

## Australian and New Zealand Society for Comparative Physiology and Biochemistry 27<sup>th</sup> Annual Meeting Australian National University

December 3-5 2010



Thank you to the journals and industries that sponsored our student prizes, Comparative Biochemistry and Physiology, Journal of Comparative Physiology B, Physiological and Biochemical Zoology, Sable Systems, and StatisiXL. The meeting also benefited from a grant from the ANU College of Medicine, Biology and the Environment, Australian National University.









THE AUSTRALIAN NATIONAL UNIVERSITY



## Acknowledgements

Running meetings are never an easy task, but usually there are several people that are helping behind the scenes to aid and abet the undertaking. Audra Johnstone and Sharyn Wragg have been absolutely tremendous with helping on the design and improvements of the web site and this book. Sarah Portelli of Bruce Hall ensured that accommodation was available on campus, and helped with meeting various requirements of the attendees. Thanks go to Hudson's Catering in recognising the challenges of delivering tea and lunches in the chaos of the building program at the university. Neil Bayley, Eric Li and Mary McDonald helped with the financial issues that arose within the new organization of the ANU. Thanks again to Sharyn Wragg for agreeing to take charge of the group photo. The meetings were assisted by a grant from The ANU College of Medicine, Biology and the Environment given by Professor Andrew Cockburn, as well as being allowed to use the buildings by Professors Kiaran Kirk and William Foley. Thanks to Helen Muirhead and Jan Elliott for helping with housekeeping (literally) matters. Finally, I have to thank both Mike Thompson and Stuart Linton for being patient with my questions regarding previous meetings and always having the answers.

Paul Cooper



### Campus Map Detailed map is indicated by red rectangle

### **Detailed Map with directions**



| Thursday 2<br>December                          | Time        | Friday 3<br>December                                    | Time        | Saturday 4<br>December                                       | Time        | Sunday 5<br>December |
|---|-------------|---|-------------|--|-------------|----------------------|
|   | 9:00-10:15  | Welcome<br>Session 1                                    | 9:00-10:30  | Session 5  | 9:15-10:30  | Session 9            |
|   | 10:20-10:40 | Morning tea +<br>Posters                                | !0:35-10:55 | Morning tea  | 10:35-10:55 | Morning tea          |
|   | 10:45-12:15 | Session 2   | 11:00-12:30 | Session 6  | 11:00-12:30 | Session 10           |
|   | 12:30-13:50 | Lunch   | 12:35-13:45 | Lunch  | 12:30-14:00 | Lunch                |
|   | 14:00-15:30 | Plenary Lecture<br>Prof Stephen<br>Simpson<br>Session 3 | 14:00-15:30 | Session 7  |             |                      |
|   | 15:35-16:15 | Afternoon tea +<br>Group Photograph                     | 15:35-15:55 | Afternoon tea  |             |                      |
|   | 16:20-17:20 | Session 4   | 16:00-17:30 | Session 8 +<br>Annual general<br>meeting                     |             |                      |
|   | 17:20-18:00 | Posters   |             |  |             |                      |
| 18:30-20:30<br>Registration +<br>Pizza + Drinks | Evening     | Barbecue at Bruce<br>Hall                               | Evening     | Conference dinner-<br>Australian National<br>Botanic Gardens |             |                      |

## Program

| Friday 3 <sup>rd</sup> December Morning |  |  |
|---|--|--|
| Session 1                               | Chair: Michael Kearney   |  |
| 9:00 am                                 | Welcome  |  |
| 9:10                                    | <b>Piyankarie Jayatilaka</b> , Ajay Narendra, Samuel F Reid, Paul<br>Cooper and Jochen Zeil<br>Does temperature restrict bulldog ants to discrete temporal   |  |
| 0.05                                    | niches?  |  |
| 9:25                                    | James D. Woodman<br>Upper thermal limits in first-instar nymphs of the Australian<br>plague locust, <i>Chortoicetes terminifera</i>  |  |
| 9:40                                    | Mariana A. Micheli-Campbell, David Booth and Craig E.<br>Franklin<br>Staying cool, keeping strong: incubation temperature affects  |  |
| 9:55                                    | performance in a freshwater turtle.<br>Jessica A. Roberts, Graeme Coulson, Adam Munn, and<br>Michael Kearney<br>How extensively and effectively do kangaroos behaviourally<br>thermoregulate?  |  |
| 10:15                                   | Morning tea (Ground and 1 <sup>st</sup> floor, Gould Wing, Building<br>116)  |  |
| Session 2                               | Chair: Bill Buttemer   |  |
| 10:45                                   | <b>Michael Kearney</b><br>Metabolic theory, life history and the fundamental niche of a<br>terrestrial ectotherm   |  |
| 11:00                                   | <b><u>Caragh B. Heenan</u></b><br>The influence of climate and altitude on bird nest conductance   |  |
| 11:15                                   | Alex Little and Frank Seebacher<br>Cold-acclimation and thyroid hormone in zebrafish   |  |
| 11:30                                   | <b>Christopher Johnstone</b> , Richard Reina and Alan Lill Using tree-based modelling to examine ecophysiological data   |  |
| 11:45                                   | <b>Tim Jessop</b><br>Assessing physiological responses of animals to aberrant<br>trophic interactions.   |  |
| 12:00                                   | <u>Madeleine Barton</u> , Melanie Norgate, Paul Sunnucks and<br>Michael Kearney.<br>Responding to climate change: flight capacity and behavioural<br>buffering of the common brown butterfly ( <i>Heteronympha</i><br><i>merope</i> ). |  |
| 12:15-13:50                             | Lunch  |  |

|             | Friday 3 <sup>rd</sup> December Afternoon                        |
|-------------|--|
| Session 3   | Chair: Mike Thompson   |
| 14:00       | Plenary Lecture: Professor Stephen Simpson                       |
|             | Graphic Nutrition: A tale of sex, cannibalism, aging and obesity |
| 15:00       | Todd J. McWhorter and Patricia A. Fleming                        |
|             | Life on the edge: digestive capacity in nectarivorous birds      |
| 15:15       | Stuart M. Linton, Reinhard Saborowski and Alicia J. Shirley      |
|             | Digestive ability of four sympatric land crab species:           |
|             | Geacrcoidea natalis, Discoplax hirtipes, Birgus latro and        |
|             | Coenobita perlatus.  |
| 15:35-16:15 | Afternoon Tea and Group Photograph                               |
| Session 4   | Chair: Stuart Linton   |
| 16:20       | Eran Levin, Amos Ar, Abrham Hefetz, Yoram Yom-Tov and            |
|             | Noga Kronfeld-Schor  |
|             | Some like it hot: Hibernatiom patterns of the greater mouse-     |
|             | tailed bat ( <i>Rhinopoma microphyllum</i> ).                    |
| 16:35       | Martha Patricia Ramírez-Pinilla, Scott L. Parker, Christopher R. |
|             | Murphy, and Michael B. Thompson                                  |
|             | Uterine angiogenesis and cell surface changes during             |
|             | pregnancy in a viviparous lizard, Niveoscincus conventryi        |
| 17:00-18:00 | Poster Session   |
| Evening     | BBQ at Bruce Hall  |

|             | Saturday 4 <sup>th</sup> December Morning  |  |
|-------------|--|--|
| Session 5   | Chair: Phil Withers  |  |
| 9:00 am     | Jenny Sprent, Sue Jones, Rob Gasperini and Stewart Nicol                                     |  |
|             | The role of leptin in the annual cycle of the echidna  |  |
| 9:15        | Damian D'Souza, Klaus Altland, Leonard Pattenden and   |  |
|             | Samantha Richardson  |  |
|             | Mechanisms of Transthyretin Amyloid Formation  |  |
| 9:30        | Melissa S. Cameron, Sofie Trajanovska and John A. Donald                                     |  |
|             | Nitric oxide regulation of blood vessels in amphibians                                       |  |
| 9:45        | BriAnne Addison, Robert Ricklefs and Kirk Klasing  |  |
|             | Testing the maternal immune imprinting hypothesis using direct                               |  |
|             | manipulation of yolk antibodies.   |  |
| 10:00       | Edward J. Narayan, Jean-Marc. Hero, John F. Cockrem and                                      |  |
|             | Jane E. Candy  |  |
|             | Urinary corticosterone responses to capture and captivity in the                             |  |
|             | cane toad (Rhinella marina, previously Bufo marinus)   |  |
| 10:15       | Morning tea (Ground and 1 <sup>st</sup> flood, Gould Wing, Building                          |  |
|             | 116)   |  |
| Session 6   | Chair: John Donald   |  |
| 10:45       | Suzanne Munns  |  |
|             | Pregnancy makes breathing expensive in lizards.  |  |
| 11:00       | Taryn S. Crispin and Craig R. White  |  |
|             | Reptile metabolic rate is correlated with altitude, but not mean                             |  |
|             | environmental temperature or annual precipitation.   |  |
| 11:15       | Philip C. Withers and Christine E. Cooper  |  |
|             | Non-invasive partitioning of cutaneous and respiratory                                       |  |
| 11.20       | evaporative water loss in small mammals.   |  |
| 11:30       | Sean Tomlinson, Phillip Withers and Shane Maloney  |  |
|             | The Role of Thermoregulation in Energetics and Water   |  |
| 11.45       | Economy: A case study of Western Australian dunnarts.  |  |
| 11:45       | Amanda J. Page, <b>Christine E. Cooper</b> and Philip C. Withers                             |  |
|             | Effects of experiment start time and duration on measurement                                 |  |
| 1.0.00      | of metabolic rate, evaporative water loss and body temperature.                              |  |
| 12:00       | K. Vesterdorf, D. Blache and <b>S. K. Maloney</b>  |  |
| 10.15       | Can we measure respiratory heat loss in a free ranging animal?                               |  |
| 12:15       | lan Gerard van Tets and Kalb Thayer Stevenson  |  |
|             | Using Dual-energy X-ray absorptiometry (DXA) to accurately                                   |  |
|             |  |  |
|             | and nondestructively measure the body composition of small,                                  |  |
| 12:30-13:50 | and nondestructively measure the body composition of small,<br>free-living rodents.<br>Lunch |  |

