

Australian and New Zealand Society for Comparative Physiology and Biochemistry 26<sup>th</sup> Annual Meeting Deakin University

November 27th-29th, 2009



# We appreciate the support of the following organisations who have contributed to the meeting

School of Life and Environmental Sciences, Deakin University

Journal of Comparative Physiology B

### Physiological and Biochemical Zoology

### **Comparative Biochemistry and Physiology Part B**

StatistiXL





### Organising committee:

### Stuart Linton, John Donald, Jan West, Tes Toop

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## Geelong Campus at Waurn Ponds





accurate as at 29 November 2007

### 2009 ANZSCPB meeting program overview

Thursday 26 <sup>th</sup> November	Time	Friday 27 <sup>th</sup> November	Time	Saturday 28 <sup>th</sup> November	Time	Sunday 29 <sup>th</sup> November
	9:00-10:20	Welcome Session 1 (4 talks)	9:00-10:40	Plenary lecture Prof Berry Pinshow Session 4 (2 talks)	9.30	Session 8 (3 talks)
	10:30- 11:00	Morning tea	10:40- 11:10	Morning tea	10:30	Morning tea
	11:00-12:40	Session 2 (5 talks)	11:10-12:50	Session 5 (5 talks)		Session 9 (3 talks)
	12:40- 14:00	Lunch	12:50- 14:00	Lunch	12:30	Lunch
	14:00- 15:40	Session 3 (5 talks)	14:00- 15:40	Session 6 (5 talks)		
	15:40- 16:30	Afternoon tea and group photograph	15:40- 16:10	Afternoon tea		
	16:30 - 17:00	Memorial for Steve Morris Mike Thompson USyd	16:10 - 17:10	Session 7 (3 talks) Annual general meeting		
	17:00 - 18:30	Poster session				
17:00- 20:30 Registration + Pizza +drinks	Evening	BBQ in the court yard/ East end dining room	Evening	Conference dinner- Deakin management centre		

### PROGRAM

Friday 27 <sup>th</sup> November		
Session 1	Chair: Mike Thompson	
9.00	Welcome	
9:10	David J Cannata, Zoe Ireland, Hayley Dickinson, Aaron Russell, Rod Snow, Jan West and David Walker	
	Maternal creatine supplementation prevents hypoxia-induced damage to the diaphragm.	
9:30	Frank Seebacher, Isabel Walter, Rob S. James	
	Mechanisms underlying the sprint-endurance trade-off in rat muscle.	
9:50	Domenic LaRosa, David Cannata, John Arnould & Jan West	
	Changes in muscle composition during development in the Australian fur seal.	
10:10	Clare Stawski and Fritz Geiser	
	Summer torpor is enhanced by good body condition in a subtropical bat.	
10:30	Morning tea (East dining room (jb1.103))	
Session 2	Chair: John Donald	
11:00	Toni-Ann Alsop, McLeod B.J, and Grant Butt	
	Epithelial HCO3 <sup>-</sup> secretion and mucus hydration in the vaginal cul-de-sac of the brushtail possum.	
11:20	MW Gill, Ray Bartolo, and Grant Butt	
	Electrogenic anion secretion in the small intestine of the common Australian brushtail possum, <i>Trichosurus vulpecula</i> .	
11:40	Caragh Heenan	
	The energetic cost of reproduction in birds.	
12:00	Scott Nankervis, Mark Powell, Janet McLeod, Tes Toop.	
	The mRNA expression and protein localisation of guanylyl cyclase-linked natriuretic peptide receptors in freshwater and chronically seawater-acclimated rainbow trout.	
12:20	Leonard K. Pattenden, Klaus Altland and Samantha J. Richardson	
	Unravelling transthyretin amyloid – bounding ahead using wallabies.	
12:40	Lunch (East dining room (jb1.103))	

Friday 27 <sup>th</sup> November	
Session 3	Chair: Jan West
14:00	Paul Cooper, Stuart Dennis, James Woodman, Ann Cowlings and Christine   Don   Effect of opioid compounds on feeding and activity of the cockroach,   Derinlanete americana
14:20	Periplaneta americana.     Kelly George, Melanie Archer, Lauren Green, Xavier Conlan and Tes Toop
	Effect of morphine on the growth rate of <i>Calliphora stygia</i> (Fabricius) (Diptera: Calliphoridae) and possible implications for forensic entomology.
14:40	Samuel Parry, Stuart Linton, Michael O'Donnell and Tes Toop
	Accumulation and excretion of morphine by <i>Calliphora stygia</i> , an Australian blow fly species of forensic importance.
15:00	Philip Matthews and Craig R. White
	Rhinoceros beetles modulate spiracular opening to regulate haemolymph pH.
15:20	Gerhard Körtner, Daniella Rojas, and Fritz Geiser
	Thermal energetics and activity patterns of the Kaluta ( <i>Dasykaluta rosamondae</i> ).
15:40	Afternoon tea and group photograph (jb1.103)
16:30	Mike Thompson
	Memorial for Steve Morris
17:00	Poster session (Foyer of the Peter Thwaites lecture theatre)
Evening	BBQ in the court yard in front of the East dining room (jb1.103)

Saturday 28 <sup>th</sup> November		
Session 4	Chair: Christine Cooper	
9.00	Plenary Lecture: Berry Pinshow	
	Water balance in desert mammals and in flying birds: multiple vs. single evolutionary paths to the same physiological end.	
10:00	Mark Chappell, Bill Buttemer and Andy Russell	
	The economy of group living: Chestnut-crowned Babblers reduce basal and thermoregulatory energy expenditure in communal roost nests.	
10:20	Andrew McKechnie and B. Smit	
	Phenotypic flexibility in avian basal metabolic rates: global variation in responses to acclimation and acclimatization.	
10:40	Morning tea (East dining room (jb1.103))	
Session 5	Chair: Bill Buttemer	
11:10	Elektra Sinclair, Ashley Ward and Frank Seebacher	
	Aggressive behaviour results in fin damage that affects swimming performance in <i>Gambusia holbrooki</i> .	
11:30	Sarah Abbott, Paul Else, and Tony Hulbert	
	The balance between dietary n-3 and n-6 polyunsaturated fatty acids has the greatest influence on membrane fatty acid composition and basal metabolic rate.	
11:50	Tony Hulbert	
	Dramatic changes in the diet of <i>Homo sapiens</i> : history of the balance between omega-3 and omega-6.	
12:10	Magdalene Trzcionka, Tony Hulbert and Bill Buttemer	
	Can the oxidative stress theory of aging explain differences in longevity between galliformes and psittaciformes?	
12:30	Ben Allardyce and Stuart Linton	
	The last piece in the cellulase puzzle, the characterisation of $\beta$ -glucosidase from the herbivorous Gecarcinid land crab, <i>Gecarcoidea natalis</i> .	
12:50	Lunch (East dining room (jb1.103))	

Saturday 28 <sup>th</sup> November		
Session 6	Chair: Ashley Edwards	
14:00	Gillian Bryant and Patricia Fleming	
	Thermal biology of free-ranging southwest carpet pythons ( <i>Morelia spilota imbricata</i> ).	
14:20	Christine Cooper, Phil Withers and S. Schmidt	
	What is the point of relative water economy?	
14:40	Phil Withers and Christine Cooper	
	Physiology of the dibber ( <i>Parantechinus apicalis</i> ): A case study using a priori contrasts with repeated measures ANOVA.	
15:00	Terry Dawson, Shane Maloney and Koa Webster	
	The under appreciated role of fur in the adaptability of large mammals to challenging thermal environments.	
15:20	<b>Patricia Fleming</b> , Luke Verburgt, Mike Scantlebury, Katarina Medger, Philip W. Bateman	
	Locomotory energetics in intact and tailless geckos.	
15:40	Afternoon tea (East dining room (jb1.103))	
Session 7	Chair: Sue Jones	
16:10	Sofie Trajanovska and John Donald	
	Endothelial nitric oxide synthase in the amphibian, Xenopus tropicalis.	
16:30	Qiong Wu, Mike Thompson, Chris Murphy	
	Adherens junctional proteins in lizard placentae.	
16:50	Fritz Geiser	
	Hibernation On Noah's Ark?	
17:10	Annual general meeting	
Evening	Conference dinner Deakin Management Centre	

Sunday 29 <sup>th</sup> November		
Session 8	Chair: Fritz Geiser	
9:30	Michael Kearney, Warren Porter	
	Size, shape, and the thermal niche of endotherms.	
9:50	<b>Craig White</b> , Philip Matthews, Sebastiaan Kooijman, Michael Kearney, Dustin Marshall	
	The mechanistic basis of metabolic allometry in colonial animals.	
10:10	Jonathan Green, Erin Aitken-Simpson, Peter Frappell	
	Heat-stress in Australasian gannets ( <i>Morus serrator</i> ): Current and future costs.	
10:30	Morning tea (East dining room (jb1.103))	
Session 9	Chair: Phil Withers	
11:00	Stuart Linton and Alicia Shirley	
	Purification and characterisation of novel lichenase enzymes from the herbivorous Gecarcinid land crab, <i>Gecarcoidea natalis</i> .	
11:20	Jessica Nealon, Stephen Blanksby, Todd Mitchell and Paul Else	
	Body Mass and Membrane Phospholipids in Mammals: Kidney and Brain.	
11:40	Matthew Dowle, Koa Webster, Elizabeth Deane	
	Faecal glucocorticoids in urban and wild bandicoots of northern Sydney.	
12:00	Close and presentation of prizes	
12:20	Lunch (East dining room (jb1.103))	

Presenter in bold

### Posters

Gillian Bryant, P.W. Bateman, and Patricia Fleming

Sniffing out Sex: Male carpet pythons use chemosensory information to find sexually attractive females.

