

Bill Buttemer

# ANZSCPB 2004



## PROCEEDINGS

*Australian & New Zealand Society for Comparative Physiology & Biochemistry*  
*21st Annual Meeting      Dec 9-12 University of Wollongong*



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**Institute for Conservation Biology, University of Wollongong**

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**Professor Philip Withers, University of Western Australia**

**ANZSCPb Proceedings**

**21st Annual Meeting, December 1-12, 2004**

**University of Wollongong, Wollongong NSW 2522**

**organised by W.A. Buttemer, P.L. Else, A.J. Hulbert, S.C. Faulks, N. Turner**

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# Sigma Delta Technologies

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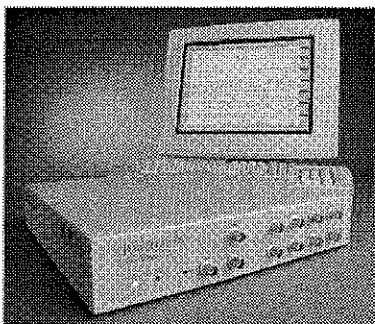
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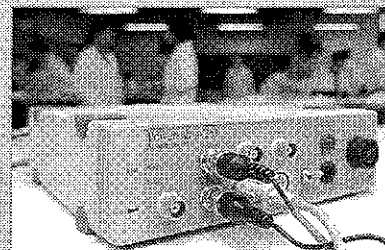
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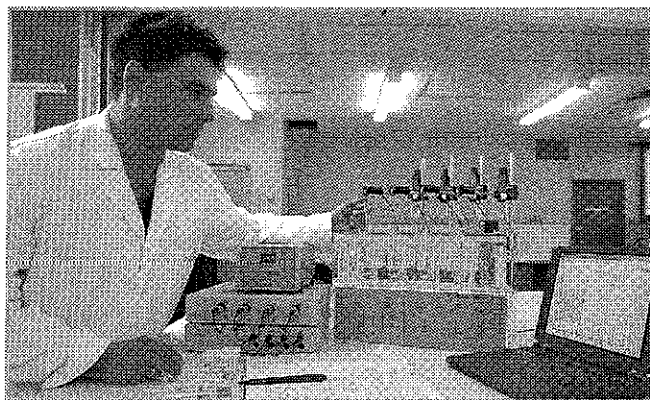
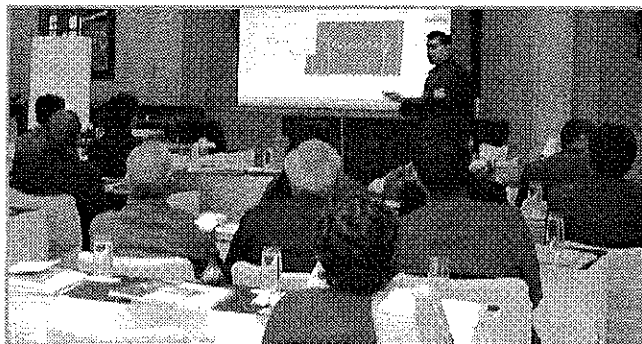
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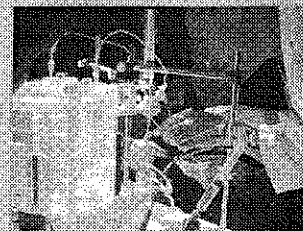
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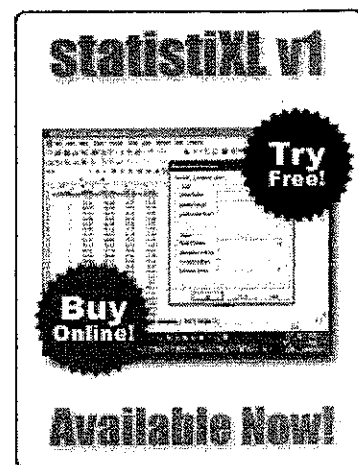
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**statistiXL** is a powerful new statistical package that runs as an add-in to Windows® versions of Microsoft's sophisticated Excel® spreadsheet program. statistiXL has been entirely designed and written by biological scientists to meet the demanding needs of anyone requiring access to a robust, versatile statistical analysis package that is quick to learn and easy to use. You no longer need to spend hours with manuals just learning how to perform the analyses you need to get to the really important bit – the results.

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For a detailed overview of the tools provided by statistiXL, check out the Features section of our website (<http://www.statistixl.com>). The best places to ask questions about statistiXL and for other support issues are the online Support Forums available on our website, although you can always email us directly at [support@statistixl.com](mailto:support@statistixl.com) if you prefer. The Forums also provide a place for you to request new features or to suggest changes to existing features that you would like to see in future versions of statistiXL. We trust that you will be impressed by the power and flexibility of statistiXL.

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# Program

## Thursday 9th December

5.00-7.00pm

registration

foyer of Building 67 (McKinnon Building)

## Friday 10th December

9.00 am	chair	registration and coffee/tea	Talks (in lecture theatre 67.104)
10.30	Paul Else	<b>Welcome and Introduction</b>	
10.40		<u>Alexander Kabat</u> and R. Swain	The metabolic costs of pregnancy in <i>Niveoscincus microlepidotus</i>
11.00		John E. Nelson and <u>Robert T. Gemmell</u>	The temperature gradient from the urogenital sinus to the pouch in the pregnant marsupial quoll, <i>Dayurus hallucatus</i>
11.20		<u>Roger Lentle</u>	Reactor theory meets rheometry: a test of chemical reactor based models of digestion using gastric and small intestinal digesta from two marsupial
11.40		<u>Stuart M Linton</u> , Peter Greenaway and David Towle	Endogenous production of cellulases by the Gecarcinid land crabs <i>Gecarcoldea natalis</i> and <i>Discoplax hirtipes</i>
12.00		LUNCH	
1.20	Sam Richardson	<u>Terry O'Dwyer</u> , W.A. Buttemer and D.M. Priddel	Relationships between parental body condition and prolactin during breeding in Gould's petrels, <i>Pterodroma leucoptera</i>
1.40		<u>Harry Battam</u> , C. Robertson and W.A. Buttemer	Dimensions and scaling of locomotor muscles of the petrels ( <i>Procellariiformes</i> )
2.00		<u>Koa N. Webster</u> and T.J. Dawson	Organ sizes and metabolic performance of marsupials
2.20		<u>Yvonne Ingen-Housz</u>	The effect of incubation temperature on hatching attributes of the freshwater turtle <i>Elseya</i> sp. from the Burnett River
2.40		<u>Elektra Sinclair</u>	Thermal acclimation in the limpet <i>Cellana tremoserica</i>
3.00		COFFEE/TEA	
3.40	Nigel Turner	A. Barber and <u>Philip Withers</u>	Ocular water loss in skinks and geckos
4.00		<u>Christine Cooper</u> , F. Geiser and B. McAllan	The effect of daily torpor on the water economy of an arid-zone marsupial, the stripe-faced dunnart
4.20		<u>Nereda Christian</u> and Fritz Geiser	Is frequency of torpor in sugar gliders ( <i>Petaurus breviceps</i> ) due to winter food shortages or restrictions to foraging?
4.40		<u>Terence J. Dawson</u> and Cyntina E. Blaney	The effects of dehydration on the thermal biology of kangaroos - differences between lab and field responses.
5.00		POSTERS	
		<u>Rachel C. Aland</u> and Sophie Baron	Histology and ultrastructure of the prostate gland of <i>Antechinus subtypicus</i>
		<u>Niels A. Andersen</u> , Christina Vedel-Smith and Stewart Nicol	Circadian and circannual patterns of body temperature and activity in the Tasmanian echidna ( <i>Tachyglossus aculeatus</i> )
		<u>Jessica Gregg</u>	Are small and large dogs different in body and membrane composition?
		Susan M. Jones, <u>Ashley Edwards</u> and Joan Whittier	Effects of environmental stress on alpine skinks in Tasmania - an Earthwatch-funded project
		<u>Susan M. Jones</u> and Ashley Edwards	Which endocrine factors influence reproductive decisions in the multiennially breeding viviparous lizard, <i>Tiliqua nigrolutea</i>
		<u>Bronwyn McAllan</u> , Steven Hobbs & David O. Norris	Presence of neurofibrillary alterations and beta-amyloid immunoreactivity in the brain of a marsupial, <i>Antechinus stuartii</i>
		<u>Michael Usher</u>	Diet, lipids and lifespan of the blowfly ( <i>Calliphora stygia</i> )
		Lisa M. Walter, Peter A.W. Rogers, and <u>Jane E. Girling</u>	Progesterone is angiogenic and oestrogen is anti-angiogenic in mouse endometrium
		Jill C. Weakley and <u>Susan L. Edwards</u>	Molecular and Immunohistochemical identification of a putative sodium proton exchanger isoform in the gills of euryhaline barramundi ( <i>Lates calcarifer</i> )
		<u>Kerry W Withers</u> and J. Billingsley	Application of machine vision to determine the density of dingo teeth
6.15		SIZZLING SOCIAL held in sports lounge in Sports & Recreation Centre (bldg 13)	

