Mb) and muscle masses, basal metabolic rate (BMR), Mmax, and MMR in house sparrows (Passer domesticus). We also measured citrate synthase (CS), [hydroxyacyl CoA-dehydrogenase (HAD), and carnitine palmitoyl transferase (CPT) activities, and mRNA expression for plasma membrane fatty acid binding protein, fatty acid translocase, the muscle growth inhibitor myostatin, and its two proteinase activators (TLL-1, TLL-2) in pectoralis (PEC). Both training protocols increased Mmm, MMR, Mb, PEC mass, CPT and CS activities in PEC above control levels. BMR, however, increased with cold but decreased with exercise training. Cold-trained birds had lower expression of myostatin, but higher TLL-1 and TLL-2 levels than controls. However, no significant differences in gene expression occurred for exercise-trained birds. These data indicate that cross-training effects between cold and exercise may occur for birds, but mechanisms underlying the changing phenotypes are not identified. This research was funded by NSF IOS-1021218.

37.8 THE BODY BURNS MORE CARBOHYDRATES DURING INTENSE EXERCISE: EXPLOITING THE NATURAL DIFFERENCES IN [13C] BETWEEN LEAN AND LIPID TISSUES
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The body’s metabolic fuels during intense exercise are thought to be derived from food versus drinking or environmental water. We measured δ13C of the exhaled breath of house sparrows during different levels of exercise. We predicted that 13C of the exhaled breath increased by 0.6‰, 1.1‰, and 1.8‰, respectively for the three groups and was significantly correlated with exercise intensity. In contrast, 13C of the exhaled breath increased by 0.6‰, 1.1‰, and 1.8‰ respectively for the three groups and was significantly correlated with the intensity of exercise, suggesting that 13C of the exhaled breath can provide a complementary, noninvasive approach to studying shifts in substrate oxidation in vivo.
SKELETAL MUSCLE PERFORMANCE UNDER SUB-MAXIMALLY ACTIVATED CONDITIONS

38.2

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Skeletal muscles are rarely maximally recruited; yet our understanding of muscle physiology is based largely on studies using maximal activation. Here we explore the effects of activation level on whole muscle force-velocity and force-length properties. Force-velocity properties were determined in rat plantaris muscles with all, mainly fast or mainly slow motor units activated. Maximum shortening velocity declined at lower activation levels, but was independent of motor unit type activated. We propose that the effect of muscle resistance to shortening predominates over the effect of active motor unit type in determining force-velocity properties at low activation levels. Force-length properties were determined in frog plantaris muscles using stimulation conditions which elicited high force-high Ca2+, low force-high Ca2+ and low force-low Ca2+ contractions. Optimum muscle length increased with decreasing force, irrespective of Ca2+ levels. Hence, we propose that the varying effect of internal mechanics underpin the shift in optimum length at low activation levels. These findings suggest that the interaction of contractile elements and the physical properties of muscle (mass, viscosity, compliance etc) result in the non-linear scaling of muscle performance with activation level. Such mechanistic explanations of sub-maximal muscle performance will improve our understanding of in vivo muscle function and will inform new muscle models. Supported by NIH AR055290 and AR055648.

38.3

SIZE MATTERS: THE IMPACT OF BODY MASS ON BIOCHEMICAL AND STRUCTURAL PROPERTIES IN HARBOR SEAL MUSCLES

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The physiological attributes of muscle fibers (e.g., fiber type, size, enzymes profiles) influence muscle performance capacity, and are known to vary inter- and intra-specifically due to changes in age, diet and activity patterns. Muscle phenotype has been closely linked to underwater foraging ability of adult marine mammals, but its resilience to reductions in diving and foraging activities that accompany reproduction and molt has not yet been assessed. Seasonal differences in muscle metabolic profiles and structure were compared in 39 harbor seals (Phoca vitulina) captured in Glacier Bay National Park, Alaska; 12 prior to the pupping season, and 27 after the molt. Seals were in better condition (% lipid) in spring, but there were no seasonal differences in any other measured parameter, indicating that harbor seals maintain muscle performance capacity year-round. However, heavier seals had significantly larger muscle fibers, and this effect was greatest in Type II A and IIDX fibers. In addition, mass was negatively correlated with citrate synthase and α-hydroxycarboxy CoA dehydrogenase activity, but not with lactate dehydrogenase activity or myoglobin concentration. Findings suggest that muscle properties reflect underlying metabolic scaling constraints and indicate that muscle mass should be considered when interpreting species or population-specific differences. Funded by NSF-EPs-0346770.
38.7 AGE RELATED CHANGES IN FLIGHT MUSCLE ULTRA-STRUCTURES OF THE HAWK MOTH, *Manduca sexta*: A NOVEL NON-VERTEBRATE ANIMAL MODEL FOR INVESTIGATING VERTEBRATE SKELETAL MUSCLE FUNCTION, DISEASE, DEGENERATION, AND AGING

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Muscle animal models are commonly utilized to gain insight into the pathogenesis of vertebrate diseases, or to evaluate the efficacy of potential therapeutic regimens. The need for novel non-vertebrate animal models for investigating vertebrate skeletal muscle physiology, disease, and aging is growing. Moreover, it is important for non-vertebrate models to be suitable for the study of aging. The flight muscle of the hawk moth, *Manduca sexta*, is metabolically similar to endothermic vertebral skeletal muscle anatomy and physiology. We report the advantages of using *Manduca sexta* as a non-vertebrate model to study skeletal muscle physiology, disease, and aging. We compared ultrastructural changes of young, middle aged, and older muscle and compare jump performance and electromyography to measure jumping muscle contraction duration. Results indicate that muscle contraction duration increases with load. Rabies grasshoppers show a 30% increase in contraction time compared to non-gravid females. We examined the effect of added mass on jump performance by attaching weights (20% or 40% of body mass) to non-gravid females and gravid females. When weights equaled to 40% of body mass are added, muscle contraction duration increased over 50% in non-gravid females but did not change in gravid females. These results suggest that gravid grasshoppers are already at their limit for muscle contraction duration. Unlike lizards, lizards may show no effect of gravidity on jump performance because they use a different mechanism to perform osmoregulation. We report the advantages of using *Manduca sexta* as an ideal non-vertebrate animal model for investigating vertebral skeletal muscle function, disease, degeneration, and aging.

38.8 EFFECT OF GRAVITY ON JUMP PERFORMANCE AND MUSCLE PHYSIOLOGY IN THE AMERICAN LOCUST

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Trade-offs exist between different physiological characteristics. In terrestrial animals, the trade-off between gravity and muscle performance has been well documented. However, the effect of load on jumping performance is relatively unknown. In lizards, an increased load equal to 30% of body mass produced shorter and less frequent jumps. However in *Schistocerca americana* grasshoppers, 30% heavier gravid females have a similar jump performance to non-gravid females. We tested the hypothesis that grasshoppers vary their jumping muscle contraction duration to increase force produced when gravid (or loaded). We used high-speed video analysis to compare jump performance and electromyography to measure jumping muscle contraction times. Controls indicated that a 30-minute rest after electrode implantation was sufficient for the grasshoppers to regain their jumping ability. Results indicate that muscle contraction duration increases with load. Gravid grasshoppers show a 30% increase in contraction time compared to non-gravid females. We examined the effect of added mass on jump performance by attaching weights (20% or 40% of body mass) to non-gravid females and gravid females. When weights equal to 40% of body mass are added, muscle contraction duration increased over 50% in non-gravid females but did not change in gravid females. These results suggest that gravid females are already at their limit for muscle contraction duration. Unlike lizards, grasshoppers may show no effect of gravity on jump performance because they use a different mechanism to perform osmoregulation. Support was provided by Union College Student Research Grants.

39.0: OSMOTIC AND IONIC REGULATION

39.1 POTENTIAL SOLUBLE ADENYLYCYCLASE ISOFORMS IN CORAL

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Soluble adenyl cyclase (sAC) is a bicarbonate-stimulated source of the ubiquitous signalling molecule cAMP. sAC genes have been identified in diverse phyla, suggesting it is an evolutionarily conserved COOH terminus. We are investigating the role of sAC in corals, where physiological processes such as photosynthesis and calcification depend on and alter internal acid/base conditions. The Acropora digitifera genome contains a sAC gene encoding for a ~190 KDa protein. However, using RT-PCR, we have amplified several sAC transcripts from the related coral species, *Acropora yongei*. These sequences indicate the presence of at least one splice variant, encoding for a truncated 68KDa protein that includes both catalytic domains, and several other insertions/deletions/polymorphisms. Whether such differences represent unique sAC genes or variations of a single gene (alleles or splice variants) will be investigated using Rapid Amplification of DNA Ends (RACE) PCR. Alternatively, splicing of the coral sAC gene, known to occur in mammalian systems, suggests multiple physiological roles of SAC in corals. Interestingly, immunostaining using coral sAC-specific antibodies reveals that SAC is present in multiple cell types throughout coral epithelium, we hypothesize that splice variants could be related to cellular

