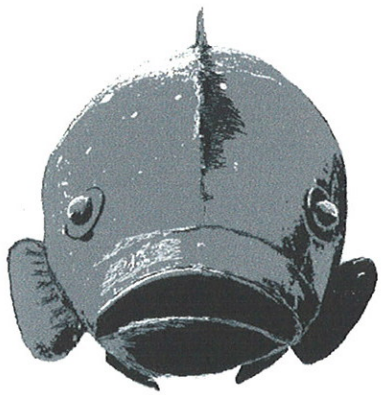
DAVID BOOTH

Australian & New Zealand Society for Comparative Physiology & Biochemistry

ANZSCPB

17th Annual Meeting

8 - 10 December 2000



Deakin University, Geelong Campus

Programme and Abstracts

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ANZSCPB

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Programme and Abstracts

Convenors:

Tes Toop and John Donald

Sponsored by:

Faculty of Science and Technology, School of Biological and Chemical Sciences and Research Services of Deakin University; University of Chicago Press (Physiological and Biochemical Zoology); Elsevier (Comparative Biochemistry and Physiology); Springer (Journal of Comparative Physiology); and Geneworks

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Programme

Thursday December 7th

4.30 - 8.30: Registration and Social at Deakin University House

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Friday December 8th

9.00 - 10.00	**Registration: foyer of Lecture Theatre SB 420**
10.00 - 10.10	**Welcome**
	Session One - Cardiovascular Physiology, Chair: Stewart Nicol
10.10 - 10.30	The principle of Laplace and scaling of ventricular wall stress and blood pressure in mammals and birds *Roger Seymour*, Amy Blaylock and Harvey Lillywhite*
10.30 - 10.50	The control of vascular tone in Notothenioid fishes is determined by phylogeny not environmental temperature *Malcolm Forster*, W. Davison and S. Egginton*
10.50 - 11.10	The renal vasculature of the agamid lizard, *Ctenophorus ornatus* *James E. O'Shea*, S. Donald Bradshaw, and Tom Stewart*
11.10 - 11.40	**Morning Tea**
	Session Two - Respiratory Physiology, Chair: Malcolm Forster
11.40 - 12.00	Development of chorioallantoic and pulmonary respiration in Australian pelican (*Pelecanus conspicillatus*) embryos and hatchlings *James Pearson*, Roger S. Seymour, Russell V. Baudinette and Sue Runciman*
12.00 - 12.20	Chasing and gassing salmon on farms: physiological insights into amoebic gill disease *Mark Powell* and Barbara Nowak*
12.20 - 12.40	The role of convection in ventilation of animal burrows *Craig White*
12.40 - 2.00	**Lunch: Union Building Dining Room**
2.00	**Session Three: Reproductive Biology and Endocrinology, Chair: Jean Joss**
2.00 - 2.20	The role of prostaglandin and mesotocin at birth in the bandicoot (*Isoodon macrourus*) *Robert Gemmell*
2.20 - 2.40	Arginine vasotocin stimulates parturition in southern snow skinks (*Niveoscincus microlepidotus*) held at different temperatures *Jane Girling* and Sue Jones*
2.40 - 3.00	Plasma mesotocin in the oestrous cycle and pregnancy of the brushtail possum *Trichosurus vulpecula* *Conrad Sernia*, Ross Bathgate and Robert Gemmell*

3.00 - 3.20	Regulation of clutch size in the skink *Niveoscinus metallicus* *Sue Jones* and Roy Swain*
3.20 - 3.40	Plasma sex steroid hormone levels in relation to the reproductive cycle of the eastern shovelnose ray, *Aptychotrema rostrata* *Peter Kyne* and Mike Bennett*
3.40 - 4.10	**Group Photo and Afternoon Tea**
	Session Four - Endocrinology, Chair: Tony Hulbert
4.10 - 4.30	Administration of oestradiol to barramundi, *Lates calcarifer*, induces protandrous sex change *Trevor Anderson* and J. Forrester*
4.30 - 4.50	Natriuretic peptide receptor signalling in the gills of fishes *Will Callahan*, John Donald, and Tes Toop*
4.50 -5.10	The effects of melatonin administration on the seasonal changes in daily activity rhythms in the marsupial *Antechinus stuartii* *Bronwyn McAllan*, W. Westman, G. Körtner and S. C. Cairns*
5.10 - 5.30	Changes in circulating steroid concentration associated with 'nipping' behaviour in the domestic dog *Adrian Rex*

5.45 - 7.15 Posters and Refreshments: SC 429

	Poster Presentations
P1	The effects of ammonia on the respiratory physiology of the juvenile big bellied seahorse *Hippocampus abdominalis* *M. Adams, Mark Powell* and G.J. Purser*
P2	Differences in heart morphology and health status of farmed Atlantic salmon *Mark Powell* and Barbara Nowak*
P3	Observations on breeding behaviour in the Tasmanian echidna, (*Tachyglossus aculeatus*) *Niels Andersen* and Stewart Nicol*
P4	Gill and swim bladder morphology, morphometry and function in the bimodal breathing tarpon, *Megalops cyprinoides* *Mike Bennett*, J. Baldwin, K. Christian, R.V. Baudinette, R. Wells & R.S. Seymour*
P5	Gait analysis and locomotor energetics in the northern brown bandicoot, *Isoodon macrourus torosus* *J.G. Garden and Mike Bennett**
P6	Vasodilator mechanisms in large arteries of the toad, *Bufo marinus* *Brad Broughton* and John Donald*
P7	Peripheral conversion of steroids in a viviparous skink, *Tiliqua nigrolutea* *Ashley Edwards* and Sue Jones*

P8	Expression of Na^+/H^+ antiporter mRNA in the gills of the Atlantic hagfish (*Myxine glutinosa*) in response to metabolic acidosis *Susan Edwards, J.B. Claiborne, and Tes Toop**
P9	Dietary fats, white adipose tissue fatty acid composition, and hibernation in free-ranging echidnas *Tachyglossus aculeatus* (Monotremata) *Frank Falkenstein, Gerhard Körtner, Ken Watson, and Fritz Geiser**
P10	The effect of water deprivation on cardiac and renal atrial natriuretic peptide mRNA expression in the Spinifex Hopping Mouse *Rachel Heimeier* and John Donald*
P11	Structure and thyroid hormone binding properties of transthyretin: Its synthesis in *Pichia pastoris* *Julie Monk*, G. Schreiber and S. Richardson*
P12	Sodium pump molecular activity and membrane lipid composition in a highly polyunsaturated species: the Octopus *Nigel Turner*, Paul Else and Tony Hulbert*

7.15 PM - LATE: BBQ AT DEAKIN UNIVERSITY HOUSE

Saturday December 9th

	Session Five - Osmotic and Ionic Regulation, Chair: Tes Toop
9.00 - 9.40	**Invited Speaker: Professor Yoshio Takei, Ocean Research Institute, Tokyo** The natriuretic peptide system and euryhalinity of eels *Yoshio Takei* and Shigehisa Hirose*
9.40 - 10.00	Natriuretic peptide receptors in the kidney of the toad, *Bufo marinus* *Stuart Meier, Shane Riddell, and John Donald**
10.00 - 10.20	Effect of transport inhibitors on fluid and ion secretion by wombat parotid glands *Michel Beal*
10.20 - 10.40	Novel aspects of transport of organic anions by the malpighian tubules of *Drosophila melanogaster* *Stuart Linton* and Michael O'Donnell*
10.40 - 11.10	**Morning Tea**
	Session Six - Water Balance and Metabolism: Chair, Ian Hume
11.10 - 11.30	Soil selection in a burrowing frog, *Cyclorana alboguttata* *David Booth*
11.30-11.50	Microenvironment conditions in Australian bats: physiology meets restoration ecology *Russell Baudinette*
11.50 - 12.10	Was Governer Grey a closet eco-physiologist? Niche partition on the basis of water economy in four species of introduced macropod marsupials on Kawau Island, New Zealand. *Roger Lentle*, I.D. Hume K.J. Stafford, M. Kennedy; M.A. Potter, B.P. Springett, and S. Haslett*

12.10 - 12.30	A seasonal comparison of energy and water requirements of long-nosed bandicoots in the Sydney region *Michele Odewahn*, Ian Hume, and Lesley Gibson*
12.30 - 12.50	Ecophysiology of four Australian Finches *Z. Carr, H. Chan, C. Cooper, A. Larcombe, J. O'Shea, S. Voss, P. Withers* & G. Zosky*
12.50 - 2.00	**Lunch: Deakin University House**
	Session Seven - Metabolism and Digestive Physiology, Chair: Sue Jones
2.00 - 2.20	Energy relations of old endemic Australian passerines: Are small clutch sizes associated with low basal metabolic rates? *Bill Buttemer*, C. Bech, M.A. Chappell, and L.B. Astheimer*
2.20 - 2.40	The Pace of Life: Molecular activity, membranes, sodium pumps, cow and crocodiles *Paul Else*, Ben Wu and Tony Hulbert*
2.40 - 3.00	Daily torpor and activity in free-ranging sugar gliders *Gerhard Körtner* and Fritz Geiser*
3.00 - 3.20	Heat balance during locomotion of the brush-tailed bettong *Koa Webster* and Terry Dawson*
3.20 - 3.40	Developing a Digesta Retention Time Model for Caecum Fermenters *Fiona Herron*, Ian Hume, Graham Faichney, and Ray Boston*
3.40 - 4.10	**Afternoon Tea**
	Session Eight - Animal Behaviour, Chair: Russ Baudinette
4.10 - 4.30	Baby please don't go: migratory constraints on reproduction in the endangered swift parrot *Lathamus discolor* *Brett Gartrell*, Susan Jones, Raymond Brereton, Lee Astheimer*
4.30 - 4.50	Fearless: Loss of startle responses in Balmain bugs (*Ibacus peronii*) *Zen Faulkes*

4.50 **ANZSCPB General Meeting**

CONFERENCE BANQUET: DEAKIN MANAGEMENT CENTRE

Cash Bar opens for pre-dinner drinks at 6.00 p.m.

