

ANZSCP



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Proceedings



UNIVERSITY
OF TASMANIA

Hosted by the
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School of Medicine
University of Tasmania

ANZSCP

Proceedings, Volume 20

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20th Annual Conference
5th – 7th December 2003

Organised by
Stewart Nicol

Hosted by Anatomy & Physiology, School of Medicine,
University of Tasmania



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Contents

Acknowledgements	3
Program overview	5
Program	6
Oral abstracts	11
Poster abstracts	41
Registrants	54
Maps	62



The 20th annual conference is supported by generous contributions from the following:

Sigma Delta Technologies

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Phil Withers

Prizes

Springer-Verlag is pleased to provide a year's subscription to Journal of Comparative Physiology – B as a student prize

Phil Withers, University of Western Australia has offered a prize consisting of a free lifetime copy of the statistics program statistiXL for the best use of statistics and graphics in a student talk. The value of the prize is \$US 75. A trial version of the software is available from <http://www.statistixl.com>

The organisers gratefully acknowledge the help and assistance of:

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Brita Hansen and Bill Halliwell, tiger logo design



Program overview

Thursday 4th December

5-7pm Registration including drinks and nibbles University Staff Club

Friday 5th December

8.30-9.00am Posters to be placed on poster boards. 3rd floor
 Medical Sciences Building
 9.00am Welcome Medical Sciences
 Lecture Theatre
 (directly in front of building)
 9.20am Session 1
 10.40am Morning tea and posters 3rd floor
 Medical Sciences Building
 11.00am Session 2
 12.40pm Lunch 3rd floor
 Medical Sciences Building
 2.00pm Session 3
 3.40pm Afternoon tea and posters
 4.00 - 5.40pm Session 4
 6.30pm - 8pm Wine and cheese Art Mob gallery
 17 Castray Esplanade

Saturday 6th December

9.00am Session 5
 10.40am Morning tea and posters
 11.00am Session 6
 12.40am BBQ Lunch "in the gully"
 2.00pm Session 7
 3.40pm Afternoon tea and posters
 4.00-5.40pm Session 8
 7.00pm Dinner University Staff Club

Sunday 7th December

9.00am Session 9
 10.40am Morning tea and prizes
 11.00am Session 10
 12.40pm Lunch

There is a location map at the back of this book.

Parking

Parking is available near the venue in College Rd. All parks bounded in yellow markers (non-permit parking) are free. Please note that parks with white markings are permit-only parks and you will be booked if you park here during office hours (8.45am-5.15pm Monday- Friday).

University Security phone 6226 7600 (24 hours)

Banks

There is a Commonwealth Bank teller machine located outside the Union Building, building number 21 on the campus map.

Taxis City Cabs 131 008

Airporter Bus Service 199 Collins St, Hobart, ph 0419 382 240



Full Program

Thursday 4th December

5-7pm Registration including drinks and nibbles University Staff Club

Friday 5th December

8.30-9.00am Posters to be placed on poster boards. 3rd floor
Medical Sciences Building
9.00am Welcome Medical Sciences
Lecture Theatre
(directly in front of building)

Session 1

9.20am *Birgus latro* excretes its nitrogenous wastes as a mixture of urate and guanine.

Stuart M. Linton and Peter Greenaway

9.40am Molecular cloning, localization and *in vivo* expression of a putative NHE2 isoform in the gills of a euryhaline fish (*Fundulus heteroclitus*).

Susan L. Edwards and James B. Claiborne

10.00am The response of the atrial natriuretic peptide system of the Spinifex Hopping-mouse to water deprivation

Rachel Heimeier and John Donald

10.20am Sympathetic perivascular nerves of the toad have both vasoconstrictor and vasodilator properties

Brad Broughton, Michelle Thacker, Susan Murphy and John Donald

10.40am Morning tea and posters 3rd floor
Medical Sciences Building

Session 2

11.00am The effect of amoebic gill disease and other stressors on natriuretic peptide receptor activity in the gills of Atlantic salmon, *Salmo salar*

Hamish McWilliam, Mark Powell, and Tes Toop

11.20am Immunolocalisation of natriuretic peptide receptors in fish kidneys

Jillian Healy, Tes Toop, John Donald, Susumu Hyodo, and Yoshio Takei

11.40am Toxicity of tannic acid and its derivative products on the growth rate of *Neoparamoeba pemaquidensis*

Tim Green, Mark Powell and James Harris

12.00pm Increased systemic vascular resistance in Atlantic salmon *Salmo salar* affected with amoebic gill disease

Melanie J. Leef, James O. Harris and Mark D. Powell

12.20pm Metabolic rate comparisons between Atlantic salmon, *Salmo salar*, infected with *Neoparamoeba pemaquidensis* or *Tenacibaculum maritimum*

James Harris, Melanie Leef and Mark Powell

12.40pm Lunch 3rd floor
Medical Sciences Building



ANZSCPB conference 2003

Program

- 10.40am Morning tea and posters
- Session 6**
- 11.00am Predicting breeding outcomes of Gould's Petrels (*Pterodroma leucoptera*) from an appraisal of parental body condition during incubation
Terence O'Dwyer, David M. Priddel, and William A. Buttemer
- 11.20am Role of VEGF and neutrophils in endometrial angiogenesis in oestrogen treated ovariectomised mice.
Jane Girling, Bambang Heryanto and Peter Rogers
- 11.40am Beauty and the Boss - the effects of age and social hierarchy on timing of oestrus in the Tasmanian devil (*Sarcophilus laniarius*)
Heather Hesterman and Susan M. Jones
- 12.00pm Timing of hibernation and reproduction in Tasmanian echidnas
Stewart Nicol, Niels A Andersen and Sue Jones
- 12.20pm Delight, decline, disease and death in a small marsupial (*Antechinus subtropicus*)
Adrian Bradley
- 12.40am BBQ Lunch "in the gully"
- Session 7**
- 2.00pm Flexible fermentation in the arctic: seasonality of ruminal digestion in muskoxen
Perry S. Barboza and T.C. Peltier
- 2.20pm BMR and FMR of the numbat: how low is low?
Philip Withers and Christine Cooper
- 2.40pm The inter-relationship between diet, digestibility, and energy and water turnover for the numbat (*Myrmecobius fasciatus*).
C.E. Cooper and P.C. Withers
- 3.00pm The influence of foraging mode and arid adaptation on burrow construction, thermoregulation and metabolism of burrowing mammals
Craig R. White
- 3.20pm A tropical rainforest possum that can't take the
Andrew K. Krockenberger
- 3.40pm Afternoon tea and posters
- Session 8**
- 4.00pm The effect of metabolic fuel availability on thermoregulation and torpor in marsupials
Wendy Westman, Bronwyn McAllan, and Fritz Geiser
- 4.20pm Metabolic and Osmotic Consequences of Aestivation in Cocoon Forming Frogs
Victoria Cartledge, Philip Withers, and Don Bradshaw
- 4.40pm Renal Physiology of Western Australian Agamid Lizards
Stewart Ford, Don Bradshaw and James E. O'Shea
- 5.00pm Evidence of a temporary adaptive shift of optimal physiological temperature of the viviparous lizard *Niveoscincus microlepidotus* during pregnancy
Alexander P. Kabat and Roy Swain
- 5.20pm Water acquisition and water conservation in two disjunct populations of the Western Tiger Snake, *Notechis scutatus*.
Mitchell Ladyman, Alex Larcombe and Don Bradshaw.



7.00pm Dinner University Staff Club

Sunday 7th December

Session 9

9.00am Resting metabolisms of adult and juvenile red kangaroos (*Macropus rufus*), how different are they?

Adam Munn and Terence Dawson

9.20am Does muscle mitochondrial and capillary morphology in red kangaroos match aerobic capacity as in placental mammals?

Terry Dawson and Brock Mifsud.

9.40am Energetics, evolution and ecology of mammalian hopping locomotion

Koa N Webster and Terence J Dawson

10.00am Does specific dynamic action offset the energy cost of heating a cold meal in wandering albatrosses?

Harry Battam, Mark A. Chappell, and William A. Buttemer

10.20am Do *Pax* genes play a role in arthropod muscle regeneration?

Robert White, Tina Lamey, Mel Ziman, & Annette Koenders

10.40am Morning tea and prizes

Session 10

11.00am Marsupial shivering and non-shivering thermogenesis: a continuum?

M Ikonomopoulou, A Kabat and **R Rose**

11.20am Heat Reward for Insect Pollinators

Roger S. Seymour Craig R. White and Marc Gibernau

11.40am The use of bioelectrical impedance to predict body water and lipid

David T. Booth and Beth L. Symonds

12.00pm Evolution of transthyretin: from bacteria to plants and humans

Samantha J. Richardson, Sarah C. Hennebry, Hannah M. Wright and Julie A. Monk

12.20pm Deiodinases in the Australian lungfish, *Neoceratodus forsteri*

Jean Joss and Margareta Sutija

12.40pm Lunch

Posters

P1 Nasal anatomy of the marsupial mouse, *Antechinus subtropicus*.
Rachel C. Aland and Adrian J. Bradley

P2 Why do musk oxen eat snow?
F. Kohl and **P.S. Barboza**

P3 The effect of water deprivation on the expression of the tonicity-responsive enhancer binding protein in the Spinifex hopping mouse, *Notomys alexis*
Ray Bartolo and John Donald



- P4 Reproductive behaviour in the Julia Creek Dunnart (*Sminthopsis douglasi*)
Alice Bjursell, Kerry Withers, Graeme Senior, Geoff Lundie-Jenkins and Petrina Kennerly
- P5 Metabolic energetics and biomechanics of pentapedal locomotion in Red Kangaroos
Donelan, JM, Rodoreda, S, Grabowski, A; Kram, R and **Dawson, TJ**.
- P6 Working to identify an exciting steroid in a viviparous skink, *Tiliqua nigrolutea*
Ashley Edwards, Susan M. Jones and Noel W. Davies
- P7 Autonomic and behavioural fever in an ectotherm
Bruce T. Firth and Ingrid Belan
- P8 Embryonic, gonadal and sexual organ development in the small viviparous skink, *Niveoscincus ocellatus*
Linda Neaves, Erik Wapstra, Debra Birch, **Jane Girling**, Jean MP Joss
- P9 How do echidnas manage to breathe when they burrow into soft soil?
Gordon Grigg, Lyn Beard, Peter Brice and Morag Wilson
Powerpoint presentation on a laptop, viewer prompted.
- P10 Faecal sex steroid monitoring reveals the reproductive cycle of the Malayan Sun bear (*Ursus malayanus*), an endangered tropical ursid
Heather Hesterman¹ and Samuel K. Wasser²
- P11 The effect of regular swimming training on basal metabolic rate of *mdx* mice
Rebecca Higgins, Kerry Withers and Andrew Hoey
- P12 The effect of water deprivation on neural nitric oxide synthase and soluble guanylyl cyclase in the cane toad, *Bufo marinus*.
Brett Jennings and John Donald
- P13 The comparative expression of uncoupling proteins in marsupials and a monotreme.
Kabat, A. P., R. W. Rose, and A. K. West
- P14 Messenger RNA expression of natriuretic peptide receptors in freshwater and seawater rainbow trout gills
Scott Nankervis, Mark Powell and Tes Toop
- P15 The influence of photoperiod on the reproductive activity of female honey possums, *Tarsipes rostratus* (Marsupialia: Tarsipedidae): assessed by faecal progestagens and oestradiol-17
Jessica Oates, Don Bradshaw and Felicity Bradshaw
- P16 Reduced sensitivity of spermatogenesis to high temperatures in *Pseudomys australis*
BP Setchell, CM Leigh and WG Breed
- P17 Molecular cloning of brain natriuretic peptide from the heart of reptiles
Sofie Trajanovska, Ray Bartolo, Yoshio Takei and John Donald
- P18 Who's been nibbling the Bunya's nuts?
Kerry Withers, Ray Marshall, John Billingsley, Kim Larsen, David Schiefelbein, Martin Jastroch, Patrick McConnell, Neil McKilligan and Geoff Lundie-Jenkins



Oral abstracts

Echidna reproduction and growth: What we currently know (or don't)

Niels A. Andersen¹, Jutta Schmid² and Stewart C. Nicol¹

¹Anatomy and Physiology, University of Tasmania, Private Bag 24, GPO, Hobart 7001, Tasmania

²Department of Experimental Ecology, University of Ulm, Germany

Despite the discovery in 1884 that echidnas were oviparous, more than 200 years later we still have only very limited information on the life history of echidnas even though they are one of Australia's most widely distributed mammal species. This lack of information is compounded by significant regional differences in many aspects of echidna biology. We will report on our findings in a Tasmanian echidna population and compare these to findings from other populations.

Since 1996 we have been studying an echidna population in the Tasmanian southern midlands, and have tagged 155 echidnas in this time. We have found a 1:1.3 sex ratio (60 males : 71 females) in contrast to Kangaroo Island where a sex ratio of 2M:1F has been reported. The use of spurs in determining the sex of adult echidnas has been long controversial but this appears to be a regional difference. In sexing Kangaroo Island echidnas Rismiller and coworkers have found no clear pattern of spur distribution between sexes, while we have never found mature female echidnas with spurs or mature males without spurs.

Mating behaviour and timing vary between the different geographic areas of echidna distribution Rismiller (2003). In Central Tasmania all echidnas hibernate each year for 3 1/2 to 7 months depending on their reproductive status (Nicol & Andersen 2002). We have never observed mating activity prior to the middle of June in contrast to Kangaroo Island where echidna mating trains have been reported as early as May (Rismiller & Seymour 1991).

Six echidnas were tagged as burrow young and one of these has been successfully followed during its first 2 years of life, providing the first information on growth rate of a free ranging juvenile of known age. Despite the high growth rates of suckling echidnas (from 0.5 to 1500g in 7 months) we could not measure any difference in daily energy expenditure (DEE) or water turnover rates between lactating and non-lactating female echidnas during spring.

Due to seemingly large regional differences, generalised statements on echidna behaviour and reproduction are difficult to make until a lot more data from different areas have been collated.



Flexible fermentation in the arctic: seasonality of ruminal digestion in muskoxen

Perry S. Barboza¹ and T.C. Peltier^{1,2}

¹Institute of Arctic Biology and Department of Biology and Wildlife, University of Alaska Fairbanks.

²Alaska Department of Fish and Game

Muskoxen consume fibrous diets such as sedges where plant growth is highly seasonal. Nutrients and energy must be accrued rapidly from a low-quality diet to meet demands for survival and reproduction through the ensuing 9 months of winter and spring. Ruminal fermentations may be unsuited to seasonal hyperphagia because digestion of high loads of fibre may be constrained by ruminal capacity, digesta flow, and by the activity of microbes.