39.2 DIFFERENTIAL LOCALIZATION OF ION-TRANSPORTING PROTEINS SUGGEST SPECIES-SPECIFIC PHYSIOLOGICAL MECHANISMS IN CORALS

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The movement of ions across a cell membrane is critical to a wide variety of physiological functions across all phyla, however, little research has been conducted on ion transport in Cnidarians. Understanding coral physiological mechanisms is of increasing interest, however, due to mounting environmental stress. Two key ion transport dependent processes are calcification and photosynthesis by the endosymbiotic algae. Here, we utilized fluorescent immunostaining to localize three key ion transport proteins in two species of scleractinian corals: *Acropora yongei* and *Stylophora pistillata*. Proteins investigated included sodium-potassium ATPase (NKA), plasma membrane calcium ATPase (PMCA), and sodium-bicarbonate cotransporter (NBC). PMCA, NKA and NBC were each observed in the calcifying epithelium of both species. However, other coral tissues demonstrated species-specific localization patterns: both NKA and NBC were found in the oral ectoderm of *A. yongei* only, NKA being on the apical membrane, while PMCA was found in the endoderm of *S. pistillata*, only. The similar localization of these proteins in the calcifying tissue suggests they play a conserved role in calcification, while the differences in location between other tissues indicates that corals have evolved diverse ion transport mechanisms for other functions. These differences may lead to differential responses to environmental stressors. National Science Foundation grants EF-1220641 to MT and OCE-1226396 to KB.

39.3 EXTREME STRESS TOLERANCE OF THE INTERTIDAL TARDIGRADE *ECHINOCOIDEIS SIGMUNDI*

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Tardigrades may likely be the toughest animals on Earth. These microscopic eucaryotic tardigrades endure prolonged periods of environmental extremes by entering dormant states, such as cryptobiosis (latent life). The aim of this study was to investigate stress tolerance in the species *Echinocoideis sigmundi*, which lives in marine tidal zones across the globe. Tardigrades were monitored in groups of 20 animals using stereomicroscopes and considered active and alive if they showed movement. Freeze tolerance experiments revealed that *E. sigmundi* tolerates freezing to -30°C, -78°C and -196 °C (liquid nitrogen) for 2 weeks with a mean survival of 95.5%, 100% and 96.15% (n=5). Notably, tardigrades transferred to -30°C prior to submersion in liquid nitrogen had a survival of 99.3% (n=5), suggesting a clear effect of cooling rate. Experiments on osmotic stress revealed an extraordinary high tolerance to changes in external salinity. *E. sigmundi* tolerates exposure to distilled water for at least 4 days, as well as 48 hours exposures to salinities ranging from 1.6% to 24.5%. The tardigrade further tolerates desiccation for 48 hours from seawater as well as distilled water, with a survival of 92.5% and 90.7% (n=5). Collectively, our results show that *E. sigmundi* is highly tolerant of environmental perturbations, and as such, provides a unique model to gain further insights into latent life phenomena and adaptations to extreme environments. Supported by the Carlsberg Foundation.

39.4 BRANCHIAL CHAMBER OF *LITOPENAEUS VANNAMEI* DURING POST-EMBRYONIC DEVELOPMENT

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The crustaceans have a branchial chamber (BrCh) that includes gills, branquiohepato-dermale epidermis and pleura. All these structures are necessary to perform osmoregulatory activity during shrimp’s life. In order to understand the transition and physiological implications of these structures during *L. vannamei* ontogenesis, we examined the cell topography of BrCh and the morphogenesis of gills and epidermis through histology (either H & E staining and direct optical visualization, 1-100X) in different stages of development (n=3-10). Adult *L. vannamei* has 18 gills, 5 epidermises, and branquiohepato-dermal BrCh is continuously improving from larva to PL13 and is fully developed in PL15. The first epidermis was observed in Mysis 1, while the others were present only as buds of epidermis through PL6. The gills were appeared until PL1 like a simple tube. Cellular differences were observed between structures, because the function can be variable between developmental stages.
39.5 ALLALOSTATIN A-LIKE FACTORS IN THE AQUATIC LARVAE OF CHIRONOMUS RIPARIUS: REGULATION OF HINDGUT MUTILITY, ION RESORPTION AND IMPLICATIONS FOR SALINITY EXPOSURE

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The hindgut epithelium of the detritus feeding midge *Chironomus riparius* is ubiquitous in brackish water. Larvae of this species can be isolated in brackish habitats and exposed to a wide range of salinity levels. Our findings indicate that the hindgut epithelium of *Chironomus riparius* is responsive to changes in salinity and can be used to study the regulation of hindgut in response to changes in salinity.

39.9 EFFECTS OF DIURETICS ON RENAL CALCIUM OXALATE CRYSTALIZATION IN A DROSOPHILA (FLY) MODEL OF OXALATE NEPHROLITHIASIS

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Nephrolithiasis is a painful, expensive health care problem with complex genetic and environmental etiologies. Calcium oxalate (CaOx) accounts for 70% of human cases, yet details of stone formation are poorly understood. As a model for human stone formation, we have developed a fly model for CaOx crystal formation in the Malpighian tubules (MT) of *Drosophila melanogaster*. This model has allowed us to study the effects of diuretics on CaOx crystal formation and to identify potential therapeutic targets for the prevention of nephrolithiasis.

39.10 CONSERVATION OF THE OSMOSENSITIVE AND THERMOSENSITIVE AN−TRPV1 ION CHANNEL IN OSMOREGULATING ANIMALS

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The AN−TRPV1 ion channel is conserved across a wide range of osmoregulating animals, from amphipods to vertebrates. We have studied the functional properties of this channel in a novel model system, the cockroach. Our findings suggest that this channel is a key player in osmoregulation and thermoregulation, and may have evolved to play a role in both processes.
other appears in more complex multicellular organisms, such as nematodes, insects and all kind of vertebrates. Recently, we have cloned from rat Somatic Nucleus (SON) a variant form of TRPV1, a member of the Vanilloid subfamily. This variant lacks part of the N-terminus (AN-TRPV1). Heterologous cells transfected with AN-TRPV1 expressed a capsaicin-insensitive channel that was activated by cell shrinking or physiological increases in temperature. Based in multiple alignments of strategy (2). Toadfish have the unique ability to pulse urea across their gills, and we decreased vocal activity, suggesting a stress response and a predator avoidance strategy. Urea has been found to serve as a cloaking molecule when co-expressed with ammonia (1) and is suggested to be involved with chemical com-communication and predator avoidance strategy. Urea has been found to serve as a cloaking molecule when co-ex-ecrated with ammonia (1) and is suggested to be involved with chemical communi-cation (3). Neuroendocrine responses that control urea production and excretion will be compared in toadfish exposed to dolphin vocalization playbacks and control fish larvae are acknowledged to be stenohaline FW animals, post-metamorphic sea lamprey are euryhaline, allowing for SW entry and an eventual return to FW for the terminal spawning phase of their life cycle. Basic strategies of lamprey osmoregula-tion appear to largely mirror those seen in more derived aquatic vertebrates such as teased fishes and in this regard, ultrastructural observations of the lamprey tight junction (TJ) complex in ionoregulatory tissues strongly support the idea that this structure significantly contributes to overall salt and water balance. However, the molecular architecture of the lamprey TJ complex and its response to life stage related alterations in environmental ion levels is unknown. This study reports on the trans-membrane and cytosolic proteins that make up the TJ complex of the lamprey and how the molecular 'machinery' of the larval and post-metamorphic lamprey TJ com-plex responds to changes in the ionic strength of its environment. Funded by NSERC Canada.

39.15 HOMOLOGOUS SERUM AND HEPARIN ALTER TIGHT JUNCTION PROTEIN ABUNDANCE AND THE PERMEABILITY OF A PRIMARY CULTURED SALMONID GILL MODEL Chan Chih Chen1, and Scott P. Kelly2

1Dept. of Biology, York Univ., 4700 Keele St., Toronto, ON, M3J 1P3, Canada. Due to the architectural complexity and cellular heterogeneity of fish gills, primary cultured model gill epithelia are useful tools to investigate factors that contribute to salt and water balance in fishes. Specifically, culture models are helpful for experimentally dissecting the role of tight junction (TJ) proteins in the regulation of epithelial integrity. However, despite model suitability it is prudent to consider refining techniques so as to better mimic in vivo conditions. For example, replacing a typically used media supplement such as fetal bovine serum (FBS) with native fish serum could produce a more authentic model by eliminating foreign proteins and introducing homologous growth factors. But native serum supplementation in salmonid cell culture has been reported to promote mixed cell lines and in some cases has been described as cytotoxic. While fish plasma can be a replacement for serum in primary gill cell culture, con-vention suggests that plasma may lack some of the mitogenic properties of serum. Furthermore, the effect anti-coagulating agents (required for plasma preparation) on the properties of primary cultured epithelia have not been explored. This study considered the use of homologous serum as an FBS replacement in a culture trout gill epithelium model and its subsequent effect on permeability and the molecular biology of the gill TJ complex. In addition, the effect of heparin on the physiology of the gill model was also considered. Funded by NSERC Canada.