Sunday program over page

Sunday December 10th

	Session Nine - Biochemistry and Population Genetics, Chair: Paul Else
9.00 - 9.20	Non-Shivering Thermogenesis in the Tasmanian bettong, *Bettongia gaimardi* identification of Uncoupling proteins 2 and 3 *Alexander Kabat*, Randy W. Rose, Julie Harris, and Adrian K. West*
9.20 - 9.40	Non-shivering thermogenesis in the Tasmanian bettong (*Bettongia gaimardi*) and the role of uncoupling proteins (UCP1, 2 and 3) *Randy W. Rose*, Alexander Kabat and A. West*
9.40 - 10.00	Molecular activity of Na^+,K^+-ATPase related to packing of membrane lipids *Ben Wu, Paul Else, L.H. Storlien and Tony Hulbert**
10.00 - 10.20	Phylogeography of the Noisy Miner throughout eastern Australia *Sarah Caple* and Janette Norman*
10.20 - 10.50	**Morning Tea**
	Session Ten - Muscle Biochemistry, Chair: Phil Withers
10.50 - 11.10	Cardiac myosins of marsupial mammals: subunit structure, developmental changes and modulation by body size *Joe Hoh*, Y. Kim, C.A. Lucas, and W.W.H. Zhong*
11.20 - 11.40	Effects of hypothyroidism on the distribution of cardiac & skeletal myosin isoforms in *Antechinus flavipes* *W.W.H. Zhong*, J.F.Y. Hoh and K. Withers*
11.40 - 12.00	Mighty Mini-Muscles in Mice *Helga Guderley*, Philippe Houle Leroy, John Swallow, and Theodore Garland Jr*
12.00	**Student Prize Presentations and Concluding Remarks**

Lunch: Deakin University House

PROGRAMME NOTES

PROGRAMME NOTES

ABSTRACTS OF ORAL PRESENTATIONS

Administration of oestradiol to barramundi, *Lates calcarifer*, induces protandrous sex change

T.A. Anderson and J. Forrester

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email: Trevor.Anderson@jcu.edu.au

Although sex change is a common phenomenon in teleosts, our understanding of the physiological processes involved is limited. Barramundi, *Lates calcarifer*, is an important species for both aquaculture and fisheries throughout SE Asia but broodfish management in hatcheries is confounded by precocious sex change. Oestradiol (E2) has been reported to be high in gonads of barramundi undergoing transition (Guigen *et al.*, 1995). The present experiment was undertaken to investigate the hypothesis that a high concentration of E2 in male barramundi induces sex change. E2 was administered in cholesterol pellets to groups of eight barramundi weighing between 1.0 and 2.9 kg at 5 doses averaging 0 mg.kg BW^{-1}, 0.2 mg.kg BW^{-1}, 1.5 mg.kg BW^{-1}, 8.4 mg.kg BW^{-1}, or 44 mg.kg BW^{-1}. Four animals per treatment were killed at 28 days post implantation. Gonads were sampled for histology and aromatase activity, and plasma testosterone (T), 11-keto testosterone (11kT) and E2 were measured. Three of four animals given 1.5 mg.kg BW^{-1} E2 and all of the animals given 8.4 or 44 mg.kg BW^{-1} E2 were either transitional or female at the end of the 28 day period. Three of four control animals and all of the animals given 0.2 mg.kg BW^{-1} E2 were male. Plasma T and 11kT declined significantly with increasing dose of E2. Gonadal aromatase was high (>130 fmol ^{3}H-androstendione converted.mg $protein^{-1}.hr^{-1}$) in transitional or female barramundi but low (<45 fmol ^{3}H-androstendione converted.mg $protein^{-1}.hr^{-1}$) in male barramundi. These data indicate a switch from androgen to oestrogen production in response to administered E2 and support the hypothesis that high levels of E2 in male barramundi initiate protandrous sex change.

Guiguen, Y. *et al.* (1995). Gen. Comp. Endocrinol. **100**, 106-118.

Microenvironment conditions in Australian bats: physiology meets restoration ecology

R.V. Baudinette

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The water balance of mammals is multifactorial but for many small species can be simplified to the ratio of metabolic water production to pulmocutaneous water loss. The ambient temperature at which this ratio (MWP/EWL) is unity has been used to explain rodent distributions in North America and parrot distributions in Australia but has not been commonly adopted. In the present study the model was expanded to include humidity. Three Northern Territory bats, the ghost bat, orange leaf-nosed and common bent-wing, were investigated and the ratio MWP/EWL was determined as a function of ambient temperature and humidity. Resulting surface plots show that under known roosting microclimates two of the bats remain in water balance but the ghost bat does not. The study also points to the vulnerability of the orange leaf-nosed bat in which rates of pulmocutaneous evaporation are about seven times that seen in rodents of similar size. Attempts have been made to re-establish bat maternity caves and the results will be related to one such attempt in SA.

Effect of transport inhibitors on fluid and ion secretion by wombat parotid glands

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Email: a.beal@unsw.edu.au

Bicarbonate concentrations are positively correlated with flow rate in parotid saliva from all marsupials investigated to date with the exception of the wombat. Wombat parotid saliva has bicarbonate levels which are essentially stable at 50-60 mmol/L (ie. about twice plasma concentrations) over most of the flow range. To assess the nature of the mechanism underpinning secretion by this gland, molecular transport inhibitors were administered by intracarotid infusion at rates sufficient to block specific ion transporters. The triple symport blocker, bumetanide (0·35-2·5 μmol/kg/min), reduced flow under stable stimulation of moderate flow and during maximal stimulation. Salivary chloride concentration fell and bicarbonate rose with bumetanide. The Cl/HCO_3 antiport blocker, SITS (4·8-5·6μmol/kg/min), caused no depression of flow nor change in anion concentrations. The Na/H antiport blocker, amiloride (4 μmol/kg/min) lowered maximal salivary flow, increased chloride and decreased bicarbonate levels. Carbonic anhydrase inhibitors (CAI), methazolamide or acetazolamide (4-22·5 μmol/kg/min), lowered salivary bicarbonate and increased chloride without any effect on saliva production. However, simultaneous administration of bumetanide (0·6-0·8 μmol/kg/min) and CAI (6-17 μmol/kg/min) was significantly more effective at blocking saliva production than bumetanide alone. The data indicate that at least 50 % of the bicarbonate is ductal in origin and CA dependent, that acinar saliva formation is supported mainly by chloride transport and that, when chloride transport is blocked, the acini can use bicarbonate secretion to partially replace chloride transport.

Soil selection in a burrowing frog, *Cyclorana alboguttata*

David T. Booth

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Email: DBooth@zen.uq.edu.au

Arid zone burrowing frog species appear to have two distinct strategies to avoid desiccation during prolonged periods of low rainfall. Species found in sandy soils dig deeper into the soil as time progresses, presumably staying below the soil drying-front and thus avoid desiccating conditions. These species can be found up to one metre below the soil surface, and can use this strategy because sandy soil is relatively easy to burrow through. Species found in heavy soils with high clay content are found in shallow burrows (15–30 cm below surface), but form a cocoon of shedded epidermis around them which acts as a water loss barrier. In this study I examined the soil drying characteristics and soil preference of the cocoon forming green-striped burrowing frog *Cyclorana alboguttata* in the laboratory. This species is typically found forming burrows in heavy clay soils in nature. The water content Vs water potential characteristic curves were typical for sandy and clay soils, and there was little difference in the rate at which the soil potential changed when soils dried on exposure to room air. Given a choice between wet heavy clay and wet sandy soil *C. alboguttata* invariably choose to burrow into the sandy soil, presumably because it was easier to dig into. Given a choice between wet heavy clay and dry sandy soil *C. alboguttata* invariably choose to burrow into the clay soil. In all cases frogs absorbed water from the soil while in burrows.

Energy relations of old endemic Australian passerines: Are small clutch sizes associated with low basal metabolic rates?

W.A. Buttemer[1], C. Bech[1,2], M.A. Chappell[1,2], and L.B. Astheimer[1,4]

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The clutch size of Australian "old endemic" passerine species is about half that of birds arriving in Australia during the Pleistocene ("new invaders") and more recently introduced Eurasian species. We examined whether these phyletic differences in fecundity corresponded with variation in basal metabolic rate (BMR). We measured BMR in 18 old-endemic species (ranging from 6 to 50 g) and 8 new-endemic or introduced species (ranging from 10 to 100 g). To correct for the influence of body mass on BMR, we first performed a linear regression on log BMR in relation to log body mass for all species measured which produced a highly significant correlation ($P<.001$). Surprisingly, old endemics tended to have higher than expected BMRs, but residual analysis revealed differences between these groups to be non significant, BMR than the other species ($P=.052$). We thus conclude that low clutch sizes in old endemic species are not due to inherent energetic constraints of the parents. However, based on our studies of White-plumed Honeyeaters, we propose that small clutch sizes benefit species that have flexible breeding schedules and live in habitats with low-amplitude variation in resources.