chair		Program	Saturday 11th December
9.00	Terry Dawson	<u>Carly Woodd</u> and WJ Sturrock	Reproductive indicators and fecal steroid analysis in the captive squirrel glider ( <i>Petaurus norfolcensis</i> )
9.20		<u>Christopher Turbill</u> , Gerhard Kortner, Fritz Geiser	Daily temperature cycles affect energy expenditure and arousal from torpor in a small, tree-roosting bat ( <i>Nyctophilus geoffroyi</i> )
9.40		<u>Beth L. Symonds</u> , Nicholas J. Hudson and Craig E. Franklin	Effect of aestivation on muscle fibre morphology in the Green-striped burrowing frog ( <i>Cyclorana alboguttata</i> )
10.00		<u>Rebecca L. Cramp</u> and Craig E. Franklin	The effect of aestivation on digestive efficiency of emergent Green-striped burrowing frogs
10.20		COFFEE/TEA	
11.00	Bronwyn McAllan	<u>Natalie Mathie</u> , Craig Franklin, Colin Limpus	The influence of body size on the diving behaviour and physiology of the bimodally respiring turtle <i>Elseya</i> sp. Nov.
11.20		<u>Lesley A. Alton</u>	Effects of aerial oxygen content and barometric pressure on biomodal gas exchange and air breathing behaviour in <i>Trichogaster leeri</i>
11.40		<u>Philip Matthews</u>	Compressible gas gills: Comparing theory with empirical data
12.00		<u>Roger S. Seymour</u> and Craig R. White	Models for embryonic respiration
12.20		LUNCH	
2.00	Sue Jones	<u>Samantha Richardson</u> , J.A. Monk, C.A. Sheperdley, L.O.E. Ebbesson, F. Sin, D.M. Power, P.B. Frappell, J. Kohrle & M. B.	Developmentally regulated thyroid hormone distributor proteins in marsupials, a reptile and fishes
2.20		<u>Sarah Hennebury</u> , H.M. Wright and S.J. Richardson	The transthyretin-like proteins of non-vertebrates
2.40		<u>Geoff Carey</u> and Craig E. Franklin	The effect of early temperature on muscle growth and development in barramundi ( <i>Lates calcarifer</i> )
3.00		<u>David T. Booth</u> , Kirsty Kiddell	The effect of temperature on the energetics of development in House crickets
3.20		COFFEE/TEA	
4.00	Bill Buttemer	<u>Nigel Turner</u> , T. Starke-Peterkovic, P.L. Else and R.J.	Electric field strength of membrane lipids from vertebrate species: Relationship with membrane lipid composition and Na <sup>+</sup> ,K <sup>+</sup> -ATPase molecular activity
4.20		<u>Paul L. Else</u> , T. W. Mitchell, N. Turner, S. Faulks and A. J. Hulbert	Membrane lipids – "regulators" or "conformers" to dietary lipid profile?
4.40		general meeting	
6.20		<u>Peter Frappell</u>	Russ Baudinette in memoriam buses leave University & International House for conference dinner at Austinmer Surf Club
			Sunday 12th December
9.00	Fritz Geiser	<u>Timothy Clark</u> , Peter B. Frappell and Patrick J. Butler	The effect of temperature during periods of increased metabolism in a varanid lizard
9.20		<u>Catriona Condon</u> and Robbie Wilson	The influence of thermal acclimation on the reproductive behaviour and swimming performance of female eastern mosquitofish ( <i>Gambusia</i> )
9.40		<u>Amanda C Niehaus</u> , Robbie S Wilson, Craig E Franklin	Thermal instability and the development and metamorphic condition of striped marsh frogs ( <i>Limnodynastes peronii</i> )
10.00		<u>Kris Rogers</u> , Mike Thompson, Frank Seebacher	Biochemical acclimation of metabolism in <i>Limnodynastes peronii</i>
10.20		COFFEE/TEA	
11.00	Tony Hulbert	<u>Frank Seebacher</u> , Cara Lowe, Bill Davison and Craig Franklin	A falsification of the thermal specialization paradigm: compensation for elevated temperatures in Antarctic fish, I. Swimming performance
11.20		<u>Craig Franklin</u> , Bill Davison, Frank Seebacher	A falsification of the thermal specialisation paradigm: compensation to elevated temperatures in Antarctic fish II. Cardiac function
11.40		<u>Robbie R. Wilson</u> and Ian A. Johnston	Testing the benefits of thermal acclimation to the sneaky-mating performance of male eastern mosquitofish
12.00		<u>Craig K. R. Willis</u> and R. Mark Brigham	Thermal physiology as a means to assess habit preferences in free-ranging, North American microbats
12.20		Awarding of Prizes and Closing of Conference	
		LUNCH	

## The metabolic costs of pregnancy in *Niveoscincus microlepidotus*

Alexander Kabat & R. Swain

School of Zoology, University of Tasmania, Hobart, Tas. 7001 Australia

*Niveoscincus microlepidotus* is a viviparous alpine lizard that has an unusually long pregnancy, which can last up to 12 months. This study examined the maternal metabolic costs associated with pregnancy in this species. Pregnant and non-pregnant female *N. microlepidotus* were tested for standard metabolic rate (SMR) at four different reproductive stages: non-pregnant, early-, middle- and late-pregnancy. The mean SMR of non-pregnant and early-pregnant females was shown to be not significantly different. However, there was an approximately 18% elevation in mean SMR during mid-pregnancy, and mean SMR during late-pregnancy was approximately 24% higher than that found in non-pregnant and early-pregnant females. This study also investigated relative changes in the active metabolic rate, and piloted a novel technique of obtaining active metabolic rates in a small animal by using the <sup>86</sup>Rubidium isotope. The distribution of the isotope revealed that 97% remained in the mother, and only 3% of the isotope was transferred to the embryos, thereby providing a strong picture of the maternal metabolic clearance. A significantly faster biological elimination rate per gram body weight ( $k_b$ ) of the isotope in pregnant females compared to non-pregnant animals was shown, thus confirming the results seen in the SMR studies. This investigation suggests that there is a significant maternal metabolic cost of pregnancy in *Niveoscincus microlepidotus* that may be exacerbated by the unusually long gestation length.

**The temperature gradient from the urogenital sinus to the pouch  
in the pregnant marsupial quoll, *Dayurus hallucatus***

John E. Nelson<sup>1</sup> & Robert T. Gemmell<sup>2\*</sup>

<sup>1</sup>Department of Biological Sciences, Monash University, Victoria 3168, Australia

<sup>2</sup>Department of Anatomy and Developmental Biology, School of Biomedical Science,  
University of Queensland, Brisbane 4072, Australia

Marsupial newborn are expelled from the uterus and they travel unaided from the sinus to the pouch and subsequently attach to the teat. The newborn of the dasyurid quoll are expelled from the uterus in a gelatinous column. The young have to swim in the gelatinous column, grasp a hair and then travel about 1 to 2 centimetres to the pouch and attach to the teat. Temperatures were monitored using an ST Pro Series noncontact thermometer (Raytek, Santa Cruz, California, USA). The infrared thermometer measured surface temperature of an opaque object. The unit's optics sense emitted, reflected, and transmitted energy which is collected and focused onto a detector. The laser is used for aiming purposes only. A temperature gradient was observed from the sinus to the pouch during pregnancy and may aid the newborn quolls to reach the pouch and attach to a teat. Further examination of birth in the quoll revealed the role the hairs play in the transfer of the young to the pouch and subsequently to the teats. The hairs formed a tunnel from the sinus to the pouch and the hairs were positioned in the pouch so that all of the young entered and proceeded to the cranial end of the pouch. However the female quoll remained motionless for about 10 minutes to allow the young to attach to the teat. Eight of the newborn attach to a teat and the excess were lost.

**Reactor theory meets rheometry: a test of the validity of chemical reactor based models of digestion using gastric and small intestinal digesta from two marsupial herbivores**

Roger Lentle

Institute of Food Nutrition and Human Health, Massey University, Private Bag 11222, Palmerston North New Zealand.

A number of optimization models of the digestive process have been based on chemical reactor theory. The test of the validity of such models is that the physical properties of digesta permit the mixing characteristics necessary for predicted reactor configuration. Thus there must be turbulent mixing in successive radial sections or areas of segmental contraction in a biological tubular reactor for it to conform with the flow requirements of a chemical plug flow reactor.

Rotational viscometry shows that the foregut digesta of tammar and parma wallabies exhibits high and non-Newtonian viscosity so as to preclude turbulent mixing and favour digesta moving as an unstirred plug with limited mixing adjacent to the mucosa. Similarly, the proximal and distal small intestinal digesta of brush-tailed possums have high apparent viscosity with non-Newtonian characteristics despite lower dry matter content.

However, further recent findings suggest that extrusion of the fluid phase from the solid phase of the digesta plug by events such as peristalsis or segmentation may lead to a degree of 'tidal' radial mixing dependent on the rheometric properties of the plug. Thus rheometric analysis shows that possum small intestinal digesta behave as weak gels having a relatively high elastic modulus and low loss modulus so as to enable them to sustain compression without flow. The apparent viscosity of distal small intestinal digesta obtained from a novel capillary viscometer lined with small intestinal mucosa is much lower than that determined by rotational viscometry suggesting that peripheral extrusion of the fluid phase occurs during flow through a tube under hydrostatic head which augments flow. Distal small intestinal digesta undergo compression and extrusion of liquid phase under hydrostatic loads that are in the normal physiological range.

Taken together these findings suggest that radial mixing may occur by extrusion but that the degree of radial mixing and thus efficiency of digestion will depend on the rheometric characteristics of the digesta and in particular on the elasticity of the solid phase. It remains to be determined whether such properties correlate with bite characteristics and food choice.



## Endogenous production of cellulases by the Gecarcinid land crabs *Gecarcoidea natalis* and *Discoplax hirtipes*

Stuart M. Linton<sup>1\*</sup>, Peter Greenaway<sup>2</sup> and David Towle<sup>3</sup>

<sup>1</sup> School of Biological and Chemical Sciences, Geelong Campus, Deakin University.

<sup>2</sup> School of Biological, Earth and Environmental Sciences, University of NSW

<sup>3</sup> Mount Desert Island, Biological Laboratory, Mount Desert Island, Maine, USA

The gecarcinid land crabs, *Gecarcoidea natalis* and *Discoplax hirtipes* are able to assimilate substantial amounts of cellulose from a leaf litter diet. This ability can be ascribed to presence in the digestive juice of total cellulase activity and the activities of the cellulase enzymes, endo- $\beta$ -1,4-glucanase and  $\beta$ -glucosidase.

These cellulase enzymes appear to be produced endogenously. Large activities of total cellulase, endo- $\beta$ -1,4-glucanase and  $\beta$ -glucosidase present within the midgut gland (The tissue responsible for the synthesis of digestive enzymes in crustacea) of both *G. natalis* and *D. hirtipes* provide indirect evidence for this. Direct evidence for the endogenous production of endo- $\beta$ -1,4-glucanase was provided by amplification of a 900 bp PCR product from the endo- $\beta$ -1,4-glucanase gene from both *G. natalis* and *D. hirtipes*. To avoid possible contamination from gut bacteria this PCR product was amplified from DNA isolated from the skeletal muscle. Given the situation for endo- $\beta$ -1,4-glucanase and the indirect evidence for the other cellulase enzymes it is likely that all of the other cellulase enzymes are produced endogenously.

The ability to produce cellulases endogenously may be present in decapods generally since the 900 bp PCR product of endo- $\beta$ -1,4-glucanase was amplified from cDNA prepared from RNA isolated from the midgut gland of the crayfish *Cherax destructor* and *Euastacus* sp. and the Australian arid zone crab *Austrothelphusa transversa*. Given this situation it is possible that the aquatic ancestors of the land crabs produced cellulases endogenously and were thus able to digest  $\beta$ -1,4-glycosidic polymers carbohydrate polymers in marine plants and algae. On adoption of terrestrial habits the presence of the cellulase enzyme may have allowed the land crabs to exploit leaf litter and other plant material readily available in the new habitat.