| Saturday 4 <sup>th</sup> December Afternoon |  |  |
|---|--|--|
| Session 7                                   | Chair: Christine Cooper  |  |
| 14:00                                       | Leonard G. Forgan and Malcolm E. Forster                         |  |
|   | Oxygen-dependence of metabolic rate in the muscles of            |  |
|   | craniates.   |  |
| 14:15                                       | Nicholas Tuckey, Malcolm Forster and Steven Gieseg               |  |
|   | Effects of rested harvesting on muscle metabolite                |  |
|   | concentrations in Chinook salmon (Oncorhynchus                   |  |
|   | tshawytscha).  |  |
| 14:30                                       | Casey A. Mueller, Jean M.P. Joss and Roger S. Seymour            |  |
|   | The low developmental cost of the Australian lungfish            |  |
|   | compared to other fishes and amphibians.                         |  |
| 14:45                                       | Roger S. Seymour and Stefan K. Hetz                              |  |
|   | The physical gill of diving-bell spiders, Argyroneta aquatica    |  |
| 15:00                                       | Imogen Munro   |  |
|   | Buoyancy regulation and the effect of selected exotic gases on   |  |
|   | the compressible gas gill of notonectids and corixids            |  |
|   | (Hemiptera).   |  |
| 15:15                                       | Lisa M. Rigby and David J. Merrit                                |  |
|   | The role of biogenic amines in regulating bioluminescence in     |  |
|   | the Australian glowworm, Arachnocampa flava.                     |  |
| 15:30                                       | Afternoon Tea  |  |
| Session 8                                   | Chair: Ashley Edwards  |  |
| 16:00                                       | Natalie J. Briscoe, Michael R. Kearney and Warren P. Porter      |  |
|   | Thinking inside the box: toward an understanding and             |  |
|   | estimation of the thermal consequences of nest box design.       |  |
| 16:15                                       | Sarah E. Norris, Todd W. Mitchell and Paul L. Else               |  |
|   | Membrane phospholipid peroxidation: effect of fatty acid pairing |  |
|   | and head group.  |  |
| 16:30                                       | Sarah K. Abbott, Paul L. Else and A.J. Hulbert                   |  |
|   | The balance of n-3 and n-6 polyunsaturated fatty acids in the    |  |
|   | diet strongly influences rat membrane fat profile and minimum    |  |
|   | metabolic rate.  |  |
| 16: 45                                      | M. Trzcionka, AJ Hulbert and WA Buttemer                         |  |
|   | The long life of birds: The rat-pigeon comparison revisited      |  |
| 17:00-18:00                                 | Annual General Meeting   |  |
| Evening                                     | Conference Dinner (Australian National Botanic Gardens)          |  |

| Sunday 5 <sup>th</sup> December Morning |   |  |
|---|---|--|
| Session 9                               | Chair: James Woodman  |  |
| 9:15 am                                 | Edward Snelling   |  |
|   | Design of the Insect Respiratory System                                     |  |
| 9:30                                    | Philip G. D. Matthews and Craig R. White                                    |  |
|   | Mechanisms underlying the occurrence of discontinuous gas                   |  |
|   | exchange in insects.  |  |
| 9:45                                    | Rebecca Morley and David J. Merritt   |  |
|   | The effect of light on bioluminescence in the glow-worm                     |  |
|   | Arachnocampa flava.   |  |
| 10:00                                   | David J. Merritt and Arthur K. Clarke                                       |  |
|   | Arachnocampa tasmaniensis (glowworms) inside caves                          |  |
|   | maintain circadian rhythmicity through visual synchronization.              |  |
| 10:15                                   | Shervin Aslanzadeh, Michael B. Thompson, Gregory Sword,                     |  |
|   | Jerome Buhl and Stephen Simpson   |  |
|   | Does isolation from group affect antipredatory behaviour of                 |  |
| 10.00                                   | Australian plague locust?   |  |
| 10:30                                   | Morning tea (Ground and 1 <sup>st</sup> flood, Gould Wing, Building<br>116) |  |
| Session 10                              | Chair: David Merritt  |  |
| 11:00                                   | Bethany J. Hoye, Ron A. M. Fouchier and Marcel Klaassen                     |  |
|   | Disease ecology in migratory birds: insights from physiology,               |  |
|   | biochemistry and behaviour.   |  |
| 11:15                                   | P. Kern, B. M. McAllan and A. J. Munn                                       |  |
|   | Coping with chaos: unpredictable food supplies intensify torpor             |  |
|   | use and induce behavioural changes in activity patterns in an               |  |
|   | arid zone marsupial, the fat-tailed dunnart (Sminthopsis                    |  |
|   | crassicaudata).   |  |
| 11:30                                   | Sarah R Pryke, Simon C. Griffith, Lee B. Astheimer and                      |  |
|   | William A. Buttemer   |  |
|   | Making the best of a bad relationship: breeding with non-                   |  |
|   | preferred males affects Gouldian finch female corticosterone                |  |
| 11.45                                   | levels and primary offspring sex ratio                                      |  |
| 11:45                                   | Thomas Wallenius, Rod Peakall, Paul Cooper and Rolf                         |  |
|   | Oberprieler   |  |
|   | Pollination ecology of the Australian cycad <i>Macrozamia</i>               |  |
| 12:30-13:50                             | communis<br>Lunch   |  |

| Posters- Friday and Saturday |   |
|------------------------------|---|
|                              | Josiane Eve and Ashley Edwards                                  |
|                              | Thyroxine effects on body condition in a viviparous reptile     |
|                              | A. Daniella Rojas, Gerhard Körtner and Fritz Geiser             |
|                              | Implanted transmitters do not affect maximum running speed of   |
|                              | two small marsupials  |
|                              | Annalise M. Stanley, Melissa S. Cameron and John A.             |
|                              | Donald.   |
|                              | The role of potassium channels in vascular regulation in        |
|                              | amphibians.   |
|                              | Nurul Wahida Othman and Paul Cooper                             |
|                              | Salivary gland activity in wild-caught yellow-winged            |
|                              | grasshoppers, Gastrimargus musicus.                             |
|                              | Sam Parry, Stuart Linton and Paul Francis                       |
|                              | Xenobiotic metabolism by the Malpighian tubules of insects, a   |
|                              | new hepatic function  |
|                              | M. Trzcionka, A.J. Hulbert and W.A. Buttemer                    |
|                              | Can the oxidative stress theory of aging explain differences in |
|                              | longevity between galliformes and psittaciformes?               |

Abstracts

Does temperature restrict bulldog ants to discrete temporal niches?

## <u>Piyankarie Jayatilaka</u>, Ajay Narendra, Samuel F Reid, Paul Cooper, Jochen Zeil

ARC Centre of Excellence in Vision Science, Division of Ecology, Evolution & Genetics, Research School of Biology, The Australian National University, PO Box 475, Biology Place, Canberra ACT 2601, Australia

Animals avoid temperatures that constrain foraging by restricting activity to specific times of the day. However, as numerous other factors vary with temperature, isolating temperature-dependent effects proves to be difficult. Congeneric, sympatric species active at different times and at different temperatures are possibly ideal models to study temperature-dependent effects on foraging.

Here, we studied the thermal biology of two congeneric and sympatric *Myrmecia* ants that occupy distinct temporal niches, investigating the effect of temperature on foraging and temporal segregation of foraging activity. We monitored foraging activity and identified the ants' temperature tolerances by determining (a) critical thermal limits and (b) walking speeds. Ants of *Myrmecia croslandi* were diurnal, with enhanced tolerance to high temperature and hibernated during winter. Their foraging onset was predicted by surface temperature. Ants of *Myrmecia pyriformis* were nocturnal throughout the year, with greater tolerance to low temperature. Foraging onset time not predicted by temperature, but by sunset time. We conclude that temperature determines the timing of foraging as well as the annual foraging patterns in *M. croslandi*, but has little effect on *M. pyriformis*. We show that the temperatures at which ant walking speeds increase in laboratory heating experiments closely reflect upper foraging limits.

Upper thermal limits in first-instar nymphs of the Australian plague locust, *Chortoicetes terminifera* 

#### James D. Woodman

Australian Plague Locust Commission, Australian Government Department of Agriculture, Fisheries and Forestry

Abstract (200 word limit, Arial 12 pt): High temperature tolerance in first-instar nymphs of the Australian plague locust, *Chortoicetes terminifera* (Walker), is an important prerequisite for survival and the formation of high-density aggregations in summer and autumn generations. This study quantified the extent of this tolerance using measures of total body water, critical upper limit, physiological and behavioural transitions, and mortality for a range of temperature regimes. The critical upper limit for fed nymphs was very high at  $53.3 \pm 1.0^{\circ}$ C, with death preceded by a clear progression of changes in behaviour, gas exchange, water loss and excretion. At more ecologically relevant temperatures, mortality from desiccation was dependent on food availability relative to exposure duration and maximum temperature as well as the rate of warming. While very high mortality was recorded at  $\geq$ 45°C in 6 h direct exposure experiments, a highly exposed and very poorly vegetated summer environment would be required for local population failures from current high temperatures and low humidity alone.

Staying cool, keeping strong: incubation temperature affects performance in a freshwater turtle.

Mariana A. Micheli-Campbell; David Booth; Craig E. Franklin School of Biological Sciences, The University of Queensland, St. Lucia, QLD, Australia

AbTime-series measurements of ground temperature in South-East Queensland have shown a 0.4 °C rise over the past decade. This region is home to many species of freshwater turtles, and this study aimed to quantify how rises in nest temperature during incubation may affect the performance of the hatchling turtles. Freshly laid Elusor macrurus eggs were collected and incubated under three constant thermal regimes (26, 29 and 32°C). Embryos incubated under the warmest regime exhibited faster development, showed a significantly reduced carapace length, body mass and growth rate compared to those hatchlings incubated under lower temperatures. There was an inverse linear relationship between the incubation temperature and the time the hatchlings took to right themselves after being placed upside down. Swimming performance trials showed that hatchlings incubated under 32°C had a lower power stroke, reduced maximum stroke force and spent the least amount of time swimming during 10 min trials, compared to the lower thermal groups. The average temperature of *E. macrurus* nests during 2009-2010 was 28.5°C. Current climate predictions for this area are for a 4.1°C rise in ambient temperature by 2070. Study results suggest that the performance of hatchling freshwater turtles may be affected over the coming decades.

How extensively and effectively do kangaroos behaviourally thermoregulate?

**Jessica A. Roberts**<sup>1</sup>, Graeme Coulson<sup>1</sup>, Adam Munn<sup>2</sup>, and Michael Kearney<sup>1</sup> <sup>1</sup>Department of Zoology, University of Melbourne, Melbourne, Victoria 3010, Australia <sup>2</sup>School of Biological Sciences, University of Wollongong, Wollongong, New South Wales 2522, Australia

Temperature regulation and homeostasis are central ideas in both ecological and comparative physiology. However, the extent that endotherms behaviourally thermoregulate in the field has been poorly quantified. Here, we modify a protocol for quantifying thermoregulation that has been wellestablished for ectotherms and apply it to kangaroos. We first parameterize a mechanistic model of heat transfer for kangaroos to investigate the thermal quality of the habitat and how well they minimize energy and water costs in different environments. This model uses physical principles to calculate the energy and water costs of maintaining a constant body temperature in a given environment. We then apply it to examine how these costs change with microclimates selected by kangaroos in the field compared with those available. We compared the predicted costs and benefits of thermoregulating for western grey and red kangaroos in summer and for eastern grey kangaroos in winter. Such a protocol for endotherms will allow us to identify the potential for kangaroos and other endotherms to buffer the effects of future climate warming by altering their behaviour and microclimate selection.

Metabolic theory, life history and the fundamental niche of a terrestrial ectotherm

### **Michael Kearney**

Department of Zoology, The University of Melbourne

Metabolic theory mechanistically captures how organisms take up energy and matter from their environment and allocate it to maintenance, development, growth and reproduction. It provides a potentially powerful framework for understanding life history patterns and geographic range constraints under fluctuating environments and across environmental gradients. However, this power depends strongly on the accurate estimation of body temperature and its effects on biological rates and activity periods. Here I integrate a metabolic theory (the  $\kappa$ -rule Dynamic Energy Budget model, DEB) with a biophysical model for inferring field body temperatures of terrestrial ectotherms (Niche Mapper) and apply it to study life history variation and geographic range limits a widespread North American lizard, Sceloprous undulatus. I use published data to estimate DEB parameters for this species. I then integrate it with the Niche Mapper system and spatial data on long-term climatic averages to predict seasonal and geographic variation in field growth rates as well as age at maturity. annual reproductive output and distribution constraints. This study illustrates the viability and power of applying DEB metabolic theory to mechanistically integrate the dynamics of growth, reproduction and varying food availability into functional traitbased models of the niche.