Melissa Cameron, Yoshio Takei, John Donald

Vasodilatory effects of adrenomedullin 2 on the vasculature of the Australian short-finned eel, *Anguilla australis* 

Rosemary Hohnen and Ashley Edwards

Effects of reproductive condition on HPG-HPA axis interaction

Keisuke Itonaga, Erik Wapstra, and Susan Jones

Evidence for placental transfer of maternal corticosterone in a viviparous lizard

Kathryn Napier, Todd McWhorter, Patricia Fleming

Paracellular absorption of glucose and xylose in the Australian frugivorous silvereye

Suzita Noor, Rowena Lewis, and Alister Ward

Conserved prolactin receptor signaling through Stat5 mediates zebrafish lateral neuromast development.

Martha Patricia Ramírez-Pinilla, Elkin Darío Rueda, and Elena Stashenko

Transplacental lipid transfer during gestation in the lizard Mabuya (Squamata, Scincidae)

Alexander Riek, Gerhard Körtner and Fritz Geiser

Thermobiology of the Queensland tube-nosed bat (Nyctimene robinsoni)

Chris Wacker and Fritz Geiser

Temperature and photoperiod affect torpor use and activity in the marsupial *Sminthopsis crassicaudata*.

Jenna Van Gramberg, Stuart Linton, Jan West

Characterization of the muscle fibre types in pristine and regenerating chelae from the Christmas Island Red Crab, *Gecarcoidea natalis*.

Anja Wollenhaupt, Kerry Withers, John Billingsley

Behaviour of juvenile Antechinus flavipes, a preliminary study

### **Oral Presentations**

Maternal creatine supplementation prevents hypoxia-induced damage to the diaphragm

**David J Cannata<sup>1</sup>**, Zoe Ireland<sup>2</sup>, Hayley Dickinson<sup>2</sup>, Aaron P Russell<sup>3</sup>, Rod J Snow<sup>3</sup>, Jan M West<sup>1</sup> and David W Walker<sup>2</sup>

<sup>1</sup>School of Life and Environmental Sciences, Deakin University, Victoria 3125, <sup>2</sup>Department of Physiology, Monash University, Victoria 3800, <sup>3</sup>School of Exercise and Nutrition Sciences, Deakin University, Victoria 3125

Hypoxia is a leading cause of morbidity and mortality in newborns. While it is known to compromise many vital organs, the effects of severe birth hypoxia on the diaphragm (an essential respiratory muscle) are unknown. To examine if the newborn diaphragm is affected by hypoxia, we developed a birth hypoxia model with an increased mortality and postnatal maturation abnormalities in a precocial species, the spiny mouse (Acomys cahirinus). Significant muscle fibre atrophy, contractile dysfunction and an increased gene expression of MuRF1 and Myostatin (promoters of proteolysis) was observed in the diaphragm 24 hours after neonates were subjected to birth hypoxia. We then hypothesized that maternal creatine supplementation from mid-pregnancy would protect the fetal diaphragm from hypoxiainduced damage. Results showed that creatine: (i) improves survival; (ii) accumulates in the fetal diaphragm; (iii) prevents hypoxia-induced fibre atrophy; (iv) restores contractile function and (v) perturbed the expression of MuRF1 and myostatin. This study demonstrated that birth hypoxia caused structural and functional damage to the diaphragm via protein degradation which could be prevented by an extra provision of creatine a few days before birth. These findings support the use of maternal creatine supplementation as a protective measure for newborns susceptible to birth hypoxia.

Mechanisms underlying the sprint-endurance trade-off in rat muscle

### Frank Seebacher<sup>1</sup>, Isabel Walter<sup>1</sup>, Rob S. James<sup>2</sup>

<sup>1</sup>School of Biological Sciences A08, University of Sydney, NSW 2006, Australia. <sup>2</sup>Department of Biomolecular and Sport Sciences, Coventry University, Coventry CV1 5FB, UK

An important constraint on locomotor performance is the trade-off between sprint and endurance performance. The aim of this study was to test the hypothesis that inter-individual variation in muscle mechanics is associated a) with tissue metabolic capacities or b) with calcium handling dynamics. We determined kinetics of rat lateral gastrocnemius muscle during maximal and sustained activities. Metabolic capacities were estimated from lactate dehydrogenase, citrate synthase, and cytochrome c oxidase activities, and we characterised Ca<sup>2+</sup> handling dynamics by determining SERCA activity, SERCA1 protein concentration, and mRNA concentrations of SERCA 1 and 2 and troponin 1.1 and 1.2 isoforms, and ryanodine receptors. There is a trade-off in muscle mechanics, and greater fatigue resistance is associated with lower tetanic stress and slower tetanus force relaxation rates. Shorter tetanus activation times were associated with increased ratio of fast to slow isoform expression of both troponin I and SERCA. Both sprint and endurance performance depend on ATP supply. Pharmacological blockade of the ryanodine receptor significantly increased muscle activation times, and decreased peak tetanus force. Hence, sprint performance is constrained by Ca<sup>2+</sup> release, and the sprint-endurance trade-off depends on troponin mediated muscle contraction and on re-sequestration of Ca<sup>2+</sup> into the sarcoplasmic reticulum to facilitate muscle relaxation.

Changes in muscle composition during development in the Australian fur seal

Domenic LaRosa, David Cannata, John Arnould and Jan West

School of Life and Environmental Sciences, Deakin University, Melbourne

During development the Australian fur seal undergoes a transition from a terrestrial pup to a predatory marine adult. Diving mammals have adaptations for diving including an increase in haematocrit, control of bradycardia and myoglobin levels. The proportion of fibre types within a locomotor muscle and their oxidative capacity was determined to see if muscles also undergo adaptations to diving.

Biopsy samples from the trapezoid muscle of pups, juvenile and adults, were snap frozen and stored at -80°C. Fibre type proportions were determined using histochemical staining for ATPase. All muscles contained Type I and Type IIa fibres and the proportions changed significantly from pup (42% Type I, 58% Type IIa) to adult (57% Type, 43% Type IIa). The oxidative capacity of Type I and Type IIa fibres was determined by staining for SDH/NADH and increased in both fibre types during development.

Myoglobin levels were quantified using western blot analysis and increased from pup to adult; only 1 isoform was expressed.

There are significant changes in muscle composition in the trapezoid muscle of the Australian fur seal throughout development, changes which can be clearly correlated to the diving behaviour of the animals at different ages.

### Summer torpor is enhanced by good body condition in a subtropical bat

### Clare Stawski and Fritz Geiser

Centre for Behavioural and Physiological Ecology, Zoology, University of New England, Armidale, New South Wales, 2351, Australia

Torpor is the most effective energy-conserving strategy available to mammals, as it substantially lowers metabolic rate, and is crucial for survival on limited resources. However, a widely held view is that torpor is avoided by mammals whenever possible because of potential costs associated with reduced body temperatures and slowed metabolic processes. We examined this hypothesis by quantifying use of torpor in relation to body condition of thirteen free-ranging northern long-eared bats (*Nyctophilus bifax*, ~10g), a species known to hibernate, from a subtropical region during the austral summer when insects were abundant. Temperature-telemetry revealed that bats used torpor on 85% of observation days and on 38% of all nights. Against predictions, individuals with a high body condition index (i.e. good fat/energy reserves) expressed longer and deeper torpor bouts and also employed torpor more often during the activity phase at night than those with low body condition index. We provide the first evidence that use of torpor in a free-ranging subtropical mammal is positively related with high body condition index. This suggests that employment of torpor is maximised and foraging minimised not because of food shortages or low energy stores, but likely to avoid predation when bats are not required to feed.

