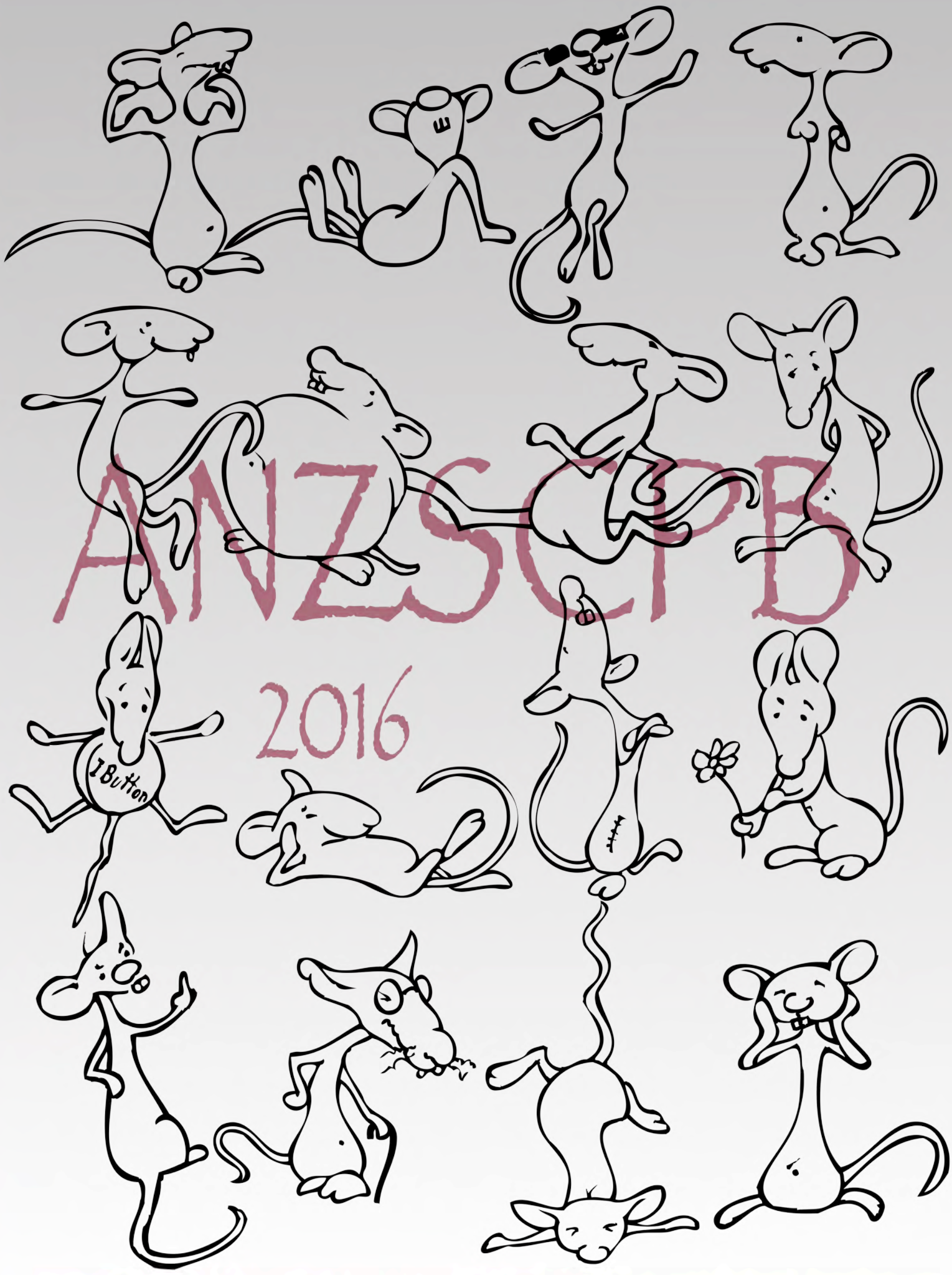
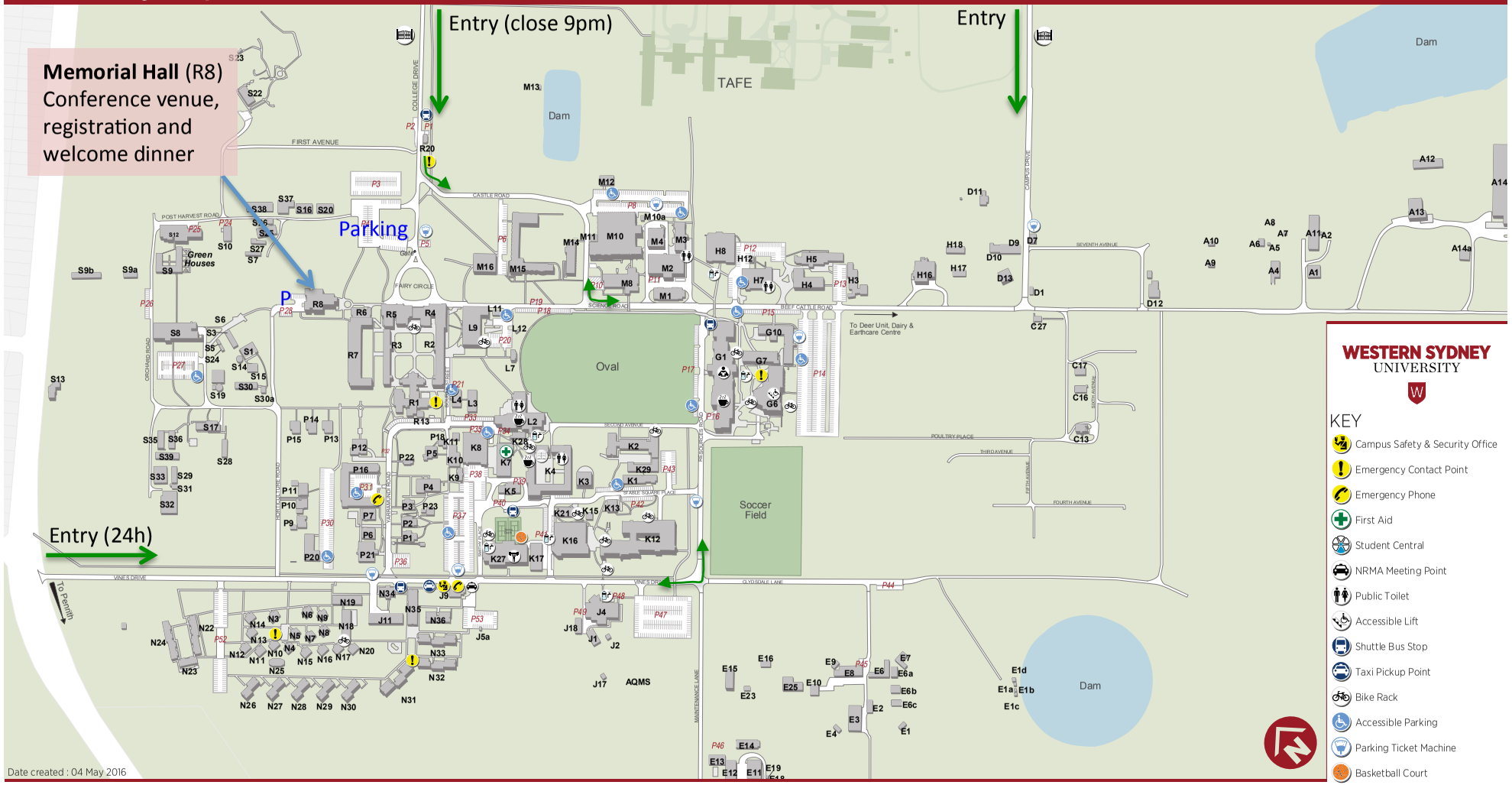


WESTERN SYDNEY UNIVERSITY

Hawkesbury Institute for the Environment

Hawkesbury campus



KEY

- Campus Safety & Security Office
- Emergency Contact Point
- Emergency Phone
- First Aid
- Student Central
- NRMA Meeting Point
- Public Toilet
- Accessible Lift
- Shuttle Bus Stop
- Taxi Pickup Point
- Bike Rack
- Accessible Parking
- Parking Ticket Machine
- Basketball Court
- Filtered Water Refill Station
- Lockers
- Kitchenette / Microwaves
- Bar

Entry Gate Opening Hours
College Drive and Campus Drive

Monday to Friday	0500 - 2000
Saturday	0800 - 1700
Sunday	Closed

Western Sydney University is a smoke-free environment

Building ID

- K8 Badanami Centre for Indigenous Education
- N34 Campus Living Villages
- H4 Campus Provost
- J9 Campus Safety and Security
- R1 Capital Works and Facilities
- E13 Farm Office
- L2 Northside Function Rooms
- R4 Hawkesbury Discovery Centre
- L9, R2 Hawkesbury Institute for the Environment
- G7 Information Technology and Digital Services
- H5 IT Procurement
- R8 Memorial Hall
- P10, P11 Men's Health Information and Resource Centre
- H3 Office of Audit and Risk Assessment
- H4 Office of Commercial and Estate Development
- R5 Office of Sustainability

Building ID

- H4 Office of the Vice-President (Finance and Resources)
- M16 Finance Office
- M16 Financial Operations
- L4 Financial Systems
- M2 Records and Archives Management Services (RAMS)
- G10, H8 School of Nursing and Midwifery
- M15 School of Science and Health
- K2 Animal Science Labs
- K12 Chemistry and Biochemistry Labs
- H16 Crime Scene Facility
- K29 Environmental Labs
- M1, M10 Food and Nutrition Labs
- K16 Forensic and Biology Labs
- S8 Horticulture and Plant Science Labs
- J4 Microbiology Labs
- K28, R6 Seminar Centre
- M8 Solar Energy Research and SIMS
- K4 Stable Square

Building ID

Student Support Services

- K4 Counselling Service
- K4 Disability Service
- K4 PASS Program
- K4 Student Campus Council
- K4 Student Representation & Participation
- K4 Student Welfare Service
- P18 Early Learning Ltd
- P12 Yarramundi House
- S9 Greening Australia
- D9, K13 Hawkesbury Community Art Workshop
- D13 Hawkesbury EarthCare
- K7 Macquarie Community College - Hawkesbury
- P21 NSW Police Leadership Centre
- S23 Secret Garden and Nursery
- S9 Turf Australia
- E8, E6c The New Secret Garden and Nursery
- E8 Riding for Disabled Richmond

POINTS OF INTEREST

Building ID

- P3, P4 Childcare Centre
- K4 Hairdresser
- P23 Multi Faith Centre, Chaplaincy
- P22 Owen Carter Memorial Chapel, Muslim Prayer Rooms
- K4 Queer Room
- L2 UWS Campus Life Office
- K4 Womens Room
- K4 Student Central
- G1 Graham Swain Library
- G1 Learning Commons
- K4 Jamaica Blue
- G1 Kiosk Cafe
- L2 Café L2, North Side Function Rooms
- K4 The University Store
- K27 Connect Fitness

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Australian and New Zealand Society for Comparative Physiology and Biochemistry

33rd Annual Meeting

1 - 4 December 2016

Western Sydney University

Hawkesbury Campus

Organising Committee

Christopher Turbill, Jenny Harvey, David Thompson and Rebecca Drury

Sponsors

Artwork by Dr Gerhard Körtner

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Conference Schedule

Registration and wood-fired pizza dinner 17:00-20:00 Thursday 1 December at Memorial Hall (R8)

	Friday 2 December		**Saturday 3 December**		**Sunday 4 December**
8:45	Welcome and announcements	8:55	Announcements		
9:00	Plenary Lecture	9:00	Plenary Lecture	9:15	Announcements
9:45	Session 1	9:45	Session 5	9:30	Session 9
10:30	Morning Tea	10:30	Morning Tea	10:35	Morning Tea
11:00	Session 2	11:00	Session 6	11:15	Session 10
12:30	Lunch	12:30	Lunch	12:30	Lunch
13:30	Session 3	13:30	Session 7		End of conference
15:00	Afternoon Tea	15:00	Afternoon Tea		
15:30	Session 4	15:30	Session		
16:05	AGM	17:00	End of day		
17:00	End of day	18:30	Conference Dinner		

Conference Program

Thursday 1 December

5:00 – 8:00pm

Registration and welcome dinner and drinks.