The influence of climate and altitude on bird nest conductance

### Caragh B. Heenan

The University of Adelaide

The nest microenvironment is a widely studied area of avian biology, however the contribution of nest conductance (the inverse of insulation) to the energetics of the incubating adult has largely been overlooked. While nest conductance scales with parent mass to the power of 0.22, there is variation within a species that may be attributed to the incubation climate and other nest-site specific variables. This study measures the thermal properties and dimensions of 200 nests across 34 species of Australian birds to determine the influence of temperature, precipitation and altitude on nest design. Altitude and mean ambient temperature are positively correlated with nest conductance, however precipitation does not directly influence nest conductance.

### Cold-Acclimation and Thyroid Hormone in Zebrafish

#### Alex Little and Frank Seebacher

School of Biological Sciences, University of Sydney

Energy metabolism is the most fundamental process affecting organismal fitness and is tightly regulated to ensure that cellular energy demand is met. even in times of physiological stress. The molecular machinery responsible for oxidative phosphorylation (OXPHOS) and ATP-production is highly conserved across vertebrates, however there is evidence that the upstream pathways regulating OXPHOS during thermal stress differ between endotherms and ectotherms. While the PGC-1 $\alpha$  axis is the major regulatory pathway of metabolic response in mammals, it does not appear to play the same key role in ectotherms. Because thyroid hormone is known to stimulate oxidative metabolism via alternate pathways, we kept hypothyroid zebrafish at warm and cold conditions (28°C and 18°C, respectively) for three weeks to determine whether it promotes cold-acclimation. While the cold-acclimated controls were found to have a significantly increased capacity for endurance swimming (as measured by uCrit) relative to warm-acclimated controls (p<0.05), this pattern was entirely abolished in the hypothyroid treatment groups. Although hypothyroidism significantly decreased the swimming performance of cold-acclimated fish, it had no significant effect on the swimming performance of warm-acclimated fish, indicating that thyroid hormone plays a specific and essential role in acclimating metabolism to overcome thermal stress.

Using tree-based modelling to examine ecophysiological data

Christopher Johnstone, Richard Reina and Alan Lill Monash University

In physiological ecology researchers often want to compare a single response variable, such as a measure of physiological stress or body condition indices, to multiple explanatory variables (e.g. microhabitat structure, floristic, climate or landscape data). Where large numbers of explanatory variables are measured or where statistical interactions in these variables occur, standard methods such as linear modelling (e.g. ANOVAs or linear mixed effect models), may produce results that are not readily interpretable. Tree-based modelling is a statistical approach used in veterinary and human health science that copes well with complex interactions among explanatory variables, but it is infrequently used in ecophysiology studies. The method allows for predictive analysis of a response variable without limiting the number of explanatory variables that may be examined. Explanatory variables can be non-normally distributed and may be nominal, ordinal, continuous or a combination of these. This talk will focus on a recently developed tree-based method, the conditional inference tree, and discuss its advantages and limitations using actual physiological ecology data from a study of a small carnivorous marsupial, the agile antechinus (Antechinus agilis).

# Assessing physiological responses of animals to aberrant trophic interactions.

#### Tim Jessop

Department of Zoology, University of Melbourne , Vic 3010

The transfer of energy and nutrients among species through food webs is vital to all scales of ecological functionality. However transfer of energy and nutrients between species is increasingly regulated by aberrant trophic interactions (eg. invasive species and human activities). The strength of these novel interactions is expected to profoundly influence fitness attributes of individuals. From a mechanistic perspective, animals could respond to trophic interactions via physiological traits that regulate trade-offs among maintenance, growth and reproduction. Here I explore two examples of how modified trophic processes influence both direct and indirect physiological responses in native animals. The first considers a "stress triangle" where introduced predators could influence trophic interactions, via predation and competition, and induce both direct and indirect physiological responses in native animals. The second example considers the effects of human mediated trophic subsidies on similar physiological traits to assess their costs and benefits to a native predator. Results indicated that both trophic interactions could influence energy acquisition and result in corresponding changes to physiology, via effects on endocrine, haematological, biochemical and immunocompetence parameters. I discuss these results in terms of understanding how trophic processes, via effects on physiology could have broader ecological and evolutionary implications for animals.

# Responding to climate change: flight capacity and behavioural buffering of the common brown butterfly (*Heteronympha merope*).

Madeleine Barton<sup>1</sup>, Melanie Norgate<sup>2</sup>, Paul Sunnucks<sup>2</sup> and Michael Kearney<sup>1</sup>.

- 1. The University of Melbourne
- 2. Monash University

Studies have shown that climate change is impacting on many ecosystems throughout the world, however the extent to which species will be able to track environmental stress remains largely unknown. Understanding the mechanisms through which climate constrains the survival of an organism will allow us to predict how it is likely to respond to future changes in climate. Flight capacity in butterflies is an important fitness component as it's required for feeding, mating and oviposition. Flight, however, is only possible within a specific thermal range, and may consequently be directly affected by changes in climate. Butterflies may be able to behaviourally buffer the impacts of thermal stress to optimise flight capacity by altering basking posture or by selecting specific locations within their habitat. This study focuses on the adult phase of the common brown butterfly (Heteronympha merope). Measurements of its themal limits for flight, and data showing how behavioural thermoregulation affects core body temperature, and flight capacity, will be presented. Incorporating these measurements with spatially explicit data-sets of climate and terrain into process-based models will enable us to predict the available time for flight, and general fitness of the common brown under current and future climate scenarios.

#### Graphic Nutrition: A tale of sex, cannibalism, aging and obesity

### Stephen J. Simpson<sup>1</sup> and David Raubenheimer<sup>2</sup>

<sup>1</sup>School of Biological Sciences, The University of Sydney NSW 2006; <sup>2</sup>Institute of Natural Sciences, Massey University, Albany, New Zealand.

Nutrition touches all aspects of biology – indeed the fundamental, interlinked triumvirate in biology is sex, death and nutrition. But nutrition is complex. Animals require numerous nutrients in particular amounts and ratios to maximise fitness. Nutrients come packaged in various ratios and concentrations in foods, which are scattered throughout the environment in time and space and may contain toxins and other non-nutrient compounds. The animal must match its multidimensional, changing nutritional requirements while minimising the costs of locating, ingesting and processing appropriate foods. We have developed a set of state-space models called the Geometric Framework (GF) to capture the multidimensional nature of nutritional requirements, the relative values of foods in relation to these requirements, the behavioural and post-ingestive responses of animals when feeding on diets of varying composition, and the growth and performance consequences of being restricted to particular dietary regimes. We have also derived the necessary theory for defining fitness in relation to nutrient intake. for describing key nutritional traits and assessing trade-offs between lifehistory responses. I will begin by introducing the models and then show how they have been used to address problems in life-history theory, immunity, human health, collective nutrition and community ecology. Along the way I will use examples spanning slime moulds to humans.

Life on the edge: digestive capacity in nectarivorous birds

### **Todd J. McWhorter**<sup>1</sup>, Patricia A. Fleming<sup>2</sup>

<sup>1</sup>School of Animal & Veterinary Sciences, University of Adelaide, Roseworthy Campus, SA 5371 AUSTRALIA. <sup>2</sup>School of Veterinary & Biomedical Sciences, Murdoch University, Murdoch, WA 6150 AUSTRALIA.

Nectar-feeding birds respond to variation in food quality by modifying their intake. Modelling of digestive function provides a tool for exploring how physiological limitations to energy assimilation (digestion, nutrient absorption, osmoregulation) might shape feeding behaviour, and for predicting digestive capacity. Simple measurements of digestive enzymes and gut morphometrics can be scaled up to predict performance by the animal using chemical reactor models of out function, and the predictions tested by challenging animals to increase their food intake in response to cold exposure or reduced feeding time. We compare digestive capacity amongst members of the three major evolutionary radiations of nectar-feeding birds: the Neotropical hummingbirds, African sunbirds, and Australasian honeyeaters. Feeding challenges suggest that capacities to digest disaccharides and absorb hexose sugars are closely matched in nectar-feeding birds. Our results further suggest that passerine nectar specialists may have greater digestive spare capacity than hummingbirds: when cold challenged acutely hummingbirds are generally unable to increase intake and lose body mass quickly, whereas honeyeaters and sunbirds increase food intake and maintain energy balance. When pushed beyond their physiological capacities to assimilate energy, hummingbirds use torpor (energy-saving nocturnal hypothermia) to attempt to balance their energy budgets, while passerines reduce activity during the day.

Digestive ability of four sympatric land crab species: *Geacrcoidea natalis, Discoplax hirtipes, Birgus latro* and *Coenobita perlatus*.

**Stuart M. Linton**<sup>1</sup>, Reinhard Saborowski<sup>2</sup> and Alicia J. Shirley<sup>1</sup> <sup>1</sup>School of Life and Environmental Sciences, Deakin University, Pigdons Road, Waurn Ponds <sup>2</sup>Alfred Wegener Institute, Am Handelshafen 12 D-27570, Bremerhaven Germany

Land crabs are catholic feeders that consume a variety of items such as leaf litter, seeds, fruits and animal material. However different species display distinct preferences for certain dietary items. The gecarcinid land crabs Gecarcoidea natalis and Discoplax hirtipes are detritivores which consume mainly leaf litter. The robber crab, Birgus latro is an omnivore with a preference for items which are high in lipid, carbohydrate and/ or protein. Its close relative, the hermit crab, *Coenobita perlatus* consumes a similar diet except that it contains lower nutrient items such as plant detritus. The activity of digestive enzymes was measured to determine if the digestive ability of G. natalis, D. hirtipes, B. latro and C. perlatus could be correlated to their dietary preferences. All species possessed enzymes involved in the digestion of plant cellulose (endo- $\beta$ -1,4-glucanase and  $\beta$ -glucohydrolase) and hemicellulose (lichenase and laminarinase), hydrolysable tannins (esterase), protein (total proteinase, endo and exo peptidase), lipid (lipase), chitin and starch/ glycogen (amylase). These activities explain the ability of these species to digest a wide range of dietary items. However enzyme activity could not be correlated to dietary preference. Perhaps others factors such as olfactory and locomotor ability and metabolic status may dictate the observed dietary preferences.

Some like it hot: Hibernatiom patterns of the greater mouse-tailed bat (*Rhinopoma microphyllum*).

**Eran Levin**, Amos Ar, Abrham Hefetz, Yoram Yom-Tov and Noga Kronfeld-Schor

Deparment of zoology, Tel-Aviv University, Israel

Many endotherms, including bats (Chiroptera), counter the energetic challenge of seasonal food shortage and low ambient temperature by performing long torpor bouts, or hibernation. Hibernation is traditionally attributed to high latitudes and cold climates and rarely been documented in the tropic or subtropics.