**Relationships between parental body condition and prolactin during breeding in Gould's petrels *Pterodroma leucoptera***

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In birds, changes in plasma prolactin (PRL) concentration coincide with changes in breeding behaviour. We measured plasma PRL concentrations throughout the breeding cycle in Gould's petrels *Pterodroma leucoptera*, a long-lived seabird. PRL concentrations increased significantly during the 4-6 week period between pre-breeding and egg laying and then remained elevated throughout incubation and into the chick-provisioning stage. Interestingly, PRL concentrations were significantly lower during incubation in birds that failed to hatch their egg in comparison to successful breeders. Because of known inhibition of PRL by glucocorticoids, and the inverse relationship between glucocorticoid secretion and body condition, we evaluated the relationship between the body condition of Gould's petrels and their plasma PRL levels. Male birds that were in poorer condition at the beginning of incubation had significantly lower PRL concentrations at the beginning of their second incubation shift. Given the known promotion of breeding behaviour by PRL, the condition-dependence of PRL suggests it may play a role in nest abandonment when body reserves are low, thereby ensuring that the bird's long-term reproductive viability is not compromised by a single breeding attempt.

## Dimensions and scaling of locomotor muscles of the petrels (*Procellariiformes*)

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The *Procellariiformes* are a globally distributed group of pelagic seabirds with a low-energy lifestyle. We undertook this biometric study, using carcasses from commercial fisheries bykill, to identify anatomical correlates of the energetically frugal *Procellariiformes*.

In petrels, the mass of the major flight muscle, *m. pectoralis*, ranged between 7.5 and 13.3% of body mass ( $M_b$ ), significantly less than the 12 to 19.4% found in other orders of soaring birds. Furthermore, these muscles scaled as  $M_b^{0.88}$ , significantly different to the scaling exponent of 1.00 for birds generally.

*M. pectoralis* is a layered muscle, with the outer (primary) layer tissue being significantly darker in colour (dark red/brown) than that of the pink, deeper (secondary) layer, suggesting differences in aerobic capabilities between these two components. The primary layer scales as  $M_b^{0.78}$ , and the secondary layer as  $M_b^{1.24}$ , thus larger petrel species appear to have reduced flapping endurance.

Take off for petrels becomes more difficult with increasing body size due to combinations of smaller pectoral muscles, higher wing aspect ratios and wing loadings. The disproportionate increase in leg (as  $M_b^{1.07}$ ) and in pectoral secondary layer muscles may be an adaptive response to the increased short-term power requirements associated with takeoff in larger animals.

Petrel locomotor muscles (leg + flight) scale as  $M_b^{1.00}$  and represent some 30% of body mass. Significantly, for over 97% of other avian species, locomotor muscles exceed 50% of body mass. Most of this difference results from the relatively small flight muscle mass of petrels, which correlates with the relatively low basal metabolic rates measured in these birds.

## Organ sizes and metabolic performance of marsupials

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Metabolism at rest is influenced not only by body size (allometric variation) but also by phylogeny, with marsupials having lower resting metabolic rates than eutherian mammals. However, marsupials are capable of reaching maximal metabolic rates that equal or exceed those of eutherians. This study investigated links between metabolic performance and sizes of internal organs. We examined the organs of a macropodoid marsupial, *Bettongia penicillata*, and collated data from the literature for other species, both marsupial and eutherian.

Allometric equations generated for the two groups showed that they differ in the relative size of some internal organs. The kidneys, which contribute substantially to resting metabolism, were smaller in marsupials than eutherians. By contrast, marsupials have significantly *larger* hearts than eutherians. Heart size regulates the transport of oxygen to exercising muscle tissue and thus can limit maximum metabolic performance.

Other organs involved in oxygen transport, the lungs and spleen, appear to be similar in size in both mammalian groups. However, the functional role of these organs is not wholly dependent on their size and in the case of the lung, the capacity for diffusion of oxygen through the lung may be greater in marsupials than in many eutherians. In combination with their large hearts, this may give marsupials the potential for greater metabolic performance.

## **The effect of incubation temperature on hatchling attributes of the freshwater turtle *Elseya* sp. from the Burnett River.**

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In reptiles, the incubation temperature experienced by developing embryos affects hatchling quality. Eggs from 8 clutches of Burnett River *Elseya* sp. turtles were incubated at 3 constant temperatures (26°C, 28°C and 30°C) to determine the influence that incubation temperature has on hatchling morphology, swimming performance and post-hatching growth rate. Results show that initial egg mass was correlated with hatchling mass. Incubation at 30°C resulted in poorer hatching success and higher post-hatch mortality and is probably the upper thermal limit to development in this species. Therefore, analysis of hatchling growth was restricted to 26°C and 28°C. Incubation temperature had no effect on hatchling mass or most morphological measures, swimming performance at 75d and post-hatching growth. However, hatchlings incubated at 26°C had longer plastrons than hatchlings from 30°C and swam faster, 3d after hatching, than hatchlings incubated at either 28°C or 30°C. There was a suggestion that clutch of origin influenced most morphological measures for 184d post-hatching and affected the swimming performance of 75d old hatchlings. Unshaded natural nests experienced greater daily fluctuations in temperature and higher temperatures overall compared with shaded natural nests, such that unshaded nest temperatures approached the upper thermal limit to development. Therefore, nesting in shaded areas may be necessary to maximize hatching success in the natural habitat. The optimal temperature range, to produce the highest quality Burnett River *Elseya* sp. hatchlings under constant temperature artificial incubation, was determined to be between 26°C and 28°C.



## Thermal acclimation in the limpet *Cellana tramoserica*

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Temperature determines all physiological responses by limiting cellular reaction rates. Daily variation differs between microhabitats which means that species subpopulations may respond differently to temperature. The aim of this study is to determine how physiological responses to temperature of the limpet *Cellana tramoserica* differ between limpets from variable and stable thermal environments. Physiological responses were measured over a range of temperatures in limpets from thermally different field sites (open and caves) in summer and winter, and in laboratory acclimation treatments. Physiological responses measured were metabolic rate, anaerobic potential and aerobic potential. Limpets from the open acclimatised anaerobic and aerobic potential in response to seasonal changes in mean temperature. These acclimatory responses did not correspond to any difference in metabolic rate. Limpets from caves, but not from the open, upregulated metabolic rate in winter. The physiological response of *C. tramoserica* to temperature depends on season and microhabitat. The magnitude of phenotypic plasticity in *C. tramoserica* encompasses physiological differences between related species. Thus, differences in physiological response to temperature in ectotherms may simply reflect individual animals' response to their immediate environment.

## Ocular water loss in geckos and skinks

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Cutaneous evaporative water loss (CEWL) is a significant component of total water loss for lizards, and it is a combination of skin evaporative water loss and ocular evaporative water loss (OWL). We provide morphological evidence that OWL would be a significant fraction of CEWL if the ocular surface evaporated as a free water surface, especially for small lizards in general and geckos in particular because they have relatively large eyes for their size. We provide physiological evidence for skinks (*Tiliqua multifasciata*, *Ctenotus* spp) and geckos (*Christinus marmoratus*) that the rate of evaporation from the corneal surface is high – the cornea evaporates nearly as a free water surface (resistance  $\sim 2\text{--}4 \text{ sec cm}^{-1}$ ) whereas the skin has a considerable resistance to evaporation ( $\sim 100\text{--}400 \text{ sec cm}^{-1}$ ). We show that OWL is considerably reduced by closing the eyelids, although this obviously compromises vision. Some squamates have a clear window in the lower eyelid, or their upper and lower eyelids are permanently fused to form a clear spectacle (brille). OWL is adaptively reduced in terrestrial squamates with spectacle, while maintaining vision, since it has an OWL (resistance  $\sim 20\text{--}40 \text{ sec cm}^{-1}$ ) intermediate between that of the corneal surface and skin. The spectacle also has an important protective role in some (*e.g.* fossorial) species.

**Effect of torpor on the water economy of an arid-zone marsupial, the stripe-faced dunnart (*Sminthopsis macroura*)**

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Reducing energy expenditure is considered to be the major role of torpor in small mammals and birds. However torpor may also have an important role in water conservation, especially for arid-zone species. Metabolic water production (MWP) and evaporative water loss (EWL) are generally the two most important avenues of water gain and loss in arid environments, where the availability of food and free water are limited, and the proportion of EWL/MWP is an indicator of the relative water economy of a species. As the effect of torpor on water economy has not been determined for marsupials, we investigated the effect of torpor on the EWL and MWP of an arid-zone marsupial, the stripe-faced dunnart (*Sminthopsis macroura*). For normothermic dunnarts, resting metabolic rate, and hence MWP, increased with decreasing ambient temperature ( $T_a$ ). Daily torpor reduced the metabolic rate of dunnarts at low  $T_a$ , and the MWP of torpid dunnarts was only 24 – 37 % of that for normothermic individuals. The EWL of normothermic dunnarts also increased at low  $T_a$ , and was significantly reduced during torpor, being 24 – 42 % of normothermic values. Torpor resulted in absolute water savings of 54-62 mg  $H_2O \cdot h^{-1}$ . The ratio of EWL/MWP was greater than 1 for normothermic dunnarts, indicating that they had an unfavourable water economy. Torpor did not have a significant effect on the ratio EWL/MWP, so although it resulted in substantial absolute water savings for the dunnarts, there was no improvement in relative water economy.

**Is frequency of torpor in sugar gliders (*Petaurus breviceps*) due to winter food shortages or restrictions to foraging?**

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Field studies on daily torpor in relation to environmental conditions are scarce and information on how differences in food availability affect daily torpor patterns in the field is non-existent. We conducted a 2-year field study to determine differences in torpor patterns among free-ranging sugar gliders using temperature telemetry at food-supplemented (FS) and non-supplemented (NS) sites. Moreover, telemetry in the field and respirometry in the laboratory were used to compare torpor frequency and energy expenditure: (i) under varying conditions of rainfall and ambient temperature; and (ii) between FS vs NS areas. Torpor occurred infrequently and in conjunction with low ambient temperature and/or rainfall (4% of all observations, 34 torpor bouts, N=10, 823 animal nights). Additionally, gliders were less active when it was cold and wet. This was not surprising as wet gliders at  $T_a$  15°C use almost twice as much energy as dry gliders at the same  $T_a$ . Declines in body mass over winter at the NS site were more pronounced than at the FS site, and significantly lower resting body temperatures in winter indicate that gliders at the NS site were under greater energetic constraints than gliders at the FS site. Nevertheless, against expectations, food supplementation had no effect on the frequency of torpor. Our study suggests that torpor in sugar gliders is used as an emergency measure to overcome cold and wet environmental conditions that make foraging unfavourable, rather than winter food shortages in general.

