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ANNUAL MEETING  
OF AUSTRALIAN & NEW  
ZEALAND COMPARATIVE  
PHYSIOLOGISTS

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THE UNIVERSITY OF QUEENSLAND  
DECEMBER 2-4, 1994

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## PROGRAM

Friday, December 2

09:30 Registration

10:30 Morning Tea

### SESSION ONE

1:30 Aquatic hypoxia as an impetus for breathing air: The Australian Yabby *Cherax destructor* as a case study.  
*Stephen Morris and Jonathan Callaghan, School of Biological Sciences, The University of Sydney*

1:50 Vasoconstriction in response to galanin in three species of elasmobranchs.  
*Clare McManus, Elaine Preston, Ann-Catherine Johnsson and Gillian Courtice, School of Physiology & Pharmacology, University of New South Wales.*

2:10 Which part of the galanin molecule is responsible for its vasoconstrictor activity in toads?  
*David Mahns and Gillian Courtice, School of Physiology and Pharmacology, University of New South Wales.*

2:30 Comparative vascular responses in elasmobranchs to different structures of Neuropeptide Y (NPY) & Peptide YY (PYY).  
*Elaine Preston, Clare McManus, Ann-Cathrine Jonsson and Gillian Courtice, School of Physiology & Pharmacology, University of New South Wales.*

2:45 Vasomotion in crab gills.  
*H.H. Taylor, Department of Zoology, University of Canterbury.*

3:00 The evolution of thyroxine distributor proteins.  
*Gerhard Schreiber, Samantha J. Richardson, Wei Duan, Charlotte M. Brack and Bridget R. Southwell, Department of Biochemistry, University of Melbourne.*

3:20 Afternoon Tea & Posters

## SESSION TWO

- 4:00** Control of gut contraction by opioid agonists and RF-amide peptides in crickets and cockroaches.  
*Paul D. Cooper and Justin Harsdorf, Division of Botany & Zoology, Australian National University.*
- 4:15** Endocrine cells in the heart of the Australian lungfish, *Neoceratodus forsteri*.  
*Lisa K. Chopin and Michael B. Bennett, Department of Anatomical Sciences, The University of Queensland.*
- 4:30** How does the fish do it? Problems of fish eye growth.  
*W.S. Jagger, Department of Ecology and Evolutionary Biology, Monash University.*
- 4:45** The role of thyroxine in the development of the brushtail possum, *Trichosurus vulpecula*.  
*W. Buaboocha and R.T. Gemmell, Department of Anatomical Sciences, The University of Queensland.*
- 5:00** The role of the fetal thyroid gland on growth and organ development in two eutherians, the rat and sheep, and two marsupials, the possum and bandicoot.  
*R.T. Gemmell and W. Buaboocha, Department of Anatomical Sciences, The University of Queensland.*
- 5:15** Function of transthyretin in thyroid hormone transport in blood and brain.  
*Bridget R. Southwell, Samantha J. Richardson, W. Duan, Charlotte M. Brack and G. Schreiber, Department of Biochemistry, University of Melbourne.*
- 6:00** **Social Mixer - Emmanuel College**

## SESSION THREE

Saturday, December 3

- 8:00 Kidney structure and function in two species of dasyurid marsupials, *Sminthopsis crassicaudata* and *S. macroura*.  
*Marcus Bayly and Julie Roberts, Department of Physiology, University of New England.*
- 8:15 Electrolyte fluxes in the main duct of the parotid gland of red kangaroos.  
*A. Michel Beal, School of Biological Science, University of New South Wales.*
- 8:30 Changes in blood electrolyte levels over the laying cycle of the hen.  
*Cindy Brackpool and Julie Roberts, Department of Physiology, University of New England.*
- 8:45 Blood gas, acid-base and ionic status of the amphibious crab *Leptograpsus variegatus*.  
*Sandra L. Butler and Steve Morris, School of Biological Sciences (A08), University of Sydney.*
- 9:00 Nitrogen metabolism in the terrestrial crab *Geograpsus grayi* (Brachyura: Grapsidae).  
*D.G. Varley and Peter Greenaway, School of Biological Science, University of New South Wales.*
- 9:15 Regulation of salt gland function in the green sea turtle *Chelonia mydas*.  
*Richard Reina, Division of Botany and Zoology, Australian National University.*
- 9:30 Autoregulation by the avian kidney.  
*Julie Roberts<sup>1</sup> and R.F. Wideman Jr.<sup>2</sup>, <sup>1</sup>Department of Physiology, University of New England, <sup>2</sup>University of Arkansas.*
- 9:45 Osmoregulatory, respiratory and acid-base responses of *Leptograpsus variegatus* to emersion and hypo-saline water.  
*Ashley Cooper and Stephen Morris, Biological Sciences, University of Sydney.*
- 10:05 - 11:00 Morning Tea & Posters