The greater mouse-tailed bat (*Rhinopoma microphyllum*) is a medium-sized subtropical bat (average weight 25g). We found that during winter in Israel, Mouse tailed bats move to warm caves (20°C) from which they do not emerge till spring (5 month). We measured bats metabolic rates using an open flow respirometry system, and analysed the composition of their body, and their pre-hibernation diet lipids. We found that during hibernation, mouse tailed bats demonstrated long periods of apnea (up to 25 minutes, in which they shut their nostrils with special valves) followed by a series of fast breaths. The fatty acid composition of these bats found to be extremely saturated. We suggest that their unique pre-hibernation diet (90% queen ants from the genus *Camponotus*) explains the high saturation level of their body fat, and provide an adaptation for hibernation in relatively high ambient temperatures.

Uterine angiogenesis and cell surface changes during pregnancy in a viviparous lizard, *Niveoscincus conventryi* 

Martha Patricia Ramírez-Pinilla<sup>1</sup>, Scott L. Parker<sup>2,4</sup>, Christopher R. Murphy<sup>3</sup>, and **Michael B. Thompson<sup>2</sup>** 

<sup>1</sup>Laboratorio de Biología Reproductiva de Vertebrados, Grupo de Estudios en Biodiversidad, Escuela de Biología, Universidad Industrial de Santander, Bucaramanga, Colombia; <sup>2</sup>School of Biological Sciences (A08), University of Sydney, NSW 2006, Australia; <sup>3</sup>School of Medical Sciences (Anatomy and Histology) and Bosch Institute, University of Sydney, NSW 2006, Australia; <sup>4</sup>current address: Department of Biology, Coastal Carolina University, SC 29528-6054, USA

During pregnancy in lizards, the vascular bed of the uterus responds to changes in the developing embryos by developing differently in the chorioallantoic and omphaloplacentae. Uterine angiogenesis during pregnancy has been postulated to be influenced by progesterone. We studied uterine angiogenesis and epithelial cell differentiation during pregnancy in the viviparous lizard, Niveoscincus coventryi, and attempted to determine the influence of progesterone by applying the progesterone receptor antagonist, mifepristone (RU486), at different stages of pregnancy. A vessel dense area develops at the embryonic pole of the eggs chamber and grows laterally to encompass 70% of the eggs surface late in pregnancy. The abembryonic hemisphere of the egg has a less dense network of vessels. An increase in vascular density occurs by intussuceptive angiogenesis and branching, with an increase in vessel length and diameter as pregnancy proceeds. The uterine surface differentiates differently in the chorioallantoic and ompaloplacental regions, with blood vessels forming luminal folds on the uterine surface in the embryonic hemisphere and a smooth uterine surface at the abembryonic pole. Ciliated cells occur throughout the uterine surface, except at the centre of the abembryonic pole. Treatment with mifipristone had no effect on development on the vasculature or uterine epithelium.

### The role of leptin in the annual cycle of the echidna

Jenny Sprent<sup>1</sup>, Sue Jones<sup>1</sup>, Rob Gasperini<sup>2</sup> and **Stewart Nicol<sup>1</sup>** <sup>1</sup>School of Zoology, University of Tasmania, <sup>2</sup>Menzies Research Institute, University of Tasmania

Leptin, a peptide hormone secreted by fat cells, is best known for its role as an adiposity signal but leptin has diverse physiological roles ranging from regulation of feeding behavior and body weight, to effects on reproduction and immune function. Leptin has been identified in all vertebrate classes, and in eutherian mammals there is a close relationship between plasma leptin and adiposity, and hibernating eutherian mammals down-regulate leptin receptors to allow seasonal fattening. The role of leptin in marsupials has received little attention but, consistent with its effect in eutherian mammals, leptin treatment of *Sminthopsis macroura* increased energy expenditure by inhibiting daily torpor (Geiser et al. 1998). The echidna is a monotreme mammal that hibernates and shows very large seasonal variations in body mass ( $\pm$  30%), and we hypothesised that echidna plasma leptin would vary predictably with body mass and season. We measure plasma leptin in free-ranging echidnas using a radioimmunoassay that we validated using Western blotting. Leptin showed a weak negative relationship with body mass, but varied predictably with season. The highest leptin levels were found during hibernation and in females during the reproductive period. The lowest levels were found in post-reproductive males.

# **Damian D'Souza<sup>1</sup>**, Dr Klaus Altland<sup>3</sup>, Dr Leonard Pattenden<sup>1,2</sup>, Dr Samantha Richardson<sup>1,2</sup>

<sup>1</sup>School of Medical Sciences, RMIT University, Bundoora 3083, Australia. <sup>2</sup>Health Innovations Research institute, RMIT University, Bundoora 3083, Australia. <sup>3</sup>Department of Human Genetics, University of Giessen, Giessen, Germany.

Transthyretin (TTR) is a homo-tetrameric protein that transports retinolbinding protein and thyroid hormones. Preliminary data has put forward an Australian story showing wild-type human TTR forms insoluble amyloid fibrils under mild acidic conditions similar to those found in vivo, however wallaby TTR under the same conditions remains stable. Each human TTR monomer contains His31, Ser46, and His90, close to the dimer interface where they help stabilise the complex. Wallaby TTR has different amino acid residues in these key positions; Lys31, Ala46, Tyr90. Our work seeks to understand why human TTR is unstable compared to wallaby TTR. We hypothesise that protonation of key Histidine residues in human TTR, absent in wallaby TTR, results in destabilising TTR facilitating amyloid formation. We produced human, wallaby, and seven cross-species mutant TTRs to determine differential stability and fibril formation rates under mild acidic conditions. Molecular modelling has shown significant structural changes between the wild-type and variant TTRs, providing good initial correlations for the stability and fibril formation assays. Currently TTR amyloidosis has only been described in humans and has never been reported in other animals. The application of evolutionary comparative biology to produce cross-species TTRs provides a great opportunity to study structure/function relationships and understand the molecular mechanisms underlying disease states.

Nitric oxide regulation of blood vessels in amphibians

**Melissa S. Cameron**, Sofie Trajanovska and John A. Donald School of Life and Environmental Sciences, Deakin University, Geelong, Australia, 3217

Nitric oxide (NO) is generated by NO synthase (NOS) of which there are three isoforms: neuronal NOS (NOS1), inducible NOS, and endothelial NOS (NOS3). Previous findings in mammals have demonstrated the presence of an endothelial NO signalling system, which mediates vasodilation. The presence of NOS3 and endothelial NO signalling in lower vertebrates is controversial with studies in amphibians demonstrating the absence of an endothelial NO system, with NO released from NOS1 in nitrergic nerves mediating vasodilation. Recently, in the amphibian, Xenopus tropicalis, NOS3 was cloned and found in a range of tissues but not in vascular endothelium. This study aimed to sequence NOS3 in the amphibian Bufo marinus. The open reading frame of *B. marinus* NOS3 encoded an 1170 amino acid protein that showed 81% sequence identity to X. tropicalis NOS3. Toad NOS3 mRNA expression was demonstrated in brain, kidney, bladder, heart, and in blood vessels with and without endothelium. Immunohistochemistry using an XtNOS3 antibody showed NOS3 immunoreactivity in the kidney, but not in the endothelium of blood vessels. The expression of NOS3 in the non-endothelial cells of blood vessels raises the possibility that it is involved in vascular regulation, which will be investigated in future research.

Testing the maternal immune imprinting hypothesis using direct manipulation of yolk antibodies.

**BriAnne Addison<sup>1</sup>**, Robert Ricklefs<sup>2</sup>, Kirk Klasing<sup>3</sup> 1 Life and Environmental Science, Deakin University, Geelong, VIC 3217 2 Biology Department, University of Missouri - St Louis, St Louis, MO, 63130, USA 3 Department of Animal Science, University of California Davis, Davis CA, 95616, USA

Immunological imprinting by maternally derived antibodies has been proposed to have both positive and negative consequences for offspring immunity in early and adult life. Using laboratory Japanese quail, we developed a novel method of directly manipulating yolk antibodies of neonates, and then followed individuals through a series of immune challenges until they were of reproductive age. Our method of directly injecting purified antibodies into the yolk sac of newly hatched chicks successfully elevated the plasma titres of specific anti-KLH IgY in neonates, to levels comparable to offspring of vaccinated hens. This allows us to test whether differences in neonatal anti-KLH IgY affect immunity at the juvenile and adult stages of life, and whether vaccination has effects on immunity independent of the effects of antibodies. We found little evidence for an effect of maternal antibodies on juvenile stage immune response, in contrast to results from previous studies. Adult immune response was also independent of neonatal IgY titres, and surprisingly only weakly predicted by previous antigen exposure. We found no evidence of carryover effects of yolk-derived antibodies on adult immunity. Our study employs new methodology for investigation of maternal antibodies.

Urinary corticosterone responses to capture and captivity in the cane toad (*Rhinella marina*, previously *Bufo marinus*)

**Edward J. Narayan<sup>a\*</sup>**, Jean-Marc. Hero<sup>a</sup>, John F. Cockrem<sup>b</sup>, Jane E. Candy<sup>b</sup> <sup>a</sup> Environmental Futures Centre, School of Environment, Griffith University, Gold Coast campus, QLD 4222, Australia <sup>b</sup> Institute of Veterinary, Animal and Biomedical Sciences, Massey University, Palmerston North, New Zealand \* *Corresponding author E-mail address*: <u>edward\_nryn@yahoo.com</u>. (E. J. Narayan)

Corticosterone is a glucocorticoid hormone secreted from the adrenal gland, and plasma corticosterone concentrations increase when animals experience a stressor. The characterisation of plasma corticosterone responses to stressors requires the collection of repeated blood samples from individual animals. This is difficult in amphibians, so we have recently developed a new method to measure corticosterone in urine. We applied this method to measure urinary corticosterone responses to capture and to confinement in captivity in adult cane toads (Rhinella marina) in Queensland. An adrenocorticotropic hormone challenge test showed that urinary corticosterone increased at 1-2 days after injection and then returned to initial values, indicating that the urinary corticosterone metabolite radioimmunoassay could detect changes in circulating corticosterone in toads. Wild toads had urinary corticosterone responses to capture and restraint in plastic bags over 8 hours, with urinary corticosterone increases first apparent 2 hours after capture. Urinary corticosterone metabolites were significantly elevated in toads five days after capture and transfer into communal housing chambers in captivity. Corticosterone concentrations declined to initial capture levels after a further seven days in captivity in individual housing chambers. The study shows the value of urinary corticosterone measurements for the assessment of physiological responses to stressors in amphibians.

Pregnancy makes breathing expensive in lizards

### **Suzanne Munns**

School of Veterinary and Biomedical Sciences, James Cook University, Townsville, QLD

*Tiliqua rugosa* is a large viviparous skink that gives birth to 1-4 young after 4-5 months gestation. The young are extremely large at birth and represent 20-40% of maternal body mass. The large embryos have the potential to compromise the function of numerous organ systems, particularly those susceptible to compressive forces such as the respiratory system. To affect pulmonary ventilation the respiratory muscles must overcome the elastic and non-elastic forces associated with expanding the lungs and chest. The energetic cost of pulmonary ventilation in this species may be relatively high due to their stiff body wall and may increase during pregnancy due to lung compression. In this study, the energetic cost of ventilation was estimated using hypercapnic gas mixtures to stimulate ventilation in sedentary pregnant (n=5) and non-pregnant lizards (n=8). The energetic cost of ventilation in pregnant lizards increased 3 fold during late gestation and remained elevated in the first week post partum. Computed Tomography analysis reveals a significant reduction in total lung volume during pregnancy due to the developing embryos. The results of this study demonstrate that T. rugosa is able to maintain minute ventilation and the rate of oxygen consumption during pregnancy but does so at significant energetic cost.