Natriuretic peptide receptor signalling in the gills of fishes

Will Callahan, John Donald, and Tes Toop

School of Biological and Chemical Sciences, Deakin University, Geelong, Victoria, 3217. Email: wcallaha@deakin.edu.au

The gill epithelium is a major site of passive and active electrolyte and water flux. The direction of these movements differs depending upon ambient salinity. Natriuretic peptides released from the heart may regulate ionic and osmotic movements across the branchial epithelium by binding to natriuretic peptide receptor (NPR) proteins which are abundant in the gill. Whilst the NPR-A and NPR-B receptor subtypes signal through the guanylyl cyclase / cyclic GMP pathway, evidence is emerging from mammalian studies that NPR-C may be coupled to the adenylyl cyclase / cyclic AMP system. This study examines natriuretic peptide receptor signalling in the gills of the euryhaline Atlantic stingray (*Dasyatis sabina*), the marine sand flathead (*Platycephalus bassensis*) and the freshwater rainbow trout (*Oncorhynchus mykiss*) to elucidate whether differences exist in guanylyl cyclase signalling between marine and freshwater fishes. Studies on preparations of isolated gill cell membranes from *D. sabina* indicate that the basal rate of cGMP accumulation is greater in marine than in freshwater animals. In addition, 1μmol l^{-1} porcine C-type natriuretic peptide stimulates cGMP accumulation above basal levels in marine animals but not in their freshwater counterparts. An *in vivo* perfusion technique reveals that 0.1 μmol l^{-1} rat atrial natriuretic peptide stimulates cGMP accumulation in *P. bassensis* but not in *O. mykiss* and that basal levels of cGMP are greater in the marine species. Interestingly, forskolin, a direct activator of adenylyl cyclase, acts synergistically with atrial natriuretic peptide to elevate cGMP accumulation in the perfused gill of the trout. This study demonstrates some interesting differences in both basal cGMP concentrations and natriuretic peptide-stimulated cGMP production between the gills of marine and freshwater fishes and suggests that natriuretic peptide receptor activity is implicated in osmoregulatory control.

Phylogeography of the Noisy Miner throughout eastern Australia

Sarah Caple[1] and Janette Norman[2]

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Noisy Miners (*Manorina melanocephala*) are largish, honeyeaters commonly found in open forests and grassy woodlands throughout eastern Australia, from north Queensland to South Australia and Tasmania. Classification of *M. melanocephala* into four subspecies has been based on morphological variation in size and plumage colour. The distribution of genetic variation, within the geographic range of the Noisy Miner, was investigated to evaluate evolutionary relationships among different regional populations and to compare patterns of genetic variation with patterns of morphological variation. Both mitochondrial DNA and nuclear DNA markers were compared. Portions of two mitochondrial genes (cytochrome-b and NADH dehydrogenase subunit 3) were sequenced from birds from throughout the range of *M. manorina*, and were found to be very similar (sequence divergence 0.3 – 1.6%), indicating a close relationship among all populations sampled. The distibution of mitochondrial DNA haplotypes indicated limited phylogenetic structure within the species, with only one group (Victoria/South Australia) consistently demonstrating geographic separation. The relative distributions of alleles at four nuclear microsatellite loci showed that geographically close populations were more closely related than geographically distant populations, suggesting that gene flow between nearby populations has been more significant than long distance dispersal in the recent demographic history of this species.

Ecophysiology of four Australian Finches

Z. Carr, H. Chan, C. Cooper, A. Larcombe, J. O'Shea, S. Voss, P. Withers & G. Zosky

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Aspects of water balance were examined for four Australian granivorous finches, two white-rumped grass finches *Taeniopygia guttata* (Zebra Finch) and *Taeniopygia bichenovii* (Double-barred Finch), and two crimson-rumped grass finches *Emblema pictum* (Painted Firetail) and *Neochmia temporalis* (Red-browed Firetail), to determine whether the more xeric species (Zebra Finch and Painted Firetail) were better able to tolerate water deprivation, and if so why.

All finches had a similar water budget with *ad lib.* drinking water, but the Zebra Finch was the only species capable of maintaining water balance without drinking (at 30 °C and 50% RH); the Double-barred Finch was less susceptible to water restriction than the Painted Firetail, and the Red-browed Firetail was the least tolerant. The Zebra Finch had a reduced food intake and a lower faecal water content when water restricted, but it would appear that the major mechanisms responsible for its tolerance of water restriction are related to the major avenues for water gain (metabolic water production) and water loss (evaporation).

Examination of metabolic and hygric physiology at ambient temperatures of 20, 30 and 35 °C indicated few differences amongst the species. Body temperature rose from about 38 °C at T_a = 20 to nearly 40 °C at T_a = 35, except for the Red-browed Firetail which did not tolerate well the high ambient temperature. The Zebra Finch had the highest metabolic rate, hence metabolic water production (MWP), and the Red-browed Firetail had the lowest metabolic rate (and MWP), at all T_as. The Red-browed Firetail had the lowest thermal conductance at 20 and 30 °C, and the highest conductance when heat-stressed at 35 °C. Evaporative water loss (EWL) was highest for the Painted Firetail and lowest for the Red-browed Firetail, and was intermediate for

the Zebra and Double-barred Finches; however, the Zebra Finch was the only species for which EWL did not increase at 35 °C. The ratio of MWP/EWL was highest (and always above 1) for the Zebra Finch at all T_as; the Double-barred Finch was intermediate, and the Firetails had the lowest MWP/EWL (EWL was adjusted to an ambient RH of 50%). The point of relative water economy (PRWE, the ambient temperature at which MWP = EWL) was highest for the Zebra Finch (> 35 °C), was 28.3 °C for the Double-barred Finch, was 27.3 °C for the Painted Firetail, and was lowest (26.9 °C) for the Red-browed Firetail.

Many particular aspects of the water budget, thermoregulation, metabolism and evaporation of these four finches indicate reasons for their sequence of tolerance to water deprivation, and presumably therefore their relative adaptation to arid environments. However, it is primarily the more favourable ratio of metabolic water production to evaporative water loss (MWP/EWL) and point of relative economy (PRWE) that best describe their respective tolerance to water deprivation. In particular, the high metabolic rate of the Zebra Finch (hence high MWP/EWL) suggests that this its major adaptation to survival in arid environments.

Natriuretic peptide receptors in the kidney of the toad, *Bufo marinus*

Stuart Meier, Shane Riddell, and John Donald

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The natriuretic peptides are involved in the control of hypervolaemia in mammals, but it is still unclear what their role is in the lower vertebrates. Amphibians in fresh water are prone to osmotic fluid loading and will encounter hypervolaemia if compensatory mechanisms are not invoked. In *B. marinus*, isoosmotic fluid loading causes a diuresis[1]; we have hypothesised that the diuresis is mediated by atrial natriuretic peptide (ANP). The present study used the toad, *B. marinus*, and had three components: i) to determine the distribution and nature of natriuretic peptide receptors (NPR) in the kidney; ii) to determine the effect of frog ANP (fANP) on renal function in a perfused kidney preparation; and iii) to determine how intra-vascular volume loading affects the expression of NPR mRNA. Receptor analysis techniques showed that high affinity ANP binding sites were predominately found on glomeruli and renal blood vessels, and that the kidney contained two types of NPR; guanylyl cyclase receptors (NPR-GC) and clearance receptors (natriuretic peptide receptor-C [NPR-C]). Partial NPR-GC and NPR-C cDNAs were obtained using PCR based cloning. Perfusion of the kidneys with fANP caused a vasodilation and a diuresis and natriuresis. It was determined that the diuresis and natriuresis were due to an increase in the glomerular filtration rate, and that fANP had very little effect on tubular reabsorption. Finally isoosmotic volume loading caused a decrease in the expression of NPR-GC and NPR-C mRNA, compared to control animals. These data indicate that fANP is a diuretic and natriuretic hormone in the toad, and that its affects are occurring primarily in the glomerulus. In addition, the expression of renal NPR mRNA is linked to volume status. The down-regulation of NPR mRNA is probably due to an increase in plasma ANP.

[1]Hillman, S.S., and Schimpf, B. (1994). Renal responses to blood volume expansion of varying osmotic concentration in two species of amphibian, *Bufo marinus* and *Rana catesbeiana*. *Physiol. Zool.* **67**(4):995-1005.

The Pace of Life: Molecular activity, membranes, sodium pumps, cow and crocodiles

Paul L. Else[1], Ben J. Wu[1] and A. J. Hulbert[2]

Metabolic Research Centre, Departments of [1]Biomedical & [2] Biological Science, University of Wollongong, N.S.W. 2522, Australia. Email: pelse@uow.edu.au

The metabolic intensity at which animals experience life varies enormously. Metabolic pace varies during development and between species and animals of different body mass. Recently we proposed the hypothesis that *variation in animal metabolism is due to membranes setting the overall pace of a small number of common cellular processes.* This hypothesis is largely based on our reported findings associated with ion pumps and leaks occurring across membranes and correlated membrane compositional differences. The present study, using the sodium pump, will show that the rate at which each pump operates (ie molecular activity in units of ATP/min.) in a large mammal (cow, *Bos taurus*) and reptile (crocodile, *Crocodylus johnstoni*) differ several fold (3,000 versus 800 ATP/min respectively). Furthermore, that using a simple membrane reconstitution, sodium pump activity could be incrementally stepped up or down dependant upon the membrane used to reconstitute. Changes in the pace of sodium pump activity measured in these experiments supports the proposition that the major explanation for the differences in the molecular activity of the sodium pumps resides within the innate properties of membranes.

Fearless: Loss of startle responses in Balmain bugs (*Ibacus peronii*)

Zen Faulkes

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Many decapod crustaceans (e.g., crayfish) have short-latency (< 20 ms) startle responses mediated by two sets of identified giant interneurons. The medial giant (MG) interneurons respond to sudden stimuli at a crayfish's anterior end, and cause a single tailflip that propels the animal backwards. The lateral giant (LG) interneurons respond to stimuli at a crayfish's posterior end, and cause a single tailflip that pitches the posterior end of the animal up, effectively somersaulting the animal into the water column. The different kinematics of these two startle behaviours are partly explicable by their synaptic connections to specialised abdominal fast flexor motor giant (MoG) neurons. In addition to these two single tailflip responses, crayfish also swim by repetitive tailflipping, which has a long latency (> 100 ms) and is mediated by a network of unidentified non-giant neurons. Balmain bugs (*Ibacus peronii*) are proficient swimmers and tailflip spontaneously or in response to gradual stimuli. These tailflips are apparently non-giant mediated, however: *Ibacus* never tailflip in response to sudden taps. Histological sections of the abdominal nerve cord revealed neither MG nor LG interneurons. Furthermore, backfills of abdominal flexor nerves show *Ibacus* have neither MoG neurons nor equivalent non-giant cells. Thus, *Ibacus* have lost startle responses and deleted the neural circuitry needed to produce them. The loss of these behaviours and associated neurons are correlated with the unusual morphology (dorso-ventrally flattened, flexed abdomen) and behaviour (alternative predator avoidance strategies; i.e., digging) of *Ibacus*.