Epithelial HCO<sub>3</sub><sup>-</sup> secretion and mucus hydration in the vaginal cul-de-sac of the brushtail possum

### Alsop, T-A<sup>1</sup>, McLeod, B.J<sup>2</sup>, Butt, A.G<sup>1</sup>

<sup>1</sup>Department of Physiology, School of Medical Sciences, University of Otago, PO Box 913 Dunedin, New Zealand <sup>2</sup> AgResearch, Invermay Agricultural Centre, Private Bag 50034, Mosgiel, New Zealand

The vaginal cul-de-sac of the brushtail possum increases in size leading up to oestrous, with an associated proliferation of the epithelial layer and secretion of mucus-rich luminal fluid. We have used measurements of the luminal composition and short circuit current (lsc) to investigate the mechanism of fluid secretion. The luminal fluid had high Ca<sup>2+</sup> (85.5±6) mMol/kg H<sub>2</sub>O, mean±SEM, n=20) but low Cl (7.6±2 mMol/kg H<sub>2</sub>O) content, suggesting an appreciable HCO<sub>3</sub><sup>-</sup> (≈185.3±10 mMol/kg H<sub>2</sub>O) content. The epithelium had a spontaneous, Isc (64±12 µA cm<sup>-2</sup> mean±SEM, n=7) that was inhibited by the anion channel blocker 5-Nitro-2-(3-phenylpropylamino)benzoic acid (NPPB, 100µM mucosal) consistent with anion secretion. The NPPB-sensitive lsc was dependent on serosal HCO<sub>3</sub><sup>-</sup> and mucosal Na<sup>+</sup>. The carbonic anhydrase (CA) inhibitor, acetazolamide (0.5mM mucosal and serosal), inhibited 34±8% (n=5) of the lsc and mucosal, but not serosal, 5-(N-ethyl-N-isopropyl) amiloride (EIPA, 200 $\mu$ M), an inhibitor of Na<sup>+</sup>/H<sup>+</sup> exchangers (NHE), inhibited 58.3±4% of the lsc. These data are consistent with a model of mucus expansion in which secreted HCO<sub>3</sub><sup>-</sup> complexes with Ca<sup>2+</sup> bound to mucus oligosaccharides to un-shield hydrophilic charges on the mucus. Ion transport data suggests that HCO<sub>3</sub><sup>-</sup> secretion is driven by CA and an apical NHE.

Electrogenic anion secretion in the small intestine of the common Australian brushtail possum, *Trichosurus vulpecula* 

### Gill MW<sup>1</sup>, Bartolo RC<sup>1&2</sup>, and Butt AG<sup>1</sup>

<sup>1</sup>Department of Physiology, School of Medical Sciences, University of Otago, PO Box 913 Dunedin, 9054, New Zealand and <sup>2</sup> AgResearch, Invermay Agricultural Centre, Private Bag 50034, Mosgiel, 9024, New Zealand

In the possum ileum fluid secretion is driven by HCO<sub>3</sub><sup>-</sup> secretion, not Cl<sup>-</sup> secretion as in eutherian mammals. We have used measurements of electrogenic ion transport with the short circuit technique and expression profiles of transport proteins to investigate the secretory mechanisms in the duodenum and jejunum of the possum. Prostoglandin-E<sub>2</sub>, stimulated the ileal short circuit current (Isc) by 94.5±7.7 µA.cm<sup>-2</sup> (X±SEM., n=8), but had little effect on the duodenum ( $\Delta$ Isc=2.7±5.7  $\mu$ A.cm<sup>-2</sup>, P>0.05) and jejunum ( $\Delta$ Isc=14.5±5.4  $\mu$ A.cm<sup>-2</sup>, P<0.05). Western blots revealed high levels of NaHCO<sub>3</sub> cotransporter (NBC) expression in the ileum and duodenum, but negligible amounts in the jejunum, while expression of the anion channel CFTR was low in the duodenum and jejunum compared with ileum. No region expressed the NaK2Cl<sup>-</sup> cotransporter NKCC1. Immunohistochemistry localised NBC to the basolateral membrane of the villous and crypt cells in the duodenum and ileum and CFTR to the apical membrane of ileal crypt and lower villous cells. These results indicate that electrogenic Cl<sup>-</sup> secretion does not occur in the small intestine of the possum, due primarily to an absence of NKCC1, and electrogenic  $HCO_3^-$  secretion is restricted to the ileum. Presumably electroneutral transport drives fluid secretion in the duodenum and jejunum.

### The energetic cost of reproduction in birds

### Caragh Heenan

Ecology and Evolutionary Biology, The University of Adelaide, Adelaide, SA, Australia

Maintenance of an appropriate nest temperature is important for the development and growth of young, however this requires the deployment of parental resources in terms of both time and energy. There may be trade-offs in resource allocation such that there is a cost of reproduction – defined as the extent to which investment in one reproductive event reduces the capacity of a parent to invest elsewhere. In the case of incubation, the energetic cost is represented by the excess heat generated by the parent to initiate and continue embryonic development within the egg, above what would normally be produced by the non-incubating bird. This is known as the incubation metabolic rate (IMR), expressed as a multiple of the basal or resting metabolic rate. This study aims to determine the IMR for the Gouldian finch (*Erythrura gouldiae*) in captivity and the Australian owlet-nightjar (*Aegotheles cristatus*) in the field using respirometry techniques. Video surveillance is used to determine nest attendance by the parents. The IMR for both species is correlated with ambient temperature diurnally, with a gradual increase during the incubation period due to growth of chicks. Fluctuations also occur that coincide with levels of activity and specific behaviours.

The mRNA Expression and Protein Localisation of Guanylyl Cyclase-Linked Natriuretic Peptide Receptors in Freshwater and Chronically Seawater-Acclimated Rainbow Trout

Scott Nankervis, Mark Powell, Janet McLeod, Tes Toop.

School of Life and Environmental Sciences, Deakin University, Pigdons Road, Waurn Ponds, 3217, Geelong, Australia

Rainbow trout, Oncorhynchus mykiss, migrate between freshwater (FW) and seawater (SW) environments. The natriuretic peptides (NPs) are a class of hormones that help to maintain osmolarity in fish when they are confronted with salinity change, particularly in the acute phase, and their actions include vasodilation, inhibition of drinking, inhibition or promotion of salt uptake and stimulation of cortisol release. These effects are mediated by the natriuretic peptide receptors (NPRs) some of which use the cGMP signaling pathway. We investigated the regulation of NPR-GC mRNA in the major osmoregulatory organs of FW and chronically SW-acclimated rainbow trout, and subsequently identified sites of NPR-GC protein expression in the same tissues. Real-time PCR was used to measure the relative expression of NPR-GC mRNA in FW control and SW-adjusted rainbow trout. NPR-A mRNA expression was consistently low and unaffected by salinity in any tissue, while NPR-B mRNA was typically expressed in greater quantities and its abundance increased in FW compared to SW in some tissues (body kidney P = 0.017; posterior intestine P = 0.05). NPR-B protein expression was localized to the smooth muscle component of structures within the tissues examined. NPR-B appears to be the predominant NPR-GC involved in the response of rainbow trout to salinity challenge and this is likely achieved through mediation of cardiovascular homeostasis as well as osmoregulation.

Unravelling transthyretin amyloid- bounding ahead using wallabies

### Leonard K. Pattenden<sup>1</sup>, Klaus Altland<sup>2</sup> and Samantha J. Richardson<sup>\*1</sup>

<sup>1</sup>School of Medical Sciences, RMIT University, Bundoora, Victoria, Australia; <sup>2</sup>Department of Human Genetics, University of Giessen, Giessen, Germany.

Transthyretin (TTR) is a thyroid hormone distributor protein. For unknown reasons in humans, TTR can change from a soluble protein to insoluble amyloid fibrils that deposit principally in the heart, disrupting normal cellular function. TTR amyloidosis disease in the aging population is named senile systemic amyloid (SSA), which is prevalent in ~25% of people over the age of 70. The only therapy for SSA is a heart transplant, which is usually not performed due to the age of the patient.

Many models of TTR SSA initiate amyloid formation under non-physiological conditions (typically pH 4.6), which dissociates the TTR tetramer into unfolded monomers before assembling as amyloid fibrils. However, we have demonstrated human TTR amyloid formation at pH 6.5; but curiously, under identical conditions, wallaby TTR remains stable. The pH is within the range for protonation of His residues, and there are interesting sequence variations between these species. We suggest that protonation of His31 results in H-bond breakage between His31 and Ser46 in humans, which results in destabilization of the TTR tetramer. Wallaby TTR does not have His31 or Ser46, thus is not destabilized at pH 6.5.

We therefore propose wallaby TTR is an important model for understanding the mechanism of TTR amyloid formation.

Effect of opioid compounds on feeding and activity of the cockroach, *Periplaneta americana* 

**Paul D. Cooper**, Stuart R. Dennis, James D. Woodman, Ann Cowlings and Christine Donnelly

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

Opioid peptides have been implicated in regulation of feeding in invertebrates. Studies have suggested that receptors for opioids are present in cockroaches and that these receptors play roles in affecting both behaviour and feeding. We examined the effect of  $\mu$ ,  $\delta$ , and  $\kappa$  opioid receptor agonists and antagonists on feeding, mass changes and activity in the cockroach, *Periplaneta americana*. The  $\kappa$  antagonist, nor-binaltorphimine, significantly increased food intake, while naltrexone (general antagonist) and naloxonazine ( $\mu$  antagonist) both reduced feeding. Interestingly, a large mass loss was observed in cockroaches treated with nor-binaltorphimine, but males and females lost mass differently during the studies. Time of activity (%) was not influenced by any drug. Water loss experiments suggested that nor-binaltorphimine increased water loss, accounting for the mass loss while also stimulating feeding. We suggest that two populations of opioid receptors are present as previously reported, with one affecting feeding and the other involved with water loss.