Memorial Hall (R8), Hawkesbury Campus, Western Sydney University, Richmond

Conference Program

Friday 2 December

*****indicates students eligible for prizes

Chair: Fritz Geiser	
8:45	Announcements
9:00	**Plenary Lecture: Claudia Bieber** and Thomas Ruf Connecting ecology and physiology: the life history strategy of a pulse resource consumer
9:45	**Chris Friesen**, Nicky Rollings, Randolph W. Krohmer, Emily J. Uhrig, Heather L. Waye, Robert T. Mason, Mats Olsson and Camilla M. Whittington Sex and death in the Canadian bush: Sex-specific telomere dynamics in the red-sided garter snake
10:00	**Christopher Turbill** and Lisa Stojanovski Giving predators the cold shoulder: torpor reduces predation risk in mice
10:15	**Nicolas Martin***, A. J. Hulbert, J. E. Bicudo, T. W. Mitchell and P. L. Else Long live the queen: influence of diet on longevity of honeybees (*Apis mellifera*)
10:30-11:00	Morning Tea
Chair: Michael Thompson	
11:00	**Lisa Bromfield***, Paul Rymer and Christopher Turbill Metabolic rate of house mice in response to air temperature and food availability
11:15 SPEED TALK	**Jacinta Kong***, Ary Hoffmann and Michael R. Kearney Predicting egg development in the parthenogenetic grasshopper *Warramaba virgo* (Orthoptera: Morabidae)
11:20	**Jessica Dudley***, Bronwyn M. McAllan, Christopher R. Murphy and Michael B. Thompson Early pregnancy in the American desert rodent Merriam's Kangaroo rat (*Dipodomys merriami*)
11:35	**Henrique Braz**, Selma M. Almeida-Santos, Christopher R. Murphy and Michael B. Thompson Uterine and eggshell changes associated with the evolution of viviparity in South American water snakes (*Helicops* spp.)
11:50	**Camilla Whittington,** Kevin Danastas, Georges Grau, Christopher R. Murphy and Michael B. Thompson Angiogenesis in amniote pregnancy
12:05 SPEED TALK	**Mohammad Khan***, Michael B. Thompson, Maria Byrne and Camilla Whittington Evolution of maternal fetal relationships in matrotrophic viviparous invertebrate: *Parvulastra parvivipara* and *Cryptasterina hystera* as model organisms

12:10	**Melanie Laird***, Hanon McShea, Bronwyn M. McAllan, Christopher R. Murphy and Michael B. Thompson Uterine cell adhesion dynamics during pregnancy in *Macropus eugenii* (Macropodidae) and *Trichosurus vulpecula* (Phalangeridae)
12:25	Announcements - Craig Franklin: Funding opportunities from Society of Experimental Biology and Company of Biologists
12:30-1:30	Lunch
Chair: Christine Cooper	
1:30	**Craig White**, Dustin Marshall and Daniel Ortiz-Barrientos Selection drives metabolic allometry
1:45	**Stewart Masson***, Christopher Hedges, Jules Devaux and Anthony Hickey Mitochondrial Glycerophosphate Dehydrogenase: a new bumblebee thermogenesis hypothesis
2:00	**Elle McDonald*** and Christopher Turbill Resting metabolic rate of mothers predicts offspring growth rate in mice
2:25	**Thomas Nelson*** and Roger S. Seymour Avian brain metabolism: Are birds bird-brained?
2:30	**Sean Tomlinson,** Kingsley Dixon and S. Don Bradshaw The buzz on honeybee energetics: Mismatches between models and measurement
2:45	**Karl Jones***, Steve Cooper and Roger S. Seymour Respiration in subterranean diving beetles
3:00-3:30	Afternoon Tea
Chair: Christine Cooper	
3:30	Announcements – Christine Cooper: IMC12 Perth July 2017
3:35	**Hugh Winwood-Smith***, Craig White and Craig E. Franklin Metabolic depression on a low carb diet: a mechanism to conserve glycogen?
3:50	**Daniel Gomez-Isaza***, Rebecca L. Cramp, Richard Smullen and Craig E. Franklin Coping with climate change: Can diet improve the thermal resilience of Barramundi, *Lates calcarifer*?
4:05	AGM

Conference Program

Saturday 3 December

*****indicates students eligible for prizes

Chair: Christopher Turbill	
8:45	Announcements
9:00	**Plenary Lecture: Steve Swoap,** Maria Vicent, Jake Bingaman and Elissa Hutt Utilizing the mouse model to examine neural mechanisms of daily torpor
9:45	**Phillipa K. Beale***, Karen Ford, Ben Moore and Bill Foley Interaction between plant secondary compounds and thermoregulation in mammalian herbivores
10:00	**Zenon Czenze***, Mark Brigham, Anthony Hickey and Stuart Parsons Aren't you cold? Seasonal torpor expression and roost choice differ between populations of New Zealand bats
10:15	**Fritz Geiser**, Kristina Gasch, Claudia Bieber, Gabrielle L. Stalder, Hanno Gerritsmann and Thomas Ruf Do hamsters bask?
10:30-11:00	Morning Tea
Chair: Claudia Bieber	
11:00	**Steven Portugal** Bright nights, costly mornings: night-time body temperature increases correspond with moon phase and cloudless nights in wintering Barnacle Geese (*Branta leucopsis*)
11:15	**Clare Stawski**, Taylor Hume, Gerhard Körtner, Shannon E. Currie, Julia Nowack and Fritz Geiser Post-fire recovery of the behaviour and physiology of a small marsupial
11:30	**Christine Cooper** and Philip Withers Control of evaporative water loss by a heterothermic dasyurid marsupial
11:45	**Qiaohui Hu**, Roger S. Seymour and Edward P. Snelling Ontogenetic scaling of femoral blood rate in western grey kangaroo (*Macropus fuliginosus*), eastern grey kangaroo (*Macropus giganteus*) and red kangaroo (*Macropus rufus*)
12:00	**Elia Pirtle***, Christopher R. Tracy and Michael R. Kearney Quantifying the influence of physiology and behaviour on reptilian evaporative water loss rates
12:15	**Nicholas Wu***, Rebecca L. Cramp and Craig E. Franklin Living with a leaky skin: Upregulation of ion transport proteins during sloughing
12:30-1:30	Lunch

Chair: Craig White	
1:30	**Pieter Arnold***, Phillip Cassey and Craig White Experimental evolution of dispersal-related traits in a model insect: morphological, physiological, and behavioural responses to spatial selection
1:45	**Gerhard Körtner** and Fritz Geiser Does aridity affect home range size? The spatial ecology of Australia's dasyurid marsupials
2:00	**Edward Narayan**, Asumi Willis, Corinne van den Hoek, Mandala Hunter-Ishikawa, Richard Thompson and Tuan Bendixsen Conservation physiology of Asiatic black bears (*Ursus thibetanus*): Monitoring stress and behaviour in bears rescued from bile farms in Vietnam
2:15	**Philip Withers**, Luke Kealley, Christine Cooper, Harriet Mills and Dominique Blache Effects of behavioural and physiological traits on release behaviour for ash-grey mice (*Pseudomys albocinereus*)
2:30	**Kristen Petrov***, James Van Dyke, Natasha Malkiewicz, Jessica Lewis, Michael B. Thompson and Ricky-John Spencer Diet generalisation and specialisation in a freshwater turtle (*Emydura macquarii*)
2:45	**James Van Dyke**, Michael Kelly, Kristen Petrov, Fiona Loudon and Ricky-John Spencer The mechanistic bases for maternal effects in turtles
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Chair: James Van Dyke	
3:30	**Julian Beaman***, Craig White and Frank Seebacher Evolution of plasticity: mechanistic link between development and reversible acclimation
3:45	**Rebecca L. Cramp**, Edward A. Meyer and Craig E. Franklin Life at low pH: The mechanistic basis for tolerance of extremely low pH by *Limnodynastes terraereginae* larvae
4:00	**Jules Devaux***, Anthony Hickey and Crystal James Hypoxia tolerant species take advantage of intracellular acidosis to maintain mitochondrial function
4:15	**Ensiyeh Ghanizadeh Kazerouni**, Frank Seebacher and Craig E. Franklin Parental environment affects offspring's responses to UV-B
4:30	**Crystal James***, Anthony Hickey, Jules Devaux, Christopher Hedges and Stewart Masson Is hypoxia tolerance reflected in New Zealand Triplefin brains? (Tripterygiidae): Analysing enzyme activities and metabolites
4:45	**Jessica K. McGlashan***, Michael B. Thompson, Fredric J. Janzen, James Van Dyke and Ricky-John Spencer Synchronous hatching in freshwater turtles: metabolic and endocrine mechanisms
6:30	Conference Dinner at The Sebel Resort, Windsor

Conference Program

Sunday 4 December

*****indicates students eligible for prizes

Chair: Camilla Whittington	
9:15	Announcements
9:30	**Yugo Watanabe***, Bert De Groef and Sylvia V. H. Grommen Regulatory elements that drive corticotropin-releasing hormone receptor 2 gene expression in avian thyrotropes
9:45	**Melissa Cameron** and John Donald Does the spotted gar, *Lepisosteus oculatus*, express a functional endothelial nitric oxide synthase?
10:00	**John Donald**, Noor Khalidah Abdul Hamid and Janet McLeod The role of leptin and ghrelin in appetite regulation in the Australian Spinifex hopping mouse, *Notomys alexis*, during long-term water deprivation
10:15 SPEED TALK	**Lesley A. Alton**, Rebecca L. Cramp, Emma Ceccato, Frank Seebacher and Craig E. Franklin DNA damage induced by ultraviolet radiation is repaired faster by enzymatic photoreactivation than by nucleotide excision repair in an amphibian species
10:20	**Ellyse Noy***, Melissa Scott, Diana Rayment, Sylvia V. H. Grommen, Kylie Robert and Bert De Groef The hypothalamo-pituitary-adrenal axis in the fat-tailed dunnart (*Sminthopsis crassicaudata*), an Australian marsupial
10:35-11:15	Morning Tea
Chair: Rebecca Cramp	
11:15	**Craig E. Franklin**, Essie Rodgers, Lily Bentley and Ross Dwyer Diving in a warming world: Environmental and physiological determinants of dive duration in crocodiles
11:30	**Michael Kearney**, Warren P. Porter and Stephen A. Murphy An estimate of the water budget for the endangered night parrot of Australia under recent and future climates
11:45	**Pippa Kern**, Rebecca L. Cramp and Craig E. Franklin Stressor interactions shape upper thermal limits
12:00	**Gillian Renshaw**, Jules Devaux and Anthony Hickey Mitochondrial plasticity as a determinant of surviving climate change.
12:15	Final announcements and award presentations
12:30-13:30	Lunch and end of conference

Abstracts

PLENARY LECTURE

Connecting ecology and physiology: the life history strategy of a pulse resource consumer

Claudia Bieber and Thomas Ruf

Department of Integrative Biology and Evolution, University of Veterinary Medicine, Vienna, Savoyenstraße 1-1a, 1160 Vienna, Austria

Torpor in mammals, i.e., hibernation, aestivation and daily torpor, is a state of metabolic depression that reduces energy expenditure during unfavourable environmental conditions. Here, we show for the first time that hibernation can last > 11 months in a free-living small mammal. Edible dormice (*Glis glis*) are strongly adapted to the availability of seed trees (i.e., beech nuts *Fagus sylvatica*). Only in so-called mast years dormice invest into reproduction and raise a single litter per year. In mast failure years, however, dormice gain weight but the food seems not sufficient to allow juveniles to grow and fatten prior to their first hibernation season. We show here that dormice in mast failure years retreated early into hibernacula when environmental conditions were mild but food availability was insufficient to raise young. We suggest that dormice opted for early hibernation onset to avoid predation and to increase survival.

Furthermore, recent evidence suggests that hibernators are particularly long lived and that the use of torpor may attenuate senescence, i.e. decreased cellular aging in terms of relative telomere length (RTL) shortening. This could also explain why dormice opted to prolong hibernation in a year without reproduction. Surprisingly however, RTL shortening was largest in animals showing extended hibernation. Specifically, RTL shortening was associated with the frequency of periodic arousals, which was highest during extremely long hibernation seasons starting in early summer. We therefore conclude that hibernation must affect longevity indirectly, by decreasing the risk of external mortality. This benefit apparently outweighs the potentially negative effect of increased senescence, especially because dormice are able to re-elongate telomeres during the summer-active season.