The control of vascular tone in Notothenioid fishes is determined by phylogeny not environmental temperature

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Animals living in extremely cold environments must show adaptations to the cardiovascular system to maintain biological activity at low temperatures. We used myography to examine the potential vasomotor control mechanisms in an Antarctic fish (*Trematomus bernacchii*; usual body temperature ca. −1°C), comparing the sensitivity to noradrenaline (NAd), carbachol (CBC) and serotonin (5-HT) by means of the cumulative dose-response, and potency with reference to depolarisation by 50 mM KCl. For the efferent branchial artery (EBA) NAd produced ca. 20% of the maximal KCl tension, and ca. 40% in the presence of 10^{-3}M sotalol, suggesting a modest but equal contribution of α and β adrenergic tonus (pEC_{50}=6.29±0.37M). CBC and 5-HT had different sensitivities (pEC_{50}=4.50±0.40M and 6.82±0.08M, respectively) but similar potencies (21.6±11.1 and 31.1±5.3% of KCl).In order to explore the origins of this unusual vascular reactivity we also examined related species. *Paranotothenia angustata* (family Nototheniidae) which inhabits the warmer waters around New Zealand, gave similar results for NAd (pEC_{50}=5.48±0.31M), CBC (pEC_{50}=4.94±0.22M) and methysergide-sensitive vasoconstriction with 5-HT (pEC_{50}=6.22±0.40M). Agonist potencies were 9, 65 and 45% that of KCl, respectively. Data for another New Zealand species, *Bovichtus variegatus* (family Bovichtidae) broadly paralleled those of *P. angustata* but with a higher potential adrenergic tonus. In contrast, EBAs from *Dissostichus mawsoni*, a pelagic Antarctic notothenid, showed a dominance of vasodilatation over vasoconstriction, with sensitive isoprenaline (pEC_{50}=6.66±0.05M) but weak serotonergic (5.2±1.5 % of KCl) responses. These data suggest that the unusual dominance of serotonergic over adrenergic control in notothenioids is primarily a consequence of evolutionary lineage rather than low environmental temperature, and that the pattern may be modified according to functional demand.

Baby please don't go: migratory constraints on reproduction in the endangered swift parrot *Lathamus discolor*

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The swift parrot is a small, endangered parrot that has an annual migratory cycle between its breeding grounds in Tasmania and its over-wintering grounds in southeastern Australia. We are studying the migratory orientation preferences of the swift parrot using Emlen funnel cones, with captive birds exposed to a full range of solar and magnetic cues. Initial results suggest that orientation preferences correspond to known seasonal movement of the wild population. These results support the hypothesis that there is an endogenous rhythm to migration in the swift parrot. Field observation suggest that this rhythm can be modulated by nutritional cues, such as the large scale flowering of eucalypts, but that there is no ability to predict such events. Experimental studies are needed to investigate these hypotheses. We suggest that migration acts as a temporal constraint on reproduction in the swift parrot and discuss the implications of this for the conservation of the enigmatic parrot.

The role of prostaglandin and mesotocin at birth in the bandicoot (*Isoodon macrourus*)

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As with eutherians, the maturation of the marsupial fetal pituitary and adrenal glands allows for the secretion of cortisol that in turn leads to increased prostaglandin production and along with mesotocin initiates birth in marsupials. In this study prostaglandin (Lutalyse) or oxytocin (Syntocinon) were administered to pregnant bandicoots at 5.00am on the calculated day of birth and the resultant effects filmed for analysis. The administration of prostaglandin caused the bandicoot to adopt the birth position several minutes after injection (N=2). However the bandicoot did not give birth for several hours, birth occurring at a similar time of the day, between 8.00am and 12.00pm, as observed for untreated bandicoots. Following the injection of oxytocin the bandicoot assumed the birth position and birth occurred within several minutes. The young were alive while still connected to their allantoic stalks. However they were unable to attach to a teat and the young did not survive (N=4). The colour of the induced young would indicate that an under-developed cardio-pulmonary system may be the cause of death. Prostaglandin is required to prepare the bandicoot for birth and mesotocin is required for the contraction of the uterus and birth to occur.

Mighty Mini-Muscles in Mice

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After 14 generations of artificial selection for high levels of voluntary wheel-running behavior, mice from the four replicate selected lines ran, on average, twice as many revolutions per day as in the four unselected control lines. To examine possible heterogeneity in the correlated responses of hindlimb muscle size and metabolic capacities, we studied mice from both selected and control lines, housed in cages with access to running wheels that were either free to rotate ("active" group) or locked("sedentary"). Thirteen of 20 individuals in one selected line (line 6) and two of 20 in another (line 3) showed a drastic, almost 50% reduction in total hindlimb muscle mass. Access to wheels that were free to rotate did not affect expression of the mini-muscle phenotype but did result in typical mammalian training effects for enzyme activities (e.g., an increased oxidative capacity). In selected lines 6 and 3, individuals with a reduced hindlimb muscle mass showed a concomitant increase in muscle aerobic capacity as revealed by the specific activities of citrate synthase and cytochrome C oxidase. Moreover, these mice showed the highest specific activities of phosphofructokinase and hexokinase. As shown by total-muscle enzyme activities, the increase in mass-specific aerobic capacity in the semini-muscles almost completely compensated for the reduction caused by the "loss" of muscle mass. However, the mini-muscle mice exhibited the lowest lactate dehydrogenase contents. Compensation was limited to glucose metabolism, given that no major increase was observed in mass-specific levels of carnitine palmitoyl transferase or glycogen phosphorylate phosphorylase. In selected line 3 (but not in line 6), the 18 of 20 individuals that did not have mini-muscles actually had hindlimb muscles that averaged 10% larger than the norm for their body mass. Our results demonstrate that during our selection for a voluntary wheel running, a variety of adaptive paths, which differentially exploit the underlying morphological and physiological traits have been followed.

Arginine vasotocin stimulates parturition in southern snow skinks (*Niveoscincus microlepidotus*) held at different temperatures

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Southern snow skinks, *Niveoscincus microlepidotus*, are found in alpine regions of southern Tasmania, Australia and exhibit an unusual biennial reproductive cycle due to the cool and variable climate they experience. Females ovulate in the spring and pregnancy extends over the summer and winter; parturition occurs the following spring. Prior to the winter, females contain fully developed embryos in utero; the mechanism preventing parturition at this stage is not known. It was hypothesised that the temperatures experienced by pregnant females prior to the winter inhibit the hormonal cascade causing parturition. We aimed to determine whether arginine vasotocin (AVT) could trigger parturition prior to winter in pregnant females held at various temperatures (28, 22, 15 and 8°C). At each temperature, females were divided into three groups (n=6) and received either a single, intramuscular injection of 2.0mg AVT/0.05 ml saline or 0.05 ml saline, or no injection. All females receiving AVT gave birth, whereas none of the control females gave birth. The time between the AVT injection and parturition increased as temperature decreased (28°C: 0-2 h, 22°C: 2-3 h,15°C: 3-6 h, 8°C: 9-60 h). The results suggest that pregnant females are capable of responding to an AVT stimulus at the temperatures that they would experience during autumn. It is now necessary to determine how the production and secretion of AVT varies in response to changing environmental conditions.

Developing a Digesta Retention Time Model for Caecum Fermenters

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The ability to model digesta retention times has application in the development of knowledge of nutrient utilisation in all animals, especially those being fed on artificial diets in captivity. The aim of this model is to predict accurately parameters of digesta passage through the gut without the need for invasive procedures. Three caecum fermenters with similar digestive strategies are of interest - the Rabbit (*Cuniculus oryctolagus*), Common Ringtail Possum (*Pseudocheirus peregrinus*) and Koala (*Phascolarctos cinereus*). Highly evolved digestive processes have been recognised in these animals including a ColonicSeparation Mechanism (CSM) which leads to the selective retention of solutes and very small particles in the caecum, and caecotrophy which is the ingestionof high-nutrient faeces derived from caecal contents (practised by rabbits and ringtail possums). This model will allow the quantitative significance of selective digesta retention (internal recycling of digesta) and caecotrophy(external recycling) to be evaluated. To construct the model, new techniques for inert markers of solutes, small and large particles of digesta that more closely fit the concept of an "ideal marker" are being developed. Concentrations of the markers at levels as low as ppb are being assayed by ICPMS (Inductively Coupled Plasma Mass Spectrometry). Data derived from patterns of appearance of the various markers in the faeces are being used to construct a digesta retention model with the aid of the computer simulation package WINSAMM. The model is being constructed initially using rabbits, confirming with ringtail possums and finally it will be tested with koalas. Ultimately, for this project, these results will provide a more quantitative explanation for how the koala is able to survive on a sole diet of *Eucalyptus* foliage.

Cardiac myosins of marsupial mammals: subunit structure, developmental changes and modulation by body size

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Atrial and ventricular myosin isoforms in small and large marsupial mammals were examined by native gel electrophoresis, SDS-PAGE and Western blotting using myosin heavy chain (MyHC) specific antibodies. Ventricular myosin in macropodids showed three native components, V_1, V_2 and V_3, and Western blots using specific anti-α and anti-β cardiac MHC antibodies showed their MHC structures to be αα, αβ and ββ respectively. Atrial myosin showed a single component with the same MyHC composition as V_1, but differed in light chains. Ventricular myosin in small marsupials (*Sminthopsis crassicaudata, Antechinus stuartii*) showed only V_1, while larger marsupials (*Pseudocheirus peregrinus, Trichosurus vulpecula*, various large macropods) showed predominantly V_3. Ventricular myosin in early pouch joeys of *Macropus eugenii* showed little or no α-MyHC, this component rose sharply around the time of pouch exit to approximately adult level. These changes lag slightly behind the previously reported developmental increase in serum levels of thyroid hormones. The results suggest that cardiac myosins in marsupial mammals are substantially the same as their eutherian counterparts in structure and responsiveness to thyroid hormones. It is suggested that ventricular myosin genes may have played a very important role in the evolution of mammals by modulating cardiac contractility to match metabolic rate in relation to body size and the thyroid state.