Effect of morphine on the growth rate of *Calliphora stygia* (Fabricius) (Diptera: Calliphoridae) and possible implications for forensic entomology

### Kelly George<sup>1</sup>, Melanie Archer<sup>2</sup>, Lauren Green<sup>1</sup>, Xavier Conlan<sup>3</sup> and Tes Toop<sup>1</sup>

<sup>1</sup>School of Life and Environmental Sciences, Deakin University, Pigdons Road, Waurn Ponds, Victoria, 3217, Australia; <sup>2</sup>Department of Forensic Medicine, Monash University, 57-83 Kavanagh Street, Southbank, Victoria 3006, Australia; <sup>3</sup>Institute for Technology, Research and Innovation, Deakin University, Pigdons Road, Waurn Ponds, Victoria 3217, Australia.

We investigated the effects of morphine in a model of decomposing tissue on growth rates of the forensically important native Australian blowfly Calliphora stygia (Fabricius) (Diptera: Calliphoridae). Various morphine concentrations were incorporated into pet mince to simulate post mortem concentrations from morphine, codeine and/or heroin-dosed corpses. Treatments for feeding maggots were: T 1 (control: no morphine); T 2 (2 µg/g morphine); T 3 (10 µg/g morphine); and T 4 (20 µg/g morphine). Ten replicates of 50 maggots each were grown at 22°C and compared at four comparison intervals: CI 1 (four day old larvae); CI 2 (seven day old larvae); CI 3 (pupae); and CI 4 (adults). Length and width were measured for larvae and pupae; costae and tibiae of adults were measured. Additionally, day of pupariation, day of adult eclosion and survivorship were calculated for each replicate. Continued presence of morphine in meat was verified using high performance liquid chromatography with chemiluminescence detection. Growth rates of C. stygia fed on morphine spiked mince did not differ significantly from those fed on control mince for any comparison interval or parameter measured. These results allow more confident usage of this species to determine minimum death time in corpses with tissue morphine concentrations at the levels studied.

Accumulation and excretion of morphine by *Calliphora stygia*, an Australian blow fly species of forensic importance

### **Samuel Parry**<sup>1</sup>, Stuart M. Linton<sup>1</sup>, Michael J. O'Donnell<sup>2</sup> and Tes Toop<sup>1</sup>

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The developmental stages of the blow fly, *Calliphra stygia* are used to estimate post mortem interval (PMI) in forensic investigations. If morphine is consumed by the fly its rate of development and hence the PMI may be changed. This change will be dependent upon the concentration of morphine within the body and the excretory ability of the animal. To examine this, larval stages of *C. stygia*, were raised upon meat containing morphine at low (7 pmol g<sup>-1</sup>) and high (17.5 pmol g<sup>-1</sup>) concentrations and the morphine content and excretory ability measured. Morphine accumulated within the bodies of maggots ( $\approx$ 70% within the tissues) at concentrations which were lower than that of the meat (3 to 24%). In the initial developing stages (2<sup>nd</sup> and 3<sup>rd</sup> instars) the morphine content of animals in the high morphine group was higher than that of the low morphine group. Animals in both groups cleared the morphine exponentially at similar rates. The Malpighian tubules of maggots were able to actively secrete morphine against a calculated electrochemical gradient using a transport mechanism that transports small type II organic cations, such as morphine and quinine. The rate of morphine secretion by the Malpighian tubules could explain the clearance of morphine by the maggots.

Rhinoceros beetles modulate spiracular opening to regulate haemolymph pH

### Philip G. D. Matthews and Craig R. White

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Insects are the most diverse and numerous terrestrial air-breathing animals. But despite this, a comprehensive understanding of their respiratory physiology is still lacking. This is clearly seen in the debate surrounding the origins and function of discontinuous gas exchange cycles (DGCs) displayed by many insects at rest. While many hypotheses have been proposed to explain the function of DGCs, no general underlying mechanism driving patterns of insect ventilation has been universally accepted. In this study we measured in vivo pH fluctuations within the rhinoceros beetle Xylotrupes ulysses coincident with CO<sub>2</sub> emission. DGCs caused haemolymph pH to fluctuate by 0.1 units, with internal pH steadily declining while the spiracles were closed and rapidly climbing when the spiracles opened. Exposure to hypoxia (5% oxygen in nitrogen) caused the spiracles to open widely and elicit a large burst of CO<sub>2</sub>, causing the haemolymph pH to increase from 7.0 to 7.3. If the spiracles were held open by continued exposure to hypoxia, the pH remained high. Once normoxia was restored, the beetles held their spiracles shut, only beginning to resume normal opening behaviour once internal pH had been restored to pre-hypoxia levels. The protracted breath hold following hypoxic exposure indicates that the beetles preferentially retain CO<sub>2</sub> in order to restore their haemolymph pH. We therefore propose that insects, like all other airbreathing animals, regulate gas exchange to maintain an internal pH balance.

### Thermal energetics and activity patterns of the Kaluta (Dasykaluta rosamondae)

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

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Many small desert dasyurid marsupials employ torpor almost daily during winter, likely because cold nights and low food availability impose high energetic costs. However, in western Australia the arid zone extends into tropical, coastal regions, where thermal conditions in winter are mild. Therefore, we studied the thermal energetics of Kalutas (~27q), a dasyurid restricted to these tropical deserts, during the Austral winter. Unlike most dasyurids, Kalutas were almost exclusively diurnal and retreated into burrows during the night. Despite being active during the warmer part of the day, Kalutas entered torpor daily. However, torpor patterns differed remarkably between the sexes. While females spend most of the night torpid at body temperatures as low as 21°C, close to soil temperature, males entered multiple short and shallow bouts throughout the night. Males also occupied larger home ranges and covered greater distances while foraging than females. Hence, males appear to spend more energy than the similar-sized females while active and also during the rest phase when they maintain an on average higher body temperature than females. Consequently, physiological as well as behavioural preparations for the September mating season might impose energetic costs for males already during winter, foreshadowing postreproductive male die-off.

### **Plenary Lecture**

Water balance in desert mammals and in flying birds: multiple vs. single evolutionary paths to the same physiological end

### **Berry Pinshow**

Mitrani Department of Desert Ecology, Jacob Blaustein Institutes for Desert Research, Ben-Gurion University of the Negev

In order to function under stressful, desiccating environmental conditions, both mammals and birds must maintain their plasma volume. In mammals, plasma volume maintenance has been studied in many desert-dwelling species and it is apparent that adaptation to exsiccating conditions has taken place in numerous taxa that exploit, to various degrees, the diverse repertoire of behavioral and physiological mechanisms available, including evading stressful environmental conditions; increasing urine concentration, thus reducing its volume; forming insoluble nitrogenous end products; desiccating feces; recycling exhaled water vapor by temporal counter-current heat exchange; reducing transcutaneous evaporation; having labile body temperatures; and redistributing blood flow. Birds too may use these mechanisms to save water, since like mammals, they closely defend plasma volume when heat and water stressed. Like mammals in desiccating circumstances, during flapping flight maintenance of plasma volume is imperative to all birds. Data show that birds do this as well as the best-adapted desert mammals. I suggest that adaptations to withstand dehydration in birds, as an order, evolved as requirements for long distance flapping flight and are "exaptations" to living and functioning in other desiccating environments.

The economy of group living: Chestnut-crowned Babblers reduce basal and thermoregulatory energy expenditure in communal roost nests

### <sup>a</sup>Mark A. Chappell, <sup>b</sup>William A. Buttemer and <sup>c</sup>Andy F. Russell

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Many of Australia's old-endemic passerine species live in cooperative social groups the year round. This results in costs in the form of competition for food and resources, but it also provides benefits in rearing young and providing protection from predators. These costs and benefits would be expected to produce an optimal group size that varies dynamically with environmental conditions. We examined the effect of group size on overnight roosting energetics at temperatures of 5 °C, 15 °C, and thermoneutrality (25-29 °C). Birds exposed to 5 °C expended energy at 2.3 times their basal metabolic rate, whereas those sharing a nest with 6 or more conspecifics had metabolic rates 34% of this value. Similar energy savings were experienced at 15 °C when 4 or more birds shared a roost nest. Surprisingly, birds experienced a 22% lower metabolic rate at thermoneutrality when sharing a nest with 3 or more individuals. Because winter months are typified by low temperatures and reduced insect abundance, the magnitude of energy savings from group roosting would reinforce group cohesion in this species.

Phenotypic flexibility in avian basal metabolic rates: global variation in responses to acclimation and acclimatization

### McKechnie, A.E. and Smit, B.

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Avian basal metabolic rate (BMR) exhibits a high degree of phenotypic flexibility. A major determinant of BMR in birds held under artificial conditions is temperature; almost without exception, birds increase BMR when acclimated to cold conditions. On the basis of data from laboratory studies of acclimation, one would predict that free-ranging birds should elevate BMR during winter. While this prediction holds for species resident at high latitudes in the northern hemisphere, species from the southern sub-tropics appear instead to decrease BMR in winter. We observed winter BMR reductions of 17-35% in five species occurring in the Kalahari Desert of southern Africa, namely Crimson-breasted Shrikes (Laniarius atrococcineus), Fork-tailed Drongos (Dicrurus adsimilis), White-browed Sparrowweavers (Plocepasser mahali), Pearl-spotted Owlets (Glaucidium gnoma) and African Scops-owls (Otus senegalensis). We analysed the available data on avian seasonal metabolic variation, and found that the magnitude and direction of BMR adjustments are correlated with latitude and temperature. Species that experience cold winters at high latitudes increase BMR during winter, likely reflecting the metabolic demands associated with enhanced cold tolerance. In contrast, species from sub-tropical latitudes exhibit winter decreases in BMR, which we interpret as evidence of energy conservation, possibly driven by winter reductions in food availability.