We measured food intake and digestive efficiency of muskoxen (n=8) in a repeated measures design without changing the quality of the diet across seasons. We fed grass hay (*Bromus* sp.) with two mineral supplements of different protein content during spring (April - May), autumn (August - September) and winter (February - March) to mimic energy and protein content of the wild diet without constraining the supply of other nutrients. All animals were castrated to remove the effect of reproduction, and fistulated at the rumen to measure degradation of each dietary component within permeable bags.

Muskoxen increased food intakes by 74% between spring and autumn (2.75 vs. 4.75 kg.d⁻¹) as body mass and body fat increased by 17% and 58% respectively from spring to winter. Ruminal substrate loads of cellulose and N were increased by 59% and 135% respectively from spring to autumn. Degradation rates within the rumen increased with food intake from spring to autumn for dry matter (0.20 to 0.50/d) and cellulose (0.29 to 0.59/d) in hay. Accretion of N on ruminal residues of hay indicated greater microbial attachment to particles in spring and winter than in autumn. Ruminal degradation of supplements also increased from spring to autumn for both C (0.52 to 0.75/d) and N (0.32 to 0.55/d). Ruminal pH probably reflected short chain fatty acid production because pH was lower in autumn (6.7) than winter (6.9), and declined within 6h of feeding supplements in each season.

These data suggest that microbial activities increase with seasonal hyperphagia in muskoxen independent of changes in diet quality, rumination or particle flow. Increased fermentative digestion may allow muskoxen to maximize net gains of both energy and protein with minimal foraging effort in small home ranges during autumn. The cause of subsequent declines in microbial activity during winter is unclear but may reflect changes in the size and composition of the microflora in response to substrate flow, temperature or secretions from the host.



Does specific dynamic action offset the energy cost of heating a cold meal in wandering albatrosses?

Harry Battam¹, Mark A. Chappell², and William A. Buttemer¹

¹Institute for Conservation Biology, Department of Biological Sciences, University of Wollongong

²Biology Department, University of California, Riverside.

Albatrosses are large marine carnivores, with the majority of species inhabiting the cold and temperate regions of the southern oceans. They use low-cost transport to locate food that may be spaced by up to several thousand kilometres. Food is vigorously contested and individuals typically engorge when finding prey, with meal sizes of 25 per cent of body mass being typical for albatross species > 2kg. Because their prey are ectotherms, albatrosses must warm a substantial mass of near-freezing food, an energy requirement equivalent to ca. 7% of the metabolisable energy content of the food. This energy may be supplied by endogenous stores, or possibly from heat associated with the specific dynamic action (SDA) of feeding.

Cephalopods are a significant component in albatross diets, and a large cuttlefish, *Sepia apama* is a regular food source for albatrosses off Wollongong NSW. In this study the SDA of albatrosses fed *S. apama* was measured and compared with heating requirements predicted by a thermal model of heating a cold meal. For albatrosses, SDA can contribute significantly to the warming of large, cold meals when digestion is commenced soon after ingestion. To contribute to meal warming, SDA must be coincident with meal heating requirements. For 33% of birds measured, SDA was adequate to meet the full energy cost of heating the meal. In the remaining 66%, SDA was delayed to the extent that some or all of the energy cost of meal warming would require use of endogenous stores.

The use of bioelectrical impedance to predict body water and lipid

David T. Booth¹ and Beth L. Symonds

Department of Zoology & Entomology, The University of Queensland.

The aims of many wildlife projects include the non-destructive measurement of body condition as reflected by body water and fat content. To date such assessments have been made by "indexes" usually involving the ratio of a linear body measurement divided by body mass, or by dilution of isotopically labelled water. Here we report the application of bioelectrical impedance technology to assess body water content and by inference body fat content. We developed algorithms for estimating total body water in cane toads, freshwater turtles, crocodiles and koalas from whole body electrical impedance measurements. We also developed algorithms for estimating body fat in toads and crocodiles. The bioimpedance algorithms predicted total body water with a high degree of accuracy, but the ability to predict fat content from water content was less reliable, principally because of the low fat content of the animals being analysed. We conclude that bioimpedance is a safe and rapid way of un-invasively measuring total body water content of animals, but extrapolating body fat content from body water is somewhat unreliable, especially in animals with low fat content.



Delight, decline, disease and death in a small marsupial (*Antechinus subtropicus*)

Adrian Bradley

Department of Anatomy & Developmental Biology, The University of Queensland, Queensland

Antechinus subtropicus is a small insectivorous dasyurid marsupial found in SE Queensland. The species shares the unusual semelparous life history strategy that occurs in several other dasyurids. In the wild all males disappear from the population by the end of September showing evidence of Selye's stress related *Diseases of Adaptation*. This study was undertaken to describe endocrine and CNS involvement in the stress related pathologies.

In males, but not in females, an increase in plasma androgens during the reproductive period depresses plasma corticosteroid-binding globulin resulting in concomitant increases in plasma cortisol (GC) being expressed in the free (biologically active) compartment. A failure of GC feedback control results in positive feedback that further elevates free cortisol. The brain of males undergoes atrophy with the hippocampus, which is vulnerable to GC excess, showing a decrease in dendritic spine density consistent with reduced connectivity. Cognitive abilities, including short-term memory and spatial ability, are compromised. Other pathological changes occur in gastrointestinal and renal systems. In the former, intravascular injection of FITC-albumin reveals that the mucosal microvasculature becomes leaky during combined physical and psychological stress while in the latter, necrosis of the proximal convoluted tubule occurs in conjunction with glomerular endothelial abnormality, basement membrane thickening, podocyte secondary foot process fusion and mesangial overgrowth into the glomerular capillary lumen. These pathological changes are consistent with the reliance of males upon the use of body tissues to provide energy for the considerable reproductive effort, an effective adaptive strategy that in the longer term results in the development of pathologies and ultimately death.

Sympathetic perivascular nerves of the toad have both vasoconstrictor and vasodilator properties

Brad Broughton¹, Michelle Thacker¹, Susan Murphy² and John Donald¹

¹School of Biological and Chemical Sciences, Deakin University, Geelong, Victoria

²Department of Anatomy and Cell Biology, University of Melbourne, Parkville, Victoria

Previously in the systemic vasculature of the toad, *Bufo marinus*, we have demonstrated via anatomical and physiological approaches that neural nitric oxide synthase (nNOS) is located within perivascular nerves (called nitrergic), and produces NO regulation of vascular tone. Thus, it was important to classify whether the nNOS was located in sympathetic, parasympathetic or sensory nerves. Using nNOS and tyrosine hydroxylase (TH: a marker for sympathetic nerves) antibodies, double labelling immunohistochemistry (IHC) revealed co-localisation between sympathetic and nitrergic nerves in the central blood vessels. In addition, we examined whether the cell bodies that give rise to the perivascular nerves in the systemic vasculature were chemically coded for nNOS and TH. Double labelling IHC in the sympathetic paravertebral ganglia demonstrated that 95% of the cell bodies that expressed TH immunoreactivity, also showed nNOS immunoreactivity. Approximately half the TH/nNOS cell bodies also showed neuropeptide Y immunoreactivity in the cell bodies of the sympathetic paravertebral ganglia. These results show for the first time that sympathetic vasoconstrictor neurones have the capacity to act as vasodilator neurones.



Aerobic performance as a functional biomarker of sublethal pesticide exposure.

William A. Buttemer¹, Paul G. Story^{1,2}, Karen J. Fildes¹, and Lee B. Astheimer¹

¹Institute for Conservation Biology, Dept. of Biological Sciences, University of Wollongong, Wollongong, NSW, Australia 2522

²Australian Plague Locust Commission, Canberra, ACT, Australia 2601

A large proportion of the vertebrate fauna of Australia is highly endemic, but very little is known about their sensitivity to pesticides. Such information is important, as locust control operations occur in habitats that support a range of native Australian vertebrates. Accordingly, we are investigating the sublethal consequences of pesticide exposure in selected native vertebrates. Included in our physiological measures is the appraisal of aerobic exercise performance.

We measured aerobic metabolism during cold exposure and while running at 1.1 m sec⁻¹ in the fat-tailed dunnart *Sminthopsis crassicaudata*, before and after ingestion of 30 mg kg⁻¹ of fenitrothion, an organophosphate pesticide. Plasma cholinesterases returned to pre-dose levels within 5 days of pesticide ingestion. Running endurance declined by over 50% for up to 5 days after dosing, but metabolic rate while running at 1.1 m/sec (8.9 x BMR) and cost of transport were unaffected. Peak metabolic rate (PMR) and cumulative oxygen consumption during a 1-h exposure to conditions equivalent to -20 °C did not change following OP ingestion, with PMR averaging 10 times BMR. We conclude that fenitrothion-induced exercise fatigue is not due to limitations in oxygen or substrate delivery or muscle uptake per se, but more likely relates to decreased ability to sustain high-frequency neuromuscular function.

We have also evaluated flight PMR in birds before and after organophosphate exposure and have found, as in dunnarts, that aerobic performance takes longer to recover than does plasma cholinesterase activity.

These results demonstrate that animals exposed to organophosphate pesticides are physiologically compromised much longer than suggested from their plasma cholinesterase levels. This has important implications for predicting the persistence and magnitude of pesticide effects on free-living animals.

Metabolic and Osmotic Consequences of Aestivation in Cocoon Forming Frogs

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Twenty seven of Western Australia's 77 frog species survive in arid environments. All WA members of the burrowing genera *Neobatrachus* (7) and *Cyclorana* (7) form a body surrounding covering known as a cocoon during aestivation. The cocoon, formed by the successive layering of skin, serves as an effective barrier to water loss. Many *Cyclorana* species of Western Australia inhabit areas where rainfall is associated with unpredictable cyclonic events. These frogs must trade-off dedicating resources to reproduction against maintaining resources to ensure they survive for the maximum possible length of time. This presentation will address the metabolic and osmotic consequences of aestivation in the cocoon forming species *Cyclorana platycephala* and *Cyclorana maini* which occur in unpredictable rainfall environments and *Cyclorana australis* which is found in the Kimberleys where they experience an extended wet season. Metabolic rates were used in conjunction with the level of energetic reserves to calculate how long these species are capable of surviving in a state of aestivation. Electrolytes, urea and the antidiuretic hormone, arginine vasotocin (AVT) were assayed in plasma from active and laboratory aestivated frogs. These data were used to evaluate whether there is a relationship between AVT and the concentration of the body fluids in frog species with a relatively water-impermeable cocoon.



Aerobic scope is independent of temperature and primarily modulated by heart rate in exercising Murray cod (*Maccullochella peelii peelii*)

Timothy Clark¹, Pat Butler² and Peter Frappell¹

¹Adaptational and Evolutionary Respiratory Physiology Laboratory, Department of Zoology, La Trobe University, Melbourne, VIC 3083, AUS.

²School of Biosciences, University of Birmingham, Edgbaston, Birmingham B15 2TT, UK

Populations of Murray cod (*Maccullochella peelii peelii*) in the Murray-Darling Basin of southeastern Australia have declined to such a degree that the species has recently been listed nationally as vulnerable. In an attempt to understand the energy requirements and associated cardiovascular adjustments of this species, heart rates (f_H) and oxygen consumption rates ($\dot{V}O_2$) of six Murray cod (mean mass \pm S.E., 1.89 ± 0.12 kg) were determined during exposure to varying levels of swimming speed and temperature. Both $\log f_H$ and $\log \dot{V}O_{2, ADJ}$ (normalised $\dot{V}O_2$ to a mass of 1 kg) increased with swimming speed up until approximately 1.2 body lengths s^{-1} (~ 0.6 m s^{-1}), after which both variables increased to a lesser extent suggesting that such a swimming velocity is close to the aerobic maximum. An increase in water temperature from 14°C to 29°C resulted in linear increases in both f_H and $\dot{V}O_{2, ADJ}$ during mild exercise, but a decrease in the amount of oxygen extracted per heart beat (termed 'oxygen pulse'). Similar relationships were determined during maximum exercise, suggesting that the total factorial scopes of f_H , $\dot{V}O_{2, ADJ}$ and oxygen pulse remain the same across the temperature range. The total factorial aerobic scope (3.7) of the Murray cod determined during exercise was primarily modulated by increases in f_H (2.2-fold increase) rather than oxygen pulse (1.7-fold increase), which is in contrast to the established suggestion that, during exercise, the teleost heart predominantly alters stroke volume rather than frequency. While fish were in holding tanks, the effect of feeding was an increase in average f_H due to an increase in the incidence of spontaneous activity, thus suggestive of a higher energy expenditure during periods of food availability. A systematic change in f_H and $\dot{V}O_{2, ADJ}$ occurred with body temperature (T_b) and exercise, thus enabling a prediction of the metabolic rate of free-swimming fish using a combination of f_H and T_b . It is calculated for this species that up to 78% of metabolizable energy may be available for growth, and this supports previous observations that the Murray cod is a fast-growing species.



The inter-relationship between diet, digestibility, and energy and water turnover for the numbat (*Myrmecobius fasciatus*).

C.E. Cooper and P.C. Withers

Zoology, School of Animal Biology M092, University of Western Australia, Crawley Western Australia 6009

The numbat is a small-medium sized marsupial, now found only in the south-west of Western Australia. It is unique as it is the only exclusively termitivorous marsupial, and as a consequence is exclusively diurnal. The restricted termite diet of the numbat allows me to examine in detail the interrelationships between diet, digestibility, metabolic rate and water turnover rate. The FMR of the numbat ($269 \pm 30.5 \text{ kJ day}^{-1}$) was lower (but not significantly) than that of a generalised marsupial, and this reflects its slightly (but not significantly) low BMR. The numbat's FMR was comparable with that of other arid-habitat Australia marsupials. The field water turnover rate (FWTR) of free-living numbats ($84.1 \text{ ml H}_2\text{O d}^{-1}$) was typical (89-98 %) of that for other marsupials. The high water economy index (WEI) for the wild numbats ($\text{FWTR}/\text{FMR} = 0.30 \pm 0.03$) suggests that either the numbats were drinking during the study, the water content of their diet was especially high, or the digestibility of their termite diet was especially low. To examine the interrelationship between WEI, digestibility and water content of termites and ants, a theoretical model for a myrmecophagous diet was constructed to evaluate the measurements for field numbats. Digestibility by captive numbats for termites was determined by feeding trials to be 64 % (*Nasutitermes* sp.) to 81 % (*Coptotermes* sp.), which correspond to a water economy index of 0.25 (*Nasutitermes* sp.) to 0.20 (*Coptotermes*). The WEI for wild, free living numbats of 0.30 corresponds to a lower digestibility of 58 %. For numbats, and other termitivorous mammals as well as reptiles, the WEI is higher than would be expected for an animal-based diet, due to the similar water content, but relatively low digestibility (58 to 81 %) of termites and ants compared to other animal-based foods.

Does muscle mitochondrial and capillary morphology in red kangaroos match aerobic capacity as in placental mammals?

Terry Dawson and Brock Mifsud.