Regulation of clutch size in the skink *Niveoscincus metallicus*

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In many reptile species, atresia of vitellogenic follicles is a major factor determining final clutch size. However this does not hold for the viviparous metallic skink *Niveoscincus metallicus*. Comparison of the mean number of vitellogenic eggs and mean number of oviductal eggs/embryos shows that in this species clutch size is fixed before vitellogenesis is established, and is not altered by follicular atresia or embryonic loss. As the number of follicles recruited is dependent upon circulating concentrations of gonadotrophins, then clutch size may be dependant on the endocrine status of the female during a critical period early in follicular development. We tested this hypothesis by administering exogenous ovine FSH to female metallic skinks at different stages of the reproductive cycle. Pre-vitellogenic females showed no ovarian response to exogenous FSH. In early vitellogenic females, FSH induced follicular recruitment: follicles were enlarged and clutch size increased by recruitment of a second cohort of follicles; and some females also ovulated. If females were treated in mid-vitellogenesis no follicular recruitment was observed, but most of these animals ovulated. Females treated with a range of doses of FSH in late vitellogenesis ovulated at least one month before natural ovulation, again without recruitment of extra follicles. Some of these females produced live young that were born small after a reduced gestation. We conclude that in this species clutch size is determined by gonadotrophin levels early in follicular recruitment, and cannot be increased once vitellogenesis is established.

Non-Shivering Thermogenesis in the Tasmanian bettong, *Bettongia gaimardi* :identification of Uncoupling proteins 2 and 3

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This is the first study that has been able to characterise uncoupling proteins in a marsupial using molecular techniques. *Bettongia gaimardi*, a rat-kangaroo increases non-shivering thermogenesis in response to Norepinephrine (Rose *et al.*, 2000), although neither Brown Adipose Tissue (BAT) nor Uncoupling Protein 1 (UCP1) are present. Uncoupling Protein 2 or 3 are also specialized protein pathways found in the mitochondria of various tissues that are thermogenetic specialists. Similar to UCP1 both UCP2 and UCP3 are believed to partially uncouple mitochondrial respiration to produce heat however the expression of UCP 2 and 3 is not associated BAT, UCP2 has been probed at 1.7 kb and is widely expressed and has been identified in White Adipose Tissue (WAT), cardiac and skeletal muscle in rodents and humans. UCP 3 has been probed at 2.5 kb (major band) and at 2.8 kb (minor band) and has been expressed at the highest level in skeletal muscle, moderately in BAT, and only weakly in WAT. This study attempted to ascertain if this species expresses Uncoupling protein 2 or 3. Tissue samples from four *Bettongia gaimardi* were taken prior to and after cold exposure at 4-5 °C for 2 weeks. The tissues were then examined for UCP 2-3 expression through western blots, and sequenced through RT-PCR, and cloning methods. These data suggest that *Bettongia gaimardi* express uncoupling protein 2 and 3. The implications of this are not yet understood, although it would present an argument for how marsupials maintain body temperature using NTS.

Daily torpor and activity in free-ranging sugar gliders

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The sugar glider (*Petaurus breviceps*) enters daily torpor only reluctantly in the laboratory. Since torpor in this species had never been observed in the wild, it was questionable whether torpor has any ecological significance. We therefore investigated torpor and activity patterns in free-ranging sugar gliders using temperature telemetry. We measured body temperature (T_b) and/or skin temperature and activity patterns for 12 gliders from autumn to spring 1998 on the Northern Tablelands. Activity was strongly affected by weather and gliders, which would be normally active from sunset to sunrise, reduced activity substantially on cold and/or rainy nights. Gliders also responded commonly to inclement weather conditions by entering torpor. Torpor frequency was highest between June and August when, on average, gliders employed torpor more than once/week. Torpor often commenced during the night and continued sometimes until the beginning of the next activity phase. Torpor bouts lasted between 2 and 23h (avg. 13h) and T_b dropped as low as 10.4°C (avg. 12.9°C). Torpor in free-ranging gliders was therefore more frequent, longer and deeper than previously recorded in the laboratory. Torpor was apparently employed to save energy when inclement weather compromised foraging. This suggests that in wild sugar gliders daily torpor is an important survival strategy.

Plasma sex steroid hormone levels in relation to the reproductive cycle of the eastern shovelnose ray, *Aptychotrema rostrata*

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The eastern shovelnose ray, *Aptychotrema rostrata* (family Rhinobatidae), is an endemic inshore batoid common to the east coast of Australia. The reproductive cycle of this species was studied in Moreton Bay, south-east Queensland, over a fifteen month period. Reproductive hormone profiles were developed for 17β-oestradiol, progesterone and testosterone in mature females and for testosterone in mature males. *Aptychotrema rostrata* is an aplacental yolk sac viviparous species with an annual seasonal reproductive cycle in Moreton Bay. Gravid females are observed during September to November and parturition occurs in November and December. Vitellogenesis does not occur in parallel with gestation in this species, with ovulation occurring during July – September. Plasma oestradiol concentrations were found to correlate with follicular development. Progesterone levels were low throughout the study period (<0.5 ng/ml), except for a distinct peak in December, suggesting a role for this hormone in the onset of follicular development. There was no distinct pattern in female testosterone concentrations over time, however levels were elevated slightly during December, March and April. The earliest observation of mature sperm in the testes of male rays is in February, however it is not until April that they became a dominant stage of spermatogenesis. Males may be ready to copulate at this time, however copulation does not appear to occur until July. Male testosterone levels increased as testicular development proceeded, declining following the reproductive season.

Was Governer Grey a closet eco-physiologist? Niche partition on the basis of water economy in four species of introduced macropod marsupials on Kawau Island, New Zealand

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A survey of the relative distribution and abundence of four species of wallaby introduced to Kawau Island New Zealand by Governer George Grey between 1858 and 1870, namely the tammar wallaby (*Macropus eugenii*), parma wallaby (*Macropus parma*), brush-tailed rock-wallaby (*Petrogale penicilliata*) and swamp wallaby (*Wallabia bicolor*) shows stability over the past four decades despite the islands small size. Environmental factors associated with the distribution of tammar and parma wallabies suggest niche partition on the basis of the availability of fresh water. Interspecies comparisons of relative renal size, mass and cortico-medullary area ratios indicate that tammars have relatively greater renal efficiency in the conservation of water than parma and rock-wallabies. Analyses of the relative water content of faecal and of distal digesta suggest greater colonic water resorption by tammars and by brush-tailed rock-wallabies, associated with a relative increase in length of the colon and, in the case of tammar wallabies, a decrease in the functional diameter of the colon. A laboratory comparison of tammars and parmas supports this hypothesis, tammars drinking significantly less than parmas. We suggest that relative lack of renal specialisation by rock-wallabies may be a consequence of the need to avoid a high concentration of browsed plant toxins in the distal nephron.

Novel Aspects of Transport of Organic Anions by the Malpighian Tubules of Drosophila *melanogaster*

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Larval and adult fruit flies, *Drosophila melanogaster*, feed on rotting fruit and may ingest large quantities of organic acids that are produced by micro-organisms or excreted by other larval or adult flies. This study characterised and elucidated mechanisms of organic anion transport by the Malpighian tubules of *D. melanogaster*. Competition experiments indicate that two types of organic anion transporters were present in the Malpighian tubules. One that transports carboxylate compounds such as para-amino hippuric acid (PAH) and fluorescein and another that transports sulphonate compounds such as amaranth and indigo-carmine. The organic anion PAH was actively transported by the tubules (K_m = 3.05 mmol L^{-1}, V_{max} = 95.5 $pmol^{-1}$). Tubules had the highest transport rate and concentrative noted to date for insects. In the renal systems of vertebrates and the bladder of the crab *Cancer borealis*, PAH is transported into cell in exchange for α-keto acids. Insect tubules appeared not to possess such a PAH/α-keto acid exchanger since PAH transport was unaffected by low concentrations (100 µmol L^{-1}) of α-keto acids (α-ketoglutarate, glutarate, citrate and succinate). Basolateral PAH transport was dependent on the Na^+ gradient. PAH transport may be directly coupled to the Na^+ gradient, perhaps via Na^+/PAH co-transport. Confocal microscopy showed that cytoplasmic transport of the carboxylate fluorescein occurred by diffusion and in vesicles. Accumulation of punctate fluorescence in the lumen of the tubules is consistent with exocytosis of the cytoplasmic vesicles at the apical membrane.

The effects of melatonin administration on the seasonal changes in daily activity rhythms in the marsupial *Antechinus stuartii*

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The carnivorous marsupial *Antechinus stuartii* relies on photoperiodic changes to time its seasonal reproduction, characterised by a brief highly synchronised mating period, followed by complete male mortality. The present study sought to explore the changes in locomotor activity from the winter solstice in both males (body mass 35 g) and females (body mass 20 g). As the administration of the hormone melatonin is known to affect the synchronisation of activity rhythms in other mammals, we also examined the influence of the oral administration of melatonin on activity patterns in *A. stuartii* in comparison to control individuals. Total daily activity differed between sexes, where males were more active than females, irrespective of whether or not melatonin had been administered. The pattern of day/night activity did not significantly differ between female treatment groups, although control females were more active than melatonin treated groups. The pattern of day/night activity differed between males and females, and between male treatment groups. Control male activity patterns reflected the profound changes in their reproductive physiology, and melatonin was found to influence the activity patterns in males.