Sex and death in the Canadian bush: Sex-specific telomere dynamics in the red-sided garter snake

Chris Friesen[1], Nicky Rollings[1], Randolph W. Krohmer[2], Emily J. Uhrig[3], Heather L. Waye[4], Robert T. Mason[5], Mats Olsson[6] and Camilla M. Whittington[1]

[1]School of Life and Environmental Sciences, University of Sydney
[2]Department of Biological Sciences, Saint Xavier University
[3]Department of Physics, Chemistry and Biology, Linköping University
[4]Division of Science and Mathematics, University of Minnesota
[5]Department of Integrative Biology, Oregon State University
[6]Department of Biological & Environmental Sciences, University of Gothenburg

Life history strategies vary dramatically between the sexes, which may drive divergence in sex-specific senescence and mortality rates. Telomeres are tandem nucleotide repeats that protect the ends of chromosomes from erosion during cell division. Telomeres have been implicated in senescence and mortality because they tend to shorten with stress, growth and age. We investigated age-specific telomere length in female and male red-sided garter snakes, *Thamnophis sirtalis parietalis*. We hypothesized that age-specific telomere length would differ between males and females given their divergent reproductive strategies. Male garter snakes emerge from hibernation with high levels of corticosterone, which facilitates energy mobilization to fuel mate-searching, courtship, and mating behaviours during a 2-4 week aphagous breeding period at the den site. Conversely, females remain at the dens for only about four days and seem to invest more energy in growth and cellular maintenance, as they usually reproduce biennially. As male investment in reproduction involves a yearly bout of physiologically stressful activities, while females prioritise self-maintenance, we predicted male snakes would experience more age-specific telomere loss than females. We investigated this prediction using skeletochronology to determine the ages of individuals and qPCR to determine telomere length. For both sexes, telomere length was positively related to body condition. Telomere length decreased with age in male garter snakes, but remained stable in female snakes. There was no correlation between telomere length and growth (size/age) in either sex, suggesting that our results are a consequence of divergent selection on life histories of males and females. Different selection on the sexes may be the physiological consequence of the sexual dimorphism and mating system dynamics displayed by this species.

Giving predators the cold shoulder: torpor reduces predation risk in mice

Christopher Turbill and Lisa Stojanovski

Hawkesbury Institute for the Environment, Western Sydney University, Hawkesbury Campus, Richmond 2753, New South Wales, Australia

Torpor provides a mechanism for mammals and birds to reduce their daily energy requirements. Prolonged deep torpor, combined with food or fat storage, allows hibernating mammals to remain dormant over the entire winter season. At the other extreme, even short and shallow torpor bouts while resting provide energy savings that reduce daily foraging requirements. Our research aims to integrate thermoregulatory effects on resting energy expenditure with foraging behaviour and its life-history consequences. In this study, we measured the body temperature of wild-caught house mice in response to variation in perceived predation risk. We found that mice use torpor even when food is available to facilitate a reduction in foraging effort and hence exposure to a perceived risk of mortality from predation. Our experiment shows that energy savings from torpor are relevant in modulating 'decisions' by mice about foraging activity in response to environmental conditions.

Long live the queen: influence of diet on longevity of honeybees (*Apis mellifera*)

Nicolas Martin[2], Anthony J. Hulbert[2], J. Eduardo Bicudo[2], Todd W. Mitchell[1] and Paul L. Else[1]

[1]School of Medicine, University of Wollongong, NSW 2522, Australia
[2]School of Biological Sciences, University of Wollongong, NSW 2522, Australia

Social insects, such as honeybees and ants, have exceptionally long-living queens and are excellent models to investigate the biology of ageing. Female honeybees are genetically identical yet can become either long-lived queens (up to 8 years) or short-lived workers (normally 2-6 weeks). We are investigating the role of dietary lipids, and their oxidation (i.e. peroxidation), to explain this longevity difference in female honeybees. Only polyunsaturated fatty acids (PUFA) are capable of significant peroxidation with monounsaturated (MUFA) and saturated fatty acids highly resistant to peroxidation. The membrane lipids of larvae, pupae, emergent workers and adult queens all have a low proportion of PUFA and high proportion of MUFA. However, by day 4 following emergence, worker bees increase the proportion of PUFA in their membranes by 5-fold (with a consequent decrease in MUFA) unlike queens that retain a low proportion of PUFA in their membranes. This change is due to the consumption of pollen in worker bees following emergence whereas queens do not consume pollen throughout their life and are fed "royal jelly" mouth-to-mouth by workers. Royal jelly has a negligible level of PUFA. We postulate that this diet-induced increase in membrane PUFA is responsible for the much shorter lifespans of worker bees compared to queens. Thus, by feeding emergent worker bees a diet low in PUFA a queen-like membrane lipid composition should be produced and lifespan extended. Preliminary results show that worker bees fed on a diet with negligible PUFA maintain a low proportion of PUFA in their membrane lipids and live longer compare to worker bees feeding on diet containing PUFA. The ability to extend bee lifespan by nutritional manipulation will provide an important experimental tool to investigate the process of aging.

Metabolic rate of house mice in response to air temperature and food availability

Lisa Bromfield, Paul Rymer and Christopher Turbill

Hawkesbury Institute for the Environment, Western Sydney University, Hawkesbury Campus, Richmond 2753, New South Wales, Australia

Research is still needed to understand the ecological significance of metabolic rate and its relation to other key traits that determine animal performance and evolutionary fitness. Past efforts to integrate metabolism with behaviour and life-histories and have relied on basal metabolic rate (BMR) as a single index of individual differences in metabolism. Yet, for small endotherms, metabolic rate is strongly affected by thermoregulatory behaviour and food availability, and individual differences in these responses might also be important repeatable metabolic traits. We measured the metabolic rate of wild-caught house mice in home cages exposed to a daily temperature cycle (15, 20, 31 °C) and alternate-day food withdrawal. Respirometry trials lasted six days and were repeated three times over three month periods for all individuals. We used these detailed longitudinal measurements to examine the mean effects of temperature and food availability, individual differences in responses, and the interrelations among individual variation in minimum daily values of resting metabolic rate (RMR) at 15, 20, 31 °C and integrated energy expenditure over the daily cycle. In this talk, I will present an initial exploration of our results, which have only just been completed. We use these results to suggest how consistent individual differences in thermoregulatory metabolic responses to food availability could play an important role in the defining variation in 'pace-of-life' (i.e. metabolic-behavioural-life-history) syndromes.

SPEED TALK

Predicting egg development in the parthenogenetic grasshopper *Warramaba virgo* (Orthoptera: Morabidae)

Jacinta Kong, Ary Hoffmann and Michael R. Kearney

School of BioSciences, the University of Melbourne, Parkville, Australia 3010

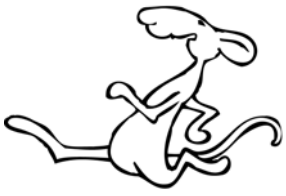
Understanding the insect life cycle is a key problem for predicting insect responses to climate, and for the management and conservation of species. The egg stage represents the fecundity of one generation and the survival of the next. Physiological adaptations, such as dormancy, of immobile eggs can regulate survival and development under variable local environmental conditions. Complex insect lifecycles can arise when developmental traits vary depending on environmental conditions and underlying genetic traits. Parthenogenetic insects allow us to investigate developmental responses of eggs to climate in a simplified genetic system. Here, we characterised egg development under constant and fluctuating temperatures in the parthenogenetic and wingless grasshopper *Warramaba virgo*. We examined the adaptive significance of egg dormancy for populations of *W. virgo* across Eastern Australia. Variation in egg development under various temperature regimes highlights the limitations of generalising laboratory experiments to the field, and the challenges for developing mechanistic models of insect responses to climate.

Early pregnancy in the American desert rodent Merriam's Kangaroo rat (*Dipodomys merriami*)

Jessica Dudley[1], Bronwyn M. McAllan[1], Christopher R. Murphy[1] and Michael B. Thompson[2]

[1]School of Medical Sciences, The University of Sydney, NSW 2006, Australia
[2]School of Life and Environmental Sciences, The University of Sydney, NSW 2006, Australia

The uterine surface undergoes significant remodeling during pregnancy to allow for implantation of the blastocyst (cell mass subsequently forming the embryo). These changes, collectively termed the 'plasma membrane transformation' (PMT), occur regardless of the placentation type that follows. Similarities in morphological and molecular changes during the PMT in viviparous lizards and marsupials suggest that common molecules play an important role in attachment of the trophoblast across species. Although placentation has evolved only once in mammals there are several different anatomical variations of the placenta in mammals. Kangaroo rats (*Dipodomys* spp.) do not exhibit the typical hemochorial (highly invasive) placenta of species within the superorder Euarchontoglires (rodents, rabbits and hares, tree shrews, flying lemurs and primates). Instead they exhibit a less invasive form, endotheliochorial (slightly invasive) placenta with little known about the molecular mechanisms underpinning its formation. Thus, we characterised the changes that occur to membrane molecules and to the cellular ultrastructure of the uterine epithelium during early pregnancy in Merriam's kangaroo rat, *Dipodomys merriami*. We used electron microscopy and immunofluorescence microscopy to describe changes to transmembrane proteins and the structural and luminal surface of uterine epithelial cells during pregnancy. These adhesion molecules included cadherins, which form the adherens junction and desmosomes which form adhesion 'spot welds' along the lateral plasma membrane, providing structural integrity to the tissue. Cadherins decrease in expression in the uterine epithelium during the pre-implantation period and there is a redistribution of desmosomes to the apical region of the lateral plasma membrane. The shift in desmosome and cadherin distribution before implantation suggests that there is a reduction in lateral adhesion between epithelial cells to allow for invasion by the blastocyst. Despite Kangaroo rats forming a less invasive placenta these same changes occur during pregnancy in species with highly invasive placentation such as the lab rat and human.



Uterine and eggshell changes associated with the evolution of viviparity in South American water snakes (*Helicops* spp.)

Henrique Braz[1], Selma M. Almeida-Santos[2], Christopher R. Murphy[3] and Michael B. Thompson[1]

[1]School of Life and Environmental Sciences, University of Sydney
[2]Laboratory of Ecology and Evolution, Butantan Institute, Brazil
[3]School of Medical Science and Bosch Institute, University of Sydney, Australia

The evolution of reptilian viviparity requires that the eggshell is reduced to bring together the uterine epithelium and extraembryonic membranes to form placentae for physiological exchanges. The mechanism by which the eggshell is reduced in thickness likely involves reducing the activity of the glands that secrete it. We tested this hypothesis using the Neotropical water snakes (*Helicops*). *Helicops* species exhibit intrageneric and intraspecific variation in reproductive mode, and viviparity has evolved three times independently in the genus. Thus it is an excellent model for investigating oviparity-viviparity transitions. The uteri of oviparous and viviparous *Helicops* are structurally similar and parallel cyclic variations in morphology. During primary vitellogenesis, the luminal epithelium is thin and glands are poorly developed. The epithelial thickness and uterine glands significantly increase during secondary vitellogenesis, but oviparous species always show the largest increases in uterine gland size. Uterine gland dimensions are similar among species with the same reproductive mode. During pregnancy, the epithelium is low and glands are depleted in the distended uteri. An eggshell is present in both oviparous and viviparous species. Nevertheless, the eggshell in viviparous *Helicops* lacks an external mineral layer and is thinner than in oviparous relatives. Both the uterine glands and epithelium secrete the eggshell, with the epithelium secreting the inner boundary, and the glands secreting the proteinaceous fibres of the eggshell. Our results support the hypothesis that eggshell thinning is associated with the evolution of viviparity and that such thinning results from less developed glands in viviparous than in oviparous taxa.

Angiogenesis in amniote pregnancy

Camilla Whittington[1], Kevin Danastas[2], Georges Grau[2], Christopher R. Murphy[2] and Michael B. Thompson[1]

[1]School of Life and Environmental Sciences, University of Sydney
[2]School of Medical Sciences, Bosch Institute, University of Sydney

Vascular endothelial growth factor is a major mediator of angiogenesis, a critically important process in vertebrate growth and development, and in pregnancy. The splice variant VEGF111 is a rare isoform found previously only in DNA-damaged human cells, until its discovery in the uterus of Australian skinks. We measured expression of VEGF111 and two major VEGF-A splice variants in the uterus of pregnant rats, showing that the three variants have different expression patterns across pregnancy. We have now identified this unusually potent splice variant in vivo in both marsupial and eutherian mammals. Our results suggest that viviparous mammals possess a precisely regulated milieu of VEGF isoforms producing the angiogenesis required for successful pregnancy and that VEGF111 may be common to all mammals, and potentially widespread in amniote pregnancy. The discovery of VEGF111 in rat uterus paves the way for the development of in vivo models of VEGF111 activity in a highly tractable laboratory animal, and is particularly significant in the context of early pregnancy loss and cancer research.