School of Biological, Earth and Environmental Sciences
University of New South Wales, Sydney NSW 2052.

Weibel, Taylor and Hoppeler and co-workers have established that for many mammals their aerobic capacity, as represented by their VO_2max , is similarly matched to the mitochondrial and capillary morphology of the bulk of skeletal muscle, with humans possibly being an exception. There are at least 125 million years of separate evolution between the marsupials and placentals as well the unusual locomotory energetics of macropodid marsupials. Consequently, we considered that an examination of the red kangaroo would clarify the generality such features across therian mammals. We examined the mitochondrial and capillary morphology of skeletal muscle of red kangaroos using electron microscopy and the techniques established by Weibel, Taylor and Hoppeler and co-workers.

The red kangaroo is a highly muscular mammal, about 50% of body mass is muscle, and it has a high VO_2max . Samples from muscles throughout the body show a high capillary volume density and capillary blood volume. In fact, despite being a marsupial and having unusual locomotor energetics the red kangaroo matches very closely the basic aerobic characteristics of placentals. It has overall features similar to those seen in athletic placental mammals, such as dogs and horses. Apparently the essential features of mammalian aerobic energetics have a very long history.



Fever in marsupials: Thermoregulatory responses of *Monodelphis domestica* and *Petaurus breviceps* to bacterial endotoxin

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It is well known that bacterial endotoxin will generally induce a hyperthermic response in eutherian mammals and birds. However, such a response has not been well documented in marsupial species. The purpose of this study was to determine whether two small non-macropod marsupial species exhibit a febrile response to bacterial endotoxin (*E.coli* LPS). Five laboratory-bred *Monodelphis domestica* and one captive *Petaurus breviceps* were implanted with temperature-sensitive radio transmitters and placed in a linear temperature gradient ranging from 15°C to 40°C. Recordings of body temperature and preferred ambient temperature were measured simultaneously after an injection of saline (i.m.) and, 24 hours later, after an injection of LPS (i.m.). A typical mammalian hyperthermic fever response was seen in response to LPS in both species and a significant reduction in preferred Ta was observed in *P. breviceps*. However, preferred Ta was not significantly affected by fever in *M. domestica*. This study clearly indicates that marsupials exhibit fever and some species may have the ability to utilise their environment to modulate their febrile response.

Molecular cloning, localization and *in vivo* expression of a putative NHE2 isoform in the gills of a euryhaline fish (*Fundulus heteroclitus*).

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The current models for branchial acid excretion in fishes include Na⁺/H⁺ exchange and the electrogenic excretion of H⁺ via H⁺-ATPase. The predominant route of acid excretion in some freshwater fishes is widely thought to be via the H⁺-ATPase/Na⁺ channel system. We hypothesize that in marine and some brackish water fishes this mechanism plays a lesser role with the predominant mechanism being the Na⁺/H⁺ exchanger (NHE). In this study using molecular biology techniques we have cloned the full-length transcript of a gill NHE from the seawater adapted mummichog. The cDNA encodes a polypeptide of 717 amino acids, which shows a 57% homology to the rat NHE2 isoform. Protein expression analysis using mammalian NHE antibodies has identified multiple NHE-like immunoreactive proteins in gill membrane preparations from both freshwater and seawater-adapted mummichogs. Acidosis induced by ambient hypercapnia, resulted in an increase in gill NHE2 protein in freshwater adapted mummichogs while in seawater adapted animals there was no consistent NHE2 protein expression.

**Eat big, die young: the biochemistry of calorie restriction in mice.**Sally C. Faulks¹, Nigel Turner², Paul L. Else² and A.J. Hulbert¹.^{1,2}Metabolic Research Centre & ¹Department of Biological Sciences & ²Department of Biomedical Science, University of Wollongong, Wollongong, Australia 2522

Dietary calorie restriction is the only treatment known to extend lifespan in a wide range of animals. The mechanisms behind this anti-aging ability are unknown, but metabolism and oxidative stress are both thought to play a role. In this study, parameters relating to both these theories were examined to gain insight into important aspects of calorie restriction's action. Weanling male mice were fed one of four food energy intakes: 40, 50, 85 or 125 kcal/wk, and their physiology and biochemistry investigated after 1 and 3 months of treatment. Body mass and growth were significantly greater ($p < 0.05$) with increased calorie intake. The increased body mass was in part due to a greater body fat content in the non-calorie restricted mice. Total oxygen consumption varied directly with calorie intake, however mass-specific metabolic rate was not different between groups. There were also no consistent changes in daily activity levels. At 3 months, calorie restriction had no significant effect on either production of Reactive Oxygen Species by liver and muscle mitochondria or the NADPH/NADP⁺ ratio in liver, suggesting that these aspects of oxidative stress do not change with such short term calorie restriction. The most significant effects of calorie restriction were observed in the membrane phospholipid fatty acid composition. In liver, kidney and brain tissue and liver mitochondria, the levels of 18:2n-6 (linoleic acid) were higher in calorie restricted mice, whereas the opposite trend was observed with 20:4n-6 (arachidonic acid), 22:6n-3 (docosahexaenoic acid) and the peroxidation index. These alterations represent a shift towards membrane fatty acids with less double bonds, reducing the susceptibility of the membrane to damage from lipid peroxidation. The early appearance of these significant changes suggests that decreased vulnerability to lipid peroxidation may be a primary mechanism of calorie restriction's lifespan-extending ability.

Renal Physiology of Western Australian Agamid LizardsStewart Ford¹, Don Bradshaw¹ and James E. O'Shea¹¹School of Animal Biology, The University of Western Australia
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Agamid lizards are a diverse family inhabiting a range of environments from mesic to arid. Our research aims to characterise the relationship between their renal physiology and morphology and to determine whether renal function is correlated with the environment inhabited by a given species. Classic measures of renal clearance have been coupled with morphological analysis to examine whether such patterns exist. We have estimated renal physiological parameters including urine flow rate, glomerular filtration rate and renal blood flow in a range of species using [³H]inulin and [¹⁴C]PAH. Additionally, morphological data such as the scaling of kidney mass with body mass have been collected which allow us to examine the form-function relationship in the renal system of these animals. This information will aid in our understanding of how different species have adapted to their particular environment.



Physiological responses to hypoxia: modulation by *Siah2*

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Hypoxia-inducible factor-1_α (HIF1_α) is a central regulator of the cellular response to hypoxia (for example, angiogenesis, vascular remodelling, erythropoiesis, glucose transport and cell survival, to name a few). Cellular O₂ concentration is a central determinant of HIF1_α expression and in non-hypoxic conditions HIF1_α is constantly degraded through ubiquitination and proteasomal destruction. In brief, the degradation of HIF1_α in normoxia requires O₂-dependent hydroxylation of HIF1_α as a prerequisite for binding of the von-Hippel-Lindau protein which forms an E3 ubiquitination ligase complex, resulting in targeting of HIF1_α for proteasomal destruction. *Siah2* limits the hydroxylation during hypoxia and is one mechanism underlying the increase in HIF1_α expression during hypoxic conditions.

Siah2 null mice subjected to chronic hypoxia exhibit a defect similar to that of HIF1_α heterozygous mutant mice in possessing an attenuated polycythemia and reduced levels of hemoglobin. Different to wild type mice, *Siah2* null mice exposed to acute hypoxia showed a complete lack of hyperneic response and instead achieved an appropriate level of hyperventilation through a dramatic decrease in metabolic rate. These results suggest that *Siah2* is important for the ventilatory response to acute hypoxia. Presumably the absence of *Siah2* changes the expression of various genes encoding receptors or neurotransmitting enzymes in the chemoreceptors involved in the transduction of the hypoxia signal to the central nervous system. Following three days in hypoxia *Siah2* null mice had slightly improved their hyperventilatory response but more importantly had restored metabolic rate to pre-hypoxic values. This finding supports the notion that hypometabolism is not a long-term survival strategy during exposure to hypoxia.

A population of captive bandicoots with individuals showing differing reproductive capability

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Is the bandicoot, *Isodon macrourus*, a seasonally breeding marsupial? Births have been observed throughout the year. Nevertheless the majority of births take place from July through to February and births are infrequent in the months March to June. In this study the births in a captive population of bandicoots were monitored from 1977 to 2002 and evidence will be presented showing that individuals having differing reproductive capabilities.

A total of 240 female bandicoots gave birth to 444 litters over the 26 years. The majority of births, 90%, were observed in the months July through to February, the breeding season. The remaining 10% of births were observed from March to June, the non-breeding period. The population of bandicoots can be divided into two groups, the 90% having a definite anoestrous period. However the remaining 10% of the bandicoots do not display this definite anoestrous period and may give birth throughout the year.

This variation in reproductive ability may be due to genetic difference and/or response to environmental factors. The bandicoot has been said to be an opportunistic breeder increasing the length of the breeding season when conditions are favorable. If conditions continue to be favorable over several years the bandicoot becomes a continuous breeder. Needless to say if conditions deteriorate then the bandicoot shows seasonality in its breeding pattern.



Role of VEGF and neutrophils in endometrial angiogenesis in oestrogen treated ovariectomised mice.

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Based on observations in human endometrium, it has been hypothesised that estrogen-induced endometrial angiogenesis is mediated by the potent endothelial cell mitogen vascular endothelial growth factor (VEGF) that is delivered to the endometrium by intravascular neutrophils. The aim of this study was to investigate the role of intravascular neutrophils in initiating endothelial cell (EC) proliferation in ovariectomised mouse endometrium. Uterine tissues were collected from ovariectomised C57/CBA female mice 24 h after oestradiol treatment with or without systemic neutrophil depletion. Neutropenia was achieved with either an in-house anti-neutrophil serum (ANS) or Gr-1 antibody. All mice received an i.p. injection of bromodeoxyuridine (BrdU) 4 h prior to dissection to allow visualization of proliferating cells using immunocytochemistry. Endometrial sections were immunostained for BrdU, vascular endothelial growth factor (VEGF), and neutrophils (using ANS). Oestrogen treatment of ovariectomised mice significantly increased the number of intravascular neutrophils, whereas induction of neutropenia with either ANS or Gr-1 in conjunction with oestradiol treatment prevented this increase. Oestrogen treatment of ovariectomised mice also significantly increased the number of intravascular VEGF-positive cells; however, whereas induction of neutropenia with ANS significantly reduced this increase, Gr-1 did not. In both studies, neutropenia significantly reduced, but did not eliminate, the amount of endometrial endothelial cell proliferation. These results suggest a role for neutrophils in endometrial angiogenesis following acute oestrogen treatment; however, the presence of VEGF-positive cells even after induction of neutropenia suggests another leucocyte may also be involved.

Toxicity of tannic acid and its derivative products on the growth rate of *Neoparamoeba pemaquidensis*

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Amoebic gill disease (AGD) is the most significant disease of the Tasmania Atlantic salmon industry and is caused by the marine parasitic amoeba *Neoparamoeba pemaquidensis*. Current commercial treatment for the disease is a freshwater bath for 2-6 h. It is known that water chemistry affects the efficacy of freshwater as a treatment for AGD; increasing total water hardness (Ca^{2+} and Mg^{2+} ions) reduces the amoebocidal properties of freshwater. To date no one has examined other water chemistry parameters that chelate Ca^{2+} and Mg^{2+} ions. Tannins occur naturally in plants as natural defence mechanisms against microbial infection and are water-soluble compounds. Tannins leach out of decaying plant material and wash into creeks and streams where they have been shown to reduce the concentration of free Ca^{2+} and Mg^{2+} ions and inhibit the growth of a wide range of aquatic pathogenic bacteria. The purpose of this study was to determine if tannins have amoebocidal properties towards marine amoebae.

The toxicity of tannin was determined by isolating *N. pemaquidensis* from the gills of deceased Atlantic salmon showing gross signs of AGD and determining their growth rates in seawater with varying concentrations and types of commercially available forms of purified tannins using a seawater culture toxicity assay. It was found that tannic acid (20 mg.l^{-1}) and propyl gallate (50 mg.l^{-1}) were amoebostatic to marine amoebae. Gallic acid and methyl gallate at the highest concentrations tested (50 mg.l^{-1}) had no effect on the growth rate of *N. pemaquidensis* in seawater. Therefore, tannins may have potential as agents for the control of AGD.



The oesophageal groove reflex in adult sheep and goats

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The oesophageal groove mechanism allows milk to bypass the fermentation of the forestomachs of neonatal ruminants. The groove closure reflex can still be initiated in adult animals, but its control and function is not well understood. There are substantial differences between sheep and goats in their water economy. This difference is driven largely by differences in the dynamics of fluid movements in the gut.

The aim of this experiment was to see whether sheep and goats differ in the extent of liquid bypass of the rumen via the oesophageal groove.

Five merino sheep, 5 Angora and 5 Australian feral goats all with rumen fistulae were fed a roughage diet *ad libitum* and allowed free access to water. A comparison of orally and intraruminally administered CrEDTA marker dynamics was made.

Based on differences in marker concentrations and the subsequent calculations for rumen volume and turnover rate, it was concluded that about 20% of ingested fluid bypasses the rumen in all three animal types. The much lower water requirement of goats compared to sheep is not related to differences in fluid bypass of the foregut.

Monitoring the impact of cane toads on native frogs in the Northern Territory

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Lack of reliable data about the impact of cane toads on native fauna prompted us to develop a novel automated method which can monitor calling activity of native frogs every night for a year or more without attention. The software relies on machine learning technology and identifies frogs to species by their calls, logging the information to flash card along with temperature and rainfall. We installed 10 solar powered and tamperproof systems along 100 kilometres of the Roper Valley Highway east of Mataranka in 1997-98. These sites were overrun progressively by toads, the first pair in the first wet season, with the last pair being overrun by early 2000. At every site the number of frog species recorded calling decreased markedly over the five year period. There have been significant and substantial declines in the number of days of records for at least seven of the 21 monitored species, with possible increases in two. Although these data suggest that toads may have a detrimental effect on frogs, we cannot unequivocally attribute the declines we have observed to the impact of toads. We are collecting data also from six sites within Kakadu National Park, just now being invaded by toads.

We are at present extending the methodology to include image as well as acoustic logging. The method has a wide range of potential applications, including outside biology.