## SESSION FOUR

- 11:00 Breeding Australian lungfish in captivity.  
*Jean Joss and Greg Joss, School of Biological Sciences, Macquarie University.*
- 11:15 Primary and secondary stress responses to line capture in the marine teleost, Blue Maomao (*Scorpius violaceus*).  
*Timothy E. Lowe and Rufus M.G. Wells, School of Biological Sciences, University of Auckland.*
- 11:30 The respiratory and acid base response of the Southern Rock Lobster *Jasus edwardsii* to low temperature and air exposure: an application to the shipping methods in the live seafood market.  
*Susan W. Oliver and Stephen Morris, Biological Sciences, University of Sydney.*
- 11:50 A lactic acid "ceiling" arrests profound acidosis in the haemolymph of an ion-conforming crab, (*Ranina ranina*), during emersion at 25°C.  
*Brian D. Paterson, Paul S. Exley and Ross A. Smith, International Food Institute of Queensland, Department of Primary Industries.*
- 12:05 The physiological dependency of the Christmas Island blue crab, *Cardisoma hirtipes*, on freshwater.  
*Jocelyn Dela-Cruz and Stephen Morris, School of Biological Sciences, University of Sydney.*
- 12:25 Natriuretic peptide receptors in fish gills.  
*John A. Donald<sup>1</sup>, Tes Toop<sup>2</sup>, and David H. Evans<sup>2</sup>, <sup>1</sup>School of Biological and Chemical Sciences, Deakin University, <sup>2</sup>Department of Zoology, University of Florida.*
- 12:45 - 2:00 Lunch & Posters

## SESSION FIVE

- 2:00 KEYNOTE ADDRESS:**  
The evolution of haemoglobin function: adaptation and the panslectionist paradigm.  
*Rufus M.G. Wells, Biological Sciences, The University of Auckland.*
- 2:45** Oxygen uptake by amphibian egg masses.  
*Roger S. Seymour, Department of Zoology, The University of Adelaide.*
- 3:00** Oxygen availability and embryonic development in sand snail egg masses.  
*David T. Booth, Department of Zoology, The University of Queensland.*
- 3:15 - 4:00 Afternoon Tea & Posters**
- 4:00** Does erythrocyte size make any bloody difference?  
*J. Baldwin, K. Breuer, A. Lill and P. Lay, Department of Ecology and Evolutionary Biology, Monash University.*
- 4:15** Scaling of respiratory variables and the breathing pattern in adult marsupials.  
*Peter Frappell<sup>1</sup> and Russ Baudinette<sup>2</sup>, <sup>1</sup>School of Zoology, La Trobe University, <sup>2</sup>School of Biological Sciences, Flinders University.*
- 4:30** Oxygen carriage in embryonic blood of marsupial and eutherian mammals.  
*Robert A.B. Holland, School of Physiology and Pharmacology, University of New South Wales.*
- 5:00 Conference Dinner - Mirimar "Batty" Boat Cruise**  
Buses leave Emmanuel College at 5:00 pm for boarding and departure at North Quay by 5:30 pm.