Reptile metabolic rate is correlated with altitude, but not mean environmental temperature or annual precipitation.

### Taryn S. Crispin and Craig R. White

School of Biological Sciences, The University of Queensland.

### Abstract (200 word limit, Arial 12 pt):

The rate at which animals transform ingested material into useable energy (metabolic rate) scales allometrically with body mass and body temperature. There remains, however, residual variation in metabolic rate that is influenced to varying degrees by a range of other intrinsic (biological) and extrinsic (environmental) factors. This presentation will examine whether a few specific extrinsic factors, measurement body temperature, mean annual temperature, altitude and annual precipitation, have a significant influence on reptile resting (RMR) and maximum (MMR) metabolic rates. By examining the correlations of reptile metabolic rate with these environmental parameters, we show that reptile RMR and MMR are positively correlated with altitude and measurement temperature, but not mean annual environmental temperature or annual precipitation.

Non-invasive partitioning of cutaneous and respiratory evaporative water loss in small mammals.

### Philip C. Withers<sup>1,2</sup> and Christine E. Cooper<sup>1,2</sup>

<sup>1</sup>School of Animal Biology M092, University of Western Australia, Crawley, WA 6009; <sup>2</sup>Department of Environment and Agriculture, Curtin University, Bentley, WA 6845

We investigate two strategies to non-invasively partition total evaporative water loss into its cutaneous and respiratory components; (1) using the slope (respiratory component) and intercept (cutaneous component) for the relationship between total water loss and metabolic rate, and (2) an iterative solution of a simple model for expired air and skin temperature to compare actual total loss with calculated cutaneous and respiratory components. If there is sufficient variation in metabolic rate and water loss (e.g. reflecting sleep state) then strategy 1 can be useful. For numbats, total evaporative water loss at air temperatures between 15 and 25 °C was equally divided between respiratory and cutaneous avenues. For a variety of small marsupials, strategy 2 provided a useful indication of the potential roles of cutaneous and respiratory avenues for water loss below thermoneutrality, again with approximately equal partitioning. Both strategies were ineffective above thermoneutrality. We conclude that it is possible to non-invasively partition total water loss for some species under some circumstances. More refined approaches might provide further discrimination power for better partitioning.

The Role of Thermoregulation in Energetics and Water Economy: A case study of Western Australian dunnarts

### Sean Tomlinson, Phillip Withers and Shane Maloney

School of Animal Biology, University of Western Australia

Thermoregulation involves a balance between heat lost *via* evaporative water and the heat generated by metabolic activity. Thermoregulatory responses of different species may be different depending upon their climatic origin and the breadth their geographic distributions. The thermoregulatory patterns of six Stripe-faced Dunnarts (Sminthopsis macroura) and eight Ooldea Dunnarts (S. ooldea) were measured between acute T<sub>a</sub>s of 10, to 35 °C. Sminthopsis macroura showed strong propensity to maintain T<sub>b</sub> across the T<sub>a</sub> gradient, while S. ooldea was more thermolabile. The metabolic rate of both S. macroura and S. ooldea decreased from  $T_a = 10$  °C to 30 °C, with comparable BMRs; there was no common regression of the metabolic rate below the TNZ. Differences in their energetic responses to low T<sub>a</sub>, probably relate to different thermoregulatory responses. A strong propensity to thermoregulate may allow S. macroura to exploit a broad range of climatic envelopes, albeit at the cost of a higher energetic and water requirement. Since S. ooldea does not expend as much energy on thermoregulation it is better adapted to the very low productivity, "hyperarid" conditions of its Centralian distribution. This suggests that it has physiological adaptations analogous to its most highly derived, arid-adapted morphology of any Sminthopsis.
Effects of experiment start time and duration on measurement of metabolic rate, evaporative water loss and body temperature

Amanda J. Page<sup>1</sup>, **Christine E. Cooper<sup>1,2</sup>** and Philip C. Withers<sup>1,2</sup> <sup>1</sup>Environment and Agriculture, Curtin University, <sup>2</sup>Animal Biology, University of Western Australia

Duration and start time of respirometry experiments have significant effects on the measurement of basal values for several commonly measured physiological variables (metabolic rate, evaporative water loss, and body temperature) in budgerigars (Melopsittacus undulatus). Α longer measurement duration reduced values for all variables for all start times, and this was an effect of actual animal activity rather than random sampling. Experiment start time had a significant effect on time taken to reach minimal values for all physiological variables, ranging from 4:00 h ± 38 min (body temperature, start time 23:00 h) to 8:54 h  $\pm$  52 min (evaporative water loss, start time 17:00 h). It also influenced the time of day that minimal values were obtained, ranging from 22:24 h  $\pm$  40 min (CO<sub>2</sub> production, start time 15:00 h) to 06:00 h  $\pm$  57 min (O<sub>2</sub> consumption, start time 23:00 h), and the minimum values measured. Consequently both measurement duration and experiment start time should be considered in experimental design. We suggest that experiments to measure standard physiological variables for small diurnal birds should commence between 17:00 h and 21:00 h, and measurement duration should be at least 9 h.

Can we measure respiratory heat loss in a free ranging animal?

### K. Vesterdorf<sup>1</sup>, D. Blache<sup>2</sup> and **S. K. Maloney<sup>1</sup>**

<sup>1</sup>Physiology M311, School of Biomedical, Biomolecular, and Chemical Science, and <sup>2</sup>School of Animal Biology M085, Faculty of Natural & Agricultural Sciences, The University of Western Australia, 35 Stirling Highway, Crawley WA 6009, Australia.

Because respiratory evaporative heat loss (REHL) uses body water it is physiologically and ecologically relevant to know under what conditions freeranging animals use panting. We investigated whether the cranial arteriovenous temperature difference could provide information about REHL. We exposed sheep to environments varying in ambient dry bulb temperatures (Env 1: ~15°C, Env 2: ~25°C, Env 3: ~40°C, Env 4: ~40°C + infra red radiation) and measured REHL simultaneously with carotid arterial ( $T_{car}$ ) and jugular venous ( $T_{jug}$ ) blood temperatures, as well as brain ( $T_{brain}$ ) and rectal  $(T_{rec})$  temperatures. REHL increased with ambient temperature. The difference between cranial arterial and venous blood temperatures ( $T_{a-v} = T_{car}$  $-T_{iug}$ ) increased from Env 1 to 2 and from Env 3 to 4.  $T_{a-v}$  reached a maximum of 0.7±0.2°C at Env 4 and was positively correlated with REHL across environments. The increase in REHL maintained homeothermy when dry heat loss decreased. While REHL could increase without generating an increase in  $T_{a-v}$ , any increase in  $T_{a-v}$  was always associated with an increase in REHL. We conclude that the cranial  $T_{a-v}$  provides useful information about REHL in panting animals.

Using Dual-energy X-ray absorptiometry (DXA) to accurately and nondestructively measure the body composition of small, free-living rodents.

Ian Gerard van Tets and Kalb Thayer Stevenson Department of Biological Sciences, University of Alaska Anchorage, 3211 Providence Drive, Anchorage, Alaska 99508, USA.

Dual-energy x-ray absorptiometry (DXA) is a nondestructive technique that can potentially measure specific components of whole-body composition in free-living and lab-raised animals. Our aim was to test the ability of DXA to measure the composition of a common arvicoline rodent, the northern redbacked vole (Clethrionomys rutilus). We used a DXA apparatus to obtain measurements of fat mass (FM), lean mass (LM), bone mineral content, bone mineral density, and fat-free mass(FFM) in carcasses of free-living and labraised voles. We then used chemical carcass analysis to derive predictive algorithms for actual values of FM, total body water, total protein, total mineral, LM, and FFM. Unexplained error in the equations for all voles arouped collectively ranged from R(2) = 0.82 to R(2) = 0.98. The DXA FM measurement had the highest coefficient of variation, and it was higher for free-living voles than for lab-raised voles. However, FM can be determined by difference with excellent precision by using the FFM equation (R(2) = 0.98). We also derived corrective terms for passive integrated transponder-tagged animals. Thus, DXA is a nonlethal, nondestructive tool capable of precisely and accurately measuring many specific parameters of whole-body composition in small free-living and lab-raised rodents.

Oxygen-dependence of metabolic rate in the muscles of craniates

### Leonard G. Forgan<sup>1</sup>\* and Malcolm E. Forster<sup>2</sup>

<sup>1</sup>New Zealand institute for Plant & Food Research Limited, P.O. Box 5114, Port Nelson, Nelson 7043, New Zealand <sup>2</sup>School of Biological Sciences, University of Canterbury, Private Bag 4800, Christchurch 8140, New Zealand

Oxyconformance of metabolic rate is generally thought to be confined to those animals capable of entering torpor. However, we present evidence that oxygen-dependence of metabolic rate by striated muscles is a conserved response in representatives of three craniate taxa: two teleost fishes, a hagfish and a rat. Oxygen consumption  $(VO_2)$  by striated muscle slices from all species related linearly to oxygen partial pressure (PO<sub>2</sub>) between 0 and 125 mmHg, despite VO<sub>2</sub> varying greatly between species and muscle types. Lactate concentrations fell in slices incubated at both 30 and 100 mmHg, suggesting aerobic rather than anaerobic metabolism. Consistent with this finding, potential energy - a proxy of ATP turnover - was PO<sub>2</sub>-dependent. Loss of tissue viability and diffusion limitation of the preparation were ruled out, as subsequent reoxygenation of the Ringer bathing slices following the initial PO<sub>2</sub> depletion resulted in unchanged VO<sub>2</sub>. Furthermore, VO<sub>2</sub> increased in all muscles when chemically uncoupled and mitochondrial succinate dehydrogenase activity was unchanged over the course of the experiments. Our data suggest that the observed reduction in VO<sub>2</sub> with falling PO<sub>2</sub> results in a decrease in ATP demand, suggesting that the hypoxic signal is "sensed" and cellular changes effected.

## Key words:

Muscle, Hypoxia, Oxygen consumption, Craniate, ATP

Effects of rested harvesting on muscle metabolite concentrations in Chinook salmon (Oncorhynchus tshawytscha)

**Nicholas Tuckey<sup>1</sup>**, Malcolm Forster<sup>2</sup>, Steven Gieseg<sup>2</sup> <sup>1</sup> The New Zealand Institute for Plant & Food Research Limited, Box 5114, Port Nelson, Nelson 7043, New Zealand <sup>2</sup> University of Canterbury, Private Bag 4800, Christchurch, New Zealand

Harvesting procedures for cultured fish are known to affect the quality and storage properties of the resulting fillets. This is due to the depletion of intracellular energy stores and the accumulation of metabolic waste products. The measurement of ATP-related compounds provides an effective indicator of the metabolic state and quality of tissue postharvest. The breakdown of these compounds is also associated with the production of ammonia. superoxide and hydrogen peroxide; the latter two are known to cause lipid peroxidation. Fillets from fresh rested Chinook salmon (anaesthetized with AQUI-S<sup>™</sup> without stress or exhaustion) had significantly higher concentrations of ATP and creatine phosphate than those from exhausted salmon. These concentrations declined in fillets from rested salmon after 12 h in storage at 15°C while in fillets from exhausted salmon they were depleted following harvest. The concentration of the taste-enhancing compound inosine monophosphate was significantly reduced in fresh rested tissue, but increased during storage, and was significantly higher following 12 h in storage. The accumulation of uric acid was also significantly reduced in rested tissue and increases in the k-value were slowed. Results indicate that rested harvesting has the potential to improve the guality and shelf life of cultured Chinook salmon.