Metabolic rate comparisons between Atlantic salmon, *Salmo salar*, infected with *Neoparamoeba pemaquidensis* or *Tenacibaculum maritimum*

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Atlantic salmon, *Salmo salar* L., have been farmed successfully in Tasmania for many years, however, there are diseases that may limit future expansion of this industry. In seawater, salmon are susceptible to the protozoal condition known as Amoebic Gill Disease (AGD), and to a lesser extent, the bacterial disease caused by *Tenacibaculum maritimum*. Both conditions have implications for gas exchange across the gills. The metabolic rate and scope of Atlantic salmon were measured in four respiratory chambers, containing 100 l of recirculating seawater. Inlet oxygen levels were measured daily, and outlet oxygen levels were measured continuously. Three experimental runs were conducted, firstly with naive salmon inoculated with AGD on day 5, then naive salmon, followed by naive salmon inoculated with *T. maritimum* on day 5. On days 2 and 8 of all trials, active metabolic rate was estimated after exhaustively exercising the fish for 10 min in hyperoxic conditions. On day 10, fish were bathed in seawater containing 10 mg.l⁻¹ chloramine-T to assess the metabolic cost of such a treatment to diseased salmon. Standard metabolic rate was estimated from the lowest hourly mean during each night, and routine metabolic rate was estimated from the 24 h mean. There were no changes to Rs after each chase, however, for Atlantic salmon infected with AGD a rise in Rs was observed from day 5 onwards. Results for salmon infected with *T. maritimum* will be included in the presentation.

Immunolocalisation of natriuretic peptide receptors in fish kidneys

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Natriuretic peptide hormones are linked to osmoregulation and cardiovascular regulation in fishes. The peptides bind to two guanylyl cyclase (NPR-GC)-linked membrane bound receptors, NPR-A and NPR-B, to elicit their physiological effects via the second messenger, cyclic GMP. The fish kidney has an important role in osmotic homeostasis and volume regulation and, therefore, the location of the NPR-GCs in the kidney can provide insight on the potential renal function of natriuretic peptides. This study used affinity purified polyclonal antibodies to NPR-A and NPR-B to determine their location in the kidney of the Japanese eel, *Anguilla japonica*. Kidneys from freshwater and seawater-acclimated eels were fixed overnight in 4% paraformaldehyde before paraffin embedding and immunostaining. NPR-A immunoreactivity was found on the apical membrane of proximal tubule 1 and the vascular endothelium, including the glomerular capillaries. In contrast, NPR-B immunoreactivity was located on the smooth muscle of blood vessels including the glomerular afferent and efferent arterioles and on smooth muscle tissue surrounding the collecting ducts. No differences in the distribution of NPR-GC immunoreactivity were observed between freshwater and seawater-acclimated eels. These results indicate that, whilst utilizing the same second messenger system, ANP and CNP act on different targets within the kidney and, presumably, elicit different effects.



The response of the atrial natriuretic peptide system of the Spinifex Hopping-mouse to water deprivation

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Atrial natriuretic peptide (ANP) regulates water and salt homeostasis by mediating natriuresis and diuresis upon binding to its receptor, natriuretic peptide receptor type-A (NPR-A). In animals that live in desert environments, it may be predicted that the ANP/NPR-A system would be down-regulated when water is limited, as the promotion of water and salt excretion by ANP would be detrimental for survival. This study investigated the effect of free water deprivation (3 and 7 days) on the expression of ANP and NPR-A mRNA, the plasma ANP concentration, and the ability of NPR-A to generate cGMP, in the Spinifex Hopping-mouse, *Notomys alexis*. To determine whether the ANP/NPR-A system in water-replete *Notomys* differs to that of a non-desert rodent, a comparison was made with a laboratory rodent, *Mus musculus*.

The levels of ANP mRNA expression in the heart remained unchanged, but in the kidney ANP and NPR-A mRNA levels were significantly increased in *Notomys* water-deprived for 7 days. Water deprivation for 3 days decreased the plasma ANP concentration, but no change was observed after 7 days. In addition, the ability of ANP to stimulate cGMP production was significantly increased in water-deprived *Notomys* in comparison to controls. The expression of ANP and NPR-A mRNA in the heart and kidney were lower in water-replete *Notomys* than in *Mus*, while renal ANP mRNA levels did not differ. Radioimmunoassay showed no significant difference in ANP plasma concentrations between control *Notomys* and *Mus*, but the ability of ANP to stimulate cGMP in kidney membranes was greater in *Notomys* than in *Mus*.

These observations show that water deprivation does not uniformly down-regulate the ANP/NPR-A system to conserve water, and that there was no consistent difference in the status of the system between *Notomys* and *Mus*.



Beauty and the Boss - the effects of age and social hierarchy on timing of oestrus in the Tasmanian devil (*Sarcophilus laniarius*)

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The devil (*Sarcophilus laniarius*) is the world's largest extant carnivorous marsupial, once also found on mainland Australia but now restricted to the island of Tasmania. Numbers in the wild were considered relatively stable until the recent outbreak of a contagious disease, which is currently devastating populations throughout the state. Although *S. laniarius* has been the subject of past research interest and also well represented in zoos and wildlife parks, our understanding of the species' reproductive biology is rather limited. The current concern for the status of wild devil populations, and their poor captive breeding record highlight the need for improved understanding in this area. Female devils from free-ranging and captive populations were sampled to provide detailed information on reproductive cycles and behaviour. Measurement of plasma progesterone (P4), assessment of vaginal smears and pouch development were used to characterise and monitor the ovarian cycle. To investigate breeding behaviour a concurrent study was undertaken for animals housed at a wildlife park. P4 levels, cytological and physical changes during the oestrous cycle were similar for wild and captive devils. Onset of breeding began in February and continued through to June and timing was closely correlated with each age cohort. The oestrous cycle (follicular and luteal phase) lasted ~35d with gestation being ~21d, and an inter-oestrus interval of ~56d in females that were unmated or lost their young. A characteristic pro-oestrus peak in progesterone was associated with a predominantly cornified vaginal smear (>95% mature epithelial cells), a maximal pouch score, and oestrus behaviour/copulation for captive devils. Groups of females housed together exhibited a distinct hierarchy of oestrus that was positively correlated with both age and individual dominance status.



Lifespan and metabolism: experiments with fruitflies and blowflies.

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In 1908 Max Rubner suggested the lifespan and the mass-specific metabolic rate of mammalian species were inversely related such that different mammal species consume the same amount of energy per unit body mass during their lifetime (their "lifetime energy potential") irrespective of their size, maximum lifespan and mass-specific metabolic rate. In the 1920s this was expanded by Raymond Pearl into the "rate of living theory" of aging. While there is equivocal evidence supporting this theory between species there has been little examination of it within a species. Here we report the results of a series of experiments examining the "rate of living theory" of aging in two insect species; the fruitfly *Drosophila melanogaster* and the blowfly *Calliphora stygia*. A population (n=65) of fruitflies were housed individually at 25°C and resting oxygen consumption of individual fruitflies was measured weekly throughout their life and their lifespan noted. Individual lifespan varied from 4 to 45 days (mean \pm SEM = 26.1 \pm 1.1 days) and resting oxygen consumption varied from 2.9 to 6.2 $\mu\text{O}_2 \cdot \text{h}^{-1}$. Mass-specific resting metabolism was constant throughout the lifetime of fruitflies (mean \pm SEM = 3.52 \pm 0.07 $\mu\text{O}_2 \cdot \text{mg}^{-1} \cdot \text{h}^{-1}$). There was no significant correlation between resting metabolic rate and lifespan and no suggestion of a "lifetime energy potential". Furthermore, calorie-restriction (the only treatment known to extend lifespan in a wide range of species) had no effect on mass-specific metabolic rate of fruitflies and the long-living *Chico* mutant strain of fruitflies had the same mass-specific metabolic rate as wild-type fruitflies. In a separate series of experiments on blowflies, daily sucrose consumption and lifespan of individuals were measured. There was no significant relationship between the food consumption and lifespan of individual blowflies. The results from all these experiments suggest that the "rate of living theory" of aging does not apply within a species.

Nitrogen metabolism in the northern brown bandicoot (*Isodon macrourus*)

Ian D. Hume and Vanesa Young

School of Biological Sciences A08, University of Sydney, NSW 2006

Maintenance nitrogen (N) requirements are determined with captive animals but they have direct relevance to the nutritional ecology of free-living animals. The maintenance N requirement of the northern brown bandicoot, *Isodon macrourus*, was determined with captive animals given diets based on a commercial small carnivore mix and shredded sweet potato, with 20% sand included to simulate the soil ingested by bandicoots during natural foraging. Regression of N balance on N intake yielded maintenance N requirements of 438 mg. kg^{-0.75}. d⁻¹ on a dietary basis and 413 mg. kg^{-0.75}. d⁻¹ on a truly digestible basis. These are high when compared with the range reported for other marsupial omnivores (46 – 146 mg. kg^{-0.75}. d⁻¹) and the eutherian mouse (265 mg. kg^{-0.75}. d⁻¹). Possible reasons include an energy deficiency on the lowest N diet which could have resulted in an over-estimate of up to 45%, an amino acid imbalance in all diets (but amino acid analyses suggested no marked deficiencies), high metabolic faecal nitrogen (MFN) due to the abrasive effect of sand on the digestive tract mucosa (but MFN was no lower without sand in the diets), or high rates of N loss in both faeces and urine as seen in mammalian carnivores. The northern brown bandicoot is perhaps the most insectivorous of all peramelid marsupials, suggesting that the high maintenance requirements for N are an intrinsic characteristic of the species. Low levels of recycling of endogenously produced urea to the gut, only 28% on the low N diet, are consistent with this suggestion. High N requirements restrict northern brown bandicoots to relatively productive coastal and sub-coastal areas throughout its range in New South Wales, Queensland, Northern Territory and Western Australia.



The metabolic and behavioural responses of the burrowing shrimp, *Trypaea australiensis* to hypoxia.

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Animals inhabiting hypoxic environments commonly have behavioural and physiological mechanisms that help them to cope with these conditions. Thalassinidean shrimp, which burrow in intertidal to subtidal estuarine regions, are routinely exposed to hypoxic conditions and are therefore likely to possess mechanisms for hypoxia tolerance. The metabolic responses of *Trypaea australiensis* to a range of oxygen tensions was examined to better understand how these shrimp deal with hypoxic exposure. Oxygen consumption rates and lactate levels were measured over a range of oxygen tensions and over varying periods of exposure. During both gradual and persistent hypoxic exposure to 10% oxygen saturation, *T. australiensis* dynamically reduced oxygen consumption rates to approximately 17% of that during normoxia, which is consistent with metabolic depression. Additionally, animals exposed to 10% oxygen saturation did not accumulate lactate over a 6h exposure period. Activity rates of animals decreased during hypoxic exposure and during a simulated tidal exposure further contributing to energy conservation. In their natural habitat animals showed a small accumulation of lactate, however only in the final hours of a 5-6h tidal exposure period. Following a severe hypoxic exposure of 6h, metabolic recovery was rapid, with oxidative balance restored and lactate levels reduced to control values by 2h. Therefore, a combination of metabolic depression and rapid recovery ensures the survival of *T. australiensis* during extended hypoxic conditions and that the shrimp will be metabolically replenished 2h after re-immersion of the burrow assuring readiness for the next low tide.

Deiodinases in the Australian lungfish, *Neoceratodus forsteri*

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Deiodinase enzymes regulate metabolism of the thyroid hormones at the cellular level in extrathyroidal tissues. They play an essential role in the local activation and deactivation (enzymatic deiodination) of the non-bioactive T4 prohormone. Three deiodinase enzymes (types I, II and III) have been characterised in all vertebrates except amphibians, which have only types II and III. All three deiodinases are selenoproteins, which are a family of proteins characterized by the presence of a selenocysteine amino acid in their active centre.

We have characterised the deiodinase enzymes in *N. forsteri*, by kinetic studies, sequencing and expression of the deiodinase genes. We have shown that in juvenile *N. forsteri*, both deiodinase type II (D2) and deiodinase type III (D3) occur in most tissues. D3 from liver has a Km in the nM range. The mRNA of this enzyme has the typical selenoprotein characteristics of an in-frame UGA codon and a SECIS element in the 3'-UTR. D2 has a Km also in the nM range, which is typical of all known D2s. D3 activity is higher than that of D2 but the activity of both is generally lower than that found in most vertebrates. To date we have no evidence for deiodinase type I (D1) presence in *N. forsteri*. The consequence of low D2 activity and no D1 would be very low tissue levels of the active thyroid hormone, T3. Since high T3 is required for metamorphosis, this is further evidence for neoteny in *N. forsteri*.



Evidence of a temporary adaptive shift of optimal physiological temperature of the viviparous lizard *Niveoscincus microlepidotus* during pregnancy

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Niveoscincus microlepidotus is a viviparous alpine lizard with a long pregnancy that lasts approximately 14 months. This provides significant experimental advantages because pregnant and non-pregnant animals can be studied simultaneously, thus avoiding confounding effects related to season. This long gestation requires the pregnant female to carry an increasing load as the clutch grows; which may have an escalating impact on her mobility. Female *Niveoscincus microlepidotus* in four reproductive states (non-pregnant, early-, middle-, and late-pregnancy) were tested for mobility (sprinting, climbing, jumping ability, and endurance capacity – all relevant to normal behaviour) at different temperatures. Early- or mid-pregnancy females did not show significant differences in sprinting, climbing and jumping performance, when tested at body temperatures at or below those previously described as optimal (26°C) for this species. There was a significant reduction in performance during late pregnancy at 26°C. However, performance was maintained at lower temperatures. Pregnant females also showed an apparent decrease in endurance capacity during pregnancy, which was most significant during the later stages, and this decrease in endurance capacity was greatest at temperatures above 26°C. These results indicate that changes in performance for *N. microlepidotus* are related to pregnancy and temperature. We suggest that pregnant female *Niveoscincus microlepidotus* temporarily decrease ecritic body temperature, by approximately 2°, which may be necessary a trade-off to facilitate the unusually long pregnancy.

A tropical rainforest possum that can't take the heat.

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The green ringtail possum (*Pseudocheirops archeri*) (Marsupialia: Pseudocheiridae) is a small marsupial folivore restricted to upland rainforest in Australia's Wet Tropics, possibly due to thermal intolerance. The lower limit of the altitudinal range of *P. archeri* is around 3-400 m, and it is abundant only at sites where the average maximum temperatures of the hottest week of the year are less than 30°C (Kanowski, unpublished). In the laboratory, *P. archeri* responded to ambient temperature in a normal mammalian way; increasing metabolism at low temperatures to maintain body temperature, and increasing evaporative water loss at high temperatures. However, at ambient temperatures above 30°C they did not evaporate sufficient water to maintain a constant body temperature and their body temperature rose linearly over time. I interpret this as a water conservation strategy. Highest annual temperatures occur in the late dry season when the canopy is dry. Water used in evaporative cooling could only be replaced by increasing water intake in foliage, also increasing intake of plant toxins and the risk of intoxication. I present the hypothesis that the distribution of this species is limited by the interaction between the duration of extreme temperatures, water availability and plant toxins. Such limitation also puts this species at great risk from the effects of climate change.



Water acquisition and water conservation in two disjunct populations of the Western Tiger Snake, *Notechis scutatus*.