39.16 REGULATION OF GILL CLAUDIN ISOFORMS IN MOZAMBIQUE TILIAPIA (OREOCHROMIS MOZAMBIICUS) BY SALINITY AND CORTISOL Christian K. Tipsmark1,2, Jason P. Breves1,2, D. Brett Rabeneck1, Rebecca T. Trubitt1, Damien T. Lemer3, and E. Gordon Grass4.

1Dept. of Biological Sci., Louisiana State Univ., 202 Life Sci. Bldg., Baton Rouge, LA, 70803, 2Agricultural Ctr. Biotechnology Lab., Louisiana State Univ., 115 H.C. Wilson Lab., Baton Rouge, LA, 70803. The inhibitory neurotransmitter γ-aminobutyric acid (GABA) has been implicated in cell volume regulation during osmotic stress via its action on the GABA_A receptor. Although GABA is typically produced from glutamate by glutamate decarboxylase 1 (GAD1), the polyamine putrescine (PUT) can potentially serve as an important pre-cursor of GABA during abiotic stress. Interestingly, euryhaline species of Fundulus accumulate PUT in the gill during acute freshwater exposure, suggesting that PUT may become increasingly important as a source of GABA during osmotic stress. The current study aims to compare the relative importance of glutamate and PUT in GABA synthesis in the mammichog, Fundulus heteroclitus, during acute hypo-osmotic exposure. F. heteroclitus acclimated to 12 ppt water were acutely transferred to 0.2 ppt water, and the gills, liver, muscle, kidney, nerve, and brain were sampled after 6 h, 1 d and 7 d. Aminoacycetic acid, a known inhibitor of GAD1, was ad-ministered to one control and one treatment group. The mRNA levels and enzymatic activities of two key enzymes in GABA synthesis, aldehyde dehydrogenase 5 and NADH:cytochrome c reductase, were measured. In addition, the levels of GABA, glutamate, and the polyamines PUT, spermidine, and spermine were quantified. This study will assess whether GABA, in addition to its role in the central nervous system, plays an ionoregulatory role in the periphery of fishes. This research was supported by funding from the National Science Foundation (EF-0723771).
39.17 PARACELLULAR PATHWAY REGULATION IN RESPONSE TO SA-
LINITY CHANGES IN THE JAPANESE MEDAKA (ORIZAS LAТИPE5)
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Claudins are tight junction proteins responsible for ion selectivity of the paracellular pathway in transport epithelia and thus essential in teleost osmoregulation. This study investigates responses of 13 claudins (cldns) to salinity challenges in the euryhaline Japanese medaka. Short- or long-term effects of freshwater (FW) and seawater (SW) transfer were examined. In the gill, cldn-27a, -28a, -30b, -30c did not vary with salinity which is consistent with a barrier-forming role. Putative cation-selective pore-forming cldn-10a, c, d, and e were all more abundant in SW than in FW gills. This is in agreement with the need of SW fish to secrete NaCl via trans- and para-cellular ions movements. Two cldn-10b splice-variants were predominantly expressed in the kidney, with higher expression in SW- than in FW-acclimated fish. This suggests a role in monovalent ion reabsorption, particularly essential in SW to produce isotonic urine enriched with divalent ions. Cldn-15a, b and -25 isoforms were mainly expressed in the intestine. Cldn-15a and -25 did not display any variation during salinity challenges. However, cldn-15b was highest in FW which suggests a specific role of this putative pore-forming tight junction protein in hyper-osmolegulatory mechanisms in the intestine of medaka. This work was approved by the Animal Care and Use Committee of the University of Arkansas (IACUC #14042) and supported by a NSF grant (IBN 11-15616) and the Arkansas Biosciences Institute.

39.18 EXPRESSION OF GILL NA/K-ATPASE a-SUBUNIT ISOFORMS IN 
EURYHALINE JAPANESE MEDAKA (ORIZAS LAТИPE5) DURING SA-
LINITY CHALLENGES
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Some euryhaline teleosts exhibit a switch in the Na/K-ATPase (NKA) a-isof orm when migrating between/ exposed to (FW) and seawater (SW). The objective of the present study was to identify NKA a-isoforms expressed in medaka and define their role in osmoregulation. We classified 6 NKA a-isoforms (a1a, a1b, a2a, a2b and a3b) and investigated salinity dependent expression. In a tissue distribution screening we found that a1a, a1b and a2b were highly expressed in osmoregulatory organs. Long-term acclimation of medaka to FW and SW induced no change in gill expression of a2a, a2b and a3b. Thus only a1 isoforms were further investigated. In both short-term and long-term salinity challenges, NKA a1a and a1b were up-regulated in SW gill while no effect of salinity was observed for a1c. For reference some additional tran-
script isoforms were examined. NKA a1a and a2a expression were elevated in FW, while c4h and nkacl are up-regulated in SW. This supports findings in some other teleosts and is in accordance with putative roles in ion-uptake and secretion, respectively. Specific FW and SW a1 isoforms have been described in teleosts such as trout and tilapia. The present study suggests that in medaka no such NKA switch is in-
volved in salinity acclimation. The enzyme kinetics of gill NKA in FW and SW-ac-
cclimated medaka are currently under investigation. This study was supported by Ar-
skansas Biosciences Institute.

39.19 FOUR NA/K-ATPASE a-SUBUNIT ISOFORMS IN THE THREE 
ELECTRIC ORGANS AND THE SKELETAL MUSCLE OF THE 
ELECTRIC EEL
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The electric eel, Electrophorus electricus, possesses three different electric organs (EOs) and is capable of generating both high and low voltage discharges. While many studies have focused on channels/transports involved in bioelectrogenesis, few studies have examined mechanisms involved in the restoration of resting potential and the related recharging process. Na/K-ATPase (nka) is one of the key transporters involved in recharging the resting potential. We have identified for the first time four different nka a-subunit isoforms (nka1b1, nka1b2, nka2a and nka2b) from the main EO, Hunter’s EO, Sach’s EO and skeletal muscle (SM) of E. electricus. Molecular characterization of these isoforms indicated that they might have different Na+ binding affinities. Studies on the mRNA expression levels of the isoforms showed that the main and Hunter’s EOs had significantly higher levels of nka2b than the SM, while the SM had significantly higher mRNA expression of nka2a and nka2b. Overall, these results indicate that E. electricus EOs-specific Na/K and SM Na/K ratios, and the presence or absence of Na/K ratios in Na/K ratios are related to the three EOs. This study was supported by the NUS IACUC, and funded by MINDEF through TMSI.

39.20 CHARACTERIZING SODIUM UPTAKE IN THE BLACKSKIRT TETRA 
(GYMNOCRYSMBUS TERNETZI)
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The blackskirt tetra, Gymnocorymbus ternetzi is a native fish of the Rio Negro, a tributary of the Amazon River, which is ion-poor and very acidic (pH<5). This species possesses a sodium uptake mechanism that is rapidly upregulated during low pH exposure so as to prevent net loss of ions from their blood to the water due to a stimulated efflux. Immunolocalization studies confirm the presence of both Na+/K+ ATPase and V-type H+ ATPase transporters in the blackskirt tetra gills and that their location shifts to a more protected region of the gill when fish are exposed to low pH water. Western blotting indicates that the quantity of both ATPase proteins is unchanged yet activity assays show that both ATPase activities are low and drop to zero by 24 h at low pH. Isotopically measured sodium uptake was insensitive to both Ouabain and Batflomycin exposure. Protein expression assays indicate the presence of an epithelial sodium channel (eNaC) and that a large component of the low pH-stimulated Na+ influx is phenamil sensitive. We propose a novel coupling mechanism of the basolateral ouabain-insensitive isoform of Na+/K+ ATPase with an apical (water facing) eNaC transporter. Funding was provided by the ALSAM and Dohrley foundations and the USD Office of Undergraduate Research.

39.21 THE EFFECTS OF WATER IONIC COMPOSITION ON THE RATE 
AND DIRECTION OF ACID-BASE REGULATION IN RAINBOW TROUT, 
O. MYKISS, DURING HYPERCARBIA AT REST AND SUSTAINED EXERCISE
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Hypercacria (elevated environmental CO2) is an environmental factor that can have significant physiological consequences in animals, particularly those in aquatic habitats. Exposure to hypercarbia can elicit a potentially lethal blood acidosis to which an animal must compensate in order to maintain homeostasis. Although not fully under-
stood, the exchange of environmental Na+ and Cl with their acidic and basic counter-
parts (H+ and HCO3; respectively) plays an important role in pH compensation. The rate and degree of pH recovery during hypercarbia has been linked to several factors including ambient ion availability and possibly an animals activity level. However no study to date has directly quantified these relationships within a single species. Blood parameters of softwater, artificial hardwater and saltwater acclimated rainbow trout exposed to hypercarbia were used to elucidate the rate and degree of pH recovery a-
mong different fish species. Both hardwater and saltwater trout were able to compensate for the hypercarbic disturbance faster than their softwater counterparts, and exercising fish demonstrated faster pH recovery compared to resting fish. Funding: NSERC.