The low developmental cost of the Australian lungfish compared to other fishes and amphibians

**Casey A. Mueller<sup>1</sup>**, Jean M.P. Joss<sup>2</sup>, Roger S. Seymour<sup>1</sup> <sup>1</sup>Ecology and Evolutionary Biology, University of Adelaide, Adelaide, SA, Australia <sup>2</sup>Biological Sciences, Macquarie University, Sydney, NSW, Australia

The rate of oxygen consumption throughout embryonic development is used to indirectly determine the 'cost' of development. In fish and amphibian embryos this cost is affected by temperature and the duration of incubation. The influences of temperature on embryonic development rate, respiration rate and energetics were investigated in the Australian lungfish. *Neoceratodus* forsteri, and compared with published data. Developmental stage and oxygen consumption rate were measured until hatching, upon which dry gut-free masses were determined. A measure of the cost of development, the total oxygen required to produce 1 mg of embryonic dry tissue, increased as temperature decreased. The relationship between the oxygen cost of development (C, ml mg<sup>-1</sup>) and dry hatchling mass (M, mg) in fishes and amphibians is described by C =  $0.30M^{0.22 \pm 0.13} (95\% \text{ CI})$ , r<sup>2</sup> = 0.52. At 15 and 20 °C, *N. forsteri* cost of development is significantly lower than the regression mean for all species, and at 25 °C is lower than the allometrically scaled data set. Unexpectedly, incubation of *N. forsteri* is long, despite development under relatively warm conditions, and may be related to a large genome size. The low cost of development may be associated with construction of a rather sluggish fish with a low capacity for aerobic metabolism. The metabolic rate is lower in *N. forsteri* hatchlings than in any other fishes or amphibians at the same temperature, which matches the extremely low aerobic metabolic scope of the juveniles.

The physical gill of diving-bell spiders, Argyroneta aquatica

**Roger S. Seymour**<sup>1</sup> and Stefan K. Hetz<sup>2</sup> <sup>1</sup>University of Adelaide and <sup>2</sup>*Humboldt-Universität zu Berlin* 

The diving bell spider Argyroneta aquatica is a unique spider that lives its entire life under water. It creates a hydrophobic hemisphere of web and fills it with air carried from the surface. We studied the gas exchange properties of this diving bell to assess its ability to take up dissolved  $O_2$  from the water thereby acting as a 'physical gill'. Without the spider, O<sub>2</sub> conductance of the bell was calculated from the rate of change of Po<sub>2</sub> difference across the wall, measured with optodes, and chamber volume, measured by dilution of injected gas. When the spider entered the bell, its respiration rate could be calculated from the rate of  $Po_2$  change and  $O_2$  conductance. These rates were confirmed by direct, flow-through respirometry. Under representative conditions, O<sub>2</sub> uptake through the diving bell wall can account for 70% of the requirements of the occupant. Most bells in these experiments limited the Po<sub>2</sub> gradient across the wall to less than 10 kPa, and spiders voluntarily endured internal Po<sub>2</sub> about 2 - 3 kPa before renewal of air from the surface. Renewal is ultimately necessary, due to  $N_2$  loss from the bell, but spiders can easily remain in the bell for more than 24 h without renewal.

Buoyancy regulation and the effect of selected exotic gases on the compressible gas gill of notonectids and corixids (Hemiptera).

#### Imogen Munro

#### University of Adelaide

Compressible gas gills are a simple adaptation used by some secondarily aquatic insects, such as Hemipteran families Corixidae (waterboatman) and Notonecteids (backswimmers), to respire underwater. The addition of an air bubble to the insect's abdomen, covering the spiracles, delivers oxygen to the respiratory system while removing carbon dioxide. Corixids rely on gas gills to extend dive time beyond that provided by the initial oxygen content of the bubble by the development of a partial pressure gradient extracting oxygen from the surrounding water. Notonectid subfamily Anisopinae uses the air bubble primarily for buoyancy regulation. By relying on additional oxygen, stored in haemoglobin cells in their abdomen to stabilize the air store's volume, Anisopinae can remain in near neutral buoyancy with ambient water. Investigation of the buoyancy change in free dives of Anisopinae, found that the stabilisation of buoyancy occurs significantly below equilibrium with ambient water. Thus, Anisopinae are not achieving neutral buoyancy in the water column; rather, very gradually sinking. Substitution of nitrogen within gas stores of corixids and notonectids for other metabolically inert gases, sulphur hexafluoride and helium, saw alteration in dive time as a result of changes in Krogh's coefficient of diffusion of the bubble. Sulphur hexafluoride increases dive times compared to atmospheric air, whereas helium decreases dive times.

The role of biogenic amines in regulating bioluminescence in the Australian glowworm, *Arachnocampa flava*.

#### Lisa M. Rigby and David J. Merritt

School of Biological Sciences, The University of Queensland, Brisbane, Australia

Glowworms are the bioluminescent larvae of a fly in the genus Arachnocampa. Neuroactive biogenic amines have been implicated in the initiation and regulation of bioluminescence in other arthropods, however no information is available on the role of these neuroactive compounds within Arachnocampa. Immunohistochemistry revealed that the cells of the light organ are innervated by nerves projecting from the terminal abdominal ganglion suggesting that bioluminescence could potentially be regulated directly by a neurotransmitter. The effects of octopamine, phentolamine, serotonin and several other biogenic amines on light output by Arachnocampa *flava* were determined by administering the amines orally, by injection and by bathing the light organ in biogenic amine solutions. Treatment with highconcentration octopamine caused larvae to emit bursts of bioluminescence at levels significantly greater than controls. The octopamine antagonist, phentolamine, produced high levels of bioluminescence when orally administered through feeding on Drosophila adults injected with phentolamine solution, however, when the light organ was bathed in a solution of phentolamine, a reduction in bioluminescence was recorded. Treatment with serotonin caused no significant alteration in light output, ruling out a role in the regulation of bioluminescence. The results suggest that the octopaminergic system plays a role in the regulation of bioluminescence in Arachnocampa.

Thinking inside the box: toward an understanding and estimation of the thermal consequences of nest box design

**Natalie J. Briscoe<sup>1</sup>**, Michael R. Kearney<sup>1</sup>, Warren P. Porter<sup>2</sup> <sup>1</sup>Department of Zoology, The University of Melbourne, Melbourne, Australia, 3010 <sup>2</sup> Department of Zoology, The University of Wisconsin, Madison WI 53706, USA

Nestboxes are widely used as a conservation tool, providing shelter for species in the absence of natural tree hollows. Both hollows and nestboxes have traditionally been viewed as providing protection against predators and climate extremes. For endotherms, appropriately designed nest boxes should minimize the energy and water costs of thermoregulation. However, in these species heat produced by the organism inhabiting the box may strongly influence its thermal environment. We monitored temperatures within occupied and unoccupied nestboxes in Melbourne during winter and spring, and found that temperatures within nestboxes containing common brushtail possums, *Trichosurus vulpecula*, were between 1.5-2.5°C warmer than temperatures within empty nestboxes. We illustrate how heated model possums and biophysical models (NicheMapper<sup>TM</sup>) can be used to assess how properties of nestboxes influence the thermal environment of animals inhabiting them, providing novel insights into nest box design and installation.

Membrane phospholipid peroxidation: effect of fatty acid pairing and head group

Sarah E Norris<sup>1,2</sup>, Todd W Mitchell<sup>1,2</sup> and Paul L Else<sup>1,2</sup> Metabolic Research Centre within the Illawarra Health & Medical Research Institute<sup>1</sup>, School of Health Sciences, University of Wollongong NSW 2522<sup>2</sup>

Phospholipids (PLs) are the major lipids associated with membranes. Phosphatidylcholine (PC) and phosphatidylethanolamine (PE) constitute about 90% of PLs. Natural fatty acids (FAs) occur as pairs on PLs, and usually consist of a saturated (SAT) with either a monounsaturated (MUFA) or a polyunsaturated fatty acid (PUFA), or MUFA with PUFA pairings; but virtually never two PUFAs together on the one molecule. We hypothesised that this may be due to a double PUFAs possessing a much higher capacity to undergo peroxidation (lose an electron and generation lipid radicals in a cascade type reaction) than SATs or MUFAs. Two PUFAs paired together would potentiate peroxidative damage by reducing diffusion distances. whereas the coupling of a PUFA to a SAT or a MUFA would interfere with radical generated (i.e. acting as a peroxidative 'fire-blanket'). Testing this hypothesis involved peroxidising lipids with the same concentration of peroxidisable FAs present but either as 16:0/18:2 or as 18:2/18:2 pairings for both PC and PE (to also examine the effect of head group) using ferrous iron/hydrogen peroxide as the initiator. No significant differences were found between PC 16:0/18:2, PC18:2/18:2 and PE16:0/18:2. Surprisingly, PE18:2/18:2 was found to produce less peroxidation product than the other lipids. This is interesting since in natural membranes such as mammalian kidney and brain, PE has 2-6x the PUFA content compared to PC respectively.

The balance of n-3 and n-6 polyunsaturated fatty acids in the diet strongly influences rat membrane fat profile and minimum metabolic rate

**Sarah K. Abbott**<sup>1</sup>, Paul L. Else<sup>2</sup>, A.J. Hulbert<sup>1</sup> <sup>1</sup>School of Biological Sciences, University of Wollongong, Wollongong, NSW, Australia <sup>2</sup>School of Health Sciences, University of Wollongong, Wollongong, NSW, Australia

This study quantifies the relationship between diet fatty acid (FA) profile and membrane composition of various rat tissues. Membrane composition was then related to minimum metabolic rate (MMR). Rats were fed one of 12 diets differing only in lipid profile. Diet saturates (SFA) ranged from 8-88%, monounsaturates (MUFA) 6-65%, polyunsaturates (PUFA) 4-81% with n-6 PUFA 3-70% and n-3 PUFA 1-70%. PUFA Balance (= n-3 PUFA as % of total PUFA) ranged from 1-86%. Diet n-6 and n-3 PUFA were 18:2n-6 and 18:3n-3 respectively. FA composition of skeletal muscle, heart, brain, liver, erythrocyte and adipose tissue phospholipids were determined. Daily metabolic rate (DMR), food intake and body mass composition were also determined. Rats with increased mass specific DMR had reduced food intake, body mass gain and % body fat content. Mass specific MMR showed strong positive correlations with diet n-3 PUFA and PUFA balance. Similarly, rat MMR related most closely to membrane PUFA Balance for all tissue types, with little to no response to membrane SFA, MUFA or PUFA composition. This indicates that the balance between membrane n-3 and n-6 PUFA content has the strongest influence on metabolic rate.