SPEED TALK

Evolution of maternal fetal relationships in matrotrophic viviparous invertebrate: *Parvulastra parvivipara* and *Cryptasterina hystera* as model organisms

Mohammad Khan, Michael B. Thompson, Maria Byrne and Camilla Whittington

University of Sydney

Matrotrophic brooding in aplacental viviparous animals involves a diverse range of maternal-fetal relationships in different phyla, and includes intragonadal lecithotrophic brooding in the Echinodermata. The evolution of viviparity requires internal fertilization and retention of offspring, but the evolution of matrotrophy requires extra embryonic nutrition to be provided to the offspring within the mother's body. In invertebrates, viviparity is associated with specialized physiological and morphological mechanisms. I aim to identify the mechanisms that are responsible for the evolution of viviparity in marine invertebrates. I will describe the brooding mechanisms, gonadal morphological changes and molecules involved in evolution of viviparous matrotrophic and broadcast spawning starfishes to assess the morphological changes associated with evolution of viviparity in two intragonadal viviparous matrotrophic invertebrates *Parvulastra parvivipara* and *Cryptasterina hystera*. Advanced non-invasive imaging techniques (μCT), microscopy, laboratory observation and transcriptomics will be used to describe the cellular and molecular mechanisms that enable matrotrophic brooding. Brood arrangement will be characterized by using micro-computed tomography (μCT) of the whole sea star. Morphological modification in the gonadal epithelial cell that may be required for viviparous larval development will be studied using transmission electron microscopy. I will also locate membrane molecules in different stages of birth through immunofluorescence microscopy and western blotting to confirm the presence of specific membrane molecules in *P. parvivipara* and *C. hystera*. RNA-Seq will be applied to identify and quantify differences in the genes expressed in the reproductive tissues between broadcasters and viviparous sea stars.

Uterine cell adhesion dynamics during pregnancy in *Macropus eugenii* (Macropodidae) and *Trichosurus vulpecula* (Phalangeridae)

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Successful mammalian pregnancy requires remodelling of the uterus to become receptive to embryonic attachment. While similar morphological changes to the uterine epithelium occur in both eutherian (placental) mammals and marsupials, the marsupial uterus employs maternal defences, including reinforcement of the uterine epithelium, to regulate embryonic invasion. Since non-invasive embryonic attachment in marsupials likely evolved secondarily from invasive attachment, uterine defences in these species may even prevent embryonic invasion. To test this hypothesis, we identified patterns of fluorescence of a key molecule involved in maintaining lateral cell-cell adhesion of the uterine epithelium (desmoglein-2) throughout pregnancy in the brush tail possum (*Trichosurus vulpecula*; Phalangeridae) and the tammar wallaby (*Macropus eugenii*; Macropodidae), each from a marsupial clade in which non-invasive attachment has evolved independently. In eutherian and marsupial species with invasive placentation, apical redistribution of desmoglein-2 pre-attachment reduces cell-cell adhesion of the uterine epithelium and facilitates invasion. Interestingly, both *M. eugenii* and *T. vulpecula* undergo this same apical redistribution, suggesting that cell adhesion, and thus integrity of the uterine epithelium, is reduced during the attachment period regardless of placental type. However, both species show additional unique patterns of desmoglein-2 localisation, including strong basal localisation for M. eugenii and nuclear localisation for *T. vulpecula*, which suggest that desmoglein-2 plays additional roles in these species. Hence, species-specific localisation of desmoglein-2 in *M. eugenii* and *T. vulpecula* may be involved in preventing embryonic invasion of the uterus by compensating for the reduced cell adhesion and maintaining an intact epithelial barrier.

Selection drives metabolic allometry

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Living species vary in size from ~0.1 pg single-celled micro-organisms to trees weighing several thousands tonnes. Put in perspective, this ~1021-fold range is similar to the difference in mass between an elephant and the Earth itself. The influence of mass on biological processes is pervasive, but is usually allometric: a 10-fold increase in mass is typically accompanied by just a 4-to-7-fold increase in metabolic rate. Understanding the basis of allometric scaling is a long-standing problem in biology. Here, we show the interspecific relationship between metabolic rate and body mass arises as a consequence of correlational selection on these traits, coupled with negative directional selection on absolute metabolic rate. This pattern of selection explains not only the covariance between metabolic rate and body mass (the allometric scaling of metabolic rate), but also explains the magnitude of the conditional variance in metabolic rate. The correlational selection we document constraints the evolution of mass-specific metabolic rates (MSMR) such that the observed range of MSMRs is just 50-fold among species that differ in size by ten billion-fold. Our results link microevolutionary processes to macroevolutionary patterns to describe the evolution of metabolic allometry in animals.

Mitochondrial Glycerophosphate Dehydrogenase: a new bumblebee thermogenesis hypothesis

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Bumblebees (*Bombus terrestris*) are an enigmatic species as they can fly at temperatures lower than many other insects. While bumblebees have adaptations that advantage heat retention, i.e. thermal inertia through large body mass and greater insulation, they require a mechanism to warm flight muscles without shivering, as this does not occur until 20oC. Precise mechanisms of heat generation are yet to be fully elucidated, with previous work suggesting that futile cycling of glycolytic and gluconeogenic intermediates releases sufficient heat for shivering to then warm muscles. However, predictions of flux through these pathways can only account for 10% of the heat required to heat the thorax muscles for flight.

Here we present a new hypothesis based on the mitochondrial glycerophosphate dehydrogenase (mGPDH) pathway. Analysis of respiration uncovered poor coupling of this pathway to ATP synthesis, as well as significant respiration rates in the absence of exogenous ADP; comparable to pre-flight conditions in the tissue. Complimentary analyses with a purpose built calorimeter showed significant heat generation from flight muscle respiring in the absence of ADP with mGPDH substrate glycerol-3-phospahe (G3P) relative to Complex I substrate pyruvate. Other analyses conducted at low temperature showed that G3P-supported respiration is less affected by low temperature than other mitochondrial respiratory pathways. Furthermore, the apparent affinity of mGPDH for G3P suggests that temperature may play a role in the passive regulation of mGPDH as a thermogenic mechanism.

We conclude that mGPDH may provide a temperature-sensitive mechanism additive to futile substrate cycling to warm bee flight muscle prior to shivering.

Resting metabolic rate of mothers predicts offspring growth rate in mice

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Current hypotheses make contrasting predictions regarding how resting metabolic rate (RMR) relates to total daily energy expenditure (DEE). The compensation hypothesis proposes a negative relationship between RMR and DEE because low maintenance energy costs would allow more energy to be allocated to other costs such as activity, growth and reproduction. In contrast, the increased intake hypothesis postulates a positive relationship between RMR and DEE because high maintenance energy costs could be indicative of a larger 'metabolic engine' that is capable of processing food into energy at a faster rate, thereby increasing the amount of energy that can be allocated to production. We aimed to test these hypotheses by first determining the repeatability of RMR and DEE, and then assessing the ecological consequences of variation in RMR by measuring reproductive output.

We measured the RMR and DEE of 'diversity outbred' (J:DO) laboratory mice (*n*=97) which exhibited a wide range of metabolic phenotypes. Metabolic rate was measured in response to variable temperature and food availability during three respirometry trials, each lasting four days, over one year. RMR at 31 °C, equivalent to basal MR (BMR), exhibited repeatability across the three trials (ICC, 0.26). RMR at 15 °C (RMR15), which accounts for thermoregulatory responses such as torpor use, showed lower repeatability across trials (ICC, 0.13) and total DEE displayed the highest repeatability across trials (ICC, 0.34). A subset of female mice (*n*=47) were bred once to determine if RMR affects offspring production. We found a negative relationship between a mother's BMR and the growth rate of her offspring, after accounting for effects of RMR15, body mass, litter size, and sex ratio. On average, offspring from mothers with BMR in the lowest 10^{th} percentile were 4.46 g lighter at the time of weaning than offspring from mothers with BMR in the 90^{th} percentile. Conversely, RMR15 had a positive effect on growth rate.

These results suggest that BMR represents an energy cost allocated away from growth, therefore providing support for the compensation hypothesis. The positive effect of RMR15 might be explained if RMR is representative of metabolic scope. Increased offspring growth rates in mothers with low BMR (low maintenance and heat production) and high RMR15 (high thermal conductance) is also predicted by the heat dissipation limitation hypothesis. These preliminary results provide a solid foundation for determining the ecological function of variation in metabolic rate.

Avian brain metabolism: Are birds bird-brained?

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Many comparisons have been drawn between the intelligence and cognition of primate and avian species, with some studies putting forth primate and avian intelligence as a case of convergent evolution. Cerebral perfusion is directly related to the metabolic rate of the brain and cognitive ability. Recently, a technique of estimating cerebral perfusion from the size of the bony foramina of the skull has been developed. Cerebral blood flow in birds is principally derived from the internal carotid and vertebral arteries which anastomose at the base of the brain. We used X-ray computed tomography to take cranial measurements of brain volume and carotid foramina radius from a range of avian species. Better understanding of the rates cerebral perfusion rates in different groups of birds can lead to interesting correlations between cranial arterial size and animal behaviour. This approach is so far unique and promises to provide further insights into both living and extinct species.

The buzz on honeybee energetics: Mismatches between models and measurement

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With a few exceptions, the measurement of field metabolic rate (FMR) in free-ranging insects has proven impossible thus far. Here we provide a review of the use of radio-isotopic turnovers to measure metabolic rate (VCO2), and confirm that VCO2 of the Honeybee *Apis mellifera* was significantly predicted by 86Rb kb (r2 = 0.57, p = 0.002), and conformed to expectations for an ectothermic species. The mass of honey solution consumed was significantly related to 22Na kb (r2 = 0.48, p = 0.008). We applied these calibrations to the study of free-ranging worker bees in landscapes with different levels of anthropogenic disruption. There were unexpected differences in FMR and food intake between the two different landscape contexts. Honeybees in deforested landscapes probably foraged less and depended upon stored resources during our study. We conclude that radio-isotopic techniques can be particularly useful for estimating FMR of insects. The use of such techniques can inform ecophysiologically-based questions on ecosystem function, productivity and conservation and land management that have previously been beyond reach in insect systems.

Respiration in subterranean diving beetles

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Respiration in many surface dwelling diving beetles (Coleoptera: Dytiscidae) has been well understood for a century. Beetles collect an air bubble which is stored underneath the elytra from which oxygen is consumed during the dive. A small bubble pushed from the tip of the abdomen can also be used to extract oxygen from the water. However, this air store still requires periodic replenishment at the surface. Several epigean submergent tolerant dytiscids have been identified and recent studies suggest these beetles may utilise pores or setae on their surfaces for oxygen uptake negating the need to return to the surface. However, respiration in subterranean dytiscids is not understood. We investigated respiration in stygobitic dytiscids from calcrete aquifers of the Yilgarn region in Western Australia. There have been numerous independent evolutionary incursions into the subterranean environment by ancestral surface dytiscids, as well as in situ speciation within calcretes, resulting in the most diverse assemblage of subterranean dytiscids in the world. We used microscopy, respirometry, fibre-optic oxygen sensors, and submergence experiments to explore respiration in two sympatric sister species, *Paroster macrosturtensis* (3.6–4.1 mm long) and *Paroster mesosturtensis*, (1.9–2.3 mm long) and an independently evolved subterranean species *Limbodessus palmulaoides* (4.2 mm long). All three species can consume oxygen from water, and have an oxygen boundary layer, but lack structures that could have respiratory function such as pores or setae. *P. macrosturtensis* has a low metabolic rate, only 25% of that predicted by other insects, and can tolerate at least 12 days of submergence. These results indicate that these beetles respire cutaneously and that this mode of respiration has evolved independently at least twice in subterranean dytiscids. This adaptation limits beetle size due to a mismatch between metabolism and surface area, but has led to an extraordinary radiation of subterranean beetles.

Metabolic depression on a low carb diet: a mechanism to conserve glycogen?