A seasonal comparison of energy and water requirements of long-nosed bandicoots in the Sydney region

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The long-nosed bandicoot (*Perameles nasuta*) is potentially a continuous breeder but in the Sydney region there is a trough in breeding activity in late autumn to mid-winter. It has been hypothesised that this strong seasonality of breeding is linked to increased energy costs for thermoregulation in winter, coupled with a lower abundance of invertebrates, their preferred food, at this time. We therefore compared field metabolic rate (FMR) and water turnover rate (WTR) of free-living long-nosed bandicoots in summer (n = 10) and winter (n = 10) using the doubly-labelled water technique. We also estimated diet from faecal scat analysis (percent volume of each dietary category) and invertebrate availability (numbers per trap) from pitfall trapping. There was no seasonal difference in WTR, but FMR was significantly higher in winter (985 +/- 363 kJ per day) than summer (696 +/- 272 kJ per day). Invertebrate availability was significantly lower in winter, but there was no difference in diet between seasons. These results indicate that there are increased energy demands in winter, but this was not coupled with a shift in diet even though invertebrate availability was lower in winter. We suggest that the increased FMR in winter may be a combination of increased costs of thermoregulation and increased foraging costs in the form of digging for underground larvae, leaving insufficient energy available for reproduction at this time of the year. We were unable to detect any significant difference in FMR between lactating (n = 3) and non-lactating females (n = 5) in summer, possibly because of small sample sizes and females at different stages of lactation.

The renal vasculature of the agamid lizard, *Ctenophorus ornatus*

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The agamid lizard, *Ctenophorus ornatus experiences* seasonal water deprivation with a simultaneous diet-related high electrolyte loading. It tolerates very significant hypernatræmia in summer and responds to this with a progressive inhibition of renal function. Filtration ceases completely during extended periods of drought. This study was performed to establish whether the functional specialisations of osmoregulation and electrolyte maintenance displayed by *C. ornatus* are reflected in anatomical specialisations of the kidneys. The structure and ultrastructure of the vasculature and nephric tubules was investigated using light microscopy and scanning electron microscopy of resin casts. The arterial and venous supplies to the kidney are unusual by vertebrate standards. The glomeruli do not appear specialised. The findings of this study suggest that these lizards may have a reduced reliance on filtration for homoeostasis and a greater reliance on tubular secretion. Such an osmoregulatory strategy could be advantageous, particularly in arid environments, where persistent filtration may necessitate some loss of water.

Development of chorioallantoic and pulmonary respiration in Australian pelican (*Pelecanus conspicillatus*) embryos and hatchlings

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To date the only avian species for which the development of lung function has been investigated is the chicken. In this study we examined the development of pulmonary respiration in another avian species, the altricial Australian pelican, which has a low degree of morphological and physiological maturity at hatching. The ontogeny of O_2 consumption and CO_2 production of whole pelican eggs showed developmental patterns unlike that previously reported in altricial species, in that a clear plateau in metabolism occurred between days 28 and 31 of the 33 day incubation. As in all avian embryos, respiration of the chorioallantoic membranes (CAM) was entirely responsible for providing the respiratory requirements of the embryo until day 31, when internal pipping of the aircell initiated pulmonary respiration. However, in the pelican this resulted in an immediate reduction of CAM respiration in proportion to the initial rate of pulmonary respiration, whereas in the chicken no reduction in CAM respiration was reported until 9-12 h later, when the embryo externally pips the eggshell. In the pelican a lower CAM respiration rate was maintained at a stable value until 6 h before external pipping (also about 12 h after internal pipping), after which it declined rapidly while pulmonary respiration became the dominant mode of respiration during the remaining 15-16 h until hatching. In contrast to the chicken, in which CAM respiration does not cease until immediately before emerging from the egg, in the pelican the CAM circulation is cut off long before the chick hatches.

Chasing and gassing salmon on farms: physiological insights into amoebic gill disease

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Amoebic Gill Disease (AGD) is the moss significant disease problem facing Australian salmon aquaculture. AGD and its treatment by freshwater bathing accounts for approximately 15% of gross annual production. AGD is caused by the protozoan parasite *Paramoeba pemaquidensis*, and results in multifocal hyperplastic lesions on the gills, with a subsequent fusion of adjacent lamellae and a hypersecretion of branchial mucus. To test the hypothesis that mortality from AGD was as a consequence of acute respiratory dysfunction, AGD affected and non-affected controls were exposed to a progressive hypoxia. Blood gasses, pH and ventilation frequency were measured during the hypoxic challenge. Under normoxic conditions, AGD affected fish had a significantly lower blood PO_2 compared to non-affected controls. Additionally, AGD affected fish had significantly elevated blood PCO_2 and lower blood pH compared with non-affected controls under normoxic conditions. However under hypoxia, respiratory and acid-base differences between the two groups were mitigated. In another experiment, AGD affected fish were acutely stressed by chasing for 5 min. During recovery, mortality occurred in fish that were unable to defend a blood pH of 7.5. In those fish, plasma lactate concentrations escalated and until mortality, whereas fish that did not die showed and effective clearance of lactate by 9 h post-chasing. These data suggest that respiratory dysfunction is an unlikely cause of mortality in AGD affected salmon, but acute acid-base disturbances appear to play a significant role.

Non-shivering thermogenesis in the Tasmanian bettong (*Bettongia gaimardi*) and the role of uncoupling proteins (UCP1, 2 and 3)

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The Tasmanian bettong (*Bettongia gaimardi*) is a small marsupial able to increase its metabolic rate by non-shivering thermogenesis. Oxygen consumption increases in response to various stimuli: vasopressin, non-epinephrine, both α1 and β adrenergic stimulation thyroid hormone and cold-acclimation (CA)(4-5°C for 14 days). Moreover, the metabolic rate response to nor-epinephrine is enhanced after CA (71% increase 'v' 47% prior to CA). The ability to respond to these stimuli only appears after week 10 of the 15-week pouch life. We have attempted to locate the source and cause of the metabolic response. Brown adipose tissue (BAT) is totally absent in this species as in most marsupials. In addition, we have failed to locate the uncoupling protein (UCP1) associated with BAT. Our research led us to investigate skeletal muscle as a source of heat production in this species and we have located both UCP2 and UCP 3 in this tissue in cold-acclimated bettongs.

Plasma mesotocin in the oestrous cycle and pregnancy of the brushtail possum *Trichosurus vulpelcula*.

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The oxytocin-like peptide mesotocin (MT) is secreted by the pituitary of the brushtail possum. In eutherian mammals, oxytocin has a major role in the contraction of the uterus during parturition. The presence MT receptors at high densities in the uterus and median vagina of the possum indicates a capacity to respond to circulating MT. However it is presently not known if and when these uterine receptors are exposed to changes in plasma concentrations of MT. In this study we investigated changes in plasma MT in pregnant and cyclic possums by removing pouch young (RPY) and then housing the females in the presence or absence of males. Jugular catheters were inserted and blood sampled daily from day 8 to 28 post RPY. Blood was assayed for MT and progesterone. MT in pregnant possums showed a single peak on the day before birth, with a plasma concentration of 44.7±14.1 pg/ml (n=6) compared to 8.3±1.6 pg/ml on the day of birth. The pattern was qualitatively similar in cyclic possums with a peak at 24 days post RPY of 32.5±13.5 pg/ml (n=4) compared to 4.3±0.6pg/ml at day 25 post RPY. Progesterone concentrations were similar in cyclic and pregnant possums. These results suggest that the most likely role for MT is uterine contraction at parturition. Any additional more speculative role may be in the degeneration of the corpus luteum in both cyclic and pregnant possums. Further studies using OT receptor antagonists are needed to test these suggested roles of MT.

The principle of Laplace and scaling of ventricular wall stress and blood pressure in mammals and birds

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Maximum left ventricular wall stress is calculated at end-diastolic volume and systemic arterial diastolic blood pressure, according to a thick-walled model for the principle of Laplace. Stress is independent of body mass and averages 13.9 kPa (± 2.3; 95% CI) in 24 species of mammals weighing 0.025 to 4000 kg, and 15.5 kPa (± 4.7) in 12 birds weighing 0.014 to 110 kg. Birds have higher arterial blood pressures and larger hearts than mammals. Systolic and diastolic arterial blood pressures increase with body mass according to $M^{0.05}$ in mammals, and heart mass increases according to $M^{1.06}$ in the same species, further supporting the principle. However, blood pressure in birds is independent of body mass, and heart mass scales isometrically. End-diastolic stress values, calculated according to Laplace, are about one-third of peak stresses recorded in isolated mammalian myocardial preparations. Hypothesized upright neck postures in sauropod dinosaurs require systemic arterial blood pressures reaching 700 mm Hg at the heart. Their left ventricles would have weighed 15 times that of similarly sized whales. Such dimensionally, energetically, and mechanically disadvantageous ventricles were highly unlikely in an endothermic sauropod.

The natriuretic peptide system and euryhalinity of eels

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Three types of natriuretic peptides (ANP, VNP and CNP), and four types of NP receptors (NPR-A, -B, -C and -D) have been identified in a euryhaline teleost, *Anguilla japonica*. ANP is secreted transiently after transfer to seawater (SW) and acts on NPR-A to inhibit drinking and subsequent intestinal absorption of Na^+ from the drunk SW, thereby inhibiting a sudden increase in plasma osmolality. ANP also acts on the kidney to decrease urine volume and increase urine Na^+ concentration. Furthermore, ANP stimulates the secretion of cortisol, a long-acting, SW-adapting hormone. In fact, ANP infusion decreases plasma Na^+ concentration in SW eels but not in freshwater (FW) eels. Thus ANP appears to be is a primary hormone for SW adaptation in eels. By contrast, plasma CNP concentration is always higher in FW eels than in SW eels. Furthermore, CNP-specific receptor, NPR-B, is expressed more abundantly in the gill of FW eels than in that of SW eels. In fact, CNP infusion increases plasma Na^+ concentration in FW eels but not in SW eels. The increase is most probably due to an increase in Na^+ uptake by the gill because a ^{22}Na influx from the environmental FW is enhanced by CNP infusion. Thus, CNP may be an important hormone for FW adaptation in eels. These results show that, although ANP and CNP belong to the same hormone family and share more than 60% sequence identity, they exhibit opposite effects on adaptation to osmotic environments. VNP may assist ANP and CNP in adaptation to either environment, since it has high affinity to both NPR-A and NPR-B. Therefore, it appears that the natriuretic peptide system is a key endocrine system for euryhalinity of eels, particularly in the initial phase of adaptation.