## **The effects of dehydration on the thermal biology of kangaroos – differences between laboratory and field responses.**

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Mammals adjust thermoregulation in the face of water deficits (dehydration) to restrict further loss. This presents special problems at high  $T_{\text{air}}$ , particularly above  $T_{\text{body}}$ , because evaporation is the only mechanism by which  $T_{\text{body}}$  can be maintained. We examined the thermoregulatory responses of arid-zone kangaroos (Red, *Macropus rufus*) and mesic-zone kangaroos (Eastern grey, *M. giganteus*) to dehydration in the laboratory and compared this with field data. In response to laboratory dehydration (15% loss of body mass) both species at  $T_{\text{air}}$  of 45°C allowed  $T_{\text{body}}$  to rise significantly from normal levels, 36-37°C, to near 40°C, with the rise in *M. giganteus* being greater. In these conditions evaporative loss of the arid-zone *M. rufus* was concurrently lower. A range of other responses to dehydration occurred and were similar in the two species, notably changes in total ventilation and oxygen extraction. Field situations are more complex because of behavioural responses. In the arid-zone during summer, with water available,  $T_{\text{body}}$  cycled during the day, more so in *M. giganteus*. With dehydration (5 days water restriction)  $T_{\text{body}}$  cycles increased, with the more mesic *M. giganteus* showing the largest mean daily range (34.2°C–38.9°C) as compared with *M. rufus* (35.5–37.8 °C).



## **The histology and ultrastructure of the prostate gland in *Antechinus subtropicus***

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The histology and ultrastructure of the prostate gland was investigated as part of a study into the life history of the marsupial mouse, *Antechinus subtropicus*. *A. subtropicus* is a small dasyurid marsupial with an extreme life history. Leading up to the short, synchronised breeding period, the males display elevated blood levels of cortisol and testosterone. Following breeding is a complete male die-off at 11.5 months. At death, males suffer from various stress-related pathologies, such as gastric haemorrhage and renal dysfunction. Females live and may breed in subsequent years.

In several related *Antechinus* species, the prostate has been reported to undergo hypertrophy and pathologies associated with breeding and cytomegalovirus infections.

Males were sampled prior to breeding and during the breeding season. Additionally, males that had survived the breeding period in isolation, and castrated males were sampled.

Light microscopy was performed on plastic embedded sections stained with a variety of histological stains. The ultrastructure was examined using transmission electron microscopy.

The prostate gland was composed on two types of glands, an outer basal cap and an inner core. There were two distinct types of glandular cell present. Secretion increased from prior to breeding to breeding, and was accumulated with distension of the prostatic acini in older and castrate males. The acinar cells adjacent to the retained secretion appeared dysplastic. Some apparently solidified secretion was present in some males, resembling corpora amylacea. Irregular growth of some prostatic glands was associated with breeding, although neoplasia was not found. There was no evidence of cytomegalovirus infection in the prostates of *A. subtropicus*.

**Circadian and circannual patterns of body temperature and activity in the Tasmanian echidna (*Tachyglossus aculeatus*)**

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**The echidna is one of the most widely distributed Australian mammals, occupying habitats ranging from mild coastal areas to hot deserts, to alpine areas, and the degree and timing of hibernation differs between habitats. So far some degree of hibernation or torpor has been found in all echidna populations studied.**

**We have been studying a population of Tasmanian echidnas where all echidnas hibernate for part of the year, every year.**

Due to the elusive nature of echidnas, levels and patterns of activity have been difficult to measure accurately. In previous studies activity has been estimated using a variety of techniques, including visual observations, capture rates and evidence of digging activity. Gross levels of activity have been estimated using doubly labelled water.

We have, for the first time, been able to record activity directly using Actiwatch activity loggers on 5 echidnas (2 male and 3 female). From some echidnas we obtained almost a whole year of activity data, showing clear seasonal differences. An unexpectedly high proportion of activity took place at night throughout the year. This is surprising because none of climate, predation or activity of prey seems to necessitate nocturnality in our study area. Nocturnality or crepuscularity have previously been suggested for echidnas living in areas where high daytime air and soil temperatures would be physiologically stressful. We have simultaneous recordings of body temperature and activity from 2 echidnas (1 female, 1 male) with good correlation between body temperature and activity. Patterns of hibernation, circadian and circannual rhythm in body temperature were recorded using implanted temperature loggers.

## **Are Small and Large Dogs Different in Body and Membrane Composition?**

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Metabolic rates, organ size and membrane fatty acid composition have previously been shown to vary with body size among mammal species. Small mammalian species have more polyunsaturated membranes, faster metabolic rates compared to their larger counterparts. Some of the metabolically significant organs are proportionally larger in the small mammals. It is not known if membrane composition varies with body size within a species. The large variation in body size of dogs makes them a good model species for such research.

The present study measured organ mass and membrane composition of various tissues of a small (<7.5kg) and large (>18kg) group of dogs. The small dogs had proportionally larger organs. Little variation in membrane fatty acid composition was observed between the small and large dogs. The only trend seen in a number of tissues was that of 20:3n6, which was higher in the small dogs, however this was not an abundant fatty acid overall. The liver was more monounsaturated in the small dogs, and the large dogs had more n-6 polyunsaturates in their membranes. Differences in organ size were estimated to be of a similar magnitude to the difference in calculated BMR between the two size groups. Based on the absence of consistent changes in membrane fatty acid composition of tissues, it appears that the difference in proportional organ size largely explains the difference in basal metabolic rate of dogs.

## **Effects of environmental stress on alpine skinks in Tasmania: an Earthwatch-funded project**

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Earthwatch is a global organisation that "promotes sustainable conservation of our natural environments and cultural heritage by creating partnerships among scientists, the general public, educators, and businesses." Earthwatch-funded projects involve volunteers who assist scientists to carry out research projects of high conservation value.

Our project focuses on the spotted skink, *Niveoscincus ocellatus*, an abundant, medium-sized species which inhabits relatively undisturbed habitats in the World Heritage Wilderness in Central Tasmania, but is also distributed along the east coast where populations are highly impacted by human activity. Due to their specific reproductive adaptations, these animals are potentially threatened by climate change: alpine populations of spotted skinks could face reproductive failure if the weather at the end of summer is unseasonably poor. The project aims to evaluate whether potential climate change or local habitat alteration, including possible effects of persistent agricultural chemicals, may affect the lizards' ability to cope with short term stressors, and/or their patterns of reproduction and development.

In our first field season (Summer 2004), we focused on measuring plasma corticosterone concentrations and obtaining preliminary data on diurnal activity patterns in lizards from disturbed and undisturbed sites at the Central Plateau and East Coast. The Earthwatch volunteers assist in numerous ways such as helping with capture and marking of animals, observing behaviours in the field, or assisting with vegetation surveys. All of these tasks can be done with minimal guidance and training in the field, which is a fundamental requirement for a successful Earthwatch project.

**Which endocrine factors influence reproductive decisions  
in the multiennially breeding viviparous lizard, *Tiliqua nigrolutea*?**

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An animal's energy intake must be partitioned between the requirements for growth and maintenance and the requirements of reproduction. For females in particular, a successful reproductive episode may result in an energy debt such that those animals are not able to reproduce again until energy stores are restored. Male blue-tongued lizards (*Tiliqua nigrolutea*) do become reproductively active every year; however females reproduce only at intervals of two, three, or even four years. Vitellogenesis occurs rapidly after spring emergence: thus reproduction depends on resources accumulated before hibernation. This implies that in females there is some physiological mechanism that signals to the reproductive system when sufficient fat reserves are available to fuel reproduction. The adrenal steroid corticosterone is a major metabolic hormone in reptiles, and appears to be important for regulating lipid cycling. In this study we compared the annual cycle of plasma corticosterone in male and female blue-tongued lizards. In males, plasma corticosterone is high during the spring mating period, lowest during summer, and rising to a significant peak during late hibernation. In both reproductive and non-reproductive females, plasma corticosterone is minimal in spring. In pregnant females corticosterone peaks during late gestation, falling sharply around the time of birth: this pattern is not apparent in non-pregnant females. Both groups showed a peak during late hibernation but this was not as marked as that seen in males. We suggest that these patterns primarily reflect the role of corticosterone in regulation of metabolic reserves.



**Presence of neurofibrillary alterations and beta-amyloid immunoreactivity in the brain of a marsupial, *Antechinus stuartii***

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In humans the development of Alzheimer's disease is associated with increasing neuronal pathology that parallels the increase in dementia. The pathology includes the development of neurofibrillary tangles, neuronal death, and presence of beta-amyloid plaques, especially in the cerebellum and limbic system, and these changes advance throughout the brain as the disease progresses. There is some controversy as to whether the neuronal pathology is also an indicator of other forms of dementia, or can be related to changes in stress and reproductive hormones, or of an aging brain.

We investigated the brain cytoarchitecture in *Antechinus stuartii*, a marsupial (males 25-40g, females 18-25 g), which exhibits a highly synchronized seasonal biology within any population, culminating in the death of all males at the end of the breeding season. Male "die-off" is related to increased circulating cortisol and testosterone and the effects of these hormones on organ systems. Silver staining and immunohistochemistry were used to detect potential neurofibrillary alterations, dendritic loss and cell death, presence of tyrosine hydroxylase, and beta-amyloid immunoreactivity in non-breeding, breeding, and post-breeding animals as well as in "die-off" males.

Neurofibrillary alterations, beta amyloid immunoreactivity and changes in the pattern of tyrosine hydroxylase staining, dendritic loss and cell death were found in breeding animals of both sexes, with the most widespread pathological changes occurring in "die-off" males and 2-3 year old post-breeding females. The changes are coincidental with changes in stress and sex steroid status, and with aging, indicating that these may play a role in the development of certain neuropathologies.

## **Diet, lipids and lifespan in the blowfly *Calliphora stygia***

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The underlying mechanisms causing ageing are unknown. It is proposed that the blowfly will make an excellent model for the study of ageing. The aims of the study were; (i) to determine if calorie restriction extends the adult lifespan of blowflies, (ii) to determine if fatty acid manipulation of the larval diet has the ability to alter adult lifespan of blowflies.

Calorie restriction: To achieve calorie restriction adult flies were provided with between 0.7mg and 6.3mg of sucrose/day, through the provision of varying volumes (10ul-90ul) of a 0.2M sucrose solution across two experimental periods. The effect of these differing food provisions on adult lifespan was recorded. Survival peaked with flies given 3.5 mg and 4.2 mg sucrose/day. Flies given less than this showed highly significant ( $p < 0.0001$ ) declines in survival, whilst individuals provided with greater amounts of sucrose showed non-significant decreases in survival, when data from the two experiments were treated separately. When data for both experiments were combined, there was small but significant decline in survival ( $p < 0.01$ ). Whilst this result does not mirror the dramatic changes in lifespan observed in other animals exposed to calorie restriction, it does provide a hint that calorie restriction extends the lifespan of *C. stygia*. Further experiments are planned where the amount of solution provided daily will be kept constant but sucrose concentration varied to manipulate daily energy intake.