#### The long life of birds: The rat-pigeon comparison revisited

**M. Trzcionka**<sup>1</sup>, AJ Hulbert<sup>1</sup> and WA Buttemer<sup>2</sup> 1 Dept. of Biological Sciences, University of Wollongong, Wollongong, NSW, Australia 2 Centre for Integrative Ecology, Deakin University, Waurn Ponds, Victoria, Australia

Birds have on average maximum lifespans which are twice that of similarsized mammals. Rats and pigeons are an extreme example having a 7-fold lifespan difference with rats living only 5 years and pigeons up to 35 years. The nowadays most accepted theory explaining longevity differences between species is the oxidative stress theory of aging, which states that longer-living species should have (1) a lower mitochondrial ROS production, (2) a higher resistance to oxidative damage through a higher concentration of critical antioxidants, (3) membranes that are less susceptible to oxidative damage (according to the modified membrane pacemaker theory), and (4) overall less oxidatively damaged lipids, proteins and nucleic acids. Studies comparing rats and pigeons have been carried out in the past, but focussing only on selected tissues and markers, and with conflicting results. This study is up to date the most complete comparative approach, finding that rats have (1) a higher mitochondrial ROS production, but only in the heart, (2) antioxidant activities which correlate positively with superoxide production and therefore contradict the theory, (3) membranes which are highly susceptible to damage, and (4) overall more lipid, protein and mitochondrial DNA damage in correlation with ROS production and susceptibility to damage. This study shows that the oxidative stress theory of aging has certain validity, but the degree of the observed differences cannot fully explain the 7-fold longevity difference.

Design of the Insect Respiratory System

#### **Edward Snelling**

University of Adelaide

Taylor and Weibel's hypothesis of *Symmorphosis* predicts that structures of the oxygen cascade are matched to maximum functional requirements with minimal excess capacity. We tested this hypothesis in the respiratory system of migratory locust by measuring maximum aerobic respiration rate of the jumping muscles during hopping and the morphology of the oxygen cascade in the hopping legs. An intra-specific allometric analysis was performed on locusts of different body mass ( $M_b$ ) at selected lifecycle stages. Maximum aerobic metabolic rate of the jumping muscles during hopping muscles during hopping scales as  $M_b^{1.07} \pm 0.02 (95\% \text{ CI})$ .

This is not significantly different from the scaling of mitochondrial volume in hopping muscle,  $M_b^{1.05 \pm 0.06}$ , and to the surface area of inner mitochondrial membrane,  $M_b^{1.03 \pm 0.08}$ . Terminal tracheoles are the site for oxygen transfer between the tracheal system and hopping muscle. The total volume of tracheoles in hopping muscle scales as  $M_b^{1.13 \pm 0.15}$ , their surface area scales as  $M_b^{1.14 \pm 0.19}$ , their anatomical lateral diffusing capacity scales as  $M_b^{1.17 \pm 0.23}$ , and their anatomical radial diffusing capacity scales as  $M_b^{1.15 \pm 0.22}$ ; all of which have exponents congruent with the aerobic capacity of the hopping muscle. The principles of *Symmorphosis* therefore are met in the respiratory system of the migratory locust.

Mechanisms underlying the occurrence of discontinuous gas exchange in insects

#### Philip G. D. Matthews, Craig R. White The University of Queensland

What is the adaptive function of a respiratory pattern that periodically prevents gas exchange? This question is central to almost all investigations into insect discontinuous gas exchange cycles (DGCs). Several hypotheses have been proposed to explain the evolution of insect DGCs, either as a mechanism to reduce respiratory water loss, enhance gas exchange in hypoxic or hypercapnic atmospheres, or reduce oxidative damage. However, these hypotheses fail to explain the sporadic occurrence of this pattern across five distantly related insect orders. To better understand this respiratory pattern we take a mechanistic approach, hypothesising that DGCs may not be adapted for any particular purpose but arise spontaneously due to the organisation of the insect's nervous system. To this end we critique the current adaptive hypotheses in light of new experimental data, and suggest an alternative theory: DGCs occur when the insect's cephalic ganglia become inactive and respiratory control is assumed by ganglia in the thorax and abdomen. In other words, DGCs are the breathing pattern of an insect in a 'sleep-like' state.

The effect of light on bioluminescence in the glow-worm Arachnocampa flava

#### **Rebecca Morley** and David Merritt

School of Biological Sciences, The University of Queensland, Brisbane, QLD 4072

This study characterises the natural bioluminescent output of the glow-worm Arachnocampa flava and investigates the effect of extraneous light exposure upon bioluminescence. Individuals vary in their bioluminescence emission pattern however a characteristic pattern is evident whereby peak bioluminescence intensity occurs shortly after dark. Larvae respond to light exposures by reducing their bioluminescence. Once light sources are removed, larvae gradually recommence glowing to pre-light exposure bioluminescent levels. Bioluminescence shows a dosage response to light exposure, with greater reductions occurring in response to increased intensity and duration of light exposure. All wavelengths (colours) of light tested caused a reduction in bioluminescence. Light in the ultraviolet and blue regions of the spectrum had the most impact on bioluminescence, and red light had the least effect. A spectral sensitivity to blue light correlates with the bioluminescence spectral emission peak of A. flava, reinforcing recent findings that glow-worms may use bioluminescence for communication. The minimal effect of red light on bioluminescence leads to the recommendation that red filters be used in all lighting scenarios for glow-worm tourism. This will minimise impacts on A. flava bioluminescence (and hence feeding patterns) and maximise tourist satisfaction levels.

Arachnocampa tasmaniensis (glowworms) inside caves maintain circadian rhythmicity through visual synchronization

#### David J. Merritt<sup>1</sup> and Arthur K. Clarke<sup>2</sup>

<sup>1</sup>School of Biological Sciences, The University of Queensland, Brisbane, Australia. <sup>2</sup>School of Zoology, University of Tasmania, Hobart, Australia

Larvae of the genus Arachnocampa, known as glowworms, are bioluminescent predatory insects that use light to attract prey. One species, Arachnocampa flava, is known to possess true circadian regulation of bioluminescence: light entrains the rhythm of nocturnal glowing. We addressed the question of whether populations of Arachnocampa tasmaniensis, dwelling in the dark zone of caves, are also rhythmic. We found that the major cave populations maintain a high-amplitude 24-hour rhythm of bioluminescence with the peak during external daylight hours. Using in-cave and laboratory light-exposure experiments, we also show that cave larvae entrain to artificial light by matching the foregoing photophase, the opposite response to epigean larvae whose bioluminescence is inhibited by light and entrain to artificial light by matching the foregoing scotophase. The response suggests that individuals within a colony in the dark zone synchronize their bioluminescence rhythms through detection of others' light and matching their daily oscillations. The type of entrainment to light appears to be related to the degree of light exposure experienced by individuals during their development. We discuss the possibility that the daily timing of prey availability in caves could produce the afternoon bioluminescence peak observed in different caves and among different species.

Does isolation from group affect antipredatory behaviour of Australian plague locust?

**Shervin Aslanzadeh**, Michael B. Thompson, Gregory Sword, Jerome Buhl and Stephen Simpson Sydney University

It is well established that many prey species gain significant anti-predatory benefits from membership in a group. On the other hand, solitary prey animals need to be more vigilant and modify their escape behaviour in order to decrease their chance of being detected by the potential predator and increase the chance of a successful escape. This study investigates the anti-predatory behaviour of Australian plague locusts, *Chortoceties terminifer* under predation risk. Solitarious Australian plague locusts avoid the crowd when being exposed to a group (Simpson et al. 1999), and thus they do not benefit from safety gained by being in a group; i.e., encounter-dilution or confusion effect. Therefore, they might rely on different strategies from those of crowded locusts to minimize detection by predators and maximize escape-attempt.

Our results show that solitarious locusts show a higher decrease in activity in presence of a predator compared to gregarious locusts which delays the onset of attacks by the lizards. Solitarious locusts have a greater flight initiation distance compared to gregarious locusts. Size and speed of the lizards do not affect flight initiation of locusts, however locusts jump away from the lizards only if lizards approach them directly.

Disease ecology in migratory birds: insights from physiology, biochemistry and behaviour

**Bethany J. Hoye**<sup>1</sup>, Ron A. M. Fouchier<sup>2</sup> and Marcel Klaassen<sup>1,3</sup>

1. Department of Animal Ecology, Netherlands Institute for Ecology, Nieuwersluis, The Netherlands

2. Department of Virology, Erasmus Medical Centre, Rotterdam, The Netherlands

3. Centre for Integrative Ecology, School of Life and Environmental Sciences, Deakin University, Waurn Ponds, Australia

Pathogens are thought to play an important role in structuring the life-history of individuals and populations. The interactions between migratory hosts and their pathogens are particularly interesting, given that migrants have the capacity to connect disparate habitats: are exposed to a greater diversity of pathogens; and, concomitantly, need to perform long distance movement. Using avian influenza virus in migratory waterfowl as a model system, we applied physiological, biochemical and behavioural approaches to investigate the timing and location of infection cycles, as well as whether infection with this naturally-incurred disease has fitness consequences for the individual. Natural infection was found to occur during overwintering, and elicit a longlasting (but not permanent) immune response. Naturally infected individuals were seen to delay migration and, in juveniles, have a reduced probability of returning the following winter. Yet experimentally-infected individuals did not experience the same costs. Counterintuitively, naturally infected individuals were found to be in better condition at the time of capture. Using stable isotope analysis, we found nutrition (C:N ratio) and infection risk were associated with antecedent foraging habitat. Our findings underscore the role of host ecology in disease dynamics, and likewise the role of pathogens in shaping ecological trade-offs for the host.

Coping with chaos: unpredictable food supplies intensify torpor use and induce behavioural changes in activity patterns in an arid zone marsupial, the fat-tailed dunnart (*Sminthopsis crassicaudata*)

### P. Kern<sup>1,2</sup>, B. M. McAllan<sup>1</sup> and A. J. Munn<sup>2,3</sup>

<sup>1</sup>Physiology, School of Medical Sciences, and Bosch Institute, The University of Sydney, NSW, 2006. <sup>2</sup>Faculty of Veterinary Science, The University of Sydney, NSW, 2006. <sup>3</sup>Intitute of Conservation Biology and Environmental Management and School of Biological Sciences, The University of Wollongong, NSW, 2522.

Scarcity and unpredictability of food and water resources characterise arid environments. Australian arid zone endotherms must have strategies for dealing with highly variable conditions, especially with current climate trends forecasting an increase in intensity of extreme weather events over both long and short time-scales. Torpor is a key strategy in dealing with energy restriction. We investigated the use of torpor in response to unpredictability, along with behavioural strategies that may promote further energy savings in a small Australian arid zone marsupial, the fat-tailed dunnart (Sminthopsis crassicaudata). The fat-tailed dunnart showed an increasing, graded response in intensity of torpor use from animals receiving ad libitum food, through to restricted (75% food-restricted) and unpredictable diet-treatments. Animals in the restricted and unpredictable treatments displayed fewer, but longer bouts of activity, greater activity before the onset of the dark period, and slower sprint speeds than animals offered ad libitum food, although total activity time did not differ between treatments. This suggests that greater intensity of torpor use may be accompanied by a number of behavioural responses to reduce energy requirements and maintain optimum foraging activity in the fat-tailed dunnart to successfully balance energy and maintain body condition. These strategies appear to allow dunnart populations to persist through times of variable energy supply, with this species being widespread in the Australian arid zone.