## Aggressive behaviour results in fin damage that affects swimming performance in *Gambusia holbrooki*

### Elektra Sinclair, Ashley Ward and Frank Seebacher

School of Biological Sciences, University of Sydney

Aggressive behaviour is prevalent in intraspecific animal interactions and has associated benefits of resource acquisition and reproductive success. However aggressive behaviour also incurs costs, creating a trade-off between resource value and the risks of damage and energetic expense. Male *Gambusia holbrooki* are highly aggressive towards one another as more aggressive males have greater access to females and achieve more successful copulations. Typical aggressive behaviour of these fish includes chasing and nipping. These behaviours are energetically costly and being nipped mostly results in damage to the fins. This study shows that fin damage sustained during aggressive interactions affects sustained swimming and fast start speeds in *G. holbrooki*. Fin damage is also shown to decrease aggressive behaviour.

The balance between dietary n-3 and n-6 polyunsaturated fatty acids has the greatest influence on membrane fatty acid composition and basal metabolic rate

### Sarah K. Abbott<sup>1</sup>, Paul L. Else<sup>2</sup>, A.J. Hulbert<sup>1</sup>

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This study quantifies the relationship between diet fatty acid (FA) profile and membrane composition of various rat tissues. Membrane composition was then related to basal metabolic rate (BMR; ml oxygen/min/kg). Rats were fed one of 12 diets differing only in lipid profile. Diet saturates (SFA) ranged 8-88%, monounsaturates (MUFA) 6-65%, polyunsaturates (PUFA) 4-81% with n-6 PUFA 3-70% and n-3 PUFA 1-70%. PUFA Balance (= n-3 PUFA as % of total PUFA) ranged 1-86%. Diet n-6 and n-3 PUFA were 18:2n-6 and 18:3n-3 respectively. FA composition of skeletal muscle, brain, liver and adipose tissue phospholipids were determined. The slope of tissue phospholipid composition plotted against diet composition quantifies the response of membrane composition to dietary fat (0=no response; 1=conforms to diet). All tissue membranes responded most strongly to diet PUFA Balance, while diet SFA, MUFA or PUFA had less influence. Adipose tissue showed the highest response to diet fat profile. Similarly, rat BMR related most closely to membrane PUFA Balance for all tissue types, with little to no response to membrane SFA, MUFA or PUFA composition. These results may have significant implications for human disease states, as diet PUFA Balances are currently below 10%, which is likely an inadequate level.

Dramatic changes in the diet of *Homo sapiens*: history of the balance between omega-3 and omega-6

### Hulbert, A.J.

"Wise man" (*Homo sapiens*) is an omnivore and individuals of this species exhibit huge dietary diversity. Like other animal species, *H. sapiens* are unable to synthesise both omega-3 and omega-6 polyunsaturated fatty acids (PUFA) and also are unable to interconvert these fats. Consequently, both n-3 PUFA and n-6 PUFA are essential dietary components. Both PUFA types are important components of membranes and have different (and sometimes opposing) effects on membrane function. It is becoming apparent that 'hunter-gather' humans predominantly consumed animal food and that the PUFA balance of this diet averaged ~30% n-3 PUFA and ~70% n-6 PUFA. Since this time, the PUFA balance of the modern human diet has changed dramatically such that in most western countries it now averages ~ 10% n-3 PUFA and ~90% n-6 PUFA. This is largely due to a dramatic increase in fats from plants (vegetable oils) at the expense of animal fats in the modern human food chain. There are important biochemical and physiological (and thus health) consequences of this change in diet and some of these will be discussed.

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

### M. Trzcionka, AJ Hulbert and WA Buttemer

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Psittaciformes (parrots) live up to 6-fold longer than Galliformes (chickens and guails). A modification of the oxidative stress theory of aging emphasizes three major components: (1) Mitochondria produce reactive oxygen species (ROS) as a normal by-product of respiration, which damage DNA, proteins, and lipids, which, in turn, causes aging and eventually death. (2) Animals have antioxidants and repair mechanisms that protect against ROS damage. (3) Membrane polyunsaturated fats exposed to ROS form lipoxidation products that are highly reactive and produce further cellular damage. Interspecific differences in membrane fatty acid composition can therefore influence the potential extent of damage. To determine whether any of these processes account for the longevity differences between short-living galliformes and long-living psittaciformes, we have undertaken a multi-species comparison, measuring mitochondrial ROS production, intermediate and end products of ROS damage, antioxidants, and membrane fatty acid composition. While mitochondrial ROS production, ROS damage products and antioxidants could not explain the observed differences in longevity, membrane fatty acid composition tended to have a lower susceptibility to peroxidation in the longer-living species. These results suggest that the oxidative stress theory of aging is not a universal explanation for differences in longevity between species and other explanations must be considered.

The last piece in the cellulase puzzle, the characterisation of  $\beta$ -glucosidase from the herbivorous Gecarcinid land crab, *Gecarcoidea natalis* 

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Herbivorous, terrestrial crustaceans consume a plant diet that is rich in cellulose. In order to digest this structural polysaccharide, herbivorous crustaceans, like many invertebrates, are known to possess endogenous cellulases such as endo-β-1,4-glucanases, which aid in the conversion of native cellulose to glucose for metabolism. However, endo-β-1,4-glucanases alone are insufficient to produce glucose, so herbivorous invertebrates must synthesise other cellulases such as  $\beta$ -glucosidase, which are responsible for the final production of glucose. A  $\beta$ -glucosidase was partially purified for the first time from a species of crustacean, the Christmas Island red crab, Gecarcoidea natalis. The 129 kDa enzyme was able to remove glucose from cellobiose, cellotriose and cellotetraose, and had limited activity towards carboxymethyl cellulose. The activity of the  $\beta$ -glucosidase purified here, along with that of the multiple endo- $\beta$ -1,4-glucanases from G. natalis described previously, would be sufficient to release glucose from cellulose in the absence of a cellobiohydrolase, the third class of cellulase found in fungal systems, which was previosly assumed to be present in invertebrates. These results give further support to the growing body of evidence that suggests that cellobiohydrolases are absent from many invertebrate systems as they are not required for efficient digestion of cellulose.

Thermal biology of free-ranging southwest carpet pythons (Morelia spilota imbricata)

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Interactions ectotherms have with their environment are important for their thermal biology. Snakes may behaviourally adjust the rate of heat exchange with the environment by altering their body posture, basking or through microhabitat choice, and each may be affected by an individual's body size. Body temperature (T<sub>b</sub>) in the southwest carpet python (Morelia spilota imbricata) was continuously monitored through surgically implanted temperature loggers and temperature-sensitive radiotransmitters, and natural behaviour was observed weekly over a three-year study in southwest Western Australia. We tested the effects of body size upon four measures of python thermal biology. We assessed whether large and small pythons behaviourally adjusted their T<sub>b</sub> by adopting different body postures, the proportion of their bodies exposed to the sun or microhabitat choices. Absolute T<sub>b</sub> appears to be warmer in larger pythons and may be affected by these behaviours. There was also some effect of body mass on thermal differential ( $T_b$  above  $T_a$   $t_{229}$ =2.24 p=0.026), with larger pythons marginally more warm than T<sub>a</sub>. Hourly heating and cooling rates of change, however, did not differ with body mass (p>0.05). Although overall temperature relations do not change as python size increases, small and large pythons may exhibit different behavioural thermal strategies.

What is the point of relative water economy?

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Relative water economy (RWE) is the ratio of metabolic water production to evaporative water loss. There is usually a negative relationship between ambient temperature and RWE, with a higher (more favourable) RWE at low ambient temperatures and a lower (less favourable) RWE at higher temperatures. The point of relative water economy (PRWE) is the ambient temperature at which the RWE is one. PRWE has been interpreted as an index of a species' adaptation to aridity, with arid species having a higher PRWE than mesic species. We tested this hypothesis for marsupials, measuring RWE for 26 species ranging from the 5g honey possum to the 5000g tammar wallaby. There was a significant negative allometric relationship for PRWE. This presumably results from small species having a higher thermal conductance, thus higher metabolic water production, than larger species. The massindependent residuals from this allometric relationship differed significantly for marsupials with arid and mesic distributions, with mesic species having a significantly lower PRWE than arid species. Significant habitat, but not allometric, effects remained after accounting for phylogenetic history. Arid habitat marsupials presumably benefit from having a more favourable ratio of water production to water loss in an environment where free water is limited.