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Long-term studies have found low carbohydrate diets are more effective for weight loss than calorie restricted diets in the short-term, but equally or only marginally more effective in the long-term. Low carbohydrate diets have been linked to reduced glycogen stores and increased feelings of fatigue. We propose that reduced physical activity in response to lowered glycogen explains the diminishing weight loss advantage of low carbohydrate compared to low calorie diets over longer time scales. We explored this possibility by feeding adult *Drosophila melanogaster* either a standard or low carbohydrate diet for nine days and measured changes in metabolic rate, glycogen stores, activity, and body mass. We hypothesised that a low carbohydrate diet would cause a reduction in glycogen stores that recovers over time, reduced physical activity, and an increase in resting metabolic rate. The low carbohydrate diet was found to reduce glycogen stores, which recovered over time. Activity was unaffected by diet but the low carbohydrate group experienced a reduction in metabolic rate. We conclude that metabolic depression could explain the decreased effectiveness of low carbohydrate diets over time and recommend further investigation of long-term metabolic effects of dietary interventions and a greater focus on physiological plasticity within the study of human nutrition.

Coping with climate change: Can diet improve the thermal resilience of Barramundi, *Lates calcarifer*?

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Environmental temperature has been identified as the single most influential factor, directly affecting key physiological processes such as metabolism and locomotion in ectotherms, including economically important cultured species. This is becoming a major concern as some species (e.g. salmonids) are now being cultured in waters close to the upper thermal limits. Much of the current research aims to develop diets that maintain or enhance fish growth whilst increasing resilience to high temperatures. Here, we examined if diet (high fat vs. low fat) can improve the growth performance in juvenile barramundi (~3.2 ± 0.07 g) while increasing their resilience to acute thermal stress. The high fat diet increased fish growth compared to the low fat diet, but had no effect on the thermal sensitivity of performance traits. However, fish fed the high fat diet showed an overall reduction in whole animal thermal tolerance (CTMax). Together, these results indicate that high fat diets maintain aerobic performance at high temperatures and increases growth performance hence it may be beneficial for aquacultural production in a warming world.

PLENARY LECTURE

Utilizing the mouse model to examine neural mechanisms of daily torpor

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In response to food scarcity and low ambient temperature, mice enter bouts of torpor resulting in energy conservation. To mediate the torpor response, the brain requires input relaying information concerning the environment (nutrients, temperature, etc). The brain also has separate centers to evoke the physiological changes of torpor. However, the neural mechanisms and circuits involved with daily torpor are unknown. We used two approaches towards identifying the different regions within the brain that are involved in the torpor response. First, we took a comparative approach, comparing the physiological effects of torpor vs. diving, as those brainstem regions involved in mediating the diving response are known. We hypothesized that the cardiovascular effects involved in the murine dive response (drop in heart rate, fall in blood pressure, and constriction of peripheral blood vessels) are similar to those used in daily torpor. The physiological responses to torpor and diving were indistinguishable. During both diving and torpor, diastolic BP fell dropped 1.5x: the interbeat interval increased 4x, and total peripheral resistance increased 4x relative to control. These data suggest that the drivers of the cardiovascular changes during a bout of torpor and during the diving response are likely shared. Second, we used optogenetics to examine the role of Agouti-related protein (AgRP) containing neurons in the arcuate nucleus of the hypothalamus in daily torpor. As these neurons sense circulating cues of energy availability, we hypothesized that direct stimulation of AgRP neurons would decrease the minimum T_b of torpid mice and increase the time spent in torpor. To test this hypothesis, hypothalamic AgRP neurons were selectively targeted with the light-sensitive channelrhodopsin-2 transgene. Mice were calorically restricted daily (65% of normal caloric intake) until regular daily torpor bouts were achieved. On baseline days, when AgRP neurons were not activated, minimum T_b was 25.6 ± 0.8 °C and time in torpor was 233 ± 34 minutes. When AgRP neurons were stimulated for one hour during entry into torpor (20 Hz for 1 second every 4 seconds for 60 minutes), minimum T_b was significantly lower (22.8 ± 0.3 °C) and torpor bouts were significantly longer (435 ± 29 min). These data support the hypothesis that AgRP neurons directly regulate torpor physiology.

Interaction between plant secondary compounds and thermoregulation in mammalian herbivores

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Through mechanisms that control the generation, conservation and dissipation of heat, endotherms can maintain a relatively constant Tb across a broad range of ambient temperatures. The diet of mammalian herbivores is fundamentally linked to thermoregulation. However, the interplay between diet selection and ambient temperature is often overlooked in ecology. The diet of herbivores in particular contains plant secondary compounds that are able to alter the physiological processes responsible for appropriate thermoregulation in multiple ways, such as by uncoupling mitochondria, binding to thermoreceptors, or because the pathways required to metabolise plant secondary compounds are thermogenic. These interactions may become more important as ambient temperature rises since dissipating excess body heat into the environment becomes more difficult. It is likely, therefore, that the implications of a rise in ambient temperature are greater for herbivores than for other mammals, and that a need to manage internal and external heat loads under these conditions could drive changes in feeding ecology. Data on the role of the liver in detoxification at elevated temperatures and the uncoupling potential of common secondary metabolites will be described.

Aren't you cold? Seasonal torpor expression and roost choice differ between populations of New Zealand bats

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Variations in weather and food availability differentially impacts the energy budgets of endotherms. There has been considerable focus on the thermal physiology of species that experience extreme seasonal differences. However, much less is known about responses by species that experience more subtle changes in seasonal weather. We monitored ambient temperatures (Ta) and skin temperatures (Tsk) of individuals from 3 populations of New Zealand lesser short-tailed bats (*Mystacina tuberculata*) using temperature telemetry. Relative to the North Island (NI), mean summer Ta was 1°C lower in the South Island (SI), yet SI individuals used torpor on 36% of observation days compared to 11% for NI bats. None of the weather variables we recorded differed between the days bats did, or did not, use torpor. Solitary roosts were occupied on 17% of observation days for NI populations and 38% for SI populations, with individuals occupying them exclusively while using torpor. Relative to the NI, mean winter Ta was higher in Little Barrier Island (LBI). Bats in LBI used daily torpor more (51%) than in the NI (33%). Furthermore in LBI, but not the NI, bats were more likely to arouse on nights with warmer sunset Ta. In LBI bats preferred thermally labile roosts and preferentially roosted inside dead punga/silver fern-tree (*Cyathea dealbata*) which contrasts with NI individuals which prefer more thermally stable tree cavities. Punga roost thermal characteristics were no different than Ta allowing for individuals to potentially take advantage of passive rewarming. Our results provide evidence that even small differences in Ta differentially impact populations. Site-specific energetic strategies are apparent between *M. tuberculata* populations during summer and winter, demonstrating that climate affects both roost choice and torpor patterns. Clearly populations in warmer climates are under less energetic stress and use site-specific adaptations demonstrating that climate affects both roost choice and torpor patterns.

Do hamsters bask?

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Basking can substantially reduce thermoregulatory energy expenditure of mammals. We tested the hypothesis that the largely white winter fur of hamsters (*Phodopus sungorus*), originating from Asian steppes, may be related to camouflage to permit sun basking on or near snow. Winter-acclimated hamsters in our study were largely white and had a high proclivity to bask when resting and torpid. Resting hamsters reduced metabolic rate (MR) significantly (>30%) when basking at ambient temperatures (Ta) of ~15 and 0°C. Interestingly, body temperature (Tb) also was significantly reduced from 34.7±0.6°C (Ta 15°C not basking) to 30.4±2.0°C (Ta 0°C basking), which resulted in an extremely low (<50% of predicted) apparent thermal conductance. Induced torpor (food withheld) during respirometry at T¬a 15°C occurred on 83.3±36.0% of days and the minimum torpor MR was 36% of basal MR at an average Tb of 22.0±2.6°C; movement to the basking lamp occurred at Tb<20.0°C. Energy expenditure for rewarming was significantly reduced (by >50%) during radiant heat-assisted rewarming, however, radiant heat per se without an endogenous contribution by animals did not strongly affect metabolism and Tb during torpor. Our data show that basking substantially modifies thermal energetics in hamsters, with a drop of resting Tb and MR not previously observed and a reduction of rewarming costs. The energy savings afforded by basking in hamsters suggest that this behaviour is of energetic significance not only for mammals living in deserts where basking is common, but also for P. sungorus and likely other cold-climate mammals.

Bright nights, costly mornings: night-time body temperature increases correspond with moon phase and cloudless nights in wintering Barnacle Geese (*Branta leucopsis*)

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It is well known that animals respond to fluctuations in light levels, with distinct circadian rhythms apparent in physiological parameters such as heart rate (fH) and body temperature (Tab). We deployed implantable data loggers that continuously record fH and Tab, in 7 wild barnacle geese (*Branta leucopsis*) wintering in south-west Scotland. The geese had a distinct circadian rhythm in fH and Tab, with night-time values being, on average, 25 beats per min and 2.5 oC lower for fH and Tab respectively when compared to day-time values. Furthermore, we have identified rhythmic night-time peaks in Tab, with increases of 1.5 oC above normal night-time values occurring in the middle of the night at regular intervals throughout the winter period. These night-time peaks in Tab coincided with specific phases of the moon, illumination levels and cloud cover. Upon dawn, resting fH was significantly higher during these mornings following the incidences of night-time peaks in Tab than those nights where no night-time peak in Tab occurred. This suggests a 'hangover effect' of these night-time peaks in Tab, which will have consequences for daily energy budgets. It is apparent that circadian rhythms in physiological parameters can be disrupted by natural occurrences as well as artificial light sources.

Post-fire recovery of the behaviour and physiology of a small marsupial

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The initial and short-term effect of fire is often a dramatic change of the environment, however, burnt habitat eventually recovers. How animals respond behaviourally and physiologically to such environmental changes is poorly understood. Our work has shown that after a prescribed fire (post-fire) female brown antechinus (*Antechinus stuartii*) spent significantly less time active than before the fire (pre-fire) and individuals in an unburnt control area (control). This reduction in total post-fire activity by antechinus was made energetically possible by increasing the duration of torpor—a highly efficient energy conservation mechanism—and also by decreasing daily minimum body temperature in comparison to pre-fire and control individuals. We hypothesised that as vegetation and food resources recover, the behaviour and physiology of the antechinus population would return to normal. Therefore, we quantified the activity and torpor patterns of antechinus at the same time from three groups: i) the area of the prescribed fire one year post-fire, ii) an area that burned two years prior and iii) the control area. While after one and two years ground cover in the areas of the prescribed burns was still patchy in comparison to the control area, it had recovered substantially. Importantly, the duration of activity of antechinus in all three groups was similar to that measured pre-fire and in the control area the previous year. Further, torpor frequency, torpor bout duration and daily minimum body temperature in all three groups had also returned to values similar to those recorded the preceding year in the pre-fire and control areas. Therefore, our new results show that, in the case of a low intensity burn, only one year post-fire antechinus resumed normal daily activity and torpor patterns, likely in response to the return of ground cover and increased foraging opportunities.

Control of evaporative water loss by a heterothermic dasyurid marsupial

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We have recently recognised that endothermic mammals and birds appear to regulate their insensible evaporative water loss independent of external factors which would be expected to perturb evaporation from the skin or lungs. Observations of this phenomenon for both marsupial and placental mammals, and for birds, suggests it is a fundamental characteristic of endothermic animals, and we hypothesise that it may be an important aspect of thermoregulation. Here we examine the impact of ambient temperature and relative humidity on the evaporative water loss of a heterothermic dasyurid marsupial, the red-tailed phascogale (*Phascogale calura*), to characterise this regulatory response in a second dasyurid species, and importantly to quantify the impact of torpor on control of evaporative water loss. Normothermic phascogales maintained insensible evaporative water loss independent of ambient relative humidity at ambient temperatures of 20, 25 and 30°C, at least at humidities ≤ 60%. There was strong statistical evidence that their evaporative water loss, corrected for water vapour pressure deficit, differed from the expected biophysical pattern at ambient temperatures of 20°C and 30°C, but not 25°C. Phascogales entered torpor at ambient temperatures of 20 and 25°C and torpor frequency was not influenced by ambient humidity. Phascogales regulated their evaporative water loss against the expected biophysical drivers of evaporation during torpor at 20°C, but not at 25°C. Control of evaporative water loss regulation amongst dasyurid marsupials is clearly not just a characteristic of hyper-arid adapted species, and is maintained, or even enhanced, during thermoregulatory phases of torpor. However, it may not occur when heterothermic species are thermoconforming, providing support for the hypothesis that insensible evaporative water loss control serves a thermoregulatory role for endothermic animals.