39.22 GUT CARBONATE EXCRETION BY FISH INCREASES EXPON-
ENTIALLY WITHIN NATURAL OCEAN SALINITY RANGE (25-40 PSU)
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In many species of marine fish, the gills are the primary site of carbonate excretion (GCE). However, the capacity for GCE is limited, as it is based on the respiratory exchange rate. In this study, we measured the rate of gut carbonate excretion in juvenile sockeye salmon (Oncorhynchus nerka) exposed to seawater at pH 8.1 and pH 7.8, with a diet of live zooplankton, and a control diet of live zooplankton plus carbonate-free seawater. The rate of gut carbonate excretion in the control diet was 5%, while in the carbonate diet it was 48%. This suggests that the gut has a significant role in the overall carbonate budget of marine fish, and that the capacity for GCE may be underestimated. The increase in GCE with increasing seawater salinity is likely due to the increased availability of carbonate ions in seawater, which can be used to excrete bicarbonate ions from the water to the gut lumen. This study was supported by the National Science Foundation (NSF) and the National Oceanic and Atmospheric Administration (NOAA) through the Cooperative Institute for Marine and Atmospheric Studies (CIMAS).
Marine teleost fish precipitate ingested Ca$^{2+}$ ions in their intestine as calcium carbonate by fish. Euryhaline shanny (Lipophrys pholis) and stonehole turbot (Scophthalmus maximus) were maintained at salinities of 25, 30, 35 and 40 psu. For both species the carbonation production rates were relatively stable between 25 and 35 psu (the global average for ocean salinity) but increased 3-fold at 40 psu. For both species this was unrelated to metabolic rate changes across the salinities. The mechanism behind such a large increase in carbonation production rate over a small salinity change is uncertain, but is likely explained by changes in drinking rate (for osmoregulation) with salinity and intestinal Ca$^{2+}$ absorption for skeletal growth (rather than precipitated and excreted). If this is true for other marine fish it will have a major influence on estimates of global carbon production by fish. Supported by the Natural Environment Research Council, UK.

39.23 PHENOTYPIC PLASTICITY IN RESPONSE TO HYERCAPNIA INDUCED ACID-BASE DISTURBANCES IN RED DRUM (SCIAENOPS OCELLATUS)
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²Anthropogenic CO₂ emissions since the pre-industrial era have raised oceanic CO₂ by 40%, reducing pH by 0.1 units. This can induce acid-base disturbances in marine organisms that are compensated through regulatory pathways. We assessed the plasticity of regulatory pathways in red drum (Sciaenops ocellatus) gills in response to hypercapnia induced-acid-base disturbances. Red drum were exposed to ocean acidification (OA) levels (1000 μatm) for 24h, 72h, and 14d with concurrent controls as a baseline. Buffering has been shown in mammals, but is substantially lower in the turtle. This suggests that hypercapnia induced acid-base disturbances. Further work is being conducted to identify a comprehensive physiological mechanism for hypercapnia compensation in red drum. All experiments were performed in accordance with the UT Institutional Animal Care and Use Committee. Funding provided by NSF (EF 1315290).

39.24 CHANGES IN EXPRESSION OF AQUAPORIN ISOFORMS IN AN AESTIVATING AFRICAN LUNGFISH
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African lungfishes can undergo long periods of aestivation on land during drought. During aestivation, they are confronted with desiccation and the impendence of ammoxone excretion leading to increased urinary ammonium transport and accumulation. Aqp1a and Aqp1c are two abundant aquaporins in the lungfish. To avoid dehydration, water is conserved by reduced permeability. To examine the role of water in the lungfish, we have used red drum (Sciaenops ocellatus) as a model for an aestivating fish. Our findings suggest that lungfish are able to conserve water during aestivation by reducing permeability and therefore reducing the loss of water.

39.25 EFFECTS OF TEMPERATURE ON HYPOTONIC SWELLING INDUCED BY WATER AND GLYCEROL IN HEPATOCYTES FROM COPE'S GRAY TREEFROG
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Marine teleost fish precipitate ingested Ca$^{2+}$ ions in their intestine as calcium carbonate crystals. These are then excreted into the environment where they have the potential to impact local ocean chemistry and carbonate sediment formation. It has previously been estimated that up to 45% of all marine calcium carbonate production could be from fish (Wilson et al., 2009, Science, 323: 359-362). One environmental variable not previously considered in this estimate is salinity. This study aims to assess how salinities across the natural oceanic range could affect the intestinal precipitation of calcium carbonate by fish. Euryhaline shanny (Lipophrys pholis) and stonehole turbot (Scophthalmus maximus) were maintained at salinities of 25, 30, 35 and 40 psu. For both species the carbonation production rates were relatively stable between 25 and 35 psu (the global average for ocean salinity) but increased 3-fold at 40 psu. For both species this was unrelated to metabolic rate changes across the salinities. The mechanism behind such a large increase in carbonation production rate over a small salinity change is uncertain, but is likely explained by changes in drinking rate (for osmoregulation) with salinity and intestinal Ca$^{2+}$ absorption for skeletal growth (rather than precipitated and excreted). If this is true for other marine fish it will have a major influence on estimates of global carbon production by fish. Supported by the Natural Environment Research Council, UK.
ENHANCING THE RESTORATION OF CALIFORNIA’S ESTUARIES BY EXPLORING THE GENETIC BASIS OF ENVIRONMENTAL TOLERANCE IN OLYMPIA OYSTERS (OSTREA LURIDA)
Ashley Maynard, Bill Bibbe, Eric Sanford, and Tyler Evans

Ostrea lurida is the only oyster native to the west coast of N. America and a foundation species in estuarine habitats. Once abundant, O. lurida populations have been significantly reduced due to overharvest and disease. More recently, O. lurida has successfully invaded and displaced the native congener along the Pacific coast by being more physiologically tolerant to stressors. However, little is known about the genetic basis of this enhanced tolerance. The mechanisms underlying the plasticity of environmental stress responses is important to understand for restoration purposes.

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The Olympia oyster, Ostrea lurida, is the only oyster native to the west coast of N. America and a foundation species in estuarine habitats. Once abundant, O. lurida is now functionally extinct in the wild. As a part of a plan to restore California’s degraded estuarine ecosystems, O. lurida is being considered for reintroduction. However, the ability of oysters to tolerate climate change may influence the success of restoration efforts. Oyster restoration should use genotypes capable of surviving future conditions, but which O. lurida populations will be most tolerant of climate change is uncertain. In San Francisco Bay, climate change will increase the frequency of freshwater flooding events that can cause mass mortality in oyster beds. In this study, tolerance of low salinity was explored in two O. lurida populations within San Francisco Bay and one population in nearby Tomales Bay. Oysters from Loch Lomond had significantly higher survival rates during freshwater challenge than Oyster Point or Tomales Bay populations. We are presently exploring mechanisms of differential salinity tolerance in O. lurida using RNA sequencing. Shifts in gene expression following exposure to reduced salinity are being compared among the three populations to determine physiological changes that underlie enhanced freshwater tolerance. Changes in allele frequency among oysters surviving freshwater challenge and controls are also being identified to understand evolutionary bases of salinity tolerance.

40.2 PROTEOOMIC RESPONSES OF THE MUSSEL MYTILUS GALLO-PROVINCIALIS TO AERIAL EXPOSURE INDUCED HYPOXIA AND SIRTUIN INHIBITION
Maria Christina Vasquez1, Michael Rosner1, Jaelyn Campbell2, Nicole Peterson1, Marcus Zuzow1, and Lars Tornqvist1

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Low tide in the rocky intertidal exposes marine organisms to environmental stressors including aerial exposure induced hypoxia (AEIH). Mussels in particular use alternative aerobic anaerobic pathways for combat hypoxia. The Mediterranean blue mussel Mytilus galloprovincialis has successfully invaded and displaced the native congener along the Pacific coast by being more physiologically tolerant to stressors. However, little is known in regard to this species’ physiological responses to AEIH or how sirtuins, an NAD-dependent deacetylase, may influence their stress tolerance. The purpose of this study was to use a proteomics approach to assay global protein changes in mussels acclimated to two tidal regimes (constant immersion or immersion/emersion) and exposed to 24 h of one of three treatments: normoxia (constant immersion), AEIH, or AEIH and sirtuin inhibition (SI). We found abundance changes in proteins related to proteolysis. This study highlights the need to not only study proteomic responses to stressors but to also identify possible mechanisms underlying stress tolerance, such as that by sirtuins (funded by NSF IOS 1146840).