Physiology of the dibber (*Parantechinus apicalis*): A case study using *a priori* contrasts with repeated measures ANOVA

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Physiological studies often involve multiple measurements of the same individuals under different environmental conditions. Repeated measures ANOVA (RANOVA) is advocated as the analysis of choice, to account for repeated measurement of the same individuals. However, RANOVA does not allow for missing data and/or *post hoc* pair-wise comparisons of experimental factors (unlike ANOVA). Here we explore techniques to overcome these two major limitations of RANOVA, by describing and quantifying effects of ambient temperature (T<sub>a</sub>) on the physiology of a small dasyurid marsupial, the dibbler. Adjusting the error degrees of freedom of an ANOVA overcomes the problem of not accounting for repeated measurements, while allowing for missing data. *A priori* contrasts allow us to describe the significance of patterns of physiological variables with T<sub>a</sub>. All physiological variables were significantly influenced by T<sub>a</sub> by ANOVA, and all except evaporative water loss by RANOVA. There were no differences between individual dibblers. Polynomial contrasts showed that oxygen consumption decreased linearly at T<sub>a</sub> ≤ 30°C, but became quadratic when T<sub>a</sub> = 35°C was included in the model. Although the pattern of the T<sub>a</sub> effect was different for other parameters, polynomial contrasts were similarly useful for describing the T<sub>a</sub> effect.

The under appreciated role of fur in the adaptability of large mammals to challenging thermal environments

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In consideration of climate change impacts the flexibility of pelage, feathers in birds and fur in mammals, is often neglected. Fur as an insulator is appreciated, but other roles are poorly appreciated, notably when solar radiation features in an animal's living space. Then the interaction between heat flows and colouration (perhaps cryptic) can be surprising. If the fur is thin then heat inflow in sunshine is driven by simple absorption and reflection of the wavelengths in the solar spectrum. As fur becomes thicker the thermal impact of colour can reverse. This counter-intuitive situation arises because radiation penetrates deeper into coats with high reflectivity and internally reflected solar radiation can become a significant heat load. However, if the pelt is very thick, then colour may be irrelevant to heat inflow except in high winds. We have examined these complex interactions in kangaroo species from tropical savannahs to alpine regions. The measured heat flow characteristics of the coats do not always match initial predictions. In the Antilopine kangaroo, a tropical species, the heat load at the skin from solar radiation was much less than anticipated. The explanation resides in the complex colour and morphology of hairs.

Locomotory energetics in intact and tailless geckos

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Many lizard species will shed their tail as a defensive response (e.g. to escape a putative predator or aggressive conspecific). For most species, tail loss compromises locomotory performance (i.e. sprint speed, endurance, balance), while a few species may actually perform better after tail autotomy. We examined the effect of tail loss upon locomotory costs in the Cape dwarf gecko, Lygodactylus capensis (~0.9 g). We measured CO<sub>2</sub> production during 5-10 minutes of exhaustive exercise (in response to stimulus) and during a 45-minute recovery period. During exercise, we measured speed (for each metre moved) as well as total distance travelled. Contrary to our expectations, tailless geckos expended less effort in escape running overall, moving both slower and for a shorter distance, compared with when Tailless geckos also exhibited lower excess CO<sub>2</sub> production (CO<sub>2</sub>) they were intact. production in excess of normal resting metabolic rate) during exercising. This may be due to reduced metabolically active tissue as tails represent 8.7% of their initial body mass. Alternatively, a change in energy substrate use may take place after tail loss. This is an intriguing finding which warrants future investigation to enable prediction of relative costs of tail loss in lizards.

## Endothelial nitric oxide synthase in the amphibian, Xenopus tropicalis

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Nitric oxide (NO) is generated by NO synthase (NOS) of which there are three isoforms: neuronal NOS, inducible NOS, and endothelial NOS (eNOS or NOS III). The presence of eNOS and endothelial NO signalling in fish and amphibians blood vessels has been controversial. This study utilised the genome of Xenopus (Silurana tropicalis) to clone eNOS and determine its tissue expression, and then establish if eNOS is involved in vascular regulation. The open reading frame of Xenopus eNOS (XteNOS) cDNA encoded an 1177 amino acid protein that showed closest structural identity to mammalian eNOS. XteNOS mRNA expression was highest in lung and skeletal muscle, with lower expression in the liver, gut, kidney, heart and brain. No discernable XteNOS mRNA expression was observed in the lateral and dorsal aortae. Western analysis of lung protein using an affinity-purified anti-XteNOS produced a single band at approximately 140 kDa, which is similar to mammalian eNOS. Immunohistochemistry showed XteNOS immunoreactivity in the collecting duct of the kidney and the lung parenchyma, but not in the endothelium of blood vessels of the kidney or mesentery. Myography using endothelium-denuded lateral aorta of X. tropicalis found an acetylcholine-induced NO-mediated vasodilation that is most likely attributed to perivascular nitrergic nerves. Thus, Xenopus has an eNOS gene that is not expressed in the vascular endothelium.

Adherens junctional proteins in lizard placentae

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Uterine epithelial cells provide the interface between an embryo and its mother during pregnancy. Cadherins, adherens junctional calcium-dependent proteins in the uterine epithelium, show major shifts during early pregnancy to facilitate the communication between maternal cells and the embryonic milieu and mediate chemical signalling during implantation in eutherian mammals where they are important in trophoblast invasion and maintaining pregnancy. We investigated the spatiotemporal changes of cadherins in the uterine epithelium of two viviparous skinks that have a non-invasive placenta. We found that cadherins redistribute during pregnancy in viviparous lizards with non-invasive placentae in a similar way to mammals that have an invasive placenta. Cadherins decrease, lessening the attachment of uterine epithelial cells to each other, as gestation progresses, and so allowing the uterine epithelium to stretch as the embryo grows. The difference in cadherins between barren and pregnant uteri from the same mother suggests that expression of cadherins is not driven solely by maternal hormones, but that it is stimulated by the presence of an embryo. Since the breaching of maternal tissue does not occur in squamates, the transformation of adherens junctional proteins is not exclusive to mammals which have invasive placentae, and suggests that additional factors are important in invasion of the uterus by the trophoblast.

## Hibernation On Noah's Ark?

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The ability of eastern pygmy-possums (*Cercartetus nanus*) to hibernate without food for up to one year has provoked discussion about how this might be related to the survival of animals on Noah's Ark (www.creationontheweb.org). The article recognizes that employment of daily and multi-day torpor (hibernation) enable mammals to survive adverse conditions on limited food. As torpor substantially reduces food and foraging requirements and thus exposure to predators the question arises whether torpor use also could have implications for the conservation of mammals. This is especially important because recent evidence shows that torpor is much more widespread than previously thought, occurring in mammalian orders containing approximately 90% of extant mammals. Interestingly, of the 61 confirmed extinct mammals over the last 500 years, 57 were likely homeothermic (unable to enter torpor) whereas only 4 species were likely heterothermic (capable of torpor). This suggests that torpor use permits mammals to survive not only adverse conditions, but also helps them in dealing with habitat degradation and introduced competitors/predators. Thus, while the significance of torpor use on the Ark may never be resolved, it appears that the ability of mammals to employ torpor is an important factor that may affect their long-term persistence.

Size, shape, and the thermal niche of endotherms

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A key challenge in ecology is to define species' niches on the basis of functional traits. Size and shape are important determinants of a species' niche but their causal role is often difficult to interpret. For endotherms, size and shape define the thermal niche through their interaction with core temperature, insulation, and environmental conditions, determining the thermoneutral zone (TNZ) where energy and water costs are minimized. Laboratory measures of metabolic rate used to describe TNZs cannot be generalized to infer the capacity for terrestrial animals to find their TNZ in complex natural environments. We present an analytical model of the thermal niche of an ellipsoid furred endotherm that accurately predicts field and laboratory data. We use the model to illustrate the relative importance of size and shape on the location of the TNZ under different environmental conditions. The interaction between body shape and posture strongly influences the location of the TNZ and the expected scaling of metabolic rate with size at constant temperature. We show how such functional traits models can be integrated with spatial environmental datasets to calculate null expectations for body size clines from a thermal perspective, aiding mechanistic interpretation of empirical clines such as Bergmann's Rule.

The mechanistic basis of metabolic allometry in colonial animals

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The relationship between mass (M) and metabolic rate (MR) has been examined for over 100 years, but the reasons why MR scales non-isometrically with body mass (M) remain poorly understood. A prominent explanation for the allometric relationship between MR and M is that it arises as a consequence of the fractal-like design of exchange surfaces and distribution networks, but the applicability of this hypothesis for colonial organisms is questionable. We measured the metabolic rates of fragments of encrusting marine bryozoan colonies that varied in mass by over an order of magnitude, and found that MR  $\propto M^{0.47 \pm 0.11}$  (95% CI). We hypothesised that this allometric scaling may be due to oxygen limitation in the largest fragments, but this is not the case. The critical  $pO_2$  for MR is less than 2 kPa, which is lower than the  $pO_2$  measured in the hypoxic boundary layer surrounding colonies. Instead, we hypothesise that the allometric scaling of bryozoan colony MR represents a balance between the high MR of the growing border annulus, which scales in proportion to colony diameter as  $M^{0.5}$ , and the low MR of the non-growing centre that scales as  $M^1$ .