Ontogenetic scaling of femoral blood rate in western grey kangaroo (*Macropus fuliginosus*), eastern grey kangaroo (*Macropus giganteus*) and red kangaroo (*Macropus rufus*)

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A nutrient artery, which passes through a foramen located on a femur shaft, supplies more than half of the total blood supply to the femur. Nutrient foramen size correlates with artery size, thus femoral blood flow can be estimated by measuring the foramen area. Interspecific femoral blood flow has been suggested to be proportional to the animals' locomotion levels in previous nutrient foramen studies, but no study has looked into intraspecific femora blood flow of animals. This study determined ontogenetic femoral blood flow rate in three species of diprotodont marsupials, which are western grey kangaroos (*Macropus fuliginosus*), eastern grey kangaroos (*Macropus giganteus*) and red kangaroos (*Macropus rufus*). A biphasic relationship is revealed for the scaling of western grey kangaroo femoral blood flow during ontogeny, with a steep exponent of 0.97 ± 0.10, and then a shallow exponent of -0.49 ± 0.94. The breakpoint matches the pouch exit body mass (ca. 4-5 kg). Growth rate is suggested to be the main driving factor for intraspecific femoral blood flow during kangaroos' in-pouch state of development, whereas the main driving factor gradually changes from growth rate to locomotion level as the kangaroos leave the pouch, and become more active. There is no significant difference in femoral blood flow between the two sexes of kangaroos during in-pouch stage, but post-pouch male kangaroo have higher femoral blood than the females because of their higher growth rate and activity levels.

Quantifying the influence of physiology and behaviour on reptilian evaporative water loss rates

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Global climate change is expected to cause significant thermal stress for ectothermic species, potentially resulting in extinctions. Another significant source of stress may be the changes in precipitation and aridity associated with temperature changes, the effects of which are poorly understood. Despite the strong physical connections between temperature and water dynamics, the two are often considered independently. Moreover, the potential for regulatory behaviours to mitigate the effects of climate changes are often overlooked: in particular, reptilian behavioural hydroregulation. I begin by presenting a predictive model of a major component of reptilian water budgets: evaporative water loss rates. This model is validated by comparing predictions of water loss rates to measured values for 39 squamate species with separated cutaneous and respiratory water loss rate measurements. Next I present a quantitative analysis of the adaptive significance of several forms of physiological and behavioural hydroregulation on the evaporative water loss rates of two species of closely related yet ecologically distinct Australian skinks. This approach predicts an animal's responses to different environmental conditions by combining a microclimatic model with a mechanistic heat and water budget model that accounts for both physiological constraints and regulatory behaviours. I find that both physiological and behavioural hydroregulatory mechanisms can influence water budgets substantially, with some behavioural mechanisms potentially reducing evaporative water loss rates by over 70%. Moreover, the amount of water that can be saved through each hydroregulatory mechanism varies greatly across physiology, behaviour and lifestyle, suggesting that the ability to cope with changing climates may also vary greatly between species. Understanding these variations will improve our ability to identify species most at risk.

Living with a leaky skin: Upregulation of ion transport proteins during sloughing

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Amphibian skin is a multifunctional organ providing protection from the external environment and facilitating the physiological exchange of gases, water and salts with the environment. In order to maintain these functions the outer layer of skin is regularly replaced in a process called sloughing. During sloughing, the outermost layer of the skin is removed in its entirety which has the potential to interfere with skin permeability and ion transport, disrupting homeostasis. In this study we measured, in vivo, the effects of sloughing on the cutaneous efflux of ions in toads Rhinella marina kept in freshwater conditions. We also measured transepithelial potential, cutaneous resistance, active ion transport, and the distribution, abundance and gene expression of key ion transport proteins sodium-potassium ATPase (NKA), and the epithelial sodium channel (ENaC) during sloughing. We hypothesised that during sloughing, there would be a greater efflux of ions as a consequence of increased permeability and/or via a reduction in the abundance or expression of cutaneous ion transport proteins. There was a significant increase in sodium and chloride efflux during sloughing in R. marina. However, although in vitro skin resistance decreased after sloughing, active sodium transport increased commensurate with an increase in NKA and ENaC protein abundance in the skin. These changes in skin function associated with sloughing did not affect the maintenance of internal electrolyte homeostasis. These results suggest that during sloughing, amphibians actively maintain internal homeostasis by increasing cutaneous ion uptake rates.

Experimental evolution of dispersal-related traits in a model insect: morphological, physiological, and behavioural responses to spatial selection

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Dispersal ability varies significantly among individuals. Much of this variation has been attributed to dispersal syndromes – suites of covarying morphological, physiological, behavioural, and life-history traits – which correlate with dispersal and invasion in free-living populations. At population range edges and invasion-fronts, these dispersal-related traits appear to be evolving through the spatial assortment of phenotypes. Yet despite the apparent ubiquity of these observations, no experimental study has attempted to replicate the evolution of phenotypic traits under spatial assortment on dispersal. We used the red flour beetle (*Tribolium castaneum*) in a laboratory dispersal system to assess how selective processes, for and against dispersal, affected dispersal-related traits. Here I will discuss our findings that body size rapidly diverged over seven generations – dispersers became smaller and non-dispersers became larger – and that dispersers had a relatively lower metabolic rate. Small individuals were more energetically and biomechanically efficient at climbing, and were therefore the ones that dispersed more readily. The variance in dispersal rate and movement was maintained even under intensive selection for opposing dispersal behaviours. This suggests that individuals may maximise their fitness by producing offspring that exhibit a variety of dispersal behaviours; a hypothesis that is further supported by the lack of a trade-off between dispersal behaviour and reproductive success.

Does aridity affect home range size? The spatial ecology of Australia's dasyurid marsupials

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We investigated relationship between body mass and home range size in Australian carnivorous marsupials (Dasyuridae) and tested whether those species living in resource-poor desert environments have relatively larger area requirements than their mesic counterparts. The movement patterns of two sympatric species of desert dasyurids (body mass 16 & 105g) were investigated via radio-telemetry during winter in Astrebla NP, Queensland. These results were compared with published records for other Australian dasyurids. Overall, home range size of dasyurids scaled with body mass with a coefficient of 1.29, which is considerably higher than that for basal and field metabolic rates. In addition, males of especially the larger species occupied larger home ranges than females, even after accounting for the size dimorphism that is common in dasyurids. While a simple separation of hone range size between mesic and arid zone species was not statistically supported, an alternative model based on average yearly rainfall and primary production was tested and this yielded significant differences. Our study therefore indicates that habitat quality affects home range size in Australian dasyurids, albeit this effect was not as strong as anticipated. Therefore, alternative avenues for reducing energy expenditure such as adjustments of population density as well as the use of daily torpor and basking, commonly observed in desert dasyurids (including the two study species), likely play a role. However, these can apparently only partly compensate for the on average lower resource density in arid habitats.

Conservation physiology of Asiatic black bears (*Ursus thibetanus*): Monitoring stress and behaviour in bears rescued from bile farms in Vietnam

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Conservation physiology concepts, tools and knowledge can help solve conservation challenges across a broad range of taxa by understanding and predicting how organisms, populations and ecosystems respond to environmental stressors. Asiatic black bear populations are declining in-situ due to bear bile farming to source bile use in traditional medicine. Animals Asia is battling this barbaric trade through the coordination of bear rescue from farms and rehabilitation programs in Vietnam and China. We studied 16 Asiatic black bears (6 males, 10 females) after immediate rescue from bear bile farms in the Quang Ninh province, Vietnam. It was hypothesised that newly rescued Asiatic black bears will demonstrate a high number of stereotypies and health problems, and high levels of faecal glucocorticoid metabolites (a non-invasive index of physiological stress). These clinical responses would be indicative of the long-term effects of physical and psychological stress that the bears have ensued on the bear bile farms. A faecal cortisol metabolite (FCM) enzyme-immunoassay was used to quantify FCM levels over a study period of 22 weeks. The key results showed that mean FCM concentrations reduced significantly over the first 22 weeks since arrival of the bears at Animals Asia's Vietnam Bear Rescue Centre. All bears performed at least one stereotypy and had at least three different types of health issues. Head swaying and pacing were the most common stereotypies performed by the bears while skin, limb, musculoskeletal, dental and blood problems were most prevalent in the group of 16 Asiatic black bears. In conclusion, our results demonstrate that physiological stress can be reduced when bears are rehabilitated in the Vietnam Bear Rescue Centre. The results also show that some degree of stereotypic behaviour and health problems may have become inherent as a result of chronic stress.

Effects of behavioural and physiological traits on release behaviour for ash-grey mice (*Pseudomys albocinereus*)

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We examined potential relationships between behavioural and physiological traits, and post-release movement and dispersal in the field, for the ash-grey mouse (*Pseudomys albocinereus*) to determine if we can predict post-release behaviour of a mammal from easily-measured laboratory traits. Laboratory measures of personality for each individual, such as exploratory behaviour (open field) and learning capacity (Barnes maze), along with physiological variables such as faecal stress hormone levels (cortisol) and basic energetic, hygric and thermal variables (metabolic rate, water loss, body temperature, thermal conductance) were measured. Individual personality and physiology was then characterised using various approaches to combine these data, including raw variables, factor analysis, behavioural indices and mixed model assessment to determine the most useful approach to quantify these traits. Raw variables were the most successful for characterising individual personality and physiology, and therefore were used to relate laboratory physiology and behaviour, and to relate these to field movements. There were no inter-correlations between the laboratory factors for open field, Barnes maze, physiology or faecal cortisol levels, suggesting that each methodology measures quite different individual traits. The only significant relationship of laboratory measures with field movement was for the open field (raw variables, $P = 0.001$; raw variables significantly contributing to factors, $P=0.006$), indicating that if an ash-grey mouse moves more in an open field in the laboratory, it will move more when relocated into the field. We conclude that the open field behavioural test is the most likely laboratory predictor of field movement and dispersal for this small native rodent after translocation.

Diet generalisation and specialisation in a freshwater turtle (*Emydura macquarii*)

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Animals forage for different foods to maximise their rates of energy and nutrient uptake. Prey are selected based on their nutritional values, but are also selected based on how costly a food resource is to obtain. When food resources are scarce, individuals may shift their foraging strategies to focus more on abundant food resources, leading to intraspecific variation in diet. As a generalist consumer, the freshwater turtle *Emydura macquarii* is capable of utilising different food resources based on prey that is present and abundant. Because of its generalist diet, *E. macquarii* is capable of exploiting and surviving in different habitats. My study examined spatial and temporal variation in the diet of a freshwater turtle species (*Emydura macquarii*) in wetlands near the Murray River, Australia, to determine 1) if populations of *E. macquarii* vary in their diet and/or display intrapopulation variation and 2) whether the diet of individual *E. macquarii* vary over time. Using stable isotopes and gut contents, I determined whether individual turtles varied in their diet across different wetlands. I also used stable isotopes to determine whether turtles varied in their diet over long (6-12 months) and short (3-6 months) timescales. Both stable isotope and gut content data revealed among-wetland differences in the diet of *E. macquarii*, with individuals from some wetlands being more carnivorous and individuals from others being more generalist/herbivorous. Specifically, *E. macquarii* that were carnivorous appeared to be strictly carnivorous, while the generalist/herbivorous *E. macquarii* consumed animals opportunistically. The degree of herbivory of *E. macquarii* was directly correlated with increasing plant abundance, but inversely correlated with water clarity. These results suggest that *E. macquarii* consume a more generalist/herbivorous diet when plants are available, but adopt a more specialised carnivorous diet when vegetation is less abundant. Habitat and diversity of available prey constrain the diet of *E. macquarii*, however *E. macquarii* appear to respond by shifting their foraging strategy from generalist to functionally specialist.

The mechanistic bases for maternal effects in turtles

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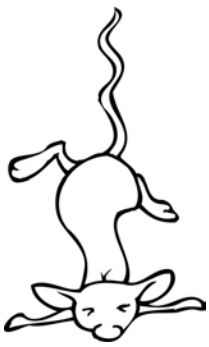
Maternal effects are the nongenetic influences mothers have on offspring phenotype. They include nest site choice, egg brooding, genomic imprinting, and the nutrients and hormones mothers allocate to their eggs. The nutrients mothers allocate to their young depend not only on the mechanisms underpinning reproductive allocation, but also on the nutritional resources available to her during reproductive allocation. We used a well-studied set of populations of declining Murray River Short-necked Turtles (*Emydura macquarii*) to investigate the mechanistic causes of maternal effects in oviparous vertebrates. *Emydura macquarii* is a generalist that consumes green algae, aquatic plants, periphyton, aquatic invertebrates, fish, and carrion. We studied *E. macquarii* in four populations in north-central Victoria that differ in adult diet. We show that females from herbivorous/generalist populations exhibit higher body condition indices than do females from more carnivorous, and potentially food-limited, populations. These constraints could limit the total amount of resources females can allocate to eggs, and thus constrain reproductive output. They could also constrain the specific macronutrients that females can allocate to yolk. Our study aims to determine how maternal diet constrains a range of reproductive variables with implications for offspring fitness. We report differences in clutch size and egg size that may indicate food constraints on reproductive output. The remainder of the study is in progress, but we aim to test for effects of population-of-origin (diet) on the following variables: egg composition, embryo metabolism, hatching success, hatchling growth rate, hatchling metabolism, and hatchling locomotor performance. Furthermore, because *E. macquarii* egg and hatchling physiology differ with laying order within females, we also aim to determine how egg composition varies with laying order. In addition to advancing knowledge of maternal effects, our results provide initial understanding of how environmental constraints on reproduction translate to fitness impacts on offspring of a declining species.