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The Western Tiger Snake, *Notechis scutatus*, is typically found in association with fresh water. However, in Western Australia, populations exist on semi-arid offshore islands with no free water. We studied two neighboring conspecific populations of tiger snakes that occur in contrasting habitats (semi-arid Carnac Island vs. swamp-dwelling Herdsman Lake) to identify differences in water economics. Carnac Island (CI) snakes demonstrate a suite of behavioural traits that enhance their capacity to acquire water. For instance, CI snakes are able to immediately detect rainfall and they respond opportunistically to the occurrence of summer rain by drinking copious quantities in a short amount of time. Swamp-dwelling Herdsman Lake (HL) snakes do not exhibit this response. Carnac Island snakes also exhibit physiological differences from HL snakes that enable a reduction in total and cutaneous evaporative water loss. Furthermore, the low catchability of CI snakes during summer indicates that these snakes aestivate to conserve water when free water is not available. The relationship between temperature and water loss was used to calculate the potential water savings a CI snake could make by aestivation in sea-bird burrows. As the two contrasting study populations show little genetic divergence, acclimatization to each of these two contrasting habitats is most likely the factor determining the difference in rates of water loss. These data support the idea that habitat, not taxonomic position, has a greater influence on rates of water loss.



Increased systemic vascular resistance in Atlantic salmon *Salmo salar* affected with amoebic gill disease

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Amoebic gill disease is currently the most significant health issue affecting the production of Atlantic salmon, *Salmo salar*, in Tasmania (Munday et al., 1990). Recent studies have suggested there is an apparent association between field AGD infection and vascular hypertension (Powell et al., 2002a). AGD-affected fish appear to have extremely high blood pressures although the actual nature of the dysfunction is not understood. In a preliminary study, which aimed to further investigate the cardio-respiratory effects of AGD using a clinical laboratory based infection model, fish were placed in a stock infection tank for 3 days, a time period known to produce characteristic AGD lesions. Fish were surgically fitted with both a dorsal aorta (DA) catheter and cardiac flow probe in order to simultaneously measure dorsal aortic pressure (DAP) and cardiac output (flow). Results showed that whilst DAP was not significantly different between the two groups, fish affected with AGD had a significantly reduced cardiac output and subsequently greater systemic vascular resistance (R_s) at 4 and 6 h post surgery compared to control (naive) animals. These results were further investigated using the same infection and surgical protocol; however, following a 6 h recovery period, fish, both control and infected were given two injections in random order of Cortland's marine saline or sodium nitroprusside (SNP), a potent vasodilator, at $20 \mu\text{g.kg}^{-1}$. DAP and cardiac output were measured 0, 2, 5, 10, 15, 30, and 60 min post each injection. Additionally blood samples of $300\mu\text{l}$ were taken via the DA prior to the injections as pre-values and then post saline and SNP injection. Arterial pH (pH_a), haematocrit (HCT) and haemoglobin (Hb) were measured. Results showed again that whilst DAP were not significantly different, cardiac output was reduced and R_s was increased in AGD-affected fish in comparison to controls; however, treatment with SNP did appear to statistically attenuate the difference. Respiratory blood parameters showed no significant differences. It is believed that while SNP did have a small vasodilatory effect, it was not dramatic in reducing the R_s of AGD-affected fish, which if left un-treated, could further reduce cardiac output by increasing resistance to ejection and ultimately lead to cardiac failure. It is also thought that the hypertension seen in field AGD-affected fish, but absent in this study, may relate to the different infection regimes. In this study fish were exposed to an acute, but highly aggressive infection, and it is possible that 3 days is insufficient to see any hypertension develop.

Munday, B.L., Poster, C.K., Roubal, F.R. and Lester, R.G.L. (1990). Paramoebic gill infection and associated pathology of Atlantic salmon, *Salmo salar*, and rainbow trout, *Salmo gairdneri*, in Tasmania. In *Pathology in Marine Science* (Perkins, P.O. and Cheng, T.C., eds), pp. 215 – 222. San Diego: Academic Press.

Powell, M.D., Forster, M.E. and Nowak, B.F. (2002). Vascular hypertension associated with amoebic gill disease affected Atlantic salmon (*Salmo salar*) in Tasmania. *Bul. Euro. Ass. Fish Pathol.*, (in press).

***Birgus latro* excretes its nitrogenous wastes as a mixture of urate and guanine.**

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The anomuran land crab, *Birgus latro* from Christmas Island, Indian Ocean excretes its nitrogenous wastes as a solid purine pellet of 61% uric acid and 39% guanine. Previously it was thought *B. latro* excreted purely uric acid. The presence of guanine in the nitrogenous excreta of *B. latro* was confirmed by its conversion to xanthine with guanase. This was not a laboratory artefact as both purines were also present in nitrogenous excreta produced by crabs in the field.

Uric acid and guanine were also present in intracellular purine deposits in *B. latro*. By contrast the purine deposits from the closely related terrestrial hermit crab *Coenobita brevipanus* contained only uric acid. Also intracellular urate levels in the midgut gland of *B. latro* were 12.7 times higher than that of the midgut gland from *C. brevipanus*. This suggests that the ability of the midgut gland to synthesise large amounts of urate and guanine for excretory purposes is an apomorphic characteristic of *B. latro*.

Given that *B. latro* excretes guanine and uric acid in significant amounts there may be selective advantages in either the production of this mixture or the production of one type of purine over another. These may lie in the metabolic cost of synthesis and/or the chemical and physicochemical properties of the two purines. Alternatively a single mutation in an enzyme that controls guanine production from inosine monophosphate (IMP), a common precursor of uric acid and guanine synthesis, may lead to the synthesis of large amounts of guanine. The presence of such a trait in an island population could then be explained by a founder effect. Both of these alternatives will be considered.

The effect of amoebic gill disease and other stressors on natriuretic peptide receptor activity in the gills of Atlantic salmon, *Salmo salar*Hamish McWilliam¹, Mark Powell², and Tes Toop¹¹School of Biological and Chemical Sciences, Deakin University, Geelong, Vic.²School of Aquaculture, University of Tasmania, Launceston, Tas.

Amoebic gill disease (AGD) is a major problem in the aquaculture of Atlantic salmon, and potentially compromises the osmoregulatory and cardiovascular systems by its damaging branchial effects. The natriuretic peptide hormone system, which is involved in maintaining osmotic balance and cardiovascular homeostasis, was hypothesised to become activated in this disease in order to ameliorate symptoms. Natriuretic peptides elicit their physiological effects by activating membrane bound guanylyl cyclase receptors and stimulating the production of cyclic GMP. Natriuretic peptide receptor (NPR) activity was measured in salmon gill membrane preparations by guanylyl cyclase assay, and compared between diseased and healthy salmon over an eleven day AGD infection. While plasma osmolality increased in AGD infected salmon, there was no evidence that natriuretic peptide receptor activity changed during infection. After the infection period, diseased and healthy seawater salmon were subjected to a freshwater bath, the common treatment for AGD. A 'handling' control group was subjected to a seawater-seawater transfer, to control for salinity transfer and the effects of AGD. Branchial NPR guanylyl cyclase activity, measured following the treatments, was elevated in all groups, possibly in response to the handling stress, as well as disease and salinity challenge. These results indicate that the natriuretic peptide system, as well as being involved in osmotic and cardiovascular regulation, may also function in the general stress response of fish.



Differences in surfactant protein sequences – an adaptation for diving?

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There are currently four surfactant proteins characterised to date, with the two hydrophobic proteins (SP-B and SP-C) being the most important with regards to surfactant function. The aim of this study was to examine the genetic sequences of SP-C in a number of aquatic mammals to determine any functional differences when compared to their terrestrial counterparts. The SP-C gene was partially sequenced from three aquatic species - ringed seal (*Phoca hispida*), blue whale, (*Balaenoptera musculus*) and Australian water rat (*Hydromys chrysogaster*) and the region encoding the functional protein was analysed. These were then compared to sequences obtained from dog (*Canis familiaris*), cow (*Bos taurus*, GenBank accession no. **NM 174462**) and rat (*Rattus norvegicus*, GenBank accession no. **U07796**). The hydrophobic alpha helical segment was highly conserved between all species. More variation was seen at the more polar N-terminal end, where the palmitoylation of cysteine residues occurs. This region is thought to be the only area of the protein able to sustain interactions with other proteins and lipid layers, and is essential for the surface active properties of SP-C. At the present time, it is unknown whether these variations in protein sequence result in functional differences related to aquatic behaviour, but this is currently being examined further.

Resting metabolisms of adult and juvenile red kangaroos (*Macropus rufus*), how different are they?

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Generally, young growing mammals have minimal resting metabolic rates (RMRs) proportionally greater than those of adult animals. This is also seen in the red kangaroo (*Macropus rufus*). Juvenile red kangaroos have RMRs 1.5 – 1.6 times that expected for adult marsupials of an equivalent body mass. When fed high-quality chopped lucerne hay, young-at-foot (YAF) kangaroos, which have permanently left the mother's pouch but are still sucking, and weaned red kangaroos had digestible energy intakes ($\text{kJ kg}^{-0.75} \text{d}^{-1}$) of $641 \pm 27 \text{ kJ}$ and $677 \pm 26 \text{ kJ kg}^{-0.75} \text{d}^{-1}$, respectively, significantly higher than the $385 \pm 37 \text{ kJ kg}^{-0.75} \text{d}^{-1}$ ingested by mature, non-lactating females. However, not growing YAF and weaned red kangaroos had maintenance energy requirements that were not different from those of mature, non-lactating females, the values ranging between 384 and 390 $\text{kJ digestible energy kg}^{-0.75} \text{d}^{-1}$. On a good quality diet, juvenile red kangaroos aged from permanent-pouch-exit until after weaning (ca. 220 – 400 d), had average growth rates of $55 \text{ g body mass d}^{-1}$. At this level of growth, juvenile red kangaroos had total daily digestible energy requirements that were 1.7 – 1.8 times those of mature, non-reproductive females. Therefore, the proportionally higher RMR of juvenile red kangaroos is largely explained by their additional energy metabolism for growth.



Timing of hibernation and reproduction in Tasmanian echidnas

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Since 1987 we have been studying aspects of hibernation in the echidna. Echidna hibernation is difficult to study on captive animals, which rarely show normal hibernatory behaviour, however when we changed our emphasis to field based studies the results suggested all Tasmanian echidnas hibernate. In 1994 we began using implanted data loggers to record body temperature, and in 1996 concentrated our field work in an area where we were able to follow individual echidnas over several years.

This has allowed us to collect good data on the timing of hibernation, which begins in late summer-early autumn (males 13 February \pm 12 days, females 5 March \pm 11 days) and most males arouse before the winter solstice (June 8 \pm 16 days). Females that subsequently produced young aroused 46 days later (July 25 \pm 16 days) while females that did not produce young hibernated for a further two months (arousal Sept 22 \pm 13). These data show that the timing of echidna hibernation is different from most hibernators, and is closely tied to the reproductive behaviour. Reproductively active animals arouse before the coldest part of the year, and reproductive activity normally begins within about a week of females arousing from hibernation.

Blood samples were taken from the rostral sinus and samples from males were analysed for testosterone, while progesterone levels were measured in females. The collated data provides an overview of the timing of reproductive activity, and by following individual animals and taking serial blood samples we have been able to relate hormone levels to specific events in the hibernation and reproductive cycles. Activity loggers on some echidnas provide further information on the timing of reproductive events.



Predicting breeding outcomes of Gould's Petrels (*Pterodroma leucoptera*) from an appraisal of parental body condition during incubation

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For the management of endangered seabird populations it would be valuable to have knowledge of which members in a breeding population are more (or less) likely to perform well in years when conditions are less than optimal. Remedial actions could then be focused on individuals most in need of attention.

Body condition of incubating Gould's petrels was evaluated over two successive breeding seasons to determine whether adult quality at this time could be used to predict breeding outcomes. An index of body condition was calculated from each adult's residual value in relation to a regression of body mass on size for all breeding pairs studied. Correlation analyses were then determined between body condition indices of parents and their provisioning performance, the growth of their chick, and the body condition of their chick at attainment of peak mass. The body condition of failed versus successful breeders was also compared.

Neither male nor female body condition during incubation was correlated with hatching success, and female condition was not related to chick provisioning rates or chick quality. By contrast, male body condition during their first incubation shift correlated significantly with chick feeding rates and the condition of the chick at its peak mass. There was also a significant correlation between the average body condition of each breeding pair throughout incubation and the provisioning rate, growth rate, and body condition of their chick at its peak mass.

Body condition indices of individual breeding males and females correlated significantly between years. Moreover, it was found that the condition of a given chick at its peak mass was correlated significantly with the condition of its sibling the following year.

Therefore, certain measures of reproductive performance and chick quality can be predicted from body condition evaluation of parents during incubation and from their chick-rearing performance the previous year.

**The innervation of the heart of the golden bandicoot (*Isodon auratus*).**

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Cardiac regulation among vertebrates is achieved through the interaction of the antagonistic autonomic nerves that run to the chambers and pacemaker cells of the heart. The pattern of cardiac innervation appears to vary both within and between the classes of vertebrate according to the priorities that each group now has for cardiac regulation. The anomalous pattern that we have previously reported for heterothermic mammals was related to their needs for torpor. We have now investigated the cardiac innervation of a metatherian homeotherm, the golden bandicoot. The pattern of innervation was investigated at 34 °C using isolated cardiac preparations. The atrial chambers and ventricular muscle were maintained under controlled conditions and allowed to beat spontaneously or were electrically paced. The innervation pattern and presence of autonomic receptors in the various chambers was investigated using the responses of these chambers to stimulation of the intramural nerves and/or applied agonists in the presence and/or absence of receptor antagonists. The findings for this homeothermic metatherian, provide yet another example of the variation that exists in mammalian cardiac innervation pattern. The relationship between a dual innervation of all chambers of the heart by antagonistic nerves and heterothermia may need to be reinterpreted.

Allometric scaling of respiratory turbinates and trachea in mammals and birdsTomasz Owerkowicz¹ and A.W. Crompton²¹Environmental Biology, University of Adelaide²Museum of Comparative Zoology, Harvard University

Respiratory turbinates, a characteristic feature of extant endotherms, augment the surface area of the nasal cavity in mammals and birds. Their epithelium acts as a temporal counter-current exchanger to reduce evaporative heat loss in expired air. So far, no quantitative relationship between respiratory turbinate surface area and metabolic rate has been established, although morphology and volume of the nasal cavity have been used to argue for, and against, the presence of turbinates in extinct vertebrates, in order to infer their metabolic status. Here we show by histomorphometric analysis that surface area of respiratory turbinates of endotherms scales to (body mass)^{0.75}, similar to their resting metabolic rate, but is threefold higher in mammals than in birds. Temperature gradients recorded from the avian trachea during oral breathing suggest that it, too, participates in counter-current heat exchange. Tracheal surface area scales to (body mass)^{0.78}, but is threefold higher in birds than in mammals. We propose that, in the absence of respiratory turbinates, tracheal elongation among theropod dinosaurs would have improved heat exchange and facilitated the evolution of endothermy.