Fatty acid composition of larval diet: Larvae of *C. stygia* were provided with diets containing elevated levels of different fatty acids, ranging from saturated, monounsaturated and both n-6 and n-3 polyunsaturated fatty acids. The effect of these different fatty acids on adult lifespan of *C. stygia* was recorded. On all occasions the addition of n-6 polyunsaturated fatty acids to larval diet resulted in significant ( $p < 0.0001$ ) declines in adult survival, when compared to other fatty acid types. Subsequent fatty acid analysis of larvae in the 3<sup>rd</sup> instar have revealed that these dietary manipulations altered membrane fatty acid composition *in vivo*.

## **Progesterone is angiogenic and oestrogen is anti-angiogenic in mouse endometrium**

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The role of progesterone (and oestrogen) in endometrial angiogenesis remains controversial. The aims of this study were to quantify endometrial angiogenesis in pregnant mice and to investigate the role of progesterone in promoting endothelial cell (EC) proliferation in ovariectomized mice; we hypothesized that EC proliferation would increase with increasing plasma progesterone concentrations in early pregnant mice, that progesterone would stimulate EC proliferation in ovariectomized mice, but only following estrogen-priming, and that progesterone-induced EC proliferation would require vascular endothelial growth factor (VEGF). Uteri were collected on days 1-4 of pregnancy when circulating progesterone concentrations were increasing but prior to implantation. Before dissection, mice were injected with BrdU enabling proliferating EC (PEC) to be quantified with CD31/BrdU double-immunohistochemistry. There was a significant increase in PEC (kruskal-wallis statistic (KW)=17.1,  $p=0.002$ ) on day 3 of pregnancy (Day 1-2: no proliferation, Day 3:  $126.6 \pm 45.6$  PEC/mm<sup>2</sup> (mean $\pm$ SE)), when plasma progesterone also increased. To determine if this EC proliferation was due to progesterone, an experiment was performed on ovariectomized mice. One group was treated with a single oestradiol injection (100 ng) on day eight after ovariectomy, followed by a no-treatment day and 3 consecutive daily injections of progesterone (1 mg). Other groups were treated with either the vehicle, oestradiol or progesterone injections only; all were dissected on day 13 following ovariectomy. Unexpectedly, mice treated with progesterone-only had the highest amount of EC proliferation ( $114.7 \pm 30.9$  PEC/mm<sup>2</sup>); estrogen priming significantly reduced EC proliferation ( $44.8 \pm 15.5$  PEC/mm<sup>2</sup>; KW=13.8,  $p=0.008$ ). In a further experiment in which VEGF anti-serum was administered concurrently with the progesterone injections, EC proliferation was significantly reduced (progesterone only:  $272.1 \pm 49.3$  PEC/mm<sup>2</sup>; progesterone plus VEGF anti-serum:  $144.8 \pm 81.3$  PEC/mm<sup>2</sup>,  $p=0.03$ ). We concluded that oestrogen priming is not required for progesterone to stimulate VEGF-dependent endometrial EC proliferation and that it inhibits angiogenesis in ovariectomized mice.

**Molecular and immunohistochemical identification of a putative sodium proton exchanger isoform in the gills of euryhaline barramundi (*Lates calcarifer*)**

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In fish, most acid-base imbalances in the short term are moderated by both blood and tissue buffering to lessen the impact of acid/base fluctuations. Limitations to buffering require that the final compensation for alterations in pH is by the transepithelial excretion of the excess acid or base from the fish to the ambient water. The model for freshwater fishes that utilizes ATP dependant proton pumps coupled with a sodium channels in the apical membrane of chloride cells and or respiratory pavement cells. In contrast, the model for marine fishes that is centered upon sodium-hydrogen exchange across the apical membrane of chloride cells. Both models are postulated to work in conjunction with chloride-bicarbonate exchangers also located in the apical membrane of branchial epithelial cells. Euryhaline fishes and moderately euryhaline species have the physiological ability to move between waters of various salinities and are often exposed to rapid changes in ionic gradients that impact on acid-base exchanges. It has recently been proposed that euryhaline fishes may utilise both the freshwater and marine model mechanisms in regulating systemic pH, and that the up regulation of the membrane transporters is governed by environmental salinity. This study has used molecular and immunohistochemical techniques to identify both mRNA and protein expression of an NHE isoform and  $H^+$ -ATPase in the gills of the euryhaline barramundi.

## **Application of machine vision to determine the density of Dingo teeth**

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To devise effective management strategies for animals in the wild and to examine their ecology, it is often useful to determine their age. For determining of the age of dingoes, methods are usually associated with measurements of the diameter of tooth pulp cavity. However, this approach is complicated in animals older than two years by closure of this cavity. Tooth density has recently been examined as a means of aging dingoes (Ellerton et al: in prep). To avoid the need for immersion of the porous tooth to use the Archimedes method, we present a method to determine tooth density through the application of machine vision technology.

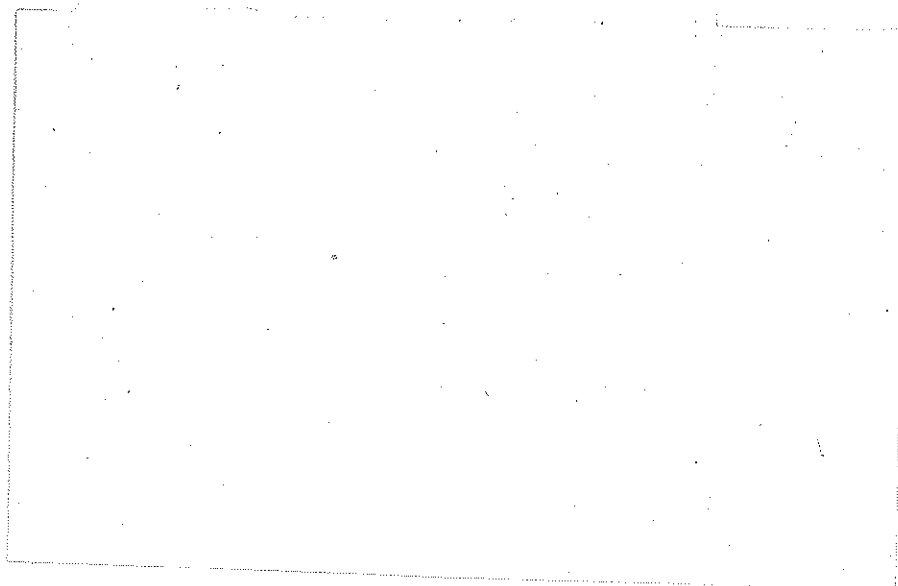
A canine tooth removed from a dingo skull of known age was mounted on a rotating vertical shaft, driven by a stepper motor controlled by a computer. Using a web camera, the computer can capture images of the tooth against a black background as the tooth rotates about the vertical axis. Active-X software was developed for analyzing images using some principles common to those used in tomography. Analysis using the green component of the image data resulted in a clear black and white silhouette of the tooth. Fifty images were captured per rotation of the tooth. These were used to compute volumes of 320 notional slices to calculate tooth volume in cubic pixels. Calibration of the system with a marble of known diameter enabled tooth volume to be expressed in mm<sup>3</sup>. Tooth density was calculated by dividing tooth mass by tooth volume.

## **Reproductive indicators and fecal steroid analysis in the captive Victorian Squirrel glider (*Petaurus norfolcensis*)**

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The Squirrel Glider (*Petaurus norfolcensis*) is a medium sized marsupial glider and is currently considered one of Victoria's ten most endangered mammalian species, listed as Threatened in Victoria and Vulnerable in New South Wales as a result of habitat destruction and fragmentation. This study was aimed towards gaining an insight into the basic reproductive biology of captive Victorian Squirrel Glider, by using non-invasive techniques. Aspects of pouch development, urinary cytology and the fecal steroids, progesterone and oestradiol-17 $\beta$ , were investigated throughout the oestrous cycle. Oestrus was detected in all reproductive females through maximal cornified epithelial cells, in urine samples, followed by the infiltration of immune cells and sperm in urine samples confirmed mating. Assay systems were validated through parallelism and charcoal stripped techniques. Thin layer chromatography identified fecal progesterone, 11- $\beta$  hydroxy progesterone, oestradiol-17 $\beta$  and oestrone. The fecal lag time was determined by establishing a 42 hr gut passage rate. Steroid hormones in fecal samples were used to characterize the reproductive cycle. A significant pre-oestrus oestradiol rise was only detected in one individual and significant oestradiol-17 $\beta$  increases post-partum ( $\pm 2$  days) ( $n = 2$ ) suggest that the Squirrel Glider may under go a post-partum oestrus. Fecal progesterone profiles followed expected patterns with significantly elevated concentrations following oestrus ( $n = 3$ ) which were maintained throughout pregnancy. Parturition coincided with decreasing progesterone concentrations ( $\pm 1$  day). This study defined the length of gestation and the luteal phase in the Squirrel Glider as 16-17 days.



## Daily temperature cycles affect energy expenditure and arousal from torpor in a small, tree-roosting bat (*Nyctophilus geoffroyi*)

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Almost all laboratory studies on thermoregulatory energy expenditure are conducted under constant air temperatures ( $T_a$ ), yet heterothermy in the wild and the daily  $T_a$  cycle are often closely linked. We measured skin temperatures and oxygen consumption of captive lesser long-eared bats (*N. geoffroyi* 7 g) during exposure to heating and cooling of  $T_a$  that simulated conditions in natural roosts under bark. To test how passive re-warming affected energy expenditure and timing of arousal, we programmed the 12-h  $T_a$  cycle (12-26-12°C) to commence at 06:00, 09:00 or 12:00 h. Use of heterothermy in captive bats resembled that of wild bats. Time of active arousal was linked to passive re-warming from rising  $T_a$ . Bats aroused at higher  $T_a$ , however, when heating commenced at 0600 h ( $25.1 \pm 2.0^\circ\text{C}$ ), than at 0900 h ( $22.9 \pm 2.6^\circ\text{C}$ ) or 1200 h ( $21.8 \pm 2.8^\circ\text{C}$ ), demonstrating a temporal affect on arousal  $T_a$ . On average, passive re-warming reduced arousal costs by 47% from  $0.502 \pm 0.07$  kJ to  $0.272 \pm 0.07$  kJ. Bats responded to cooling of  $T_a$  below  $23.7 \pm 2.9^\circ\text{C}$  by re-entering torpor, even late in the afternoon, before arousing again near lights off. Periods of normothermy were therefore restricted to the time of maximum  $T_a$  (i.e.  $>24^\circ\text{C}$ ). On average, bats spent  $2:54 \pm 1:30$  h normothermic and rest phase energy expenditure was  $1.98 \pm 0.84$  kJ, about half that estimated at a constant, average  $T_a$  of  $19.8^\circ\text{C}$ . Thus, bats can gain significant energetic savings by taking advantage of maximum heating provided in thermally unstable roosts.