## SESSION SIX

Sunday, December 4

- 08:15 Body temperature rhythms and activity in reproductive male *Antechinus stuartii* (Marsupialia).  
*Gerhard Körtner and Fritz Geiser, Department of Zoology, University of New England.*
- 08:30 Reduction of metabolic rate and thermoregulation during daily torpor.  
*Xiaowei Song, Gerhard Körtner and Fritz Geiser, Department of Zoology, University of New England.*
- 08:45 Control of respiration in hibernating echidnas.  
*Stewart Nicol and Niels A. Andersen, Department of Physiology, University of Tasmania.*
- 09:00 Plasma triglyceride and  $\beta$ -Hydroxybutyric acid levels in Red-sided Garter Snakes (*Thamnophis sirtalis parietalis*) at emergence from hibernation.  
*Joan M. Whittier<sup>1</sup> and Robert T. Mason<sup>2</sup>, <sup>1</sup>Department of Anatomical Sciences, The University of Queensland, <sup>2</sup>Department of Zoology, Oregon State University.*
- 09:15 Cellular lipid bilayers are more polyunsaturated but less monounsaturated in a mammal compared to reptiles: a basis for the evolution of endothermy?  
*Tahira Rana and A.J. Hulbert, Department of Biological Sciences, University of Wollongong.*
- 09:30 Dietary assimilation in a herbivorous terrestrial crab, *Gecarcoidea natalis*.  
*Peter Greenaway and S.M. Linton, School of Biological Science, University of New South Wales.*
- 09:45 Field measurements of respiratory and energy state of Christmas Island Red Crabs *Gecarcoidea natalis* during the annual breeding migration.  
*Agnieszka Adamczewska and Stephen Morris, Department of Biology, University of Sydney.*
- 10:05 - 11:00 Morning Tea & Posters

## SESSION SEVEN

- 11:00** Myocardial fibre orientation in the ventricle of the rainbow trout.  
*Graham Chan-Thackeray and Peter S. Davie, Department of Physiology and Anatomy, Massey University.*
- 11:15** Sensory processing in the fish brain: cerebellar circuits and synaptic plasticity discriminate signals from self-generated noise.  
*John C. Montgomery<sup>1</sup> and David Bodznick<sup>2</sup>, <sup>1</sup>School of Biological Science, University of Auckland, <sup>2</sup>Department of Biology, Wesleyan University, USA.*
- 11:30** The importance of touch in the Northern Quoll (*Dasyurus hallucatus*).  
*John Nelson, Karen Schwensen and Janell Thomas, Department of Ecology and Evolutionary Biology, Monash University.*
- 11:45** The effect of exercise training on internal oxygen convection (TO<sub>2</sub>), oxygen consumption (VO<sub>2</sub>), and swimming performance of chinook salmon (*Oncorhynchus tshawytscha*).  
*Helgi Thorarensen, Patricia Gallagher and Anthony P. Farrell, Department of Biology, Simon Fraser University, Canada.*
- 12:00** Metabolic adaptations of reptiles to dry season conditions in tropical Australia.  
*Keith Christian, School of Biological Sciences, Northern Territory University.*
- 12:15** Energetic cost of embryonic development in lizards.  
*Michael B. Thompson<sup>1</sup>, James R. Stewart<sup>2</sup> and Kylie J. Russell<sup>1</sup>, <sup>1</sup>School of Biological Sciences (A08), University of Sydney, <sup>2</sup>Faculty of Biological Sciences, University of Tulsa, USA.*
- 12:30** The nutritional consequence of small avian granivores consuming green seed.  
*L.R. (Rick) Allen, School of Environmental and Information Sciences, Charles Sturt University.*
- 12:45** **Award of Student Prizes**
- 1:00 - 2:30** **Lunch**



## KEYNOTE ADDRESS

### The evolution of hemoglobin function: adaptation and the panselectionist paradigm

*Rufus M.G. Wells, Biological Sciences, The University of Auckland*

'As my conclusions have lately been much misrepresented, and it has been stated that I attribute the modification of species exclusively to natural selection, I may be permitted to remark that in the first edition of this work, and subsequently, I placed in a most conspicuous position- namely at the close of the Introduction- the following words: "I am convinced that natural selection has been the main, but not the exclusive means of modification." This has been of no avail. Great is the power of steady misinterpretation.'

*(Origin 1872 edn. p.395)*

The mission of comparative physiology is to document physiological diversity and explain its origins. In pursuing homologous and analagous relationships between organisms and processes, a number of comparative physiologists claim that all features and processes have been scrutinised by natural selection. Physiologists have used the term "adaptation" to mean (i) *an evolutionary process by which natural selection adjusts the frequency of genes encoding the traits that affect fitness*, (ii) *a trait or feature*, (iii) *phenotypic plasticity*. Perhaps we should question whether the panselectionist paradigm has potentially misleading consequences. Furthermore, how do we test the hypothesis that a physiological trait is adaptive under a particular set of environmental circumstances? Hemoglobin function is a good example with which to discuss these questions because it shows physiological diversity among closely related taxa while serving the same role in oxygen transport.

The hemoglobin archetype imposes structural constraints in which limited mutational possibilities have the potential to alter hemoglobin function through changes to its stereochemical structure and allosteric interactions. It would therefore seem that hemoglobin has the potential to meet the oxygen requirements of animals living in diverse environments.