Heat-stress in Australasian gannets (Morus serrator): Current and future costs

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We investigated how a temperate seabird, the Australasian gannet, copes with the high temperatures it encounters at the northernmost extreme of its distribution in Victoria, Australia. We heated and cooled the gannets in the laboratory using a dynamic protocol and measured climate variables at the breeding colony to investigate how often the gannets are subject to heat-stress. The gannets had an upper critical temperature of 33.3°C above which heart rate ( $f_{\rm H}$ ), rate of oxygen consumption ( $\dot{V}_{\rm O_2}$ ) and body temperature (T<sub>b</sub>) were all elevated. During cooling, physiological variables were elevated in comparison to heating, but there was no evidence of a hysteresis in  $f_{\rm H}$  or  $\dot{V}_{\rm O_2}$  with respect to  $T_{\rm b}$ , suggesting that gannets have relatively unsophisticated physiological mechanisms to lose excess heat. Climate measurements were used to derive a relationship to predict standard operative temperature (T\_e) throughout the breeding season. T\_e would have exceeded the UCT six times during the 2005/06 breeding season with a small increase in metabolism each time. We used three possible regimes of projected increased temperature to predict how often the gannets might be heat-stressed in future years. The number of heat-stress episodes increased exponentially with increasing temperature and each episode increased in intensity and duration.

Purification and characterisation of novel lichenase enzymes from the herbivorous Gecarcinid land crab, *Gecarcoidea natalis* 

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G. natalis consumes leaf litter and is able to digest substantial amounts of cellulose and hemicellulose using cellulase and hemicellulase enzymes. The hemicellulase, lichenase which hydrolyses the hemicellulose, lichenan was purified and characterised. Lichenan is a mixed linkage polysaccharide of glucose whose carbohydrate units are joined by a mix of β-1,3 and  $\beta$ -1,4-glycosidic bonds. It is found in the cell walls of cereals, grasses, fungi, algae and protozoa. Three lichenase isoforms, termed lichenase 1a, 1b and 2, with respective molecular masses of  $53\pm0$  (3) kDa,  $43\pm0$  (3) kDa and  $47.4\pm0(3)$  kDa were purified from the midgut gland of *G. natalis*. All three isoforms were capable of hydrolysing both lichenan and carboxy-methyl cellulose, indicating they also possessed endo-β-1,4-glucanase (cellulase) activity. Lichenase 1a had the highest lichenase activity compared to endo- $\beta$ -1,4-glucanase activity and was thus deemed to be a true lichenase. All three enzymes hydrolysed cellotetrose to either two units of cellobiose or cellotriose and glucose. They could not hydrolyse cellobiose and thus lacked  $\beta$ -1,4-glucosidase activity. Kinetics of the lichenase isoforms were also measured. The presence of an enzyme with both hemicellulase and cellulase activity may explain the evolutionary origin of cellulase enzymes in terrestrial animals.

Body Mass and Membrane Phospholipids in Mammals: Kidney and Brain

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The membrane phospholipids in kidney and brain of different sized mammals (mouse, sheep and cattle) were examined. The results showed that the previously described reduction in membrane phospholipid unsaturation index (number of double bonds per 100 acyl chains) as mammals increase in body mass involves changes in the acyl composition of all phospholipid classes. That changes in membrane phospholipid acyl composition (i.e. decreased omega-3 fats, increased monounsaturated fats and decreased unsaturation index with increased body size) are not restricted to any specific phospholipid molecule or to any specific phospholipid class. That as mammals increase in body size they increase their use of both monounsaturates and less unsaturated polyunsaturates at the expense of the highly unsaturated long-chained omega-3 (n-3) and omega-6 (n-6) polyunsaturates in all phospholipid classes. Contrary to comparisons between vertebrate species, the distribution of membrane phospholipid classes in membranes of the mammals examined was essentially the same. Phosphatidylcholine (PC) and phosphatidylethanolamine (PE) combined constituted ~91% and ~88% of all phospholipids in kidney and brain respectively. The lack of sphingomyelin lipids in the membranes of mice and increased presence in the larger mammals may indicate an increased presence of membrane lipid-rafts structures in largersized mammals.

Faecal glucocorticoids in urban and wild bandicoots of northern Sydney

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Using a simple enzyme immunoassay, we measured faecal glucocorticoids in two species of bandicoots (*Perameles nasuta* and *Isoodon obesulus*) in national parks and urban backyards in northern Sydney. Females of both species showed higher faecal metabolite concentrations than males, probably due to the reproductive requirements of female bandicoots. In both sexes, a peak in corticosterone levels occurred during winter. This may be due in part to a shift in dietary preference during the winter months. Long-nosed bandicoots (*Perameles nasuta*) captured in suburban backyards had significantly higher faecal corticosterone levels than those in the neighbouring Ku-ring-gai Chase National Park. We propose that increased vigilance due to increased predation risk and competition for resources in an urban habitat leads to an increase in faecal glucocorticoid levels in this species.

# **Poster Presentations**

Sniffing out Sex: Male carpet pythons use chemosensory information to find sexually attractive females

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Snakes rely on chemoreception to find males and successfully copulate. We recorded chemoreception response (i.e. tongue-flicking) of 10 individually-housed male southwest carpet pythons to 12 randomly-assigned treatments: skin lipid pheromones (collected in hexane and presented on cotton buds) from 6 female pythons and 6 control treatments (male skin lipids, prey, cologne, hexane only, cotton bud only or the experimenters' presence only). Males showed greater interest (number of tongue flicks) during the first minute for all treatments, with less interest in minutes 2 and 3 ( $F_{2,18}$ =35, p<0.001). In minute 3 of each trial, male response was only maintained for scents collected from the three largest female pythons ( $F_{22,198}$ =1.7, p<0.05). Male carpet pythons therefore discriminate between scents and show greater interest in larger (i.e. more fecund) females. This ability to identify female scents may favour male mate searching behaviour, as previously found for garter and rattle snakes.

Vasodilatory effects of adrenomedullin 2 on the vasculature of the Australian shortfinned eel, *Anguilla australis* 

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Homeostasis of the cardiovascular system is regulated by a number of different factors, which control blood pressure and blood flow. A novel peptide has been identified as a candidate for cardiovascular regulation. and has been termed adrenomedullin (AM). AM is a member of the calcitonin-gene-related peptide family, which in mammals has been identified as a potent vasodilator. A component of the vasodilation has been attributed to the endothelial nitric oxide (NO)-signalling pathway. Until recently, no AMs had been identified in any non-mammalian species; however, recent work by Japanese researchers has determined that a family of five AMs is present in teleost fish. These AMs have been divided into three groups, AM1/4, AM2/3 and AM5, with AM1 being considered the orthologue of mammalian AM. From this finding, four homologous AMs (AM1, AM2/3 and AM5) have been cloned from the Japanese eel, Anguilla japonica, with whole animal cardiovascular studies suggesting that AM2 and AM5 are potent vasodepressors. Using *in vitro* organ bath physiology, this study aimed to determine if AM2 is mediating vasodilation of the dorsal aorta of the Australian short-finned eel, Anguilla australis, via the NO or prostaglandin signalling pathway. Application of AM2 mediated a long lasting vasodilation (72.9% ± 2.3) of preconstricted dorsal aortae, which was not significantly affected by pre-incubation of vessels with the soluble guanylyl cyclase inhibitor, ODQ (78.8%  $\pm$  8.2), or the cyclooxygenase inhibitor, indomethacin (56.7% ± 8.6). Removal of the endothelial layer also had no effect on the AM2 dilation (80.6% ± 9.4). These findings suggest that AM2 is not mediating vasodilation via the endothelium in teleost fish, and is probably acting directly to relax the vascular smooth muscle.

## Effects of reproductive condition on HPG-HPA axis interaction

#### Rosemary Hohnen and Ashley Edwards

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In vertebrates, activation of the stress response, via the hypothalamic-pituitary-adrenal (HPA) axis often results in the down regulation of processes not immediately necessary for that individual's survival including reproduction (regulated via the hypothalamic-pituitary-gonadal (HPG) axis). The nature of the relationship between reproductive (testosterone (T)) and stress (corticosterone (B)) hormones depends upon the reproductive phase of the animal. As part of a larger project, we investigated whether artificially stimulating the HPA axis would alter plasma T and B concentrations during breeding or quiescence in male blue-tongued lizards, *Tiliqua nigrolutea*. We measured several parameters relating to HPA and HPG axis function: the increase in circulating B during both breeding and quiescence confirms the role of ACTH in this species as a stimulator of B response. The increase in blood glucose levels following ACTH injection supports the role of the HPA axis in facilitating the male *T. nigrolutea* response to stress acting to mobilise energy reserves, a role B plays in many reptilian species. A decrease in plasma T in response to activation of the HPA axis suggests ACTH suppresses, directly or indirectly (via elevation of B concentrations), concentrations of T, a critical reproductive hormone.