Evolution of plasticity: mechanistic link between development and reversible acclimation

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Phenotypic characteristics of animals can change independently from changes in the genetic code. These plastic phenotypic responses are important for population persistence in changing environments. Plasticity can be induced during early development, with persistent effects on adult phenotypes, and it can occur reversibly throughout life (acclimation). These manifestations of plasticity have been viewed as separate processes. In a recent review, we argue that developmental conditions not only change mean trait values but also modify the capacity for acclimation. Acclimation counteracts the potentially negative effects of phenotype-environment mismatches resulting from epigenetic modifications during early development. Developmental plasticity is therefore also beneficial when environmental conditions change within generations. Hence, the evolution of reversible acclimation can no longer be viewed as independent from developmental processes.

Life at low pH: The mechanistic basis for tolerance of extremely low pH by *Limnodynastes terraereginae* larvae

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Aquatic pHs below 5.0 are toxic to most freshwater animals. This toxicity arises largely through the perturbation of ionic homeostasis – low pH disrupts epithelial integrity and compromises ion uptake machinery. Unusually for amphibians, embryos and larvae of the Australian frog, *Limnodynastes terraereginae* can hatch and develop in water as low as pH 3.0 making them one of the most acid tolerant vertebrates on the planet. This study explored the mechanistic basis for tolerance of extremely low pH by larvae of *L. terraereginae*. Larvae were reared from embryos at either pH 3.5 or circumneutral (6.0-6.5). Branchial morphologies, whole body sodium fluxes and epithelial ion transporter expression patterns were compared across treatments. Larvae reared at pH 6.5 and acutely exposed to pH 3.5 suffered a net loss of sodium as a consequence of the inhibition of sodium uptake; efflux rates were not significantly different from those of larvae reared and tested at pH 6.5. However, larvae reared and tested at pH 3.5 suffered no net loss of sodium, suggesting that sodium uptake mechanisms are plastic and can acclimate to restore sodium homeostasis in *L. terraereginae*. Compared with other amphibian species, larvae of *L. terraereginae* have a very high affinity sodium transport system which may allow them to take up sodium from naturally dilute waters. However, the nature of the transporters responsible for sodium uptake *in L. terraereginae* remain unclear. *L. terraereginae* larvae reared at low pH are able to resist the damaging actions of H+ on epithelial junctions, possibly through increased mucus production which serves to create a semi-permeable barrier between the tissue and the acidic environment. Taken together, exploitation of extremely low pH environments by larvae of *L. terraereginae* appears to be achieved through both physiological and morphological adaptations which protect both sodium uptake capabilities and epithelial integrity.

Hypoxia tolerant species take advantage of intracellular acidosis to maintain mitochondrial function

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Fish inhabit environments with variable oxygen supplies, in particular for intertidal fishes. Problematically, hypoxia promotes anaerobic metabolism, lactate accumulation and associated cellular acidosis. The brain is very sensitive to the accumulation of protons, and this likely impacts hypoxic brain mitochondria (mt). While mt consume oxygen (JO2) to generate chemical (ΔpH) and electrical (Δ) gradients across the inner-mt-membranes to produce ATP, the effects of extramitochondrial pH on brain mt function remains largely unexplored. We predicted that hypoxia-tolerant species (HTS) should better tolerate acidosis than hypoxia-sensitive species (HSS) in terms of buffering capacities and mt function and dynamic. Using high resolution respirometry we titrated lactic-acid to decrease extramitochondrial pH, and simultaneously follow JO2, Δm, H+ buffering capacities and mt swelling of brain mt within permeabilised brain and isolated mt. Four New Zealand triplefin fish species were compared, each with different hypoxia-tolerances and ranging from high intertidal to subtidal niches. While HTS and HSS displayed similar H+ buffering capacities (~5mU pH.mg-1), contrasting responses were found for mt function. In HSS 4 mM lactate elevated JO2, yet decreased Δm by ~5% with a mild acidosis (Δ pH -0.3) and mt were totally uncoupled mt at pH 5.8. In contrast, 10 mM lactate (Δ pH -0.6) induced a 15% inhibition of JO2 in *Bellapiscus medius*, the most HTS. In B. medius Δ remained stable and coupling capacity at pH 5.8 was maintained to 30% of that at pH 7.2. Overall, these data indicate that in the HTS B. medius decreased pH supresses JO2 yet maintains phopsphorylation integrity to extremely low pH.

Parental environment affects offspring's responses to UV-B

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The parental environment plays an important role in determining the ability of offspring to cope with their environment. Such developmental plasticity is beneficial when parental and early developmental and later offspring environments are matched, but it can be detrimental if there is a mismatch. UV-B radiation damages cells directly and by increasing reactive oxygen species (ROS) formation. Our aim was to test whether parental exposure to UV-B increases offspring ROS defence mechanism to reduce the negative effects of UV-B in offspring. To test our hypothesis, we raised juvenile guppies (*Poecilia reticulata*) under UV-B and control (no-UV-B) conditions to mature and breed. After parturition, offspring from each parental group were transferred to UV-B and control treatments and raised to maturity. Our results showed that exposing parents to UV-B increased the resilience of their offspring to the negative effects of UV-B. When exposed to UV-B, offspring from parents also exposed to UV-B had significantly greater sustained swimming performance, which was paralleled by increased catalase activity and glutathione concentrations, and reduced ROS damage to membrane and proteins, compared to offspring from control conditions. However, parental exposure to UV-B increased damage to proteins and infection rates by white spot protozoan in control offspring. There was no effect of parental exposure to UV-B on offspring superoxide dismutase activity, resting and active metabolic rates, or offspring size. We showed that parental exposure to UV-B can be beneficial only when offspring were also exposed to UV-B. The trade-off between the beneficial effects of parental exposure to UV-B on offspring also exposed to UV-B environment and the increased susceptibility to infection in offspring not exposed to UV-B can be important in determining the resilience of populations in variable environments.

Is hypoxia tolerance reflected in New Zealand Triplefin brains? (Tripterygiidae): Analysing enzyme activities and metabolites

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University of Auckland

Adult mammalian brains are extremely sensitive to reduced oxygen, failing within minutes of anoxia. Hypoxia enhances anaerobic metabolism, causing redox imbalance, and lactate and glutamate accumulation which potentially leads to excitotoxicity and cell death. In comparison, some aquatic animals routinely survive levels of less than 3% atmospheric oxygen. We have found that New Zealand triplefin fish, especially intertidal rock pool species, are robust to low oxygen repeatedly experiencing hypoxia or near anoxia, while maintaining brain function. Investigations into mechanisms enhancing survival of the brain of these fish should be informative as to how humans could increase hypoxia tolerance (HT).

As part of a larger project five New Zealand triplefin species ranging from intertidal (experience hypoxia) to subtidal zones (do not experience hypoxia) were investigated as they show different degrees of HT. Two key mechanisms thought to be impacting HT were examined. Firstly, the activity of key enzymes was assessed to determine their contributions to overall metabolism. The second mechanism to compliment this is analysis of abundance in metabolites between species subjected to a 20 minute hypoxic event. Enzymatic activity was determined spectrophotometrically, whilst Gas Chromatography-Mass spectrometry (GC-MS) was used to determine concentrations of key metabolites (sugars, organic acids, fatty acids, lipids and proteins). Preliminary data has found that anaerobic associated lactate dehydrogenase activity was surprisingly lower in HT species than hypoxia sensitive species (420±27 & 520±20 umol/min-1/g-1 respectively). However, HT species had ~22% greater creatine kinase activities compared to their hypoxia sensitive relatives. Glutamate dehydrogenase, citrate synthase, malate dehydrogenase, and adenylate kinase all showed no relationship between enzyme activity and HT. Primary results from GC-MS show hypoxia influences metabolite abundance among species. Overall these results suggest HT species may have decreased anaerobic capacities in terms of lactate formation, but may sustain hypoxic function using creatine phosphate stores.

Synchronous hatching in freshwater turtles: metabolic and endocrine mechanisms

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Synchronous hatching is a form of environmentally cued hatching (ECH) which allows embryos to alter the time of hatching in relation to the environment through phenotypic plasticity. Synchronous hatching in turtles has evolved to reduce variation in incubation time and increase an individual's chance of survival. Egg position and thermal gradients in a nest alter developmental rates of embryos, and have potential to cause asynchronous hatching times. Cues from more advanced eggs stimulate less advanced eggs to either hatch early or accelerate development through metabolic compensation. Hormones likely play a critical role in enabling metabolic compensation and early hatching. Thyroid hormones and glucocorticoids regulate embryogenesis and are vital during birth/hatching events in many species. I compared the metabolic and endocrine mechanisms of hatching synchrony in freshwater turtles. Metabolic compensation and changes in circadian rhythms enabled embryos to adjust their developmental rates and catch-up to more-advanced eggs. Hormone analyses indicated there was no difference in triiodothyronine (T3) and corticosterone concentrations during asynchronous development thus are not responsible for stimulation of metabolic compensation. There was however a clear physiological response to exogenous T3 applications, which caused neonates to hatch earlier than expected but with no developmental or metabolic costs. Triiodothyronine and corticosterone concentrations increased in the yolk of the developing embryos towards the end of incubation in both asynchronous and synchronous clutches, which coincides with hatching. These hormones might not regulate metabolic compensation, but they are likely important for synchronous hatching. Synchronous hatching has evolved independently in different turtle lineages and the specific mechanisms utilised to achieve synchronous hatching in each species likely maximise an individual's chance of survival. Comparing the mechanisms used to synchronously hatch further improves the understanding of the different ways in which ECH has evolved in reptiles.

Regulatory elements that drive corticotropin-releasing hormone receptor 2 gene expression in avian thyrotropes

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In representative non-mammalian vertebrates, CRH has a potent thyrotropin (TSH)-releasing effect in addition to its corticotropin (ACTH)-releasing effect. In chickens, the TSH-releasing activity by CRH is mediated by type 2 CRH receptor (CRHR2) in the thyrotropes (TSH-producing cells) of the pituitary gland. However, it is not known whether CRH controls TSH release in other avian species that have different thyroidal activity during their life cycle. Therefore, we investigated the TSH-releasing capacity of CRH in an altricial species, the zebra finch (*Taeniopygia guttata*). The cellular localisation of CRHR2 mRNA in the pituitary was determined by in situ hybridisation, combined with immunohistochemical staining of pituitary thyrotropes. Our study showed that CRHR2 mRNA is present in the zebra finch thyrotropes, similar to what was previously found in chicken pituitary. Furthermore, isolated zebra finch pituitaries stimulated with 100 nM CRH showed increased secretion of TSH-like activity as measured in a thyroid bioassay. These results suggest that the CRHR2 expressed on thyrotropes are responsible for TSH release by CRH in altricial avian species like in precocial species. We then attempted to identify the regulatory elements in the gene promoter of CRHR2 in chicken. Since two transcription factors, Pit1 and GATA2, determine thyrotrope differentiation and maintain expression of the TSHβ subunit, we hypothesized that the thyrotrope-specific expression of CRHR2 is also governed by the same regulatory elements and transcription factors. Fragments of the putative chicken CRHR2 promoter were fused to the luciferase reporter gene and co-transfected with Pit1 and/or GATA2 expression plasmids into COS7 or murine thyrotrope-derived TαT1 cells. Luciferase reporter assays showed that GATA2 but not Pit1 activated the CRHR2 promoter. These results indicate that CRHR2 expression is induced by GATA2 activity as is the case for the TSHβ gene, but in contrast to TSHβ, the mechanism does not require Pit1 binding.