Examples from hemoglobin are used to illustrate: the level at which natural selection operates, maladaptive and inappropriate adaptive stories from panselectionists, the role of the environment in integrating function, the difficulty of ontogenetic functional explanations, and a way of testing the adaptationist argument by falsifiability criteria.

We may conclude: that one cannot automatically assume that variation in hemoglobin function is the result of natural selection, that natural selection is not an optimising agent, that animals do not play a passive role in adaptation, and that much of what we discern as physiological diversity is epiphenomenal.

It is a significant problem that many animals possess the physiological equipment required for their particular mode of life without much reference to their phylogenetic histories. This can be resolved in cases where historical approaches become sterile by extending the evolutionary definition of adaptation to include current utility.

Field measurements of respiratory and energy state of Christmas Island Red Crabs  
*Gecarcoidea natalis* during the annual breeding migration.

Agnieszka Adamczewska and Stephen Morris  
Department of Biology, University of Sydney.

The Christmas Island red crab *Gecarcoidea natalis* is endemic to Christmas island. These land crabs undertake an annual migration from the interior of the island to the shore terraces to breed. The migration is triggered by the arrival of the monsoon rains and is synchronised with the lunar cycle, lasting 5 to 7 days. During this time the crabs may migrate > 4 km to the coast. The crabs migrate only during daylight hours and radiotracking data revealed that the red crabs may travel up to 1000 m in one day.

Blood samples were taken from the pulmonary vessel, the pericardial cavity and from the infrabranial venous sinuses and analysed for O<sub>2</sub> and CO<sub>2</sub> content, pH and P<sub>O</sub><sub>2</sub>. Crabs were sampled before the onset of the migration during normal every day foraging, and after the onset of migration just after dawn and in the late afternoon. Blood leaving the lung remained well oxygenated at all times. After a day walking the crabs did not experience blood acidosis, no L-lactate build up in the blood or in the muscles and there were no significant differences in the arterial-venous O<sub>2</sub> content. Exercise is supported aerobically. During the migration the crabs do not experience dehydration and do not appear to use muscle glycogen stores as an energy source. The red crabs do not appear to suffer from fatigue during the migration and in the field have great physical endurance and distance capacity.

The nutritional consequence of small avian granivores consuming green seed.

L. R. (Rick) Allen

School of Environmental and Information Sciences, Charles Sturt University

The faunal assemblages of terrestrial ecosystems, particularly those in the more xeric environments, include species which rely on the regular production of seeds by the higher green plants as a food source. The requirement for protein by animals is really a demand for essential amino-acids in a balance suitable for efficient utilization, a criterion rarely satisfied by cereal grains. The Australian grass-finches are however widely reported to consume in the wild large quantities of green seed when it becomes available, and the opportunistic breeding of wild Zebra Finches is known to be stimulated by the occurrence of rain.

Green and ripe seeds of seven pasture and weed grasses were harvested from experimental plots, and along with three cereal flours and whole egg powder, analysed for the amino-acid composition of their protein. The relative levels between samples of ten amino-acids essential in the diets of growing birds were compared using a dendrogram generated from Raabes Similarity Index.

The protein of all green seeds clustered with whole egg, and away from all but one of the ripe seeds and seed products. This indicated that the benefit of green seed in the diet of small avian granivores is not simply one of shorter passage time allowing more seed to be processed for the extraction of essential amino-acids, but one of accessing a source of higher quality protein.

## Erythrocyte Metabolism in the Koala (*Phascolarctos cinereus*)

Michelle Baker<sup>1</sup>, Nihal Agar<sup>1</sup>, Susan Hemsley<sup>2</sup> and Paul Canfield<sup>2</sup>

1. Department of Physiology, University of New England, Armidale, NSW 2351
2. Department of Veterinary Pathology, University of Sydney, NSW 2006

Several interesting features of the haematology of the koala have been reported; for example, the erythrocytes of healthy koalas can exhibit anisocytosis and polychromasia, Howell-Jolly bodies and Heinz bodies (Canfield *et al.*, 1989; Spencer and Canfield, 1994). In addition, circulating nucleated erythroid cells are common in healthy koalas (Canfield *et al.*, 1989). Moreover, Heinz body anaemia has been reported in a koala, with speculation on increased susceptibility to oxidant drugs and chemicals (Spencer and Canfield, 1994). In view of this, we have now investigated the erythrocyte metabolism in the koala. Parameters measured were enzyme activities, glycolytic rates and oxidant defence. Compared with some marsupial species, the koala erythrocytes :

- (i) are more sensitive to haemolysis in the presence of hydrogen peroxide.
- (ii) lose reduced glutathione much faster in the presence of low concentrations of acetylphenylhydrazine,
- (iii) have a higher activity of glucose phosphate isomerase.