Heat balance during locomotion of the brush-tailed bettong

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We have examined the locomotion of the brush-tailed bettong, a 1 kg rat-kangaroo which is an obligate hopper at high speeds. Oxygen consumption, evaporative water loss and deep body temperature were measured during exercise at speeds from 0.5 to 6.2 ms-1. In this species hopping at higher speeds is energetically cheaper than the cost of running in a similarly-sized quadruped. Rates of oxygen consumption increased only slightly as speed increased, in a pattern close to that seen in larger kangaroos. Evaporative water loss and post-exercise body temperature did not vary with hopping speed. However, the rate of change in body temperature increased with increasing speed, suggesting increased heat storage at higher speeds. A model for heat balance during locomotion for the bettong will be presented.

The role of convection in ventilation of animal burrows

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The relationship between body mass and the respiratory microenvironment of burrowing animals is examined using artificial burrows containing surrogate animals that simulate O_2 consumption by removal of air and simultaneous replacement with N_2. Allometric relationships between body mass and burrow radius, nest chamber radius and O_2 consumption rate show that published mathematical predictions of diffusion-mediated gas exchange are adequate to describe the respiratory environments of animals in small blind-ending burrows through porous substrata. Diffusion is sufficient to ventilate burrows containing small occupants with low O_2 consumption rates (<3 ml O_2 min^{-1} or 340 g) or animals weighing less than 30 kg residing in subterranean nest chambers connected to the surface by one or more tunnels. Outside of these limits, convection prevails and prevents the development of hypoxic conditions, particularly in burrows of large mammals with high oxygen consumption rates (>7 ml O_2 min^{-1} or 1300 g). The maximum mass of burrowing animals is therefore not constrained by the development of hypoxia or hypercapnia.

Molecular activity of Na^+,K^+-ATPase related to packing of membrane lipids

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The molecular activity of the Na^+,K^+-ATPase from tissues of different vertebrate species can vary several-fold. The Na^+,K^+-ATPase molecular activity was measured in microsomes from the kidney and brain of the rat (*Rattus norvegicus*) and the cane toad (*Bufo marinus*). The phospholipids surrounding this enzyme were isolated from these microsomal preparations and their pressure-area relationships measured in monolayers using a Langmuir trough. Very strong correlations were found between the molecular activity of Na^+,K^+-ATPase enzyme and parameters describing the packing of the surrounding phospholipids, namely the average area occupied by membrane phospholipids at constant surface pressure, and the average surface pressure in the monolayer at a constant average area per phospholipid. The influence of membrane lipid composition on the activity of a membrane protein (mediated by physical properties of the lipids), may represent a fundamental principle applicable to all membrane proteins and have significant implications with respect to diet and some medical conditions.

Effects of hypothyroidism on the distribution of cardiac & skeletal myosin isoforms in *Antechinus flavipes*

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Effects of hypothyroidism on myosin heavy chain (MyHC) composition of cardiac and skeletal muscles were investigated in a marsupial, *Antechinus flavipes*. Four animals were given metimazole in their food and water for 5 weeks to induce hypothyroidism. Changes in myosin isoforms in cardiac (ventricle) and skeletal (Tibialis Anterior and Gastrocnemius) muscles were studied. Electrophoretic analysis of intact ventricular myosins in most animals revealed 3 isoforms V_1, V_2 and V_3. Western blots using specific anti-α and anti-β cardiac MyHC antibodies showed their MyHC structures to be αα, αβ and ββ respectively. Surprisingly, in 3 animals intact myosins showed 4 bands, Western blots of the first 3 suggest that they are triplets arising from the dimerization of two isoforms of α MyHC, while the 4th band corresponded to V_3. Hypothyroidism resulted in a fourfold increase in β MyHC content. Four skeletal MyHCs isoforms (slow/β, fast IIA, IIX and IIB, in the order of associated fibre speed in eutherians) were separated on SDS-PAGE and identified by immunoblotting with highly specific monoclonal antibodies. Hypothyroidism elicited a significant decrease in IIB MyHC content from about 75% of total MyHC to 50% with an associated increase in the slower isoforms. We conclude that thyroid hormones have the same influence on skeletal and cardiac myosin gene expression as in eutherian mammals, and that hypothyroidism leads to a slowing of the heart and skeletal muscles. We note that *A. flavipes* has the unusual potential of expressing two distinct isoforms of α cardiac MyHC.

POSTER ABSTRACTS

The effects of ammonia on the respiratory physiology of the juvenile big bellied seahorse *Hippocampus abdominalis*

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Juvenile big bellied seahorse *Hippocampus abdominalis* were acutely and chronically exposed to elevated ammonia and nitrite {(24 h exposure: 0.01, 5.0, 10.1, 14.8 & 19.9 $mg.L^{-1}$ total ammonia-nitrogen [TA-N] & <0.001, 74.4, 99.2 & 123.6 $mg.L^{-1}$ [NO_2-N] nitrite-nitrogen) & (35 d exposure: 0.11, 0.55, 1.67 & 3.07 $mg.L^{-1}$ TAN & <0.001, 0.92, 4.67 & 9.10 mg NO_2-$N.L^{-1}$)}. Significant ($P<0.001$) increases in oxygen consumption rate and ventilation frequency occurred at 14.8, 19.9 $mg.l^{-1}$ TA-N and 99.2, 123.6 $mg.l^{-1}$ NO_2-N for acutely exposed fish. Oxygen consumption rate was significantly ($P<0.05$) elevated at 1.67 & 3.07 $mg.l^{-1}$ TA-N in chronically treated fish and ventilation frequency increased significantly ($P<0.05$) at 0.55, 1.67, 3.07 $mg.l^{-1}$ TA-N and 4.59, 9.10 $mg.l^{-1}$ NO_2-N. There were no significant differences in growth between controls and ammonia exposed fish. Mortalities occurred at 14.8, 19.9 $mg.l^{-1}$ TA-N. Recommendations for safe environmental concentrations of the above toxicants are given pertaining to a culture situation.

Observations on breeding behaviour in theTasmanian echidna, (*Tachyglossus aculeatus*)

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The echidna, is an egg laying mammalian hibernator. It has been a very successful coloniser of many different habitats despite the fact that at most it raises 1 young/year. For newborn echidnas to acquire an adequate body weight before the following winter the growing season must be as long as possible. We have been studying the pattern of hibernation using implanted dataloggers in reproductively active male and female echidnas (Nicol & Andersen 2000). Reproductively active female echidnas aroused earlier than females that did not reproduce. Mating occurs within a week of the female's last arousal. Mating is followed by a 3 weeks gestation period during which the female echidna regains some of the body weight lost during hibernation. In our study area the female echidna digs a nursery burrow in which she retreats to lay an egg into her "pouch". This incubation period and the first month after the young echidna hatches is characterised by a recognisably more stable period in the mother's body temperature. This has allowed us to make estimates of the timing of reproductive events by combining field observation and body temperature records.

Nicol, S.C., Andersen N.A. (2000) Patterns of hibernation of echidnas in Tasmania. In: G. Heldmaier, S. Klaus, M. Klingenspor (eds) Life in the Cold. 21-28

Gill and swim bladder morphology, morphometry and function in the bimodal breathing tarpon, *Megalops cyprinoides*

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The tarpon, *Megalops cyprinoides*, is an active teleost that inhabits tropical marine and freshwater environments. It combines air-breathing and aquatic gill ventilation to supply its oxygen requirements. Gill and respiratory swim bladder morphology and morphometry were examined in fish caught in freshwater. Gills were well developed with lamellar area proportional to (body mass)$^{0.865}$, equating to an A_{200} of 265 mm^2/g. Mean blood-water diffusion distance was 2.07 μm. Four, highly-vascularised bands of tissue on the internal swim bladder surface received blood from arterial branches of the dorsal aorta. Venous blood drained anteriorly, carrying potentially oxygenated blood to the posterior cardinal veins/sinus venosus. Corrosion casting revealed the accessory respiratory structures of the swim bladder to contain sheets of capillaries of about 6.5 μm diameter, with a mean harmonic blood-gas diffusion distance of 0.72 μm as determined from histological sections. The surface area for respiratory gas exchange in the swim bladder was about 10% that of the gills. Respirometry revealed that 99.7% of the resting metabolic oxygen requirement was obtained from the water via the gills, when in normoxic water (ppO_2 = 22.7 kPa). However, in hypoxic water (ppO_2 = 8.7 kPa) only 36% of the required oxygen was provided by aquatic respiration and air-breathing at the surface was frequent.

Vasodilator mechanisms in large arteries of the toad, *Bufo marinus*

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In mammals, the vasodilatory mechanisms of atrial natriuretic peptide (ANP) and nitric oxide (NO) are well documented when compared to those of non-mammalian vertebrates. This study was designed to examine the vasculature of an amphibian to determine whether ANP activates the particulate guanylate cyclase receptor (PGCR) and NO the soluble guanylate cyclase receptor (SGCR) to mediate vasodilation and ii. to identify whether NO is derived from the vascular endothelium. *In vitro* organ bath physiology experiments showed that 10^{-5} M HS-142-1 (particulate guanylate cyclase antagonist) reduced ANP vasodilation by 28 % and that 10^{-5} M ODQ completely inhibited the vasodilatory response of sodium nitroprusside (NO donor). These results indicate that ANP and NO mediate vasodilation via a PGCR and a SGCR, respectively. NADPH diaphorase histochemistry was used to identify whether NO synthase (the enzyme that generates NO) is present in the vascular endothelium. The unstained vascular endothelium of *B. marinus* revealed that NO synthase is not located in the vascular endothelium. However, NADPH staining was evident in the perivascular nerves, which suggests that NO could be a vascular neurotransmitter in amphibians.