Evidence for placental transfer of maternal corticosterone in a viviparous lizard

### Keisuke Itonaga, Erik Wapstra, and Susan M. Jones

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Physiological mechanisms may protect embryos from hormonal exposure during embryonic development. However, a number of empirical studies have shown that excessive hormonal exposure during embryonic development affects offspring phenotype in a variety of taxa. For example, glucocorticoid exposure during embryonic development usually has negative impacts on offspring phenotype. Maternal glucocorticoids may affect embryonic development directly, or by altering maternal alters maternal physiology and/or behaviour and thereby affecting embryonic development indirectly. There is experimental evidence that circulating maternal cortisol is transferred to the foetus across the mammalian placenta. However, to date, there is no direct evidence that circulating maternal corticosterone (CORT) passes through the placenta and into the embryos of viviparous reptiles. Thus, we injected <sup>3</sup>H-CORT into pregnant mothers of *Pseudomoia entrecasteauxii* to provide conclusive evidence that circulating maternal CORT passes through a lizard placenta and therefore may directly affect the developing embryos. Our results provide direct evidence that circulating maternal CORT passes through the placenta in this species. We discuss these results in terms of the relationships between the degree of CORT transfer and embryonic stage.

Paracellular absorption of glucose and xylose in the Australian frugivorous silvereye

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Small birds face strong selection pressure to digest food rapidly and thereby reduce digesta mass carried during flight. One way they may do this is by rapidly absorbing a high proportion of glucose via the paracellular (non-mediated) pathway. Paracellular absorption provides a non-saturable absorptive process that automatically compensates for acute changes in dietary nutrient concentrations. Nectarivorous birds show extensive absorption of L-glucose, which increases with diet sugar concentration, indicative of significant nonmediated glucose uptake. D-xylose is a pentose sugar that is a major component of some nectars and mistletoe fruit. The absorption mechanisms of xylose are currently unknown in small birds. We investigated the effects of food energy density and intake rate on the bioavailability of radiolabelled L-glucose and D-xylose at two dietary sugar concentrations (250 and 1000 mmol/L hexose) in the frugivorous silvereye (Zosteropidae). Bioavailability was higher for D-xylose than L-glucose, and increased with diet sugar concentration (Lglucose: 22 and 42%; D-xylose: 46 and 91% for 250 and 1000 mmol/L hexose diets respectively). The higher bioavailability of D-xylose suggests that D-xylose is absorbed by both paracellular and mediated mechanisms in the silvereye, possibly in the same manner as D-glucose.

Conserved prolactin receptor signaling through Stat5 mediates zebrafish lateral neuromast development

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The biological actions of prolactin in mammals constitute some of the most defining features of this class of organism, including mammopoiesis and lactogenesis. These processes are largely mediated by the Stat5a transcription factor, which is rapidly activated following activation of the prolactin receptor. In this study, we show that zebrafish prolactin receptor a (*prlr.a*) and *stat5.1* also interact functionally in the development of lateral line neuromasts. The *stat5.1* gene is highly expressed in the developing neuromast, while morpholino-mediated knockdown of either *stat5.1* or *prlr.a* resulted in abrogation of lateral line neuromasts formation. Further analysis showed that this was due to defective generation of neuromasts from the prolactin receptor-Stat5 signalling module despite its alternative functions across different vertebrate species.

Transplacental lipid transfer during gestation in the lizard *Mabuya* (Squamata, Scincidae)

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*Mabuya* skinks have the greatest degree of placentotrophy known among Reptilia. This skink provides most of the lipids needed for the development of its embryos as well as other organic and inorganic nutrients across the placentas. Its recently ovulated eggs are microlecithal (0.4 mg, 1-2 mm diameter) and lack of fatty yolk platelets; in contrast, the newborn mass is ~1 g and has a snout-vent length of ~35 mm. We characterize and quantify the net uptake of lipids (cholesterol, vitamin E and fatty acids in the major lipid classes - triacylglycerols, phospholipids, cholesteryl esters-), during gestation in an Andean population of *Mabuya* and compare these results with other placentotrophic skinks. Although the proportion of lipids with respect to the total dry mass does not vary among the different developmental stages, lipids are transferred from the first stages of embryonic development with maximal transference during the last stage of pregnancy. Triacylglycerols and phospholipids were the main lipid classes found in all developmental stages. However, the relative proportion of these lipid classes differs among different developmental stages, suggesting changes in the transference and in the function of each lipid class for the provision and utilisation of structural lipids and energy during embryonic development.

Thermobiology of the Queensland tube-nosed bat (Nyctimene robinsoni)

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The aim of the present study was to investigate physiological and behavioural strategies employed by the fruit bat *Nyctimene robinsoni* in winter in a tropical environment. The study was undertaken from July to August 2009 in Northern Queensland. Animals were trapped using mist nets. To measure daily body temperature (T<sub>b</sub>) fluctuations, temperature-sensitive radio transmitters were implanted in five individuals. Implanted individuals were radio tracked daily to their roosting sites. Metabolic rate was measured by open flow respirometry at ambient temperatures (T<sub>a</sub>) of approximately 15, 20, 25 and 30°C. Free-ranging bats showed a distinct 24-h circadian cycle in T<sub>b</sub>, which was significantly affected by time of the day with the lowest T<sub>b</sub> just after sunset. Average daily core T<sub>b</sub> ranged from 34.7±0.6 to 37.3±0.8°C (mean±SD) over an average daily T<sub>a</sub> range of 17.1±1.1 to 23.5±1.8°C and the lowest T<sub>b</sub> recorded was 32.6°C. Resting metabolic rate of bats was significantly affected by T<sub>a</sub> (P<0.001, *R*<sup>2</sup>=0.856). Our results indicate that tube-nosed bats do not regularly enter torpor during the tropical winter. The energy constraints experienced by tube-nosed bats in our study with relatively moderate T<sub>a</sub> fluctuations and high availability of food were likely not substantial enough to require use of torpor.

Temperature and photoperiod affect torpor use and activity in the marsupial *Sminthopsis crassicaudata* 

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At times of low ambient temperature and reduced food availability many small marsupials enter torpor to conserve energy. While it is well established that ambient temperature does influence the use of torpor and thus the amount of energy saved in the dunnart, *Sminthopsis crassicaudata*, little is known about the interrelations between temperature and photoperiod on sexual differences in torpor use and thermal biology in this species. We exposed male and female dunnarts to different temperature and photoperiod regimes and determined torpor use and depth, activity, change in body mass, and tail width. Loss of body mass was substantially reduced when torpor was employed frequently. Torpor use increased and skin temperature decreased with decreasing ambient temperature, however, photoperiod did not strongly affect these variables. In contrast, exposure to long photoperiod resulted in an increase in activity at high ambient temperatures. Overall, females were less active than males and used torpor more frequently. Our study shows that torpor and photoperiod interact in affecting thermal biology and activity in dunnarts and that males and females differ in their response to acclimation.

Characterization of the muscle fibre types in pristine and regenerating chelae from the Christmas Island Red Crab, *Gecarcoidea natalis* 

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This study investigated the relationship between chelae morphology and muscle fibre composition of the Christmas Island Red Crab. Males of this species grow larger and develop asymmetrical chelae; females however possess smaller symmetrical chelae. This species is purely terrestrial and use their chelae for many functions including burrowing, defense, courtship, feeding, grooming and walking.

Crustacean muscles have different fibre types which can be characterised by their greatly difference sarcomere length (SL). Short-sarcomere fibres have a SL  $\leq 4\mu$ m, contract quickly producing relatively low forces. Fibres with long sarcomeres (SL  $\geq 6\mu$ m) exhibit slower speeds of contraction but produce larger forces. SL was determined using the He-Ne laser and confirmed using histology. Long- and short-sarcomere fibres can also be distinguished by specific protein isoforms. P75 is only expressed in short-sarcomere fibres and the fibres differ in the isoforms of paramyosin, troponin I and troponin T.

72% of male crabs had the large chelae on the left hand side. Fibres exhibited a broad range of SL's 3-21µm, average 9.66µm (pristine) and 3-15µm, average 8.45µm (regenerate). There was only a small population of fibres with a SL  $\leq$  4µm in the pristine chelae and these fibres did not express the characteristic P75 protein. This suggests that the fibre types are a continuum with the majority of fibres expressing proteins characteristic of long-sarcomere fibres but having a broad range of SL's.

Behaviour of juvenile Antechinus flavipes, a preliminary study

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The life cycle of *Antechinus flavipes* is characterised in the wild by post-mating male mortality. This study characterises behaviour of juvenile male and female *A. flavipes*. *C*aptured in South East Queensland and maintained in separate enclosures under natural lighting, the behaviour of each *A. flavipes* was recorded using a CCD camera positioned in front its enclosure. The cameras were interfaced with a computer and a DVD recorder. Each enclosure was illuminated by infra-red light emitting diodes. The two-day observation period was divided into 15 second intervals in order to identify and quantify behaviours of brief duration. This preliminary study was limited to one male and one female in order to process the large amount of data acquired. Twelve behaviours were identified. The male A. *flavipes* was active for a greater proportion of the total observation time than the female. The male exhibited a greater number of activities per hour and also devoted a greater proportion of time to some of them than the female. This parameter varied greatly during the observation period for each sex. The behaviour patterns in this preliminary work will be utilised in the design of a more comprehensive study to examine behaviour of a larger number of *A. flavipes* at crucial stages of their life history.

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