Significance of these and other results will be discussed.

Canfield, P.J., O'Neill, M.E. and Smith, E.F. (1989) *Aust. Vet. J.* 66, 324-326.

Spencer, A.J. and Canfield, P.J. (1994) *Comp. Haematol. Int.* 4, 114-117.

### Does erythrocyte size make any bloody difference?

*J Baldwin, K. Breuer, A. Lill and P. Lay*

Department of Ecology and Evolutionary Biology, Monash University,  
Clayton, Vic.3168.

Vertebrates show differences of at least 2000 fold in erythrocyte volume. As rates of oxygen loading and unloading increase with decreasing cell size, one might predict an inverse relationship between erythrocyte volume and aerobic metabolic rate. On a gross level this appears to be so, as erythrocytes of birds and mammals generally are smaller than those of reptiles, amphibians and fish. However on closer inspection the relationship is far from clear cut. As an extreme example, shrews and whales, with mass specific metabolic rates differing by several orders of magnitude, possess similar sized erythrocytes.

A confounding factor encountered in attempting to correlate erythrocyte size with respiratory function for non-mammalian vertebrates is the well established relationship between cell size and nuclear DNA content. Two quite different approaches for unravelling these complex interrelationships are presented. 1. Monitoring seasonal changes in erythrocytes of small birds over wintering in southern Australia. 2. Investigating correlations between erythrocyte volume and DNA content in phylogenetically diverse fish differing markedly in aerobic scope.

**Kidney structure and function in two species of dasyurid marsupials,  
*Sminthopsis crassicaudata* and *S. macroura*  
Marcus Bayly and Julie Roberts  
Department of Physiology, University of New England, Armidale, NSW 2351.**

Kidney structure and function were assessed in two species of dasyurid marsupial, *Sminthopsis crassicaudata* and *S. macroura*, both of which inhabit arid and semi-arid environments. The laboratory mouse provided a comparison with a eutherian of similar body size. All species were able to produce a highly concentrated urine, over 4000 mOsm.kg<sup>-1</sup> for the *Sminthopsis* species and just over 3000 mOsm.kg<sup>-1</sup> for the mouse, even though the animals were not water-deprived. Glomerular filtration rate (GFR) was measured in the mice by two means, by bolus injection of the marker and use of a plasma disappearance curve and by use of subcutaneously implanted osmotic minipumps. In the minipump experiment, water was withheld from the mice on the last day of the experiment. The two methods of measuring GFR gave similar results in hydrated animals. However, water deprivation led to a significant reduction in GFR. The GFR for the marsupials was at the lower limit of that which would be predicted from allometric equations. Nevertheless, there were no significant differences among the three species.

The structure of the kidneys of the three species was similar, with all having a renal papilla that extends beyond the confines of the kidney. However, this was much more pronounced in the marsupial species. All species possessed comparatively high renal indices, indicating a potentially high urinary concentrating capacity. The glomerular volumes of both cortical and juxtamedullary nephrons were largest in *Sminthopsis macroura* and smallest in the mice.