Peripheral conversion of steroids in a viviparous skink, *Tiliqua nigrolutea*

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Peripheral (extragonadal) metabolism of the primary reproductive steroids, testosterone (T) and estradiol (E2), occurs in a variety of vertebrate body tissues. The primary steroids may be modified in two main ways: derivatisation to more biologically active forms, or conjugation to increase solubility. The molecular structures of these steroid metabolites vary between vertebrate classes. Such phyologenetic differences are of intrinsic evolutionary interest; furthermore some of these metabolites may function as semiochemicals, communicating location, reproductive condition and even synchronising mating behaviour in some species. However, such data is rare for reptiles. We have investigated and confirmed the occurrence of substantial peripheral steroid metabolism using *in vitro* incubations of tissues from the blue-tongued skink, *Tiliqua nigrolutea*. Additionally, we have demonstrated that the relative proportions of primary steroids undergoing each of these types of conversions vary with sex, reproductive condition and tissue type. This study has identified a group of steroid molecules that are potentially communicating information to conspecifics during the mating period.

Expression of Na^+/H^+ antiporter mRNA in the gills of the Atlantic hagfish (*Myxine glutinosa*) in response to metabolic acidosis

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Sodium/proton exchangers (NHE) are transmembrane proteins that facilitate the exchange of a Na^+ ion for a H^+ ion across cellular membranes. The NHE are present in the gills of fishes and are believed to function in acid-base regulation by taking up a Na^+ ion from the environment in exchange for the extrusion of a proton across the branchial epithelium. This study demonstrates the presence of a branchial NHE in the gills of the Atlantic hagfish, *Myxine glutinosa*, by reverse transcriptase-polymerase chain reaction (RT-PCR). The subsequent partial cDNA sequence shares homology with other vertebrate and invertebrate NHE isoforms. In addition, using semi-quantitative, multiplex RT-PCR we demonstrate that mRNA expression of the hagfish gill NHE is upregulated for 24 hours following an induced metabolic acidosis. Expression was increased to 4.4 times basal levels at 2 hours post infusion and had decreased to 1.6 times basal by 6 hours. Expression had returned to basal levels by 24 hours post-infusion. The inference from this study is that a gill NHE which is potentially important in acid-base regulation has been present in the vertebrate lineage since before the divergence of the hagfishes from the main vertebrate line.

Dietary fats, white adipose tissue fatty acid composition, and hibernation in free-ranging echidnas *Tachyglossus aculeatus* (Monotremata)

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Diets rich in unsaturated fatty acids lower body temperatures and prolong torpor bouts in herbivorous rodent hibernators, which is likely to improve winter survival. As the potential effect of dietary fats on torpor in insectivorous hibernators is unknown, we studied fatty acid composition of dietary insects and echidna white adipose tissue (WAT) during the pre-hibernation season in the field, and determined whether WAT composition changes during hibernation. Echidna WAT fatty acids during the pre-hibernation season were almost identical to those of the most abundant prey species, the ant *Iridomyrmex* sp.. Oleic acid (C18:1) was by far the most common fatty acid in both ant (60.1%) and echidna WAT (61.7%). The essential linoleic acid (C18:2) was also similar in ant (5.3%) and echidna WAT (5.4%). After about 5 months of hibernation and an 18% loss of body mass, echidna WAT fatty acids had changed significantly. The monounsaturated oleic acid (C18:1) and palmitoleic acid (C16:1) had declined from 61.7% to 50.3% and from 6.8% to 4.9% respectively. In contrast, linoleic acid (C18:2) and the saturated fatty acids (C12:0, C16:0, C18:0) had significantly increased. Our study suggests that, unlike herbivorous rodent hibernators, echidnas rely to a large extent on the common dietary monounsaturated fatty acids as fuel for hibernation. Moreover, the high concentration of monounsaturates may compensate for the moderate availability of polyunsaturates and enable them to function at low body temperatures during hibernation.

Gait analysis and locomotor energetics in the northern brown bandicoot, *Isoodon macrourus torosus*.

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The northern brown bandicoot, *Isoodon macrourus*, is a native Australian marsupial belonging to the family Peramelidae. The sub-species, *I. macrourus torosus* is the common bandicoot species along the east coast of Australia and was studied to determine the primary gaits and locomotor energetics of this species. *Isoodon macrourus torosus* used both quadrupedal and bipedal forms of locomotion. However, the two primary gaits used were a slow quadrupedal walk at speeds of about 0.3 m s^{-1} and a faster quadrupedal half-bound at higher speeds. When moving quadrupedally, bandicoots primarily increase their speed of locomotion by increasing stride length. Stride frequency remained approximately constant within quadrupedal gaits. Both gathered and extended suspension periods were used during faster half-bounding gaits. Bipedal vigilance and walking behaviours occurred on a regular and voluntary basis, with bipedal walking speed increasing through increased stride frequency. There was an obvious effect of size (body mass) on the various quadrupedal parameters with larger animals using greater stride lengths and lower stride frequencies than smaller ones. The rate of oxygen consumption, as determined from open-flow respirometry for animals moving on a motorised treadmill, increased linearly with speed. Unfortunately, practical limitations restricted energetic analysis to slow - moderate 'running' speeds. An apparent plateau of oxygen consumption at higher speeds was shown to be an artifact due to (a) changes in animal position relative to the extraction mask and, (b) exchange of gas between the chamber and the external environment. Morphological analysis of the hind limbs suggested that uncoupling of locomotor costs from speed would be unlikely in this species.

The effect of water deprivation on cardiac and renal atrial natriuretic peptide mRNA expression in the Spinifex Hopping Mouse

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The spinifex hopping mouse, *N. alexis*, is capable of tolerating an absence of free water for prolonged periods by excreting a concentrated urine and utilising metabolic water. This study was designed to investigate the linkage between water availability and the activation of cardiac and renal atrial natriuretic peptide (ANP) in *N. alexis*. The experimental animals were obtained from a breeding colony at Deakin University and were housed in glass aquaria with sand with nesting jars. They were fed either a birdseed mix or 20 g of millet seed per day. Different groups of hopping mice were subject to water deprivation (removal of free water) for periods of 7 or 14 days; control hopping mice had access to water. At the end of the experimental period, water-deprived and control mice were sacrificed and haematocrit, plasma osmolarity, tissue water, and cardiac and renal ANP mRNA levels were determined. Water deprivation did not have a significant effect on haematocrit, plasma osmolarity, and tissue water content. The expression of ANP mRNA was studied with two techniques: RNA dot blotting and a semi-quantitative PCR-based method. Using RNA dot blotting and densitometry, cardiac ANP mRNA expression was observed to increase following water deprivation, while renal ANP mRNA had a trend to decrease in expression. These observations were further examined using a more sensitive RT-PCR based expression technique. Using this approach, cardiac ANP mRNA expression was not significantly affected by water deprivation but, in contrast, renal ANP mRNA was significantly reduced ($p < 0.03$). These data indicate that down-regulation of the renal natriuretic peptide system may be an important adaptation to a xeric environment.

Structure and thyroid hormone binding properties of transthyretin: Its synthesis in *Pichia pastoris*

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Transthyretin is involved in the distribution of thyroid hormones. It is composed of four identical subunits that come together to form a central channel, the site for thyroid hormone binding. Amino acid residues implicated in the binding of thyroid hormone are identical across species. However, during evolution transthyretin affinity for L-triiodothyronine (T3) decreased while affinity for L-thyroxine (T4) increased. *Pichia pastoris* (yeast) was used as a recombinant protein expression system for synthesis of recombinant chicken transthyretin. The secreted recombinant protein's electrophoretic mobility was identical to the native chicken transthyretin. The recombinant protein was also recognised by anti-chicken transthyretin antiserum. The recombinant chicken transthyretin preferentially bound T3, $K_d=21.3\times10^{-9}$M, over T4, $K_d=35.0\times10^{-9}$M. Previously reported measurements were $K_dT3=12.3\times10^{-9}$M and $K_dT4=28.8\times10^{-9}$M (Chang *et al.*, 1999 Eur. J. Biochem., 259, 534-542).

Differences in heart morphology and health status of farmed Atlantic salmon

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Heart and gill morpohology was compared for groups of Atlantic salmon (*Salmo salar*) that had experienced different histories of Amoebic Gill Disease. Fish with histories of high levels of AGD had significantly longer ventricles compared with fish with histories of light AGD. However, there were no significant differences in heart somatic index, heart-ventricle mass ratio between fish from different AGD histories. These data suggest that the repeated and chronic nature of AGD may have significant effects on the cardiovascular system of salmon.

Sodium Pump Molecular Activity and Membrane Lipid Composition in a Highly Polyunsaturated Species: the Octopus

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Recently, a new hypothesis has suggested that the activity of cellular proteins is regulated by the membrane environment in which they reside.[1] According to this theory, polyunsaturated membranes, as found in most mammals, are generally associated with high molecular activity of proteins, while low molecular activity proteins are present in more monounsaturated membranes, such as found in many ectothermic species. Measurements of certain membrane proteins however, including sodium pumps, have found high molecular activity in some ectothermic species (primarily of specialised salt organs from coldwater species). In these ectothermic species no concerted effort has previously been made to relate the molecular activity of the proteins to that of the membrane composition. The current study has examined membrane lipid composition and sodium pump molecular activity across a variety of organs in a highly polyunsaturated species: the octopus (O. *australis*). Octopi were collected from local beaches and a variety of organs (digestive gland, kidney, gill, brain, and tentacle) were removed for the measurements. Sodium pump molecular activity, which is the turnover rate per protein, was calculated for all organs following determination of sodium pump number, and Na^+K^+ATPase activity. Phospholipid fatty acid composition was also determined for microsomal fractions from the same organs. Phospholipids from all of these organs were highly polyunsaturated, and this was generally associated with high molecular activity of their sodium pumps. These results are consistent with a pacemaker role for membranes in cellular metabolism.

[1]Hulbert AJ, Else PL (1999) Membranes as possible pacemakers of metabolism. *J. Theor Biol.* 199: 257-74.

ANZSCPB 2000 Participants

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