**Effect of aestivation on muscle fibre morphology in the  
Green-striped burrowing frog (*Cyclorana alboguttata*).**

Beth L Symonds<sup>1\*</sup>, Nicholas J Hudson<sup>2</sup> & Craig E Franklin<sup>1</sup>

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Muscle disuse atrophy is caused by prolonged disuse or unloading of skeletal muscle and it affects muscle tissue and microvasculature at the physiological, biochemical and functional level. It includes reduction of muscle cross-sectional area and mass, and results in loss of locomotor performance. The Green-striped burrowing frog, *Cyclorana alboguttata*, is an example of an amphibian aestivator that inhabits the semi-arid zone of South-East Queensland. After three months of aestivation, the wet muscle mass of several hindlimb muscles, and the locomotor and contractile properties of the gastrocnemius remained unchanged from that of control animals, indicating an absence of muscle disuse atrophy. I intend to explore the hypothesis that the lack of muscle atrophy is a function of fibre type, given that during mammalian immobilisation the fast twitch fibres show an attenuated response compared to their slow twitch counterparts. I am using enzyme histochemistry to fibre type several muscles in the hind legs and vocal chords. Secondly, I aim to determine whether the frog elevates antioxidant defences during aestivation, as this has previously been shown to reduce muscle wasting in immobilised mammals. Results of this study could have important implications in determining the mechanism/s used to inhibit muscle disuse atrophy.



## **Effects of aestivation on digestive efficiency of emergent Green-striped burrowing frogs**

Rebecca Cramp<sup>1\*</sup> and Craig E. Franklin<sup>1</sup>

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Food deprivation results in numerous changes to the digestive tracts of vertebrate animals. These changes can significantly affect the ability of animals to re-feed following protracted periods of fasting or starvation. Burrowing frogs can spend upwards of three-quarters of the year underground in aestivation, during which time they do not feed. Upon arousal from aestivation, animals have only a very short amount of time in which to restore endogenous fuel supplies before having to again enter aestivation. The ability of animals to re-feed following bouts of aestivation is therefore of the utmost importance. We examined several morphological parameters of the gastrointestinal tract, digesta passage rates and nutrient assimilation efficiencies of Green-striped burrowing frogs (*Cyclorana alboguttata*) following prolonged fasting during three months of aestivation and compared these with frogs that had been continuously fed. Whole animal digesta passage rates were significantly reduced following three months aestivation as a result of a decreased digesta evacuation rate from the stomach. Furthermore, food was selectively retained in the small intestine for an increased time following three months of aestivation. Overall digestibility of food and nitrogen, carbon and energy extraction efficiencies were not significantly different from control values following three months of aestivation. These findings suggest that *C. alboguttata* employs reduced digesta passage rates so as to maximize nutrient assimilation efficiency following prolonged food deprivation during aestivation.

## **The influence of body size on the diving behaviour and physiology of the bimodally respiring turtle *Elseya sp. nov.***

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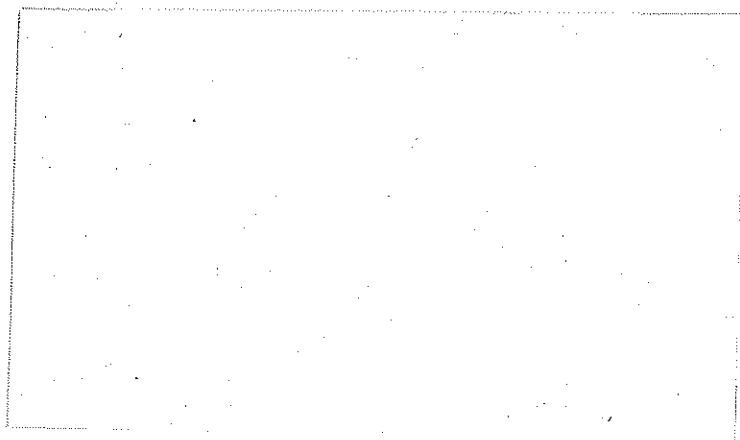
The aim of this study was to investigate the relationships between body size, aquatic respiration and diving behaviour in the bimodally respiring turtle *Elseya sp. nov.* Measures of cloacal bursae surface area, metabolic rate, capacity for aquatic respiration, and dive duration both with (normoxic) and without (hypoxic) aquatic respiration, were obtained and examined in relation to body size. The cloacal bursae surface area of *Elseya sp. nov.* increased significantly with body mass at a rate proportional to  $Mb^{0.77}$ , such that the mass-specific surface area of the cloacal bursae was significantly greater in smaller turtles. Capacity for aquatic respiration decreased with increasing body mass, as the larger mass-specific cloacal bursae surface area of the smaller turtles allowed them to extract a relatively larger amount of  $O_2$  from the water than the larger turtles. The dive duration of *Elseya sp. nov.* increased with increasing body mass in hypoxic condition but was independent of body mass in aquatic normoxia. Under normoxic conditions, the high capacity of the smaller turtles for aquatic respiration allowed the smaller *Elseya sp. nov.* to significantly increase their dive duration above that based on aerial respiration alone, to a duration equal to that of the larger *Elseya sp. nov.* The rivers that *Elseya sp. nov.* inhabit have recently been dammed with more weirs being proposed in the future. Decreases in aquatic  $PO_2$  as a result of damming will significantly decrease the dive duration of small sized *Elseya sp. nov.* and may seriously threaten the long-term survival of this species.

## Effects of aerial oxygen content on bimodal gas exchange in *Trichogaster leeri*

Lesley A. Alton

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The presence of O<sub>2</sub> chemoreceptors within the air-breathing organ (ABO) of the aquatic air-breathing fish *Trichogaster leeri* was investigated by varying aerial O<sub>2</sub> partial pressure (P<sub>aO<sub>2</sub></sub>) and maintaining aquatic O<sub>2</sub> partial pressure (P<sub>wO<sub>2</sub></sub>) near 21 kPa. *T. leeri* is an obligate air-breather that breathes air even in hyperoxic water, and it was hypothesised that if ABO-O<sub>2</sub> chemoreceptors did not exist, air-breathing frequency and ABO gas exchange would not change with varying P<sub>aO<sub>2</sub></sub>. However, fish responded to increasing P<sub>aO<sub>2</sub></sub> by decreasing air-breathing frequency, increasing aerial O<sub>2</sub> consumption rate ( $\dot{V}_{O_2}$ ), decreasing aquatic  $\dot{V}_{O_2}$ , and increasing mean O<sub>2</sub> uptake per breath, suggesting that ABO-O<sub>2</sub> chemoreceptors are present. Breath-by-breath assessment of apneic  $\dot{V}_{O_2}$  showed that with increasing P<sub>aO<sub>2</sub></sub>, apneic  $\dot{V}_{O_2}$  also increased. This indicates that the observed increase in mean O<sub>2</sub> uptake per breath with increasing P<sub>aO<sub>2</sub></sub> was facilitated not only by an increase in apnea duration, but also by an increase in the air-blood P<sub>O<sub>2</sub></sub> gradient. This data also allowed for the calculation of end-apnea ABO-P<sub>O<sub>2</sub></sub> that increased with increasing P<sub>aO<sub>2</sub></sub>. This suggests that regulation of air breathing via ABO-O<sub>2</sub> chemoreceptors is imperfect and that bimodal respiration is probably affected by several additional factors, including chemoreceptors elsewhere in the central and peripheral nervous system and mechanoreceptors in the ABO.



## **Compressible gas gills: Comparing theory with empirical data**

Philip Matthews

Environmental Biology, University of Adelaide, SA 5005, Australia

Many adult aquatic insects can survive underwater for hours by carrying a bubble of air over their spiracles. Two models have been proposed to describe how such a bubble, usually termed a compressible gas gill, can facilitate gas exchange with the water. One model predicts a rapid drop in oxygen partial pressure leading to a steady state, the other a constant decline in oxygen partial pressure for the duration of the dive. However, these models have never been validated by empirical observation. Real-time measurements have now been made of oxygen partial pressures in the compressible gas gills of submerged corixids, shedding new light on the accuracy of the models.

The corixid, *Agraptocorixa eurynome* (Hemiptera, Heteroptera), carries a large bubble of air allowing it to dive for well over an hour. Individual bugs were tethered underwater with their limbs being left free to ventilate their gas gill. An optode (optical oxygen probe) was inserted into the gas gill to measure the oxygen partial pressure. Of the two competing models, the model assuming a rapid initial  $PO_2$  drop plateauing at a stable  $PO_2$  was found to be most representative of oxygen changes within the gas gill during diving, with stable oxygen partial pressures being attained at 4 kPa for active and 2 kPa for very active individuals. However, it was found that both models made a number of incorrect assumptions regarding the impact of variables such as metabolic rate and ventilatory activity on oxygen levels within the bubble.

## **Models for embryonic respiration**

Roger S. Seymour\* and Craig R. White

Environmental Biology, University of Adelaide

Modelling has been an important tool in physiology throughout its history. In respiration physiology, for example, almost a century ago, August Krogh modelled diffusion of oxygen from a capillary. More recently, the models of Peter Scheid and Johannes Piiper set paradigms for comparative respiration in vertebrates. This presentation attempts to demonstrate the roles modelling might play in comparative developmental physiology. It discusses the types of models, presents the hierarchical levels of modelling, evaluates the applicability and limitations of each and rationalizes different approaches.

The hierarchy of modelling will be presented, with reference to historical approaches in the area of embryonic respiration. Emphasis will be placed on the practicalities of modelling with two types of model: analytical and numerical. With examples centered around embryonic gas exchange, the presentation will show how modelling can be useful (1) to estimate the constraints on a system outside of the measured range of values, (2) to measure the evolutionary constraints on a system by allometric analysis, (3) to check the sensitivity of parameters, (4) to confirm that an experimental result is reasonable or (5) to visualise a complex system in which measurements are impossible. It will also point out the dangers of modelling and emphasize the need for corroboration with experimental data.