Making the best of a bad relationship: breeding with non-preferred males affects Gouldian finch female corticosterone levels and primary offspring sex ratio

# Sarah R Pryke<sup>1</sup>, Simon C. Griffith<sup>1</sup>, Lee B. Astheimer<sup>2</sup> and **William A. Buttemer**<sup>2</sup>

<sup>1</sup>Department of Biological Sciences, Macquarie University, Sydney, NSW 2109<sup>2</sup>Centre for Integrative Ecology,

Gouldian finches have two distinctive colour morphs, a black-headed and redheaded form, that occur in a 70:30 % frequency throughout their sympatric distribution. Females prefer to mate with males sharing their head colour, but between 20-30% of free-living females are constrained to mate with males of different head colour. To evaluate the fitness consequences of intermorph breeding, 50 captive red-headed and 50 black-headed females were each randomly bred with a male of matching and one of different head colour. Offspring gender was determined in chicks and unhatched embryos using PCR methods. Females mated with preferred males had unbiased primary sex ratios (49% males), whereas females mated with non-preferred males produced 82% sons. Plasma corticosterone levels were significantly higher in females mated with non-preferred than with preferred males within days of pairing, and were substantially elevated in these females at the time of egg laying. The significant correlation between female plasma corticosterone during egg laying and the proportion of male progeny in both intramorph and intermorph pairings suggests corticosterone strongly influences pre-zygotic sex determination in this species. Such outcomes have significant fitness consequences, as male progeny from mixed-morph pairings have much higher survivorship than daughters.

#### Pollination ecology of the Australian cycad Macrozamia communis

**Thomas Wallenius**<sup>1,2</sup>, Rod Peakall<sup>1</sup>, Paul Cooper<sup>1</sup> and Rolf Oberprieler<sup>2</sup> 1. CSIRO Ecosystem Sciences, 2. ANU Research School of Biology

Cycads are dioecious gymnosperms, with both male and female inflorescences (cones) synchronizing pollen-shedding and receptive phases. Diel volatile emissions, thermogenesis and pollinator movements between male and female plants are correlated with these phases and characterize the pollination system. Regulation of specific volatiles is hypothesized to mediate pollinator movements between *Macrozamia* cycads in a "push-pull" manner, whereby high concentrations repel pollinators and low concentrations of volatiles attract them to cones. However, the "push-pull" hypothesis does not explain qualitative variation in volatile constituents, the function of thermogenesis, nor patterns of pollinator visitation of male and female plants, in relation to cone phenological stage.

My research on *Macrozamia communis* aims to test and expand existing cycad pollination hypotheses by investigating the patterns, function and specificity of volatile emissions on the behaviour of the pollinating weevil *Tranes lyterioides*. This seminar will detail the patterns of volatile emissions and thermogenesis in male and female *M. communis* cones; summarize pollinator movement between plants; present results from behavioural bioassays investigating repellent and attractant properties of volatiles; and evaluate electroantennographic (EAG) responses of *T. lyterioides* to individual and combinations of volatiles.

Posters

#### Thyroxine effects on body condition in a viviparous reptile

#### Josiane Eve and Ashley Edwards

School of Zoology, University of Tasmania

Body condition is important for reproductive success in many vertebrate taxa. Animals need to be able to assess how much stored energy is available for them to devote towards reproduction as they approach a breeding season. Thyroxine, a metabolic hormone, affects the storage and mobilisation of fats. We investigated the role of thyroxine in conjunction with the actions of reproductive (17B-oestradiol, testosterone) and metabolic (corticosterone) hormones in a viviparous, annually breeding skink, Niveoscincus metallicus. Exogenous thyroxine, alone or in combination with 17B-oestradiol, testosterone or corticosterone, was administered to Niveoscincus metallicus, by injection over several weeks. We measured changes in patterns of tail and abdominal fat storage in adult female skinks in response to these hormone treatments during two energetically important reproductive phases, post parturition (energy storage) and vitellogenesis (energy mobilisation). We calculated changes in body condition scores as the residuals of regressions between body (snoutvent) length and mass. Body condition was significantly affected by all treatments, and showed reproductive phase effects with all treatments except corticosterone. It appeared that the other hormones interacted with T4 to play a role in energy storage and mobilisation, but the effects of corticosterone on energy metabolism were independent of reproductive phase in this species.

Implanted transmitters do not affect maximum running speed of two small marsupials

#### A. Daniella Rojas, Gerhard Körtner and Fritz Geiser

Centre for Behavioral & Physiological Ecology, Zoology, University of New England, Armidale NSW, Australia

Radio telemetry is used to quantify behavioral, ecological and physiological variables of animals. Because of technological reasons, relative transmitter size generally increases with decreasing body mass of the study animal, and the recommended transmitter mass of <5% of body mass, which is not based on scientific evidence, often prohibits work on small mammals. We compared burst running speed, important for predator avoidance, in two small dasyurid marsupials the fat-tailed dunnart (Sminthopsis crassicaudata, body mass 17.4 g) and Giles' planigale (*Planigale gilesi*, 12.6 g) without and with implanted transmitters. In both species, maximum running speed was not negatively affected by the transmitters (Paired t-test, p>0.15), even though the transmitters weighed 6.4 to 14.1% of the animals' body mass. Further, relative transmitter mass (% of body mass) was not correlated with maximum running speed ( $r^2 < 0.03$ , p > 0.75). Our results show that transmitters well above 5% of body mass, do not affect locomotor performance of two small marsupials. We therefore conclude that transmitters <10% of body mass can be safely used for ethical experimentation in small terrestrial mammals (J. Mammal. 2010, DOI: 10.1644/10-MAMM-A-052.1).

The role of potassium channels in vascular regulation in amphibians.

Annalise M. Stanley, Melissa S. Cameron and John A. Donald. School of Life and Environmental Sciences, Deakin University, Australia, 3217

The involvement of K channels in vasodilation in amphibians has not been extensively studied. Previous physiological studies showed that nitric oxide (NO)-mediated vasodilation in toad, Bufo marinus, involved the activation of a range of K channels in different arteries. The first part of this study determined the mRNA expression of K channels in Xenopus tropicalis and Bufo marinus blood vessels. Three types of K channels, K<sub>ATP</sub>, K<sub>Ca</sub> and K<sub>V</sub>, were expressed in X. tropicalis blood vessels, whilst only KATP channels were expressed in B. marinus blood vessels. Ky channels were expressed in B. marinus brain but not in blood vessels. The second part of this study determined the role of K channels in cGMP-mediated vasodilation in B. marinus using dual-wire myography and specific K channel inhibitors. It was found that ANP-mediated vasodilation involved the activation of  $K_V$  and  $K_{Ca}$  channels in the femoral and brachial arteries respectively; however, no evidence was found for involvement of K channels in NO-mediated vasodilation. These results provide evidence that ANP-mediated vasodilation in amphibian blood vessels may not be mediated via cGMP.

Xenobiotic metabolism by the Malpighian tubules of insects, a new hepatic function

**Sam Parry**, Stuart Linton and Paul Francis School of Life and Environmental Sciences, Deakin University, Pigdons Road, Waurn Ponds, Victoria 3217, Australia.

Recent genetic evidence showing a high expression of genes for detoxification enzymes such as cytochrome P450, suggest that the Malpighian tubules (insect kidneys) may be capable of significant detoxification of xenobiotics. However this detoxification ability has yet to be demonstrated biochemically. The study examined if the model organic compound, morphine could be metabolised as it was transported across the cells of the Malpighian tubules from model flies, Drosophila melanogaster and Calliphora stygia, thus displaying a hepatic function in addition to the tubule's excretory and osmoregulatory functions. To do this, Malpighian tubules were dissected out of flies and set up in an organ bath which allows them to secrete urine. The urine from the tubules was pooled (100 tubules for D. melanogaster, 60 for C. stygia) and the morphine and metabolites within it analysed using HPLC with acidic potassium permanganate chemiluminescence detection. The resulting chromatograms indicated that the tubules of *D. melanogaster* metabolised morphine into 2 compounds while the tubules of C. stygia metabolised morphine into 1 compound. These compounds are hypothesised to be glutathione and gluconoride conjugates. C. stygia was able to metabolise morphine far more efficiently than D. *melanogaster* metabolising approximately 76% of the morphine.

Salivary gland activity in wild-caught yellow-winged grasshoppers, *Gastrimargus musicus*.

#### Nurul Wahida Othman and Paul Cooper

Evolution, Ecology and Genetics, Research School of Biology, The Australian National University, Canberra 0200

Yellow-winged grasshoppers (Gastrimargus musicus) are commonly found in grasslands and pasturage in south-east Australia during the summer months. We collected both green and brown morphs during their activity times by netting animals to investigate the feeding status by crop mass and the size and morphology of the glands to determine whether any difference was present between the morphs. Most animals had empty crops, but we found that green morphs (7/28) were marginally more likely to have food in their crops than the brown morphs (1/25). Insects that did not have food in their crops had smaller salivary glands. No obvious differences were present in the structure of the glands themselves between the colour morphs, and no difference was obvious between the morphs with respect to the presence of serotonin or dopamine using immunohistochemistry. However, as most animals were caught soon after flying, we cannot rule out that something may have caused the animals to eject food contents. Future work will look at the role octopamine has on salivary glands, as octopamine is known to be released with flying in Orthoptera.

Can the oxidative stress theory of aging explain differences in longevity between galliformes and psittaciformes?

<u>M. Trzcionka</u><sup>1</sup>, A.J. Hulbert<sup>1</sup> and W.A. Buttemer<sup>2</sup> 1 Dept. of Biological Sciences, University of Wollongong, Wollongong, NSW, Australia 2 Centre for Integrative Ecology, Deakin University, Waurn Ponds, Victoria, Australia

Birds have basal metabolic rates (BMR) that are on average 1.5-fold higher than those of similar-sized mammals. According to the rate of living theory. birds would be expected to live only two-thirds as long as similar-sized mammals, yet birds live up to four-times longer. Differences in maximum lifespan can be also found within the class aves, e.g. between galliformes (chickens and quails) and psittaciformes (parrots) with parrots living up to 6fold longer. A modification of the oxidative stress theory of aging, which suggests explanations for the observed differences in longevity, emphasizes three major components: (1) Mitochondria produce reactive oxygen species (ROS) as a normal by-product of respiration, which damage DNA, proteins, and lipids, which, in turn, causes aging and eventually death. (2) Animals have antioxidants that protect against ROS damage. (3) Membrane polyunsaturated fats exposed to ROS form lipoxidation products that produce further cellular damage. Interspecific differences in membrane fatty acid composition can therefore influence the potential extent of damage. To determine whether any of these processes account for the longevity differences between Galliformes and Psittaciformes, we have undertaken a multi-species comparison, measuring mitochondrial ROS production, products of ROS damage, antioxidants, and membrane fatty acid composition. Only membrane fatty acid composition supported the oxidative stress theory, tending to have a lower susceptibility to peroxidation in the longer-living species.

27th annual meeting of Australia and New Zealand Society for Comparative Physiology and Biochemistry The Australian National University, Canderra, ACT, Australia 3 December 2010