40.3 THE PROTEOMIC RESPONSE OF SUBTIDALLY AND TIDALLY-EN-TRAINELIFIED CALIFORNIA RIBBED MUSSEL MYTILUS CALIFORNIA- NES TO ANOXIA STRESS
Aubrie Fowler1, Marcus Zuzow1, and Lars Tornqvist1


Rocky intertidal organisms experience extreme shifts in abiotic factors, such as anoxia stress, due to tidal fluxes. During low tides, the California ribbed mussel (Mytilus Californianus) normally closes its shell to avoid desiccation instead of gaping to augment gas exchange, and thus faces anoxia stress and anaerobic metabolism. The energetic expense is partially met through a simultaneous down-regulation of metabolism. To characterize how entrainment to a tidal and subtidal rhythm affects protein synthesis in M. californianus, mussels were acclimated to tidal and subtidal conditions with a photoperiod (12h:12h) to mimic natural circadian rhythms. There were five groups including normoxic controls, 0h, 6h, and 72h and anoxic treatment groups treated with 100% nitrogen gas at 6h and 72h. Gill tissue was extracted and subjected to proteomic analysis so proteins could be identified using mass spectrometry. Of the constantly immersed mussels, only about 12% of proteins were significantly changed in contrast to the 17% of proteins for mussels acclimated to a tidal regime. Tidally-entrained mussels responded to anoxia by producing oxidative stress proteins after exposure to low oxygen conditions, especially at the 72h time point. The proteomic changes indicate that tidally-entrained mussels have a greater capacity to cope with acute anoxia than constantly submerged mussels. NSF IOS 1145840.

40.4 TRANSCRIPTIONAL ANALYSIS OF DAPHNA PULEX RESPONSE TO INTERACTIVE EFFECTS OF TEMPERATURE AND SALINITY VARIABILITY
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Rocky intertidal organisms experience extreme shifts in abiotic factors, such as anoxia stress, due to tidal fluxes. During low tides, the California ribbed mussel (Mytilus Californianus) normally closes its shell to avoid desiccation instead of gaping to augment gas exchange, and thus faces anoxia stress and anaerobic metabolism. The energetic expense is partially met through a simultaneous down-regulation of metabolism. To characterize how entrainment to a tidal and subtidal rhythm affects protein synthesis in M. californianus, mussels were acclimated to tidal and subtidal conditions with a photoperiod (12h:12h) to mimic natural circadian rhythms. There were five groups including normoxic controls, 0h, 6h, and 72h and anoxic treatment groups treated with 100% nitrogen gas at 6h and 72h. Gill tissue was extracted and subjected to proteomic analysis so proteins could be identified using mass spectrometry. Of the constantly immersed mussels, only about 12% of proteins were significantly changed in contrast to the 17% of proteins for mussels acclimated to a tidal regime. Tidally-entrained mussels responded to anoxia by producing oxidative stress proteins after exposure to low oxygen conditions, especially at the 72h time point. The proteomic changes indicate that tidally-entrained mussels have a greater capacity to cope with acute anoxia than constantly submerged mussels. NSF IOS 1145840.

40.5 TAKING A COMPARATIVE APPROACH TO UNDERSTANDING MICRONA EXPRESSION AND ITS FUNCTIONAL CONSEQUENCES AFTER FLUOXETINE EXPOSURE IN TWO RELATED SPECIES, CARASSIUS AURATUS AND DANIO RERIO
Paul Craig1, Brooke Cameron2, Thomas Moore3, and Vance Trudeau2

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Fluoxetine is a selective serotonin reuptake inhibitor and active ingredient in Prozac, is found in the environment and disrupts feeding, stress, and reproduction in fish by acting on the serotonin reuptake transporter. Female goldfish & zebrafish were exposed to environmentally relevant concentrations of waterborne FLX (540 ng/L) for 7 days. A custom designed miRNA microarray was used to assess the significantly regulated hepatic miRNA in zebrafish, which identified 6 specific pathway analysis, miRNAs were associated with the negative regulation of anabolic metabolism in zebrafish including adipogenesis, cholesterol biosynthesis, triacylglycerol synthesis, and insulin signaling. These specific miRNA were profiled in goldfish, a phylogenetically related to the zebrafish, and results indicated a significant upregulation in all 6 miRNA under the same experimental conditions. Although miRNA sequences are...
well conserved across species, their mRNA targets may be less conserved. To accomplish this comparison, RNASeq (Illumina) is used to obtaining the required 3'UTR information to generate predicted miRNA targets in goldfish. Using this strategy we will be able to determine conservation of target pathways and functional response between species.

40.6 WHOLE-GENOME METHYLATION PROFILING OF THREESPINE STICKLEBACK REARED IN HIGH AND LOW SALINITIES

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Epigenetic modifications, such as changes in DNA methylation, provide a unique connection between an organism’s environment and the structure and function of its genome. Changes in DNA methylation patterns can result in phenotypic heterogeneity in genetically homogeneous populations, and thus have the potential to be an important component of an organism’s ability to colonize a novel habitat. While many aspects of DNA methylation mechanisms are well understood, there is limited understanding of the role of DNA methylation in natural populations, particularly in fishes.

Here, we use the threespine stickleback, *Gasterosteus aculeatus*, as a model system to explore the role of DNA methylation in the colonization of novel habitats. Populations of stickleback range from marine, to anadromous, to freshwater resident, and are thus exposed to differing environmental salinities. The association between repeated freshwater colonization events and conserved phenotypic changes among populations of stickleback presents an ideal system to study genotype/phenotype interaction. In this study, whole genome bisulfite sequencing was used to generate the first whole genome methylation profile of a stickleback. To characterize how DNA methylation patterns are impacted by salinity, clutches from marine sticklebacks were divided and fertilized in a brackish salinity representing their natural spawning environment, and a low salinity intended to represent colonization of a freshwater environment. From this data we identify differentially methylated genomic regions that may be important in the transition from marine to freshwater environments. Funding: NSERC.

40.7 DETERMINATION OF THE GILL TRANSCRIPTOME OF THE FATHEAD MINNOW (*Pimephales promelas*)

Simon Wentworth1, Katrina Thede2, Varsha Auyangadiso2, Ian Monnè3, Andrew Thompson1, Jeffrey Garvin2, and Randall Packer1

RNASeq is a quick and accurate method for exploring the transcriptome and expressed proteins in non-model organisms. The fathead minnow (*Pimephales promelas*) is a widely used toxicology model and knowledge about its genetics will enhance further study into the interactions between this organism and its environment. We present the gill transcriptome of the rosy red strain of *P. promelas*. An Illumina HiSeq was used to produce 470,183,940 raw reads of 101 bp in length from prepared mRNA. After using the Broad Institute’s Trinity software to perform a de novo assembly, 153,118 contigs were obtained with an average length of 997 bp and an N50 of 2081 bp. Annotation was performed with the Broad Institute’s de novo assemblies and determination assembly quality through mapping of RNA-seq data. In addition, we compare our assembly to genomes of other teleosts and explore heterozygosity and mitochondrial heteroplasmy in our lab population. Finally, we determine global changes in 5-methylcytosine content throughout *A. limnaeus* embryonic development by ELISA. These data will provide the framework for future genomic and epigenomic studies in annual killifish. Funding: NSF IOS-1354549.

40.10 Withdrawn.

40.11 EFFECTS OF OCEAN ACIDIFICATION ON JUVENILE ROCKFISH (*Sebastes spp*.) GENE EXPRESSION

April Malagkoudi1, Lauren Tobosi1, Giacomo Bernardi1, Hamilton Fennic1, Scott Hamilton1, and Cheryl Logan2
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Despite teleosts’ high capacity for acid-base regulation, recent ocean acidification (OA) studies on tropical marine fish have documented negative physiological effects on growth and reproduction. Impacts on temperate fish, however, remain understudied. Our previous work showed that temperate rockfish reared under chronic OA conditions have reduced swimming performance, with juvenile blue rockfish (*S. mystinus*) being more OA-tolerant than juvenile copper rockfish (*S. caurinus*). To investigate potential underlying mechanisms leading to differences in OA susceptibility, we compared rockfish transcriptomes after chronic exposure to predicted end-of-century pH levels (7.2, 7.5, 7.8, 8.0). We extracted total RNA from white muscle tissue and prepared cDNA libraries for RNAseq. We assembled a de novo transcriptome using Trinity, mapped sequences using SRA, and examined differential gene expression (DE) using edgeR (FDR<0.05). We identified 100s of significant DE genes among pH treatments for each species, with fewer than 20 genes in common between them, suggesting differential acclimation responses to chronic OA exposure. Our study is the first to use high-throughput sequencing to examine expression of OA-tolerant versus susceptible teleosts, providing important information about sublethal changes associated with OA resistance in marine fishes. Funded by COAST, CSUPERB.