## **Developmentally-regulated thyroid hormone distributor proteins in marsupials, a reptile and fishes**

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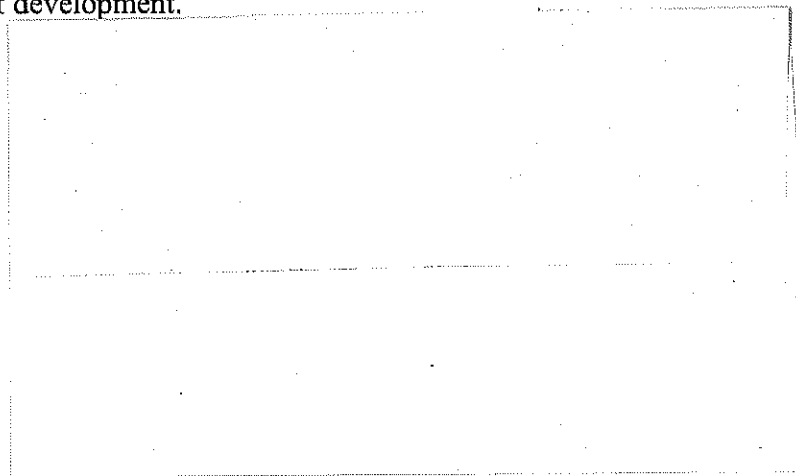
<sup>6</sup> Institut für Experimentelle Endokrinologie und Endokrinologisches Forschungs-Centrum der Charité Berlin, D-10117 Berlin, Germany;

<sup>7</sup> Department of Zoology, The University of Melbourne, Parkville 3010, Victoria, Australia.

Thyroid hormones are essential for vertebrate development. There is a characteristic rise in thyroid hormone levels in blood during critical periods of thyroid hormone-regulated development. Thyroid hormones are lipophilic compounds, which readily partition from an aqueous environment into a lipid environment. Thyroid hormone distributor proteins are required to ensure adequate distribution of thyroid hormones, throughout the aqueous environment of the blood, and to counteract the avid partitioning of thyroid hormones into the lipid environment of cell membranes. In human blood, these proteins are albumin, transthyretin and thyroxine-binding globulin.

We analysed the developmental profile of thyroid hormone distributor proteins in serum from a representative of each order of marsupials (*M. eugenii*; *S. crassicaudata*), a reptile (*C. porosus*), in two species of salmonid fishes (*S. salar*; *O. tshawytsch*), and throughout a calendar year for sea bream (*S. aurata*). We demonstrated that during development, these animals have a thyroid hormone distributor protein present in their blood (which is not present in the adult blood) which has higher affinity than the thyroid hormone distributor proteins in blood of the adult. In fish, reptile and polyprotodont marsupial, this protein was transthyretin. In a diprotodont marsupial, it was thyroxine-binding globulin.

We propose that the rise in thyroid hormone levels observed during development requires an augmented thyroid hormone distributor protein network in the blood during this stage of development.



## The Transthyretin-like Proteins of Non-vertebrates

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In vertebrates, the distribution of thyroid hormone in blood and cerebrospinal fluid is accomplished by three proteins, including the homotetramer, transthyretin (TTR). The primary structure of TTR has not altered significantly during the evolution of vertebrates, suggesting that the structure of the gene must have evolved prior to the divergence of vertebrates from invertebrates. Bioinformatic analyses have revealed that a large number of non-vertebrates including bacteria, fungi, nematodes, insects and plants may possess open reading frames whose product might code for a transthyretin-like protein (TLP).

Amplification of cDNA from *Caenorhabditis elegans* and *Arabidopsis thaliana* has confirmed the expression of the TLPs in these organisms. RT-PCR was used to confirm expression of the TLP gene in *Escherichia coli*. The presence of such an open reading frame in *Salmonella* sp. was also confirmed by amplification of genomic DNA.

The *in vitro* synthesis of the putative *S. dublin* and *C. elegans* TLPs was achieved using an *E. coli* expression system. The *S. dublin* TLP was found to be periplasmic whilst most bacterial TLPs were predicted to be cytoplasmic. Both the *S. dublin* and *C. elegans* TLPs were found to be tetrameric, as are vertebrate TTRs. Antibodies generated against the *S. dublin* TLP cross-reacted with recombinant *A. thaliana* and *C. elegans* TLPs, but not with TTRs from human, chicken or wallaby.

It is unlikely that thyroid hormones are the natural ligands of TLPs. The failure to detect thyroid hormone binding to the *S. dublin* protein, suggests that the hormone binding site of the bacterial TLP possesses subtle differences which might indicate binding of a structurally different ligand. Studies are currently underway to elucidate the structure and function of non-vertebrate TLPs.

## **The effect of early temperature on muscle growth and development in barramundi *Lates calcarifer***

Geoff Carey\* and Craig E. Franklin

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The incubation temperature of fish eggs can significantly affect subsequent muscle growth and development in fish. The aim of this study was to determine the effects of early temperature on muscle growth and development in fertilised eggs and larvae of barramundi (*Lates calcarifer*).

Eggs were incubated at three experimental temperatures within the known thermal tolerance of the animals, one close to the lower thermal limit (26°C), one at the ambient temperature of the brood tanks (29°C) and one close to the upper thermal limit (31°C). On hatching, a number of larvae were fixed and later prepared for histology, and others were on grown at experimental temperatures and sampled at later developmental stages.

Histological analysis of newly hatched larvae showed significant effects of incubation temperature on several muscle parameters in barramundi, primarily muscle cellularity (number and size of fibres), with low temperature inducing a higher number of smaller muscle fibres (hyperplasia) and high temperature inducing a lower number of larger fibres (hypertrophy). At around three months old treated barramundi juveniles were used in a growth trial lasting 12 weeks to determine any differential effects of incubation temperature on subsequent growth rates. Results showed a 22% increase in mass of barramundi incubated at low temperature and then returned to control temperature over those incubated and grown at control temperature, and a 26% increase over those incubated at high temperature and grown at control temperature. Implications of this research will be discussed.



## **The effect of temperature on the energetics of development in house crickets**

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Temperature is known to influence the rate of growth and development of ectotherms. Here we investigate the influence of temperature (25°C & 28°C) on growth rates, pattern of oxygen consumption, production costs and energy densities of the domestic house cricket, *Acheta domesticus*. Crickets reared at 25°C developed slower (taking 119 days Vs 49 days to reach maturity), were heavier at maturity (dry mass 62.7mg Vs 54.4mg), and consumed more energy during development (4.1kJ Vs 1.4kJ) compared to crickets reared at 28°C. The fastest growth rates were observed during the final instar stage at both temperatures. Peak oxygen consumption rate (250µl/h Vs 200µl/h) and total oxygen consumed (210ml Vs 70ml) and hence production cost (the total energy consumed during development per gram of dry adult tissue produced 65kJ/g Vs 26kJ/g) were greater at 25°C than 28°C. Average energy density of cricket tissue during development was greater at 28°C (33.7kJ/g) than at 25°C (26.6 kJ), however at maturity cricket tissue from both temperatures had similar energy densities (33-34kJ). Predicted pre-final instar lipid content of crickets were higher at 28°C (80%) than 25°C (40%), but similar at both temperatures in the final instar stage (~80%).

**Electric field strength of membrane lipids from vertebrate species:  
Relationship with membrane lipid composition and Na<sup>+</sup>,K<sup>+</sup>-ATPase  
molecular activity**

Nigel Turner<sup>1\*</sup>, Thomas Starke-Peterkovic<sup>2</sup>, Paul L. Else<sup>1</sup> and Ronald J. Clarke<sup>2</sup>

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Intramembrane electric field strength is a very likely determinant of the activity of ion-transporting membrane proteins in living cells. In the absence of any transmembrane electrical potential or surface potential, its magnitude is determined by the dipole potential of the membrane's lipid components and their associated water of hydration. Here we have used a fluorometric method to quantify the dipole potential of vesicles formed from lipids extracted from the kidney and brain of eleven different animal species from four different vertebrate classes. The dipole potential was compared with the fatty acid composition and with the Na<sup>+</sup>,K<sup>+</sup>-ATPase molecular activity of each preparation. The magnitude of the dipole potential was found to be relatively constant across all animal species, i.e. 236-334 mV for vesicles prepared from total membrane lipids (i.e. phospholipids + cholesterol) and 223-256 mV for phospholipids alone. The significantly lower value for phospholipids alone is likely related to the removal of cholesterol, as *in vitro* reduction of cholesterol levels with cyclodextrin also reduced dipole potential. Surprisingly, no significant dependence of the dipole potential on fatty acid composition was found. This may, however, be due to concomitant compensatory variations in lipid head group composition. The molecular activity of the Na<sup>+</sup>,K<sup>+</sup>-ATPase was found to increase with increasing dipole potential. The fact that the dipole potential is maintained at a relatively constant value over a wide range of animal species suggests that it may play a fundamental role in ensuring correct ion pump conformation and function within the membrane.

## **Membrane lipids – "regulators" or "conformers" to dietary lipid profile?**

Paul L. Else<sup>1\*</sup>, T. W. Mitchell<sup>1</sup>, N. Turner<sup>1</sup>, S. Faulks<sup>1</sup> and A. J. Hulbert<sup>2</sup>

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Departments of <sup>1</sup>Biomedical & <sup>2</sup> Biological Science, University of Wollongong.

Membranes represent complex environments where much of the metabolic chemistry of life occurs. Lipids are a major constituent of membranes and appear to be relatively consistent in their composition for any tissue of a given species despite the continuous remodelling of membranes. However, large changes in lipid composition occur between species within phylogenetic groups. In mammals and birds, smaller species with high rates of metabolism have membrane lipids that are highly polyunsaturated whereas larger species with lower rates of metabolism possess membranes that are increasingly monounsaturated at the expense of the polyunsaturated fats. The fact that increasing levels of polyunsaturated fats are present in the smaller species presents an interesting question since polyunsaturated fats (omega-6 and omega-3) need to be obtained from the diet as a result of the loss, in the distant evolutionary history of vertebrates, of the ability to produce these fats *de novo*. This study will use data from a variety of sources and employ the classic "conformer – regulator" paradigm to examine the response of membrane composition to dietary intake. The results of the analysis will then be discussed in terms of the possible physiological consequences for animals, including the human species.

## **A tribute to Russell Victor Baudinette 1945-2004.**

### **The search for generalisations in animal function: 'footnotes' for Russ.**

Russ Baudinette is the product of an esteemed academic genealogy, completing his PhD in 1972 at the University of California, Irvine under the supervision of Dick MacMillen and a post-doctoral Fellowship, for two years, with Knut Schmidt-Nielsen at Duke University.

A combination of imagination and determination allowed Russ to research generalisations in animal function and their adaptation to diverse environmental conditions. Early in his career he developed his passion for how things moved, in particular the intricacies of kangaroo hopping.

Russ had a friendly, charismatic personality that, combined with his acute questing mind, made for a fun, productive and vibrant research environment. His laboratory proved a fertile crèche for the graduate and honours students he mentored and for the array of visitors it hosted.

Russ is held in exceptionally high esteem by colleagues and the scientific community around the world. His interest in kangaroos and his legacy to all zoological is fittingly honoured in the appellation of a subspecies of the banded-hare wallaby as *baudinettei*. He died far too soon.