Evolution of transthyretin: from bacteria to plants and humans

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In humans, transthyretin (TTR) is involved in the distribution of thyroid hormones in the body and brain. TTR synthesised by the liver is secreted into the bloodstream, where it binds thyroid hormones and distributes them around the body. The choroid plexus forms the blood-cerebrospinal fluid barrier, which is part of the blood-brain barrier. TTR synthesised by the choroid plexus is secreted into the cerebrospinal fluid, where it distributes thyroid hormones. In addition, this TTR is involved in transporting thyroid hormones from the blood into the brain.

We found that the amino acid sequence of TTR has not changed significantly during vertebrate evolution. Therefore, the gene must have evolved prior to the divergence of vertebrates from non-vertebrates. We identified open reading frames (ORFs) which would code for TTR-like proteins in the genomes of bacteria, plants, and invertebrate animals. These ORFs were amplified by PCR, cloned into expression vectors and the corresponding proteins were synthesised. The recombinant proteins were tetramers, similar to vertebrate TTRs, however they did not appear to bind thyroid hormones. We are screening these TTR-like proteins for their native ligands. TTR is an example of a protein whose structure has been conserved in all kingdoms, but whose function has evolved.

Marsupial shivering and non-shivering thermogenesis: a continuum?

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Many Eutherian mammals maintain their body temperature by both shivering and non-shivering thermogenesis (NST); the latter based on the presence of brown adipose tissue (BAT) and uncoupling protein one (UCP1). Although the occurrence of non-shivering thermogenesis is controversial in marsupials, in our laboratory we have found it to occur in species from the three major marsupial groups. *Bettongia gaimardi*, *Perameles gunnii* and *Sarcophilus harrisii*. These results were determined by measuring control metabolic rates and after cold acclimation and subsequent injection by nor-adrenalin. All attempts to locate BAT and/or UCP1 in these animals failed, leading us to assume that NST in marsupials occurs in their absence.

In order to ensure our results were of NST and not merely from shivering, we also monitored the animals by electromyography. Although it was obvious that the marsupials initially shivered in response to cold and then acclimated, we were interested to note that a change occurred in muscle tone in response to nor-adrenalin injection. This response increased after cold-acclimation.

Our research suggests that both shivering and non-shivering thermogenesis are located, at least in part, in skeletal muscle and that in marsupials they may form a continuum.



Heat Reward for Insect Pollinators

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The mechanisms that flowers use to encourage visits by pollinators usually involve two aspects—attraction and reward. The cues for attraction are appearance and scent, and the rewards are usually offered as food energy in nectar, pollen or other flower parts. Some insect-plant relationships involve thermogenic flowers. While heating is almost always considered only to enhance scent production, its role as a direct energy reward has never been demonstrated. We measured the respiration rates of *Cyclocephala colasi* beetles that pollinate inflorescences of the arum lily *Philodendron solimoesense* in French Guiana. The inflorescences are protogynous and maintain thermogenesis during the 24-h period between female receptivity and pollen release. Beetles arrive after sunset in the female phase and remain active all night in the floral chamber, where they mate and consume sterile male florets. They rest during the following day and depart with pollen in the evening. The elevated temperatures in the floral chamber reduce the beetles' overall rate of energy expenditure 2.0 to 4.8-fold during their activity period, which has a major influence on their energy requirements.

Energetics, evolution and ecology of mammalian hopping locomotion

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Bipedal hopping is relatively uncommon among terrestrial mammals: it has evolved only in four families of rodents and a single super-family of marsupials. Hopping locomotion by large mammals (kangaroos and wallabies) is energetically economical at fast speeds, but experiments on smaller hopping mammals have yielded inconsistent results. Body size is therefore not the sole determinant of the cost of hopping locomotion; we suggest that both evolutionary history and ecological niche also play an important role. To gain an insight into the relative importance of these factors, we examined the hopping energetics and gait parameters of a small marsupial, *Bettongia penicillata*.

Using a treadmill and respirometry apparatus, we determined that fast hopping (above 2 ms⁻¹) by *B. penicillata* has a significantly lower cost than does running by a mammal of the same mass (1 kg). Video analysis of hopping *B. penicillata* showed that the stride frequency and length patterns of this species are also similar to those of the larger hopping marsupials. One implication of this research is that energetically economical locomotion may be a feature of hopping marsupials but not of hopping rodents.



The effect of metabolic fuel availability on thermoregulation and torpor in marsupials

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The physiological signals that trigger torpor onset are poorly understood. However, many species enter torpor when food is restricted, suggesting that torpor onset is related to metabolic fuel availability. We determined whether a reduction in either glucose or fatty acid availability initiates torpor in the marsupial heterotherms, the stripe-faced dunnart (*Sminthopsis macroura*) and the eastern pygmy-possum (*Cercartetus nanus*). *Sminthopsis macroura* uses daily torpor, has limited endogenous energy stores, and thus relies on frequent food intake. In contrast, *C. nanus* is a hibernator with substantial energy reserves and can survive without food for months during the hibernation season, but at other times of the year also may use short bouts of torpor when food is restricted. Because the two species display different torpor patterns and energy storage strategies it is likely that they will respond differently to reductions in specific metabolic fuels. Both species were administered the metabolic fuel inhibitors, 2-deoxy-D-glucose (2DG, reduces glucose availability for cellular oxidation) and mercaptoacetate (MA, reduces fatty acid availability for cellular oxidation). The metabolic rate and body temperature (T_b) of animals treated with the inhibitors was compared to those of food-deprived animals. Glucoprivation by 2DG caused substantial reductions in metabolic rate and T_b in both *S. macroura* and *C. nanus*. However, *C. nanus* treated with 2DG had much higher minimum T_b and metabolic rates than those of torpid food-deprived animals. It appears that glucoprivation reduces the hypothalamic T_b set point, but by only 8 °C rather than by approximately 28 °C as during natural torpor. 2DG administration also induced a torpor-like state in *S. macroura*. The T_b of 2DG-treated and torpid food-deprived *S. macroura* were similar, however, the metabolic rates of 2DG-treated individuals were higher than the torpid food-deprived individuals. Reduced availability of fatty acids for cellular oxidation via MA also induced a torpor-like state in some *C. nanus*, with physiological variables that did not differ from those of torpid food-deprived animals. In contrast, MA-treatment did not reduce T_b or metabolic rate in *S. macroura*. Therefore, it appears that a reduction in both glucose and fatty acid availability forms part of torpor initiation in *C. nanus*. In contrast, in *S. macroura* low glucose availability appears to be the predominant signal for torpor initiation.



The influence of foraging mode and arid adaptation on burrow construction, thermoregulation and metabolism of burrowing mammals

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Two competing but non-exclusive hypotheses to explain the reduced basal metabolic rate (BMR) of mammals that live and forage underground (fossorial species) are examined by comparing this group with burrowing mammals that forage on the surface (semi-fossorial species). These hypotheses suggest that the low BMR of fossorial species either compensates for the enormous energetic demands of subterranean foraging (the cost of burrowing hypothesis) or prevents overheating in closed burrow systems (the thermal stress hypothesis). Arid burrowing mammals have a significantly lower BMR than mesic ones, so BMRs of fossorial and semi-fossorial animals were compared within these groups. The BMRs of mesic fossorial and semi-fossorial mammals could not be reliably distinguished, nor could the BMRs of large (> 77 g) arid fossorial and semi-fossorial mammals. These findings favour the thermal stress hypothesis, as the groups appear to have similar BMRs despite differences in foraging costs. However, in support of the cost of burrowing hypothesis, small (< 77 g) arid fossorial mammals were found to have a significantly lower BMR than semi-fossorial mammals of similar size. Given the high mass-specific metabolic rates of small animals, they are expected to be under severe energy and water stress in arid environments. Under such conditions, the greatly reduced BMR of small fossorial species may compensate for the enormous energetic demands of subterranean foraging. In further support of the cost of burrowing hypothesis, fossorial mammals were found to have significantly higher wet thermal conductances and to construct significantly larger nest chambers than semi-fossorial mammals. The high thermal conductance of fossorial mammals may prolong burrowing bouts by delaying hyperthermia through facilitation of conductive heat loss to the soil. At rest, however, this high thermal conductance will become a liability and compromise thermoregulation. Fossorial mammals appear to have solved this problem by constructing significantly larger nest chambers, which presumably contain more insulative nesting material, than those constructed by semi-fossorial mammals.



Do *Pax* genes play a role in arthropod muscle regeneration?

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What makes a stem cell become a muscle cell? Our current understanding of vertebrate myogenesis is that stem cells are driven along the myogenic path by a cascade of transcription factors. The expression of each transcription factor defines a step in the differentiation process, and it appears that *Pax* group III transcription factors provide the critical first step in this pathway; that of determining which stem cells may become muscle cells. Vertebrates possess two *Pax* group III genes; they utilise *Pax3* to specify embryonic myoblasts and *Pax7* to specify adult myoblasts (muscle satellite cells), thus *Pax7* is vital for adult muscle regeneration in vertebrates. *Pax* group III genes are highly conserved and have been isolated from vertebrates, jellyfish, *Drosophila*, and others. Genes on the arthropod lineage have not yet been implicated in myogenesis, but are known to have a role in segmentation. The Australian yabby (*Cherax destructor*) provides a phenomenal model for adult muscle regeneration. Yabbies regenerate entire limbs, including the muscle contained within them. They also have a strictly regulated pattern of muscle growth, being intrinsically linked with the cyclic moulting of their growth-restricting exoskeleton. Previous research has implicated muscle-localised satellite cells as playing an important part in post-moult regeneration, however the stem cells utilised for limb regeneration must come from further afield, and thus two distinct types of stem cell appear to be responsible for muscle regeneration in the yabby. My research has involved the isolation and characterisation of a *Pax* group III gene in the yabby and the analysis of its expression during muscle regeneration. I have designed degenerate primers and used PCR and RT-PCR to isolate novel *Pax* group III sequences from the yabby and used RT-PCR to map expression in regenerating limb tissue and moult-regenerating muscle. The expression of a *Pax* group III gene during muscle regeneration invites the tantalising possibility that *Pax* group III expressing stem cells are involved in arthropod myogenesis.

BMR and FMR of the numbat: how low is low?

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In mammals, a termitivorous or myrmecophagous diet is often associated with low energy availability and consequently reduced energy turnover, both basal metabolic rate (BMR) and field metabolic rate (FMR). The numbat (*Myrmecobius fasciatus*) is a unique marsupial in being exclusively termitivorous and diurnal, and has been suggested to have a low BMR compared to other marsupials. Similarly, the aardwolf (*Proteles cristatus*) is a termitivorous hyaenid that has been suggested to have a low BMR and FMR. Both the numbat and aardwolf are useful examples to examine whether they have an adaptively low BMR/FMR as a consequence of their termitivory, because both are derived from generalist carnivores (dasyurids are the closest relatives for numbats, and Carnivora for aardwolves). But, are their BMRs/FMRs statistically lower than expected? We used two comparative methods to account for possible effects of phylogenetic inertia, and allometric correction to account for variation in body mass. We then evaluated whether BMR and/or FMR is low for numbats and aardwolves, by (1) using 95% prediction (not regression) confidence limits, and (2) comparison of residual outliers from the normalised expected residuals (dasyurids/marsupials for the numbat; carnivores/placentals for the aardwolf). It proved difficult to demonstrate with any statistical confidence whether the numbat has a lower BMR/FMR than dasyurids (or marsupials) or the aardwolf than carnivores (or placentals in general). Our approach needs to be considered by other comparative physiological studies, and the difficulties that we describe will hamper other comparative physiologists who seek to statistically conclude that a particular species has a lower or higher than expected metabolic rate, or any other physiological variable.



Poster abstracts

Nasal anatomy of the marsupial mouse, *Antechinus subtropicus*.

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Antechinus subtropicus is a small dasyurid marsupial found in south-eastern Queensland. It has an extreme life history, with a short synchronous breeding period in September, followed by complete male mortality; the males displaying pathologies consistent with chronic stress. Previous work has shown that chemosignals from conspecifics, received by the olfactory system, may significantly contribute to the state of stress. This study investigated nasal anatomy to further clarify the role of olfaction in the life history of *A. subtropicus*.

Serial sections were taken from decalcified snouts of adult *A. subtropicus*, and several different histological stains were performed. These sections were examined to describe the osseous and cartilaginous anatomy, the types of epithelia present and their extent, and the distribution and development of glandular tissue.

A. subtropicus has complex turbinate bones, and extensive areas of olfactory sensory epithelium. Glandular structures are similar to those described in other marsupials. Of interest were the large numbers of parasites in the nasal cavities and alterations in epithelial type seen in males shortly before die-off.

The anatomy of the nasal region indicates that olfaction is an important sense and will be discussed in the context of the life history of *A. subtropicus*.



Why do musk oxen eat snow?

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Wild muskoxen rely upon ruminal fermentation to utilize their fibrous diet during winter and spring when forages are still frozen and snow is the main source of water. Snow ingestion is also apparent in captive muskoxen that ingest up to 2kg of snow after consuming pelleted mineral supplements. We tested the hypothesis that ingestion of cold material disrupts the ruminal environment and the activity of its microbes.

Responses to cold water and food were measured in four ruminally fistulated muskoxen (5-9 y; castrated males; 260-310 kg). We used a repeated measures design in which muskoxen were assigned to morning or afternoon experiments to control for any diurnal effects within animals. Muskoxen were fitted with sensors for recording movement, heart rate, and temperature at the ear (tympanic), skin surface and 35cm within the rumen (Minimitter Co. Oregon). Data loggers recorded temperature every 15s whereas heart rate was monitored every 60s. Background measures were recorded for 10 min after rumen temperature stabilized to a plateau ($\pm 0.5^\circ\text{C}$). Food (1kg) was provided at ambient temperature (20°C) or frozen (-12°C) whereas water (0.5L) was injected directly into the rumen at 4°C or 40°C . Microbial activity was assayed in each animal by collecting ruminal digesta in the same time period (morning or afternoon) as the temperature experiments. Digesta fluid was incubated with either $9\text{g}\cdot\text{L}^{-1}$ saline (control) or 0.5 M glucose (substrate) at 35, 37.5 and 40°C . Net production of gas was calculated as the difference between substrate and control over 20 min. Repeated measures for gas production, feeding or water injection within experiments were compared by ANOVA.

Rumen temperature was not affected by heart rate, rumen activity or body movement of muskoxen. A drop in tympanic temperature after consuming cold food suggested that food temperature could influence feeding rate. Ruminal temperature (38.7°C) was elevated (39.1°C) for $>90\text{min}$ after feeding irrespective of food temperature. The activity of microbes and secretions probably increase ruminal temperature, but ruminal activity may be insensitive to changes in digesta temperature. Small loads ($<2\%$ of rumen fill) of cold water reduced rumen temperature (-0.5°C) for $>20\text{min}$ and reduced the effect of feeding on ruminal temperature. Microbial activity *in vitro* declined below 37.5°C but did not increase at 40°C . These data suggest that postprandial thermal loads may increase ruminal temperature above the optima for microbial activity but ingestion of cold water or snow after feeding could facilitate maintenance of optimal temperature for rumen fermentation. Snow ingestion may therefore reflect fermentability of the diet, the amount of food consumed and the accompanying solute load.