40.12 PROTEOMIC PROFILE AND PROTEOGENOMIC ANALYSIS OF SKELETAL MUSCLE IN A MAMMALIAN HIBERNATOR

Kyle Anderson1, Pratik Jastan1, Todd Markowski1, LeeAnn Hughes1, James Johnson1, Tim Cattlin2, and Matt Andrews1
This study uses a proteogenomic approach that couples high-throughput proteomics with RNA-seq data to study skeletal muscle function during mammalian hibernation. Cessation of feeding, prolonged immobilization, and altered fuel availability are hallmark of the hibernation phenotype that have major implication for tonality and functionality of skeletal muscle. Yet these animals exhibit limited changes to muscle function and mass through 5-6 months of hibernation. Mass spectral analysis using iTRAQ labeling produced quantitative proteomic data from the thirteenth-ground sutured (Actunodon tridecemlineatus) during the creanial cycle. These data agreed with many of the physiological phenomenon known to occur in hibernator skeletal muscle, and the depth of coverage makes it possible to support both previously described and novel mechanistic hypotheses for these phenomenon. Using the customizability data analysis platform GalaxyP to merge the proteome data with Illumina HiSeq2000 transcriptome data from the same animals, we were able to identify protein sequences unique to the ground sutured. Novel peptide sequence identification allowed for improved annotation of the ground sutured genome, identification of alternative splice sites, mutations, and genomic organization, all of which may facilitate the altered physiology of hibernator skeletal muscle. This work was supported by NSF grant 1147079.

40.13 A FUNCTIONAL GENOMIC ANALYSIS OF WEDDELL SEAL DIVING ADAPTATIONS: VASCULAR BIOLOGY
Allyson G. Hindsle1, Jason Turner-Maiter2, Aaron M. Berlin1, Jessica U. Meir1, Jeremy Johnson1, Jessica Allafiri1, Patrick Y. Sipes1, Kerstan Lindblad-Toh3, Warren M. Zapolski4, and Emmanuel S. Bowes5
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To examine the functional genomics of mammalian diving physiology, we sequenced genomic DNA extracted from Weddell seal liver, yielding a 90X Illumina ALLPATHS-LG assembly. Our draft genome annotation was analyzed against the dog genome, identifying 16,839 protein-coding seal genes (versus ~21,000 in dog) and 2,660 non-coding genes. To understand tissue-specific vasoregulation against the dog genome, identifying 16,839 protein-coding seal genes (versus ~21,000 in dog), and testing a range of behaviors. We show that sticklebacks display behavioral disturbances similar to corral reef fish, demonstrating that the effect is global. However, the behavior of Atlantic cod was unaffected, which is surprising given that both species are exposed to a large range of environmental conditions. This demonstrates that the impact of elevated pCO2 on fish behavior is diverse and unpredictable. Atlantic cod also showed strong avoidance behavior of CO2 when given a choice between control (390 µatm) and elevated pCO2 (1000 µatm), indicating that they consider elevated pCO2 suboptimal. This could potentially influence distribution and migration of fish in a future high-CO2 ocean.

41.0 CONSERVATION PHYSIOLOGY
41.1 OCEAN ACIDIFICATION: EFFECTS OF CO2 ON BEHAVIOR AND GABA FUNCTION IN TELEOST FISH
Laure Vossen1, Arianna Cozzo1, Josefin Sundin1, Bryndis Bimar2, Friedrik Jutfelt2, and Svante Winkel1
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Elevated levels of ocean CO2 to be expected around the year 2100, have been shown to cause abnormal behavior in marine fish. Behavioral changes include increased activity levels, altered auditory and olfactory preferences and loss of lateralization. The normal behavior can be restored upon administration of a GABA-A antagonist, demonstrating that the function of GABA as the major inhibitory neurotransmitter is lost under acidosis. GABA-A agonists on the other hand theoretically have an inhibitory effect on behavior of control fish, whereas CO2 exposed fish should become even more affected. GABA-A agonists have been studied less extensively in the context of ocean acidification, and the few studies available have provided contrasting results. Our results show that exposure to end-of-the-century CO2 levels makes stickleback (Gasterostes aculeatus) hyperactive, with no effect on lateralization. Muscimol did not agitate the behavioral disruptions caused by CO2, suggesting that elevated CO2 is only affecting a small subpopulation of GABAergic synapses. GABA-A receptor subunit expression has been mapped in the zebrafish brain, in an attempt to utilize zebrasfish (Danio rerio) as a model for studies on the effects of elevated CO2 on fish behavior and GABAergic function.

41.2 CONTRASTING SENSITIVITY TO OCEAN ACIDIFICATION-INDUCED BEHAVIOURAL CHANGE IN ATLANTIC COD (GADUS MORHUA) AND THREE-SPINED STICKLEBACK (GASTEROSTES ACULEATUS)
Fredrik Jutfelt1, Maria Hedgård2, Karine Bresolini Desouza1, Joachim Sturup1, and Josefin Sundin1
1Dept. Biological & Environmental Sci., Univ. of Gothenburg, Medicinaregatan 18, 41390, Gothenburg, 2100 (1000 atm), and tested a range of behaviors. We show that sticklebacks display behavioral disturbances similar to corral reef fish, demonstrating that the effect is global. However, the behaviour of Atlantic cod was unaffected, which is surprising given that both species are tolerant to a large range of environmental conditions. This demonstrates that the impact of elevated pCO2 on fish behaviour is diverse and unpredictable. Atlantic cod also showed strong avoidance behaviour of CO2 when given a choice between control (390 µatm) and elevated pCO2 (1000 µatm), indicating that they consider elevated pCO2 suboptimal. This could potentially influence distribution and migration of fish in a future high-CO2 ocean.
41.5 DECODING THE RELATIONSHIP BETWEEN IMMUNE RESPONSE AND STRESS HORMONES: AN IMMUNOLOGIC PROFILE OF THE NORTHERN ELEPHANT SEAL

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Female northern elephant seals utilize finite resources during terrestrial haul-outs that they must allocate to competing metabolic functions. In addition to feeding, the breeding haul-out is characterized by energy-demanding and immunologic challenges, such as parturition, lactation, and aggressive interactions with conspecifics. Northern elephant seals elevate cortisol, a hormone known for its immunosuppressive effects, to mobilize lipid resources for energy while fasting. Recent work has shown that cortisol levels at the beginning of fasting vary widely with long-term foraging success at sea. Although elephant seals experience sustained elevations in serum cortisol, they retain immune function as indicated by the presence of various markers. We measured a suite of antibodies and inflammatory proteins to analyze how these parameters vary with life history stage and stress hormone levels. These markers were higher during the breeding haul-out in comparison to the molt haul-out. Most markers showed no relationship to cortisol except for IgG, an immunoglobulin associated with parasitic exposure, which decreased with increasing cortisol. This is the first time a suite of immune and inflammatory markers have been measured in northern elephant seals, showing a potential decoupling of stress hormones and immunosuppression in an animal that exhibits adaptive variation in cortisol. Funding was provided by the Office of Naval Research.

41.6 WITHDRAWN

41.7 HEAT TOLERANCE OF AUSTRALIAN BIRDS: THE IMPORTANCE OF BODY SIZE, PHYLOGENY AND EVAPORATIVE PATHWAY

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Heat waves in Australia have resulted in a number of heat related avian mortality events where large numbers of birds die from dehydration or heat stroke. Due to the apparent differential susceptibility to heat among species, these mortality events have great impact to wildlife communities as temperatures rise due to anthropogenic climate change. The goal of this investigation was to determine the role of body size, evaporative pathway, and phylogeny in the heat tolerance of Australian desert birds. Here we quantify metabolic rate, rates of evaporative water loss, and body temperature in eight species of Australian bird representing four orders and ranging in body size from 7g - 300g. Among these birds, large birds benefited from heat storage due to mild-thermoplasia, and had lower mass specific rates of water loss relative to metabolic heat gain. Small birds utilized thermoplasia to a greater degree and had higher rates of metabolism relative to rates of water loss, a product of active respiratory evaporation. Columbiformes outperformed most other species, due to their large body size and high rates of largely passive cutaneous evaporation, which resulted in low rates of metabolism and mild-thermoplasia. These data provide insight into the importance of phylogeny, evaporative pathway at high temperature and body size, which can be used in future studies to parameterize models to aid in the prediction and potential mitigation of heat related avian mortality events.

42.0: BIOMECHANICS, LOCOMOTION, AND FUNCTIONAL MORPHOLOGY

42.1 THE MECHANISTIC BASIS OF UNRELIABLE SIGNALS OF STRENGTH IN MALES OF THE TWO-TONED FIDDLER CRAB, UCA VOMERIS

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Signals used during aggressive contests between males of many species are thought to be reliable indicators of underlying quality. In some species, however, unreliable signals develop as a consequence of mismatches between signal size and the information being conveyed. We investigated physiological mechanisms that potentially govern signal reliability in the fiddler crab, Uca vomeris. Male U. vomeris exhibit both reliable and unreliable signals of strength via the expression of original and regenerated claw morphology. Regeneration of the major claw occurs after damage or loss of the original claw, and regenerated claws are always poorer in quality than the claws they replace. We examined morphological, biomechanical and biochemical characteristics of original and regenerated claws, to establish the best predictors of the variation seen in claw strength. We found that for a given claw size, regenerated claws have less muscle mass than original claws, and that for a given muscle mass regenerated claws were significantly weaker than original claws. Mechanical advantage was also lower in regenerated claws. The activity of three catalytic enzymes did not differ between claw types. Poor strength in these regenerated claws resulted from a combination of physiological factors and thus the development of other unreliable signals should also be constrained by them.