Peter Frappell  
La Trobe University

## **The Effect of Temperature During Periods of Increased Metabolism in a Varanid Lizard**

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During aerobic activity in vertebrates, the rate of oxygen consumption ( $\dot{V}_{O_2}$ ) may be increased by the cardiovascular system through augmentation of cardiac output (the product of heart rate [ $f_H$ ] and cardiac stroke volume) and/or extraction of oxygen from the circulating blood. It has previously been postulated that  $f_H$  may be used as an indicator of metabolic rate, though most such studies have been performed on endothermic animals at the typical operant body temperature. Ectothermic vertebrates are often active at body temperatures below preferred, and we aimed to determine in Rosenberg's goanna the cardiovascular adjustments that allow the increase in  $\dot{V}_{O_2}$  during exercise at cooler temperatures. During treadmill exercise, a factorial aerobic scope of 6.5 was maintained across the temperature range of 18-36°C and, both at 25°C and 36°C, similar increases in oxygen extraction (2.2-fold),  $f_H$  (2.0-fold) and stroke volume (1.8-fold) were observed. Metabolism may also be increased during periods of digestion (specific dynamic action), and it was found for Rosenberg's goanna that  $f_H$  primarily governs the increase in  $\dot{V}_{O_2}$  during such periods. In contrast, the usually tight relationship between  $f_H$  and  $\dot{V}_{O_2}$  uncouples during periods of heating and cooling when there exists a thermal hysteresis in  $f_H$  but not in  $\dot{V}_{O_2}$ . It would appear to be possible to use  $f_H$  to predict  $\dot{V}_{O_2}$  in an ectotherm, though such predictions must take into consideration the thermal characteristics of the animal.

## **Influence of thermal acclimation on sneaky mating avoidance and burst swimming in female eastern mosquitofish (*Gambusia holbrooki*).**

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Although the range of acclimation responses and their underlying mechanisms have been well documented, the adaptive significance of seasonal acclimation has been seldom studied. I used the effect of temperature on the mating behaviour of female eastern mosquitofish (*Gambusia holbrooki*) to test the adaptive benefits of acclimation. The mating system of *G. holbrooki* is dominated by male sexual coercion, where all matings are sneaky-copulations and females never cooperate and actively avoid all attempts. The ability of female *G. holbrooki* to avoid sneaky matings was tested after 4-5 weeks acclimation to 16°C and 32°C. Thermal acclimation of burst swimming performance was also assessed as this trait is likely to underlie avoidance. I predicted that acclimation would enhance the ability of female *G. holbrooki* to avoid sneaky matings in their host acclimation temperature relative to females acclimated to another environment. In contrast with predictions, acclimation did not enhance the ability of female *G. holbrooki* to avoid sneaky matings at host acclimation temperature. At 32°C, the rate of copulations was 2.5 times greater for females acclimated to 32°C than 16°C. Thus, it appears there are no adaptive benefits for acclimation to female avoidance behaviour. Female aggression was also 2.5 times greater for 32°C-acclimated fish at 32°C than 16°C-acclimated fish. For burst swimming performance, warm-acclimation significantly increased at 32°C, while no effect of acclimation was observed when tested at 16°C. Although it has been assumed that female *G. holbrooki* never cooperate during mating, this study suggests that females may modify their avoidance behaviour after long-term exposure to warm environments, thereby increasing the probability of successful copulations. The disparity between this study and previous reports of female *G. holbrooki* behaviour warrants further investigation.

## **Thermal instability and the development and metamorphic condition of striped marsh frogs (*Limnodynastes peronii*)**

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The aquatic breeding sites for many Australian frogs are often shallow and ephemeral, and tadpoles of many species are commonly observed in seemingly impossible conditions. For example, tadpoles in small, unshaded pools may experience temperature fluctuations of greater than 20°C daily. Tadpoles depend on environmental heat for metabolic processes and growth, and extreme variation on such a short time scale has the potential to impact a number of physiological and morphological parameters, such as time to metamorphosis, body size and locomotor (escape) performance. Although several studies have evaluated the effects of temperature on development and locomotor performance, the effects of the wide temperature fluctuations on young frogs are unknown.

We conducted a laboratory experiment to examine the growth and development of striped marsh frog (*Limnodynastes peronii*) tadpoles under fluctuating and stable temperature regimes. During the larval phase, we found striking acceleration in the rate of growth and time to metamorphosis in the fluctuating-group compared with the stable-group. In addition, the larval environment appeared to influence the performance of recently-metamorphosed frogs. Despite a lack of mass differences among treatments, metamorphs raised in fluctuating temperatures possessed greater maximum jumping performance. Early metamorphosis and greater jumping ability of these frogs may aid in their survival, as pool desiccation and predator avoidance are important causes of mortality in early life. Further work will examine the metabolic and physiological causes underlying these differences in growth and performance.

## **Biochemical acclimation of metabolism in *Limnodynastes peronii* tadpoles**

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We investigated how tadpoles of the striped marsh frog (*Limnodynastes peronii*) acclimate their metabolism to lowered temperature. We measured the rate at which metabolic enzymes (lactate dehydrogenase (LDH), citrate synthase (CS), and cytochrome c oxidase (CCO)) acclimate in response to lowered temperature in the axial muscle of these tadpoles over six weeks. In addition, we also examined the activities of these enzymes in the liver tissue after six weeks, and determined if there were changes in the proportions of LDH isoenzymes between acclimation groups. We found that LDH acclimates in axial muscle; the differences between the control and cold-acclimated group became apparent after 21 days. After 42 days, the activity of LDH in axial muscle in the cold-acclimated group was 30% greater than the control group. The rate at which acclimation in this species occurs is appropriate for seasonal changes in temperature, and these animals may not be able to respond to a rapid drop in temperature. Both LDH and CS increased in activity in the liver (5 and 1.3 times greater, respectively, in the cold-acclimated group). The thermal sensitivity ( $Q_{10}$ ) of LDH between 20 and 30°C in the cold-acclimated group ( $1.2 \pm 0.01$ ) compared to the control group ( $1.6 \pm 0.15$ ). This change in thermal sensitivity is explained by the changes in proportions of LDH isoenzymes. Cold-acclimated tadpoles expressed more isozymes that retain function at lower temperatures.



**A falsification of the thermal specialization paradigm: compensation for elevated temperatures in Antarctic fish: 1. Swimming Performance**

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Specialization to a particular environment is one of the main factors used to explain species distributions. Antarctic fish are often cited as a classic example to illustrate the specialization process and are regarded as the archetypal stenotherms. Here we show that the Antarctic fish *Pagothenia borchgrevinki* has retained the capacity to compensate for chronic temperature change. By displaying astounding plasticity in cardiovascular response and metabolic control, the fish maintained locomotory performance at elevated temperatures. Our falsification of the specialization paradigm indicates that the effect of climate change on species distribution and extinction may be overestimated by current models of global warming.

## **A falsification of the thermal specialization paradigm: Compensation to elevated temperatures in Antarctic fish. II. Cardiac function**

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The cardiovascular system plays a major role in supporting sustained swimming in fish and is influenced by changes in water temperature. Here, we examined the thermal acclimation of cardiac function in the Antarctic teleost, *Pagothenia borchgrevinki* at rest and after exhaustive exercise. A Doppler flow probe was placed around the ventral aorta of *P. borchgrevinki* acclimated to -1°C and 4°C for 4-6 weeks. Cardiac output, heart rate and stroke volume were recorded at rest and after exhaustive exercise in fish acutely transferred from their acclimation temperature to -1, 2, 4, 6 and 8°C. In the -1°C acclimated fish, the factorial scope for cardiac output was maximal at -1°C and decreased with increasing temperature, such that at 8°C there was no significant difference between resting and maximal cardiac output. With acclimation to 4°C, scope for cardiac output was maintained at 4, 6 and 8°C, but was reduced at -1°C. The restoration of cardiac scope at higher temperatures with warm acclimation coincided with a change from a predominantly volume-driven (inotropic) pump to a rate-driven (chronotropic) pump.

## **Testing the benefits of thermal acclimation to the sneaky-mating performance of male mosquito fish**

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The effect of temperature on the ability of male eastern mosquito fish (*Gambusia holbrooki*) to obtain sneaky-copulations was used to test the benefits of thermal acclimation. The mating system of mosquito fish is dominated by male sexual coercion, which means males rarely display to females, females almost never cooperate during copulations, and all matings are achieved by stealth. Preliminary experiments revealed temperature markedly influenced both the propensity of males to follow females and their ability to obtain these sneaky-copulations. For the acclimation experiments, fry were raised under controlled conditions in the laboratory and, after reaching maturity, were then exposed to either 18° or 30°C for 6 weeks. The ability of males to obtain sneaky-copulations was tested at each temperature under two sets of conditions. The mating performance of each individual male was first recorded when placed in a small aquarium with two mature females (absence of male-male competition). Secondly, each male was paired with one similarly-sized male from the opposite acclimation group (each 18°C-male paired with a 30°C-male). Size-match males were then competed against each other for successful matings with only one female (male-male competition). I found the benefits of thermal acclimation to male mating performance were dependent on whether they were tested in the presence or absence of male-male competition.

## **Thermal physiology as a means to assess habitat preferences in free-living, North American Microbats**

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Studies assessing wildlife habitat preferences often employ a presence-absence approach, where sites used by individuals are compared to randomly selected, un-used sites. This approach can reveal correlations between physical landscape features and preferred habitats, but may be limited because causal motivations underlying habitat preferences cannot be identified. Our objective was to determine if physical features of preferred habitats, identified using a presence-absence approach, can be explained using thermal energetics. We radiotracked reproductive female hoary bats (*Lasiurus cinereus*), a wide-ranging North American microbat, to their open-foliage tree roosts and compared physical features of roost trees with randomly selected non-roost trees. We recorded ambient temperature and wind speed at roosts and non-roosts and entered these data into predictive models of thermoregulatory energy expenditure. Hoary bats selected roost sites on the southeast side of white spruce trees (*Picea glauca*; mean orientation  $158.6 \pm 6.3^\circ$  SSE). Roosts: (1) were more likely than random trees to be the same height as the surrounding forest canopy; (2) had less canopy cover facing out from the tree in the direction of the roost branch; and (3) had lower forest density on their southeast side. Wind speed was significantly lower at roosts sites compared to opposite sides of the same trees, presumably due to increased protection from prevailing west winds. Incorporating an estimate of convective cooling due to wind, we predicted daily thermal energy expenditure for normothermic bats and found that selected roost sites provided statistically significant energy savings (up to  $1.60 \pm 0.99$  kJ day<sup>-1</sup>) relative to the predicted expenditures if bats roosted on the opposite sides of trees. Our results provide direct evidence that hoary bats select forest roosts on the basis of microclimate and suggest that thermal physiology can help explain motivations underlying habitat preferences of free-living animals.

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