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

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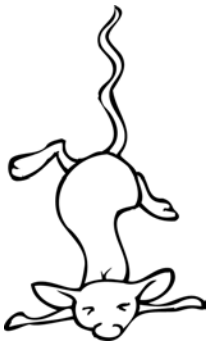
The evolution of endothelium-derived nitric oxide (NO) in blood vessels, and its associated signalling pathway, remains contentious in comparative cardiovascular physiology. The advancement of comparative genomics has provided insight into the evolution of the NO synthase (NOS) enzymes (NOS1, NOS2 and NOS3), in particular that of NOS3 that is expressed in the endothelium of mammalian blood vessels. Until recently, the parsimonious view was that NOS3 evolved early in the tetrapods, as all three isoforms are found in amphibians, but only two isoforms (NOS1 and NOS2) are found in the genomes of teleost and chondrichthyan fishes. Recently, all three NOS proteins have been predicted in the genome of the non-teleost actinopterygian, *Lepisosteus oculatus*, which reshapes our understanding of NOS3 evolution. This study aimed to characterise the nos3 gene and determine if NOS3 is a functional protein within the vasculature of *L. oculatus*. The predicted nos3 gene did not demonstrate a conserved synteny with the nos3 gene of mouse and Xenopus nos3, compared to the conserved synteny that is found for nos1 and nos2. Phylogenetic analysis showed that the predicted NOS3 protein grouped with other vertebrate NOS3 proteins; *L. oculatus* NOS1 and NOS2 grouped with their respective isoforms. To confirm the genomic sequence, we have cloned 57% of the *L. oculatus* nos3 mRNA, which was found to be 99% similar to the predicted sequence. We then designed a specific *L. oculatus* NOS3 antibody, and demonstrated NOS3-immunoreactivity in the adventitia of many blood vessels. To determine if the presence of NOS3 within the vasculature contributed to vasodilation, myography was performed on the dorsal aorta. Interestingly, addition of the NO donor, sodium nitroprusside, had no effect on vascular tone. Together, these data suggest that *L. oculatus* does express a NOS3 protein within the vasculature, but it does not appear to contribute to vascular regulation.



The role of leptin and ghrelin in appetite regulation in the Australian Spinifex hopping mouse, *Notomys alexis*, during long-term water deprivation

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Many mammals live in deserts where drinking water is absent and survive by gaining preformed and/or metabolic water from food and employing a suite of adaptations to reduce water loss. However, given the importance of food as a source of water, little is known about how desert mammals can regulate appetite to increase consumption of preformed water and substrate for metabolic water production. In the Spinifex hopping mouse*, Notomys alexis*, water deprivation (WD) induced a biphasic pattern of food intake with an initial hypophagia that was followed by an increased, and then sustained food intake. The mice lost approximately 20% of their body mass, and there was a loss of white adipose tissue. Stomach ghrelin mRNA was significantly higher at day 2 of WD but then returned to the same levels as water-replete (day 0) mice for the duration of the experiment. Plasma ghrelin was unaffected by WD except at day 10 where it was significantly increased. Plasma leptin levels decreased at day 2 and day 5 of WD, and then increased significantly by the end of the WD period. Water deprivation caused a significant decrease in skeletal muscle leptin mRNA expression at days 2 and 5, but then it returned to day 0 levels by day 29. In the hypothalamus, WD caused a significant up-regulation in both ghrelin and neuropeptide Y mRNA expression, respectively. In contrast, hypothalamic ghrelin receptor mRNA expression was significantly down-regulated. A significant increase in leptin receptor mRNA expression was observed at days 17 and 29 of WD. This study demonstrated that the sustained food intake in *N. alexis* during WD was uncoupled from peripheral appetite-regulating signals, and that the hypothalamus appears to play an important role in regulating food intake; this may contribute to the maintenance of fluid balance in the absence of free water.

SPEED TALK

DNA damage induced by ultraviolet radiation is repaired faster by enzymatic photoreactivation than by nucleotide excision repair in an amphibian species

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Ultraviolet radiation (UVR) has been an important environmental stressor and selection pressure for biological organisms since life began. UVR absorbance by important molecules within cells can initiate a range of photochemical reactions that are both detrimental and beneficial to living organisms; UVR causes injury to cells by damaging DNA, proteins and lipids, but is also necessary for vitamin D synthesis, DNA repair and vision. As a consequence of anthropogenic ozone depletion the amount of damaging UVR reaching the biosphere has increased. Increased UVR is suspected to have contributed to the rapid decline of numerous amphibian populations around the world. Exposure to UVR is known to have negative consequences for the health and survival of the embryos and tadpoles of several amphibian species. However, like other organisms, amphibians have evolved a number of defences against the damaging effects of UVR, including the repair of DNA damage. Enzymatic photoreactivation (EPR) and nucleotide excision repair (NER) are two processes by which DNA damage can be repaired. The efficacy of EPR is known to vary among amphibian species and is negatively correlated with UVR sensitivity, but less is known of NER. Here we present our findings on the rate of DNA repair by EPR and NER in tadpoles of the striped marsh frog *Limnodynastes peronii*. Our data show that EPR is considerably more effective at removing DNA damage than NER in *L. peronii* with 50% of the damage repaired after 1.4 h by EPR and 42 h by NER. Without EPR to repair DNA damage, tadpole survival was significantly reduced between 18 and 24 h, adding support to our finding that NER is not an effective repair mechanism in this amphibian species.

The hypothalamo-pituitary-adrenal axis in the fat-tailed dunnart (*Sminthopsis crassicaudata*), an Australian marsupial

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As wildlife management decisions can be informed by the understanding of how an animal responds to threats, the stress physiology of the fat-tailed dunnart (*Sminthopsis crassicaudata*), an Australian marsupial, was studied. Like most vertebrates, marsupials respond to stressors with the activation of the hypothalamo-pituitary-adrenal (HPA) axis. Upon perception of a stressor, the hypothalamus releases corticotropin-releasing hormone (CRH), which stimulates the anterior pituitary gland to secrete adrenocorticotropic hormone (ACTH) into the systemic circulation. This hormone, in turn, stimulates the adrenal cortex to release glucocorticoids, the actual stress hormones. We cloned dunnart prepro-CRH and the ACTH precursor pro-opiomelanocortin (POMC) mRNA and determined the glucocorticoid response to restraint stress. Restraint stress was found to effectively induce a response within 30 minutes, with free cortisol levels increasing more than those of corticosterone, even with the corticosterone concentrations being more abundant in the circulation in unstressed animals. The dunnart coding sequences of POMC and prepro-CRH encode peptides of 399 and 200 amino acids, respectively. While the ACTH and beta-endorphin sequences within POMC are highly conserved, the POMC sequence shows some unique features in this species, and perhaps all Australian marsupials, including the secondary loss of a gamma-melanotropin sequence and duplications of the ACTH sequence, possibly as a result of aberrant crossing-over events. Mature dunnart CRH is identical to CRH in human, mouse, rat and chicken, and as a consequence, tools used to study CRH in these species are predicted to work in the dunnart as well.

Diving in a warming world: Environmental and physiological determinants of dive duration in crocodiles

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Temperature has a pervasive effect on physiological function and organismal performance. Dive duration of ectotherms is influenced by physiological responses and capacities and has been shown to be highly thermally dependent, where elevated temperatures can significantly reduce dive times. Given that diving plays a significant ecological role in crocodiles, including foraging and predator avoidance, understanding the potential effects of predicted rises in global temperatures on this critical behaviour is of interest. Using field data and taking an experimental approach we examined the thermal sensitivity of diving in estuarine crocodiles and explored the flexibility and plasticity of physiological systems to buffer the negative impacts of elevated temperatures on dive duration. Crocodiles were exposed to long-term thermal treatments, designed to emulate water temperatures under different climate change scenarios and physiological responses and dive capacities tested. The ability of crocodiles to mitigate the effects of temperature on diving ability via thermal phenotypic plasticity and compensation will be discussed.

An estimate of the water budget for the endangered night parrot of Australia under recent and future climates

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Endangered species management must now incorporate the potential effects of climate change, but this is often in the context of limited data. The endangered night parrot was recently rediscovered in the Australian arid zone and a major effort is underway to ensure its survival. A key question is to what extent it is dependent on standing water under current and future climates, as this has major implications for understanding and managing its habitat requirements. However, very little is known about its ecology and physiology, and its conservation status precludes invasive ecophysiological studies. Here we show how the methods of biophysical ecology permit strong inferences about this problem with minimal data. We developed a biophysical model of both the parrot and its habitat at the only known site of occurrence. We used allometrically-adjusted observations of the known physiology of a closely related desert-adapted Australian parrot, the budgerigar, to infer unknown aspects of the night parrot's physiological responses, together with plumage measurements from museum specimens. We tested the microclimate model against empirical data on microhabitat temperatures and compared the endotherm model predictions against an infra-red thermograph of the bird itself. We then used the model to predict the frequency with which the parrot would need to find standing water under current and future climates depending on the water content of its food. Our field data show that air temperature in night parrot roosts during high summer typically exceeds the inferred resting core temperature (38 °C) and can exceed 45 °C. Our calculations imply that night parrots can persist on dry seed during winter conditions without exceeding dangerous levels of dehydration, but would need access to water or succulent (55% water) food during summer. Air temperature at the site is projected to increase 3 °C by 2070, which would lead to likely lethal (22% of body mass) levels of daily dehydration in some years even on succulent food, and would dramatically increase its dependence on standing water. Our findings have significant implications for the conservation management of the night parrot and provide guidance for future research priorities.

Stressor interactions shape upper thermal limits

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The vulnerability of organisms to climate warming can be predicted by how close species are to their upper thermal limits, and their capacity for plasticity in this trait. In addition to temperature, many biotic and abiotic factors can alter thermal tolerance and shape the sensitivity of organisms to rising temperatures. Importantly, it has been demonstrated that interactive effects between multiple stressors shape physiological tolerances. As such acclimation capacity of upper thermal limits should be assessed in relation to variation in multiple environmental stressors. If cross tolerance between stressors results in changes to upper thermal limits, single factor studies may poorly predict thermal tolerance under natural conditions. Here we examine the interactive effects of temperature and ultraviolet radiation on the upper thermal limits of tadpoles (*Limnodynastes peronii* and *Platyplectrum ornatum*) in the context of stable and fluctuating temperature variation. Temperature and ultraviolet radiation had interactive effects on the upper thermal limits of *L. peronii* tadpoles in stable temperature condition. Exposure to ultraviolet radiation increased upper thermal limits of tadpoles in cold temperatures, but reduced upper thermal limits of warm acclimated tadpoles. Under fluctuating temperature conditions exposure to ultraviolet radiation increased the upper thermal limits of *P. ornatum* tadpoles, but reduced upper thermal limits under stable mean temperatures. These data demonstrate that exposure to ultraviolet radiation can modulate the effects of temperature on upper thermal limits, and highlight the importance of assessing stressor interactions when predicting responses to environmental variation. Species survival in the face of climate warming will be determined by the interactive effects of temperature with multiple natural and anthropogenic factors.

Mitochondrial plasticity as a determinant of surviving climate change

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The thermal limit of cardiac mitochondrial efficiency could be a major determinant of species distribution (1). The effects of high temperature on brain mitochondria is less well explored. We examined the effect of 6 temperatures on brain mitochondrial function in homogenates from two Orectolobiform sharks which occupy shallow reef flats exposed to thermal stress during summer low tides and/or periodic nocturnal hypoxia. Epaulette sharks (*Hemiscyllium ocellatum*), can undergo metabolic depression but Grey carpet sharks (*Chilloscyllium punctatum*) cannot, yet both can withstand comparable periods of anoxia. We measured the effect of each temperature on: i) mitochondrial coupling efficiency; ii) non-phosphorylating proton leak from mitochondria; and iii) changes in substrate utilisation for complex I and complex II.

High resolution oximetry with a multiple substrate-inhibitor protocol revealed that for both species: mitochondrial coupling (efficiency) was greatest at 25oC, and maintained at 30oC but was 25% lower at 37oC and 50% lower at 40oC. Mitochondria in both species were totally uncoupled at 45oC.

Despite an exponential increased in proton leak as temperature increased, Epaulette mitochondria maintained their electron transport system in coupled mitochondria at 25-37oC, while Grey carpet shark mitochondria showed a 30% decrease in mitochondrial efficiency at 37oC compared to 25oC. Examination of substrate utilisation revealed that Epaulette shark mitochondria had a more stable complex 1 utilisation than Grey carpet sharks, especially at 37oC.

These two species of Orectolobiform sharks maintained mitochondrial coupling efficiency over a wide range of thermal challenges but failed at temperatures higher than they currently encounter in their natural environment.

1. Reference: Iftikar et al., (2014) J. Exp. Biol. 217, 2348-2357.

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