The effect of water deprivation on the expression of the tonicity-responsive enhancer binding protein in the Spinifex hopping mouse, *Notomys alexis*

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Reabsorption of water in the collecting ducts of the kidney, and the subsequent production of a concentrated urine, is dependent on increasing the osmolarity of the interstitial fluid bathing the renal tubules as they pass through the outer medulla to the inner medulla. Salt and urea are the main solutes that contribute to the osmolarity and ionic strength of the renal medulla, but cause hypertonicity, which compromises cellular function. In response to hypertonicity, cells accumulate compatible osmolytes (non-ionic osmolytes), which lower the ionic strength within the cell to isotonic levels by replacing electrolytes (ionic osmolytes). The tonicity-enhancer binding protein (TonEBP) is a transcription factor that stimulates transporters that catalyse the accumulation of compatible osmolytes. The role of TonEBP in desert mammals is interesting to consider as they have an increased requirement for the conservation of water. The Spinifex Hopping-mouse, *Notomys alexis*, is a desert rodent that has physiological adaptations that enable them to survive without access to free water. Two important adaptations are the production of highly concentrated urine (measured up to 9 M), and extremely dry faeces, which can only be accomplished by creating osmotic gradients for the reabsorption of water; however, this may cause hypertonicity in the kidney and colon.

A water deprivation experiment was set up to determine the mRNA expression of TonEBP in the kidney and colon. Immunohistochemistry will also be performed to determine the localisation of TonEBP in the kidney and colon. A 514 base pair partial TonEBP cDNA was cloned from *Notomys* that was 97 % and 96 % similar to *Mus musculus* TonEBP nucleotide and amino acid sequences, respectively. Seven days of water deprivation caused a significant increase in TonEBP mRNA expression in the kidney of *Notomys*, while in the colon TonEBP mRNA expression increased after three and seven days of water deprivation. The immunohistochemistry experiments are currently being undertaken. From the results thus far, it appears that there is an increase in hypertonicity in the kidney and colon of water deprived *Notomys* that is causing an enhanced expression of TonEBP mRNA.



Reproductive behaviour in the Julia Creek dunnart (*Sminthopsis douglasi*)

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The Julia Creek dunnart (*Sminthopsis douglasi*) is a carnivorous marsupial. The International Union for Conservation of Nature (IUCN) has classified the species endangered. This is partly due to the restricted range, which is limited to cracking soil habitat of mitchell-grass downs, northwest Queensland. Queensland Parks and Wildlife Service have initiated a recovery plan to secure the species status. One component of this plan is a captive breeding program at David Fleay Wildlife Park, Burleigh Heads. The maintenance of a population in captivity is dependent on continuous, successful breeding of the species.

One important factor in breeding *S. douglasi* is the identification of oestrous. The current method of identifying oestrous is by examining urine for the presence of cornified cells. The reproductive status of males is established by sperm counts in urine. To collect urine, the animals have to be removed from their cages and restrained. This may be stressful for the animals. This method is also very time-consuming. The aim of this study is to identify specific behaviour of *S. douglasi* and associate this with oestrous. This may provide non-invasive ways of identifying oestrous, which would facilitate breeding the species in captivity. This in turn would be useful in designing appropriate conservation programs and also provide a basis for future research.

The study includes observations in captivity and in field. Physiological records include body weight and reproductive status. Behaviour of captive animals is being recorded using video cameras with motion sensors and infrared light sources. This minimizes disturbance of the animals. Behaviour such as social activities, feeding, sleeping and grooming, etc. is being recorded. Preliminary results suggest that females are more active when they are in oestrous, with regards to intensity as well as duration of activity. There are also more encounters between males and female in oestrous.

Metabolic energetics and biomechanics of pentapedal locomotion in red kangaroos

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Kangaroos move in a biomechanically interesting way at slow speeds; they walk pentapedally using their tail. Early work on red kangaroos (*Macropus rufus*) suggested that this mode of locomotion is metabolically expensive. Kangaroos largely rely on their pentapedal gait to forage and graze. We trained 7 animals to wear a mask and walk on a treadmill. This allowed us to measure metabolic power using indirect calorimetry. In comparison to bipeds and quadrupeds of similar mass, pentapedal walking in kangaroos requires ~2.6 times the metabolic power. To determine the mechanical work required, we trained the same animals to walk along a walkway and across a force platform. A video camera recorded sagittal plane motion. By combining video and ground reaction force data, it was found that the mechanical work required to walk pentapedally was 5.5 times that of walking in bipeds. We also found that the front legs and tail have different functions. The hind legs support most of the body weight and perform most of the work. The front legs act mainly as a brake and the tail acts mainly as a motor. Surprisingly, the tail generates the same amount of mechanical power as both human legs in biped walking.



Working to identify an exciting steroid in a viviparous skink, *Tiliqua nigrolutea*

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17 β -estradiol (E2) is broadly believed to be the major circulating form of estrogen in almost all vertebrates. Its role in the stimulation of vitellogenesis in females is well established, and the assumption is that E2 stimulates other physiological and behavioural processes related, in particular, to reproduction. During incubation studies into steroid biosynthetic pathway preference in blue-tongued lizards, *Tiliqua nigrolutea*, we noted that while E2 was not produced, an alternative, highly polar steroid was synthesised in significant quantities. We have investigated this steroid further, using *in vitro* incubation with various steroid precursors, with products identified by a combination of TLC, HPLC with radiometric detection, UV spectrum comparisons and preliminary GC-MS. We know the steroid is more polar than E2, but less polar than estriol (E3). It is synthesised from pregnenolone (P5), probably via testosterone (T), but is not synthesised from androstenedione (AD). Gonadal production of this polar steroid varies with sex and reproductive condition: greater production is seen in both sexes during the spring reproductive period (greatest in vitellogenic females). We believe that this polar steroid is an alternative to E2 and could have important roles in reproductive physiology in this species.

Autonomic and behavioural fever in an ectotherm

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Many organisms respond to infection by developing a fever, which is an elevation of the set point temperature around which thermoregulation occurs. Endotherms attain this elevated set point largely by autonomic mechanisms. Ectotherms also are able to develop a fever, but utilise behavioural mechanisms. We provide evidence that in lizards, both physiological and behavioural manifestations of an elevated set point are evident.

Jacky lizards (*Amphibolurus muricatus*) were injected with *Salmonella typhosa* lipopolysaccharide at a dose of either 0.02 or 2 g/g dissolved in saline or saline only. After injection, each of the different groups were monitored for body temperature (T_b) in thermal gradients, and the T_b and skin temperature at which they panted (= panting threshold) was ascertained when the lizards were subjected to a constant rate of radiant heating. On the day following injection, lizards only developed a behavioural fever with the 2 g/g dose, while the panting threshold was significantly increased for both doses. These results show that both autonomic and behavioural mechanisms of an ectotherm are responsive to pyrogens, and suggest that the autonomic mechanisms may be more sensitive to such exposure.



**Embryonic, gonadal and sexual organ development in the small viviparous skink,
*Niveoscincus ocellatus***

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Recent evidence has suggested that viviparity and environmental sex determination (ESD) may be compatible biological attributes; this raises fundamental questions about the evolution and physiological control of sex determination in vertebrates. However, the majority of research into the timing of gonadal differentiation (and sex determination) in reptiles has focused on oviparous species. Baseline information on embryonic development in viviparous species is lacking and information on gonadal differentiation and sexual organ development is almost non-existent. Here we present an embryonic morphological development table (10 stages) that includes the timing of gonadal differentiation and development of the reproductive ducts and hemipenes in the spotted snow skinks (*Niveoscincus ocellatus*) - a small annually reproducing skink. This species was specifically chosen because skews in sex ratios, presumably as the result of some form of ESD, have been documented. Sex differentiation occurred in the middle third of development, as is typical of oviparous reptiles. The gonads are initially indifferent, with both an outer cortex layer and inner medulla present, and show no signs of organization; the Mullerian and Wolffian ducts occur in both sexes. Differentiation into the ovary begins with the degeneration of the medulla and the congregation of germ cells in the cortex (stage 2). Degradation of the medulla continues as the germ cells congregate in the thickening cortex layer (stage 5). Differentiation of the indifferent gonad into testes occurs at approximately the same stage (stage 2) and proceeds rapidly with the development of seminiferous tubules within the medulla and the onset of the regression of the cortex (stage 3). In males, there are large amounts of interstitial tissue surrounding the seminiferous tubules within the medulla by stage 5; this corresponds to maximum development of the hemipenes in males and corresponding regression in the females. Embryonic females retain both Wolffian and Mullerian ducts throughout development but with significant development of the Mullerian duct. In contrast, in males the regression of the Mullerian ducts begins once the testes have developed (stage 3) and the ducts are completely lost by stage 5. The identification of the period of gonad differentiation in this species provides the basis for more controlled investigation into environmental effects (temperature and hormones) on sex determination in viviparous reptiles.



How do echidnas manage to breathe when they burrow into soft soil?

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Echidnas are famous for their capacity to dig into soft earth and 'disappear'. We have been impressed by their capacity to manage even under very friable, dry dusty soil; you'd think they would be unable to breathe. We wondered if they might arch the body, making a small air space cavity into which they could put the snout. We decided that fluoroscopy would allow us to see the disposition of their snout and body when burrowing into soft material. Because sand or soil would be too dense for X-rays to penetrate, we filmed echidnas burrowing into kitty litter made from recycled paper. We saw no sign of an animal holding its nose in any pocket of air under the body, and conspicuous movements of the diaphragm indicated continuing respiration. Both animals burrowed with the head and snout extended, exploring ahead of them, and they maintained it in that position once they had established themselves fully burrowed. Because kitty litter is more porous than sand or fine soil it is not an ideal test medium. We offer a bottle of red wine to the person suggesting the perfect medium. An Honours student at UQ is taking this further, exploring respiratory gas exchange by burrowing echidnas and adding to Mike Augee's seminal work in this area.

Presentation: Powerpoint presentation on a laptop, viewer prompted.

Faecal sex steroid monitoring reveals the reproductive cycle of the Malayan Sun bear (*Ursus malayanus*), an endangered tropical ursid

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The Sun bear (*Ursus malayanus*) is an endangered tropical mammal inhabiting the rainforests of South East Asia. Wild populations are believed to be in severe decline mainly due to rapid loss of habitat through deforestation and the illicit trade in bear parts. Unfortunately little is understood of the reproductive biology or behaviour of *U. malayanus*, which is reflected by an inconsistent and poor breeding record in captivity. It is difficult to assess factors limiting captive breeding without an improved knowledge of reproductive processes in this species. The aim of this study was to apply faecal sex steroid monitoring to characterise and monitor the reproductive cycle of the female and male Sun bear toward more effective management of captive populations. Faecal sex steroids were analysed in samples collected from Sun bears housed in zoological parks in North America and New Zealand, over periods of < 27 months. Male samples were assayed for testosterone and female samples for oestradiol-17 β and progestagens. The mean length of the follicular and luteal phase was 11.2 \pm 1.3 days and 93.3 \pm 3 days, respectively. Increased concentrations of oestradiol were associated with the onset of oestrus behaviours and mating activity. The pattern of oestradiol and progestagen metabolite excretion during pregnancy and pseudopregnancy was similar, indicating that sex steroid monitoring is not an effective method for unequivocal diagnosis of pregnancy in the Sun bear. Testosterone levels were often elevated during mating, with peaks accompanying mating activity. There was no significant effect of season ($p > 0.05$) but cycles in androgen excretion were apparent, occurring at 4 - 6 month intervals.



The effect of regular swimming training on basal metabolic rate of *mdx* mice

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Duchenne muscular dystrophy (DMD) is a fatal muscle wasting condition occurring in 1 in 3500 live male births. A genetic defect prevents the expression of the sarcolemmal protein dystrophin, causing myocyte fragility. A similar condition occurs in the *mdx* mouse, a widely used animal model for DMD research. The metabolic consequences of muscular dystrophy are poorly understood. To date, basal metabolic rate (BMR) has not been determined in the *mdx* mouse. Regular exercise lowers BMR and exercise (swimming) has been shown to have a positive effect on skeletal muscle function in *mdx* mice at some ages. Anecdotal evidence suggests that swimming may also be beneficial to boys with DMD. This study aims to determine whether swimming is beneficial to *mdx* mice.

Body weight, food consumption and BMR were measured in 6 week old *mdx* and C57 BL10 ScSn mice. Subsequently, mice were divided into either a swimming group (15 minutes per day, 4 days per week) or a sedentary group within each strain and these parameters were measured again at 24 weeks of age. *Mdx* mice consumed more food and weighed significantly more than age matched C57 mice in both the swim-trained and the sedentary groups. A lower body weight was evident in both the swim-trained groups, with this reaching significance in the *mdx* group, but not the C57 group. Training did not alter BMR in either strain, but trained *mdx* mice have higher a BMR than trained C57 mice. In conclusion, the higher food consumption in *mdx* contributed to the higher body weight while swim-training ameliorates this increase in body weight.



The effect of water deprivation on neural nitric oxide synthase and soluble guanylyl cyclase in the cane toad, *Bufo marinus*.

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In mammals, nitric oxide (NO) has been shown to decrease vascular resistance in the kidney, whereas, renal vascular resistance is increased when the enzyme responsible for the production of NO, nitric oxide synthase (NOS) is inhibited, thus demonstrating that NO contributes to the maintenance of renal vascular tone. A number of studies have also demonstrated a role for NO in the regulation of sodium and water reabsorption within the renal medulla, as infusion with a NOS inhibitor causes an increase in arterial pressure in association with retention of sodium and water. In addition, it has been shown that all three NOS isoforms are up-regulated in the inner and outer medulla of water-deprived rats, possibly to counteract an increase of systemic and intrarenal vasoconstrictive substances (1). To date, the role of NO in the amphibian kidney is yet to be elucidated. Recently, it was reported that the systemic vasculature of the cane toad *Bufo marinus* lacks an endothelial NOS system, and instead is regulated by neurally-derived NO (2).

This study, therefore, aimed to clone and sequence neural NOS and the receptor for NO, soluble guanylyl cyclase (sGC), from *B. marinus* and measure their expression levels in the kidney and brain of control and dehydrated animals. An 860 base pair partial nNOS cDNA was cloned from *B. marinus* that was 81% and 90% homologous to *Xenopus laevis* nNOS at the nucleotide and protein levels, respectively. In addition, a 631 base pair partial sGC cDNA was cloned that was 82% and 97% homologous to *Homo sapiens* sGC at the nucleotide and protein levels, respectively. The expression analysis is currently being undertaken.