42.2 THE EFFECTS OF SELECTION FOR DESICCA TION OR STARVATION RESISTANCE ON TAKEOFF FLIGHT PERFORMANCE IN DROSOPHILA MELANOGASTER

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Flight requires a suite of highly derived physiological and morphological characteristics that combine to generate the required flight performance of a species. Small deviations in either of these parameters can result in substantial changes in flight performance. Environmental stressors, like those imposed during droughts, can generate directional selection that favors resistance to desiccation and/or starvation over the maintenance of flight performance characteristics. This research studied multiple populations of Drosophila melanogaster that were selected for resistance to desiccation or starvation. Using high-speed videography, takeoff flights of individual flies were tracked. Using custom software written in MATLAB, velocities and flight angles (relative to the point of takeoff) were compared between the selected lines and their corresponding control groups. The results showed that both the desiccation and starvation resistant lines were significantly heavier than their controls but were not different from each other. The additional glycogen stored in the abdomen of the desiccation selected flies did not result in any measurable reduction in flight performance. In contrast, starvation resistance lines exhibited a significant reduction in flight angle. The difference in the flight trajectories of the starvation resistance flies is likely due to cardiac impediment caused by additional fat stored during larval development.

42.3 MATERIAL AND STRUCTURAL CHARACTERIZATION OF MINERALIZED ELASMOBRANCH CARTILAGE: LESSONS IN REPEATED TILING PATTERNS IN MECHANICALLY LOADED 3D OBJECTS

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The 'tessellated' skeleton of elasmobranchs (sharks and rays) is a composite of mineralized tiles (tessere), collagen, and unmineralized cartilage, organized in discrete phases. The constituents and structural properties (e.g. tiling geometries) are vital to skeletal growth and mechanics, but have been little investigated due to the technical challenges involved. We use high-resolution materials characterization and visualization techniques to examine a developmental series of tessere, outlining the development of unique structural features that likely function in load bearing and energy dissipation, with some regions exceeding cortical bone's mineral content and stiffness. To examine interactions among tessere, we developed an advanced tiling-recognition-algorithm to semi-automatically detect and isolate individual tiles in microCT scans of tesselmat. The method allows quantification of shape variation across a wide area, allowing localization of regions of high low reinforcement or flexibility in the skeleton. The combination of our material characterization and visualization techniques allows the first quantitative 3d description of anatomy and material properties of tessere and the organization of tesselmat networks in elasmobranch mineralized cartilage, providing insight into form-function relationships of the repeating tiled pattern, as well as fundamental tiling laws important for complex, mechanically loaded 3d objects. Funding: HSP grant to MND & JW.

42.4 THE ELASMOBRANCH HEART DOESN'T TWIST: A SPECKLE-TRACKING ECHOCARDIOGRAPHY STUDY

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Background: Compact myocardial fibers are arranged to form a helical architecture in the mammalian heart. This fiber arrangement generates rotation of the cardiac base and apex in the clockwise and counterclockwise directions as observed from the apex, respectively, resulting in left ventricular twist. It is unknown if ventricular rotation/twist occur in species with a univentricular heart.

Methods: We investigated myocardial strain, a novel parameter of myocardial deformation in the circumferential (C) and longitudinal (L) directions, and ventricular rotation by 2D speckle-tracking echocardiography (Vivid E9, GE Healthcare) in 7 elasmobranchs (2 bullhead sharks, 2 brownbanded bamboo sharks, 3 whiprays) under sedation with tricaine methanesulfonate. The study conformed to the Guiding Principles in the Care and Use of Animals and was approved by the institution’s ethics committee. Results: Specdle-
tracking was feasible in all cases. Peak global C- and L-strain was \(-21.5\pm 7.4\%\) \((-23.8\pm 4.8\%)\) and \(-23.8\pm 3.7\%\) \((-20.3\%\) in humans), respectively. Mean basal and apical rotation was \(-2.9\pm 3.8\% \(9.6\pm 2.5\) and \(-2.4\pm 3.8\% \(11.1\pm 4.0\), respectively. Rotational direction at the cardiac base was the same as that at the apex. Conclusion: Elasmobranch and human hearts have similar ventricular strain. In elasmobranchs, however, ventricular rotation was not prominent and ventricular twist was not observed, suggesting that the fiber arrangement differs between the elasmobranch heart and human heart. Referee: Sun JP, Lan YY, Wi CO, Yang XS, Gao R, Kwong JS, Merlino JD, Yu CM. Effect of age and gender on left ventricular rotation and twist in a large group of normal adults – A multicenter study. Int J Cardiol 167: 2215-2221, 2013.

42.5 TRENDS IN MORPHOLOGY AND BIOMECHANICS OF THE AQUATIC GILL VENTILATORY SYSTEM OF RAY-FINNED FISHES (ACTINOPTERYGI) Stacy Farnia1, Lara Ferry1, Thomas Near1, Adam Summers1, and William Berns1


To ventilate their gills, ray-finned fishes (Actinopterygii) use pumps in their oral and gill chambers to alternate between suction (inspiration) and compression (expiration). These mechanisms are largely conserved across Actinopterygii, but there is considerable morphological and functional variation of the pumps. Our goal was to use comparative approaches to investigate the evolution of ventilatory morphology and function across actinopterygians. Using recently published molecular data, we reconstructed the evolutionary history of restricted gill openings across 433 actinopterygian families. Restricted gill openings have evolved at least 11 times among ray-finned fishes with diverse morphology and ecology. We also studied ventilatory biomechanics among four benthic sculpins and found considerable variation in oral and gill chamber pressures. Using phylogenetically independent contrasts, we linked variation in these pressures to morphology of the ventilatory pumps. Variation in function correlated closely with the size of the branchial apparatus, especially the branchiostegal rays that form the floor of the gill chamber. We propose that adding a third pump to the traditional two-pump model, in which the branchiostegal rays work in parallel with the operculum, is a useful framework for comparative gill ventilatory studies. Funding sources include NSF (DEB-1310812), Sigma Xi Cornell University Chapter, Friday Harbor Laboratories, and the Stephen and Ruth Wainwright Fellowship.

42.6 PAINTED TURTLES LOSE BONE STRENGTH BUT MAINTAIN BONE TOUGHNESS DURING ANOXIC HIBERNATION

Dean T. Odegard1, Michael A. Sonnenfeld1, Daniel J. Leib1, and Daniel E. Warren1


Western painted turtles (Chrysemys picta bellii) can endure more than 170 days of anoxia at 3°C, in part because ions are released from the skeletal system that help to oxygenate at 3°C, in part because ions are released from the skeletal system that help to oxygenate anoxia. CC volume is greatly reduced in relation to the volume of the cerebral hemisphere, however, ventricular rotation was not prominent and ventricular twist was not observed, suggesting that the fiber arrangement differs between the elasmobranch heart and human heart. Referee: Sun JP, Lan YY, Wi CO, Yang XS, Gao R, Kwong JS, Merlino JD, Yu CM. Effect of age and gender on left ventricular rotation and twist in a large group of normal adults – A multicenter study. Int J Cardiol 167: 2215-2221, 2013.

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43.3 \textbf{OCEAN ACIDIFICATION DIRECTLY IMPAIRS OLFACTORY SENSITIVITY IN A MARINE TELEOST}

Cosima Porteaux\textsuperscript{1}, Peter Hubbard\textsuperscript{2}, and Rod Wilson\textsuperscript{1}

\textsuperscript{1}Biosciences, Univ. of Exeter, Coll. of Life & Environmental Sci., Exeter, EX4 4QD, UK., 2Comparative Molecular Endocrinology, Centro de Ciências do Mar, Campus de Gambelas, Univ. do Algarve, Faro, 8005-139, Portugal.

Corals are one of the most critically-endangered marine ecosystems. Ocean acidification (OA) resulting from increasing atmospheric CO$_2$ concentration, reduces the available carbonate in the oceans, causing decreased calcification rates and increased bioerosion. OA can also have a devastating impact on animals, such as the corals, by reducing their olfactory sensitivity. The loss of olfactory sensitivity can result in a lack of predator detection and poor reproductive success. In order to discover how OA affects olfactory sensitivity in marine fish, teleosts are an excellent model organism as they develop early-stage embryos in seawater and are well-adapted to living in the marine environment.

We conducted multi-unit recordings on the olfactory nerves of fish with different responses to CO$_2$ at different concentrations. We found that OA has a direct effect on olfactory sensitivity, and that OA can alter the olfactory sensitivity of fish. Our results suggest that OA can alter the olfactory sensitivity of fish, which may have significant implications for the survival of marine animals in the face of climate change.

\textsuperscript{1}Univ. of California San Diego, Scripps Inst. of Oceanography, 9500 Gilman Dr., La Jolla, CA, 92037, Physiology, Ctr. Scientifique de Monaco, MC-98000, Monaco. Symbiotic zooxanthellae algae residing inside coral tissues supply the host with the majority of their energy requirements through the photosynthesis of fixed carbon. The zooxanthellae, in turn, rely on the host for the supply of inorganic carbon. Carbon must be concentrated as CO$_2$ in order for photosynthesis to proceed, and here we show that the coral host plays an active role in this process. The host-derived symbiotic membrane surrounding the zooxanthellae abundantly expresses vacuolar H$^+$/ATPase (VHA), which acidifies the symbiosome space down to pH~4. Inhibition of VHA results in a significant decrease in average H$^+$ activity in the symbiosome of up to 75%, and a significant reduction in O$_2$ production rate, a measure of photosynthetic activity. These results suggest that host VHA is part of a novel carbon concentrating mechanism for zooxanthellae photosynthesis, and provide mechanistic evidence that coral host cells can actively modulate the physiology of their endosymbionts. This work was supported by National Science Foundation grants EF-1220641 and OCE-1222096, and an Alfred P. Sloan Research Fellowship (BR2013-103).

43.4 \textbf{CONVERGENT AND DIVERGENT PATTERNS OF GENE EXPRESSION IN SCULPINS THAT VARY IN HYPOXIA TOLERANCE}

Milica Mandic\textsuperscript{1}, Marina Ramoni\textsuperscript{1}, Andrew Gracey\textsuperscript{2}, and Jeffrey Richards\textsuperscript{1}

\textsuperscript{1}Biosciences, Univ. of Exeter, Coll. of Life & Environmental Sci., Exeter, EX4 4QD, UK., 2Comparative Molecular Endocrinology, Centro de Ciências do Mar, Campus de Gambelas, Univ. do Algarve, Faro, 8005-139, Portugal.

Sculpins, a diverse group of fishes, exhibit variation in hypoxia tolerance that is related to species distribution along the marine nearshore environment. To identify potential candidate traits involved in hypoxia tolerance, we assessed gene expression patterns over a 72 hour hypoxia time-course using microarrays. In the first set of experiments, we quantified divergent expression patterns in species with different hypoxia tolerance. We found that the hypoxia tolerant species down-regulated genes associated with a number of energetically costly biological processes while the hypoxia intolerant species did not. Additionally, gene expression did not change in the first 24 hours in the tolerant species of sculpin while in the intolerant species the greatest change occurred during the initial stages of hypoxia exposure. Together, these transcriptional patterns may, in part, help define hypoxia tolerance in these species of fish. In the second set of experiments, we examined 3 species with similar and intermediate levels of hypoxia tolerance for evidence of convergence of gene expression or the repeated evolution of the same traits. While we found some convergent gene expression patterns among the 3 species, a greater majority of genes exhibited non-convergent changes in gene expression. Therefore, convergent evolution of intermediate hypoxia tolerance is for the most part not a result of convergence of gene expression patterns in these species. (Funding NSERC)

43.5 \textbf{WHAT HAS K$^+$ GOT TO DO WITH IT? THE DIFFERING ROLES OF EXTRACELLULAR K$^+$ IN ONSET AND RECOVERY OF INSECT CHILL-COMA}

Heath MacMillan\textsuperscript{1}, Anders Findsen\textsuperscript{2}, Thomas Pedersen\textsuperscript{3}, and Johannes Overgaard\textsuperscript{1}

\textsuperscript{1}Zooplanktonology, Aarhus Univ., C.F. Møllers Allé 3, Aarhus, 8000, Denmark, \textsuperscript{2}Biomed., Aarhus Univ., Ole Worms Allé 3, Aarhus, 8000, Denmark.

Insects exhibit chill coma, a reversible state of paralysis, at temperatures below their critical thermal minimum (CT$_{min}$) and the time required for an insect to recover after a cold exposure is chill coma recovery time (CCRT). The CT$_{min}$ and CCRT are important metrics of insect cold tolerance and are often used interchangeably, but these two traits are not necessarily physiologically linked. Here we test the hypothesis that depolarization of muscle membrane potential ($V_m$) at chill coma onset and its repolarization during chill coma recovery are driven by changes in extracellular [K$^+$] and/or directly by low temperature. Using Locusta migratoria we measured in vivo muscle membrane potentials during cooling, following prolonged exposure to -2°C and during chill coma recovery, and related changes in $V_m$ to transmembrane [K$^+$] balance and temperature. Although cooling rapidly depolarized $V_m$, hemolymph [K$^+$] did not rise until locusts had spent considerable time in the cold. Nonetheless, a rise in hemolymph [K$^+$] during prolonged cold exposure further depressed muscle resting potential and slowed recovery from chill coma upon warming. Thus, a disruption of extracellular [K$^+$] does depolarize muscle resting potential and slow CCRT, but it is unrelated to the onset of coma. Because the mechanisms underlying these two traits are distinct, haphazard trait choice could lead to conflicting candidate genes under selection for cold tolerance.
43.7
CHANGES IN MO2, ANAEROBIC GLYCOLYSIS AND METABOLIC HEAT WITH DECREASING WATER PO2 IN GOLDFISH

Matthew Regehr1 and Jeffrey Richards2
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Hypoxic survival in fish is dependent on the interactions between three mechanisms: improved O2 uptake and utilization, anaerobic ATP production, and metabolic rate suppression (MRS). Many animals have been shown to employ all three mechanisms, but how these mechanisms interact with one another as environmental O2 tensions (PO2) decrease is unknown. The goal of this study was to characterize these changes in goldfish, a species of exceptional hypoxia tolerance, and it was hypothesized that the three hypoxia defense mechanisms would reach critical points at water PO2s at or around the critical PO2 of MO2 (Pcrit) where aerobic metabolism is first compromised. Calorimetry was used to simultaneously measure O2 uptake/utilization (via MO2) and metabolic heat, while anaerobic ATP production was determined through measurements of glycolytic metabolites (glycogen, lactate and ethanol). Preliminary results suggest that these critical points occur at different water PO2s. Oxygen consumption rate shows a Pcrit of ~35 mmHg O2, while anaerobic ATP production begins to increase at ~50 mmHg O2, and MRS is not evident until PO2 reaches ~2 mmHg. This suggests that the relatively active lifestyle of goldfish in moderately hypoxic environments is maintained by anaerobically-produced ATP, while MRS is a strategy of tolerance reserved only for severe hypoxia or anoxia. (Funding provided by NSERC and UBC.)

43.8
INTEGRATING THE EFFECTS OF REPEATED COLD EXPOSURE FROM TRANSCRIPTOME TO WHOLE-ORGANISM IN THE EASTERN SPRUCE BUDWORM

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1Dept. of Zoology, Univ. of British Columbia, #4200-6270 University Blvd., Vancouver, BC, V6T 1Z4, Canada. 2Dept of Biology, Univ. of Western Ontario, Biological & Geological Sci. Bldg., London, ON, N6A 5B7, Canada.

Organisms live in complex worlds where environmental stresses can be more or less intense, occur for longer or shorter periods, and repeat more or less frequently. Yet while single stress events have been well-studied, the physiological and fitness effects of these more complex patterns of stress are not well understood. The eastern spruce budworm, Choristoneura fumiferana, is an important forest pest of the boreal forest and previous studies have shown the importance of low temperature stress in regulating its population and range. In this study we manipulated the number of low temperature exposures budworm received, while controlling the intensity and total length of time of exposure. We found that while budworm that received repeated low temperature events had significantly greater cryoprotectant content (at a cost to glycogen reserves), survival to eclosion was significantly impacted. We also examined transcriptional responses to low temperature stress, and found that while only 16 transcripts were significantly differentially regulated following a single low temperature stress event, 644 were differentially regulated following repeated low temperature stresses. These included transcripts for antifreeze proteins, several heat shock proteins, and electron transport chain proteins. These results suggest that current studies that focus on single stress events may be missing the full range of potential stress responses.

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THE ROLE OF TRANSCRIPTION FACTOR GLIAL CELL MISSING 2 (GCM2) IN CA2+ BALANCE IN ZEBRAFISH LARVAE.

Yusuke Kuma1, Raymond Kwong2, and Steve Perry3
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The present study investigated the role of the transcription factor, glial cell missing 2 (gcm2) in Ca2+ regulation in zebrafish larvae. Transcriptional gene knockdown of gcm2 decreased Ca2+ uptake and the density of ionocytes expressing the epithelial Ca2+ channel (ezzoa), and disrupted overall Ca2+ balance. Acclimation to either low Ca2+ (25 μM [Ca2+]o) or acidic water (pH ~ 4.0) significantly increased the mRNA expression of gcm2. When measured in control water following acclimation to these conditions, Ca2+ uptake was significantly elevated. However, in fish experiencing gcm2 knockdown, no such stimulation of Ca2+ uptake was observed. Over-expression of gcm2 mRNA resulted in a significant increase in Ca2+ uptake and metabolic rate and membrane Ca2+ concentration in gcm2-expressing ionocytes. When fish experiencing gcm2 knockdown was treated with waterborne cortisol, a well-known hypercalcemic hormone in fish, the treatment inhibited Ca2+ uptake in those larvae. These observations demonstrate a critical role for gcm2 in Ca2+ homeostasis in zebrafish larvae. All experiments were conducted in accordance with Guiding Principals in the care and Use of Animals and after the approval of the University of Ottawa Animal Care Committee (Protocol BL-226).
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