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The comparative expression of uncoupling proteins in marsupials and a monotreme.

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This study identified the expression of uncoupling proteins in several species of marsupial and one monotreme, the aim being to obtain an understanding of the evolution and possible role for these proteins. The species investigated were *Bettongia gaimardi*, *Sarcophilus harrisii*, *Sminthopsis crassicaudata*, *Trichosurus vulpecula*, *Perameles gunnii*, and *Tachyglossus aculeatus*. None of the species used in this investigation identified uncoupling protein 1, whereas uncoupling protein 2 was expressed in all species. There was a differential expression of uncoupling protein 3) in the species investigated, only *S. crassicaudata* and *B. gaimardi* expressed it although, there was no correlation with thermogenic ability. This study showed that none of the species investigated possess uncoupling protein 1, therefore presumably brown adipose tissue is absent also. Uncoupling protein 2, however, may be expressed in the entire marsupial taxa, and at least one monotreme. The close homology between all three proteins suggests that these two members of the mitochondrial anion carrier protein superfamily have evolved from UCP2. This could be an explanation for the difficulties in identifying a definitive role for UCP2, as it may be the precursor for uncoupling proteins.



Messenger RNA expression of natriuretic peptide receptors in freshwater and seawater rainbow trout gills

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The natriuretic peptide hormones of fishes are implicated in the regulation of salt and water balance in euryhaline fish encountering different salinities, and are best characterised in eels. Natriuretic peptides elicit their responses through specific membrane bound receptors. Plasma natriuretic peptide concentrations vary in eels depending on whether they are in fresh water (FW) or sea water (SW), and specific receptor expression alters in target tissues, such as the gills, under these conditions. However, little is known about this system in other euryhaline teleosts, such as trout.

This study investigated the mRNA expression of three natriuretic peptide receptors (NPR-A, NPR-B and NPR-C) in the gills of the rainbow trout, *Oncorhynchus mykiss*, in response to 1 week SW acclimation. Freshwater trout were acclimated to SW for 1 week, or maintained in FW, before tissue harvesting. Specific cDNA sequences of NPR-A, NPR-B and NPR-C were obtained and homologous PCR primers were used in a semi-quantitative PCR technique to assess mRNA expression in FW and SW fish. Gill NPR-A and NPR-C did not significantly differ in SW trout compared with FW trout. However, NPR-B significantly increased in the SW trout. This pattern of NPR expression differs to that found in eels. Our data indicate that the changes in NPR expression after SW transfer may not be general and may be species specific. Further studies are necessary in other euryhaline species to identify whether there are general patterns of NPR expression.



The influence of photoperiod on the reproductive activity of female honey possums, *Tarsipes rostratus* (Marsupialia: Tarsipedidae): assessed by faecal progesteragens and oestradiol-17

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Honey possums are considered to be opportunistic and continuous breeders in their natural habitat in south-western Australia. Fewer births have been recorded in the summer months but their reproductive activity, in particular, lactation, is associated with periods of adequate food supply. Honey possums, like most macropodid marsupials, exhibit embryonic diapause, and in an outdoor laboratory colony, females that had conceived in different months during the previous year demonstrated a synchronised period of birth during mid-February. Cessation of diapause in Bennett's wallaby and the tammar is initiated by a change in the daylight length. This study investigated whether the observed synchrony in births shown by Honey possums is under the influence of photoperiod.

Six females were placed in a CTR on a 15L: 9D light regime to simulate a long daylength over summer. After seven weeks 3 females (Group 2) were changed to a short daylength of 10L: 14D, while the other 3 females (Group 1) were maintained on the long daylength. Faecal oestradiol and progesteragen levels were measured during the experiment and females were sacrificed at the completion to determine their reproductive condition. Group 2 females were found to have very large and greatly expanded blastocysts with significantly higher levels of progesteragens after the change in photoperiod ($p < 0.05$). In contrast, the Group 1 females had very small diapausing blastocysts and progesteragen levels did not change throughout the study. Overall oestradiol levels also increased significantly in Group 2 females ($p < 0.05$) after the change to a shorter daylength, but not in Group 1 females.

These results demonstrate that a change from long to short days stimulates increased progesteragen output (and oestradiol-17 to a lesser extent), which supports the growth and expansion of the blastocysts. We suggest that photoperiod, in particular its change to a shortening day length, is the stimulus for terminating diapause in the Honey possum during its first reproduction of the year. A similar role for photoperiod later in the year cannot be attributed as subsequent breeding in September to October occurs when day length is increasing and may be more entrained to unknown factor/s in their nectarivorous diet.



Reduced sensitivity of spermatogenesis to high temperatures in *Pseudomys australis*

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The deleterious effects of high environmental temperatures or local heating on spermatogenesis are well known in laboratory rodents and farm animals. The Australian rodent, *Pseudomys australis*, the plains rat, lives in shallow burrows in the arid zone of central Australia. It was therefore of interest to determine whether this species has evolved mechanisms for maintaining fertility under hot conditions. Eleven adult male animals from a breeding colony at the University of Adelaide were exposed in a microclimate chamber to temperatures ranging from 35 to 38°C for 2 periods each of 12 h on successive days, two or three at each temperature. Nine laboratory mice were exposed to 35, 36 or 37°C for 2x12 h, three at each temperature for comparison. Two plains rats were anaesthetized, and their scrotum, tail and hind legs immersed in a water bath at 43°C for 20 min. All animals were killed 21 days after exposure to the heat, one testis and epididymis removed, weighed and homogenized in Triton X-100 in saline to count the spermatozoa, and the other testis fixed for histology.

The testes of the plains rats were not reduced in size, and the testicular and epididymal sperm counts were similar to controls if the climate chamber was set at 35, 36 or 37°C, but there was reduction in testis weight to about 50% of control, and the sperm counts were also reduced when the chamber was set at 38°C. In contrast, the testes of the laboratory mice were reduced to about 60% of control at 37°C, but unaffected at the lower temperatures. Exposure of the testes of the plains rats to direct heating in a water bath resulted in a fall in testis weight and sperm counts to about 40% of control, which is slightly greater than that reported for normal or heat-adapted laboratory mice (van Zelst et al, 1995, *Reprod. Fertil. Dev.* 7, 1117-1121) treated in the same way.

It is concluded that the plains rat, like the heat-adapted laboratory mice, has not evolved testis that are less susceptible to heat-induced damage but instead have evolved a more efficient way of keeping their testes cool in a hot environment.



Molecular cloning of brain natriuretic peptide from the heart of reptiles

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The natriuretic peptide (NP) system is comprised of a group of structurally related hormones that have an important role in the maintenance of cardiovascular and body fluid homeostasis. The cardiac NPs have been cloned and sequenced from all classes of vertebrates except reptiles. In teleost fish, amphibians, and mammals, the heart expresses both atrial NP (ANP) and brain NP (BNP). However, BNP appears to be the sole cardiac NP in birds. This study used molecular cloning to determine the type/s of cardiac NP in the atria of the saltwater crocodile, *Crocodylus porosus*, and the long-necked tortoise, *Chelodina longicollis*.

Using both 3' and 5'-RACE PCR, the entire sequence of a crocodile BNP was isolated and cloned from the atria. Crocodile preproBNP was 77% homologous to chicken preproBNP and the predicted mature BNP was identical to that of chicken. Crocodile BNP mRNA was highly expressed in the atria, ventricles, and brain, while lower levels of expression were observed in the gastrointestinal tract, kidney, and liver. RACE PCR was also used to isolate a partial BNP cDNA from the atria of the tortoise, which was also identical to crocodile and chicken. No cDNA clones of an ANP were isolated from either species.

These data provide initial evidence that BNP rather than ANP is the primary atrial NP in two species of reptiles. Furthermore, there is remarkable homology between reptilian and chicken BNP, which is not seen when comparing NP sequences of other vertebrate classes.

Who's been nibbling the Bunya's nuts?

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Bunya pine trees (*Araucaria bidwillii*) dominate the Bunya Mountains National Park of south-east Queensland. They produce large cones, which fall intact to the ground, disintegrating into scales and their nuts (seeds) within a few days. This study aimed to determine the nutrient content of the nuts and identify which fauna in the Bunya Mountains National Park consume them. Water and ash content were determined by drying, protein by Kjeldahl analysis, lipid by Soxhlet analysis, minerals by atomic absorption spectroscopy and carbohydrate by difference. Fatty acid composition of lipids was determined by gas chromatography. Video cameras with infrared illumination recorded fauna consuming Bunya nuts. Results to date indicate that Bunya nuts are consumed by mountain brushtail possums (*Trichosurus caninus*) and fawn-footed melomys (*Melomys cervinipes*). Brush turkeys (*Electura lathamii*) consume nuts broken open by other fauna. Swamp wallabies (*Wallabia bicolor*) consume the scales, but apparently not the nuts. Fresh Bunya nuts are abundant early in the year, especially every three years. They have high water (50%) and carbohydrate (46%) contents and low protein (0.25%) and lipid (1.8%) contents. The most abundant mineral is Magnesium, 102mg per 100g. The high linoleic acid content (68%) of the lipid fraction may satisfy requirements of native fauna and humans for this dietary essential fatty acid.



Registrants

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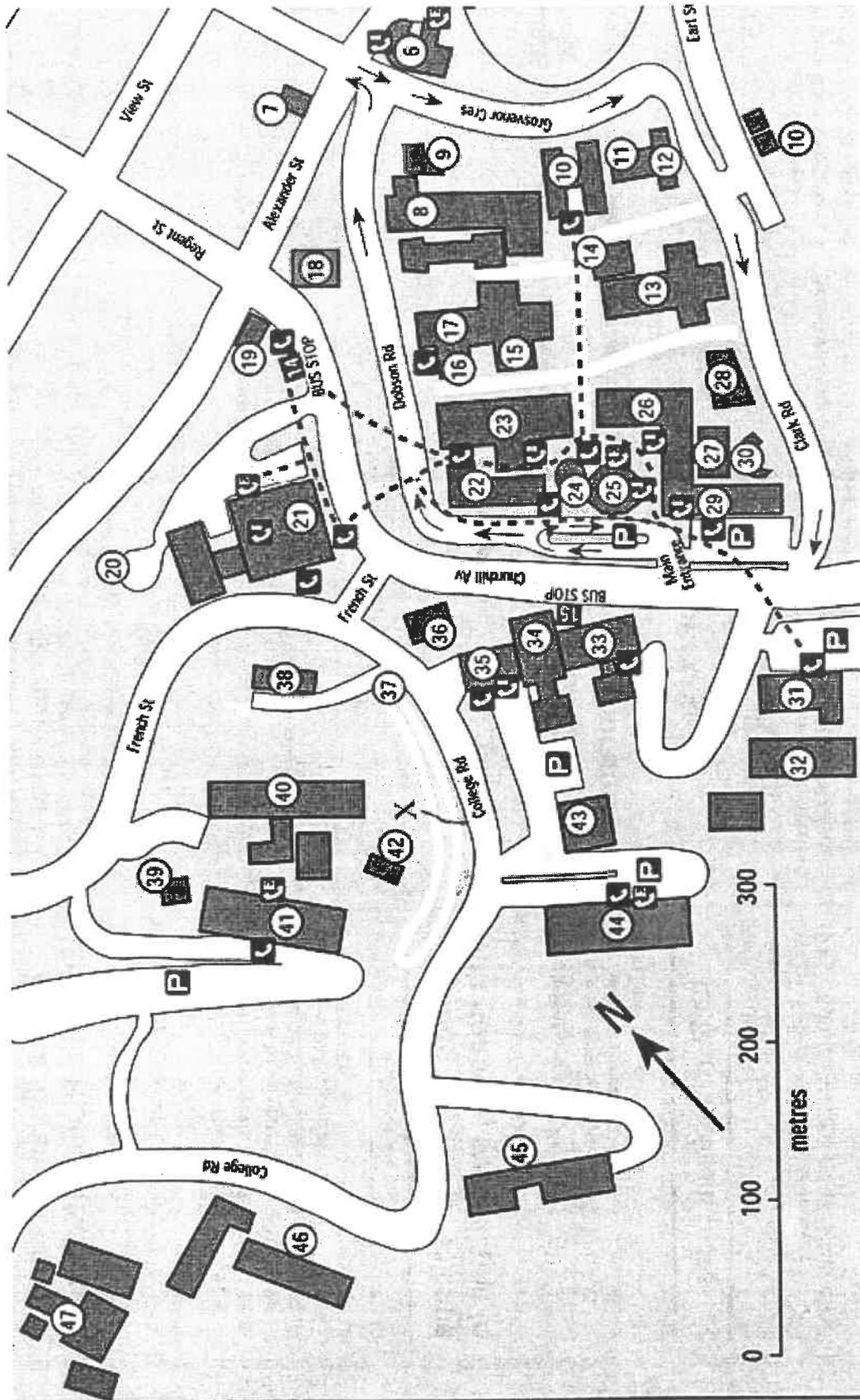


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Maps

University of Tasmania, Sandy Bay Campus

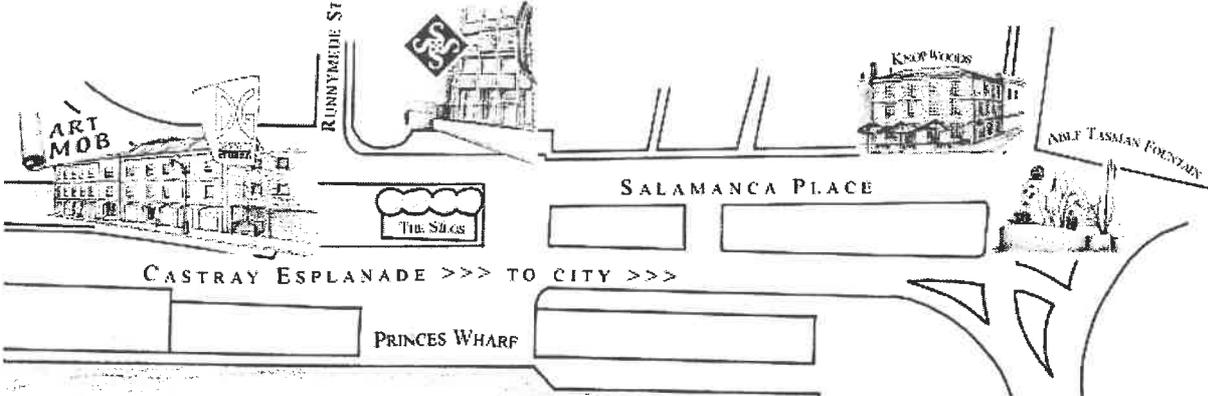


18: Staff Club
X: BBQ area "in the gully",
take walking track from College Rd

44: Medical Sciences building and lecture theatre
46: St John Fisher College
47: Christ College



Art Mob gallery



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