**Electrolyte Fluxes in the main duct of the parotid gland of red kangaroos  
A Michel Beal**

*School of Biological Science, University of New South Wales*

Over the upper half of its flow range, the parotid gland of kangaroos secretes saliva which is almost isotonic NaHCO<sub>3</sub>. Because secretion can be blocked substantially (>90%) by either carbonic anhydrase inhibitors or Na/H exchange blockers but not by loop diuretics, the primary fluid of this gland appears HCO<sub>3</sub>-based. To explain the observed flow rate dependency of HCO<sub>3</sub> and Cl excretion in this saliva, it is necessary to postulate that HCO<sub>3</sub> is reabsorbed and Cl is secreted at some region of the excurrent duct system. The direction of electrolyte fluxes across the wall of the main excretory duct of the gland was investigated by *in situ* duct perfusion at 55, 13.8 and 5.5 μl/min with a solution having ion concentrations characteristic of high-flow saliva (viz. Na, 159; K, 6; Cl, 6; HCO<sub>3</sub>, 145; PO<sub>4</sub>, 7 mmol/l plus a small amount of inulin). Decreasing perfusion rate was associated with progressive changes in concentration of all ions except Na. At 5.5 μl/min (n=7), [Cl] rose to 58.2 ± 3.43 mmol/l whereas [HCO<sub>3</sub>] decreased to 94.6 ± 4.02, [PO<sub>4</sub>] to 5.6 ± 0.16 and [K] to 5.2 ± 0.19 mmol/l. [Na] was unaltered at 160.0 ± 1.16 mmol/l. Additionally, influx of Ca and Mg resulted in [Ca] and [Mg] rising to 673 ± 48.4 and 278 ± 21.6 μmol/l respectively at the lowest perfusion rate. The ratio of inulin concentration in the collected perfusate to that of the unperfused solution was 97.9 ± 1.31. The potential between duct lumen and arterial blood averaged 4.2 ± 0.68 mV, lumen negative. At perfusion rates of 13.8 μl/min and higher, duct flux rates for all ions declined as time and/or perfusion-rate were increased. The results show that the resting excretory duct of Na-replete kangaroos, if it has the opportunity, is capable of substantial modification of [HCO<sub>3</sub>], [Cl], [Ca] and [Mg] in saliva secreted at low flow rates. The duct reabsorbs small amounts of K and PO<sub>4</sub>, and transports negligible amounts of Na.

## Oxygen availability and embryonic development in sand snail egg masses

David T. Booth

*Department of Zoology, The University of Queensland*

I investigated the oxygen transport physiology of sand snail *Polinices sordidus* egg masses using oxygen microelectrodes and open flow respirometry. *P. sordidus* eggs are laid in a jelly matrix which rapidly absorbs water and swells into a horseshoe shaped sausage. The average diameter of these sausages is 37 mm. Eggs are enclosed in capsules that are distributed throughout the jelly matrix, but 65% of the eggs are located within 3 mm of the outer surface. There is no circulatory or canal system within the matrix so all gas exchange between developing embryos and the environment must occur by diffusion through the jelly matrix.

Oxygen tension in the outer layer remains moderately high ( $P_{O_2} > 10$  kPa) throughout incubation but decreases rapidly in more centrally located regions so that by day 4 embryos in this region are exposed to extremely hypoxic conditions ( $P_{O_2} < 1$  kPa). This hypoxia limits oxygen consumption of embryos to low levels and appears to greatly slow or arrest embryonic development. From day 4 onwards the central region gradually becomes less hypoxic because the hatching of peripherally located embryos causes the outer layers of the jelly matrix to disintegrate and thus reduces the diffusion distance for oxygen between the centrally located embryos and surrounding seawater. As the oxygen tension rises, development accelerates and the embryos eventually hatch as viable veligers, apparently unharmed by their prolonged exposure to hypoxia.

## Changes in blood electrolyte levels over the laying cycle of the hen

*Cindy Brackpool, and Julie Roberts*

*Department of Physiology, University of New England, Armidale, NSW 2351.*

Blood samples were collected from laying hens, every two hours, for a period of 30 hours. Blood sample time was taken with respect to the time of the previous oviposition. Ionised calcium, sodium and potassium were analysed immediately after sample collection using an AVL Electrolyte Analyser. Plasma samples were analysed later for total calcium on a Cobas Bio Spectrophotometric Autoanalyser.

Following oviposition, plasma total calcium levels remained relatively constant until mid-cycle, then levels declined over the remainder of the cycle. However, blood ionised calcium concentrations exhibited a sigmoidal pattern, falling to their lowest levels in mid-cycle and then increasing over the second half of the laying cycle. Plasma sodium levels declined from 6 to 16 hours postoviposition and then increased during the second half of the cycle. The pattern for plasma potassium was different, showing an initial increase, falling to the lowest levels at mid-cycle and then increasing again. The changes in the plasma levels of these ions reflect the utilisation of calcium by the shell gland and the movements of sodium and potassium which accompany the transport of calcium from the blood to the developing egg shell.

Stress and metabolic response patterns in marsupials

*Adrian J. Bradley*

Department of Anatomical Sciences, The University of Queensland

\*POSTER

From a range of studies on marsupials representing different families it is apparent that in response to perceived stress, several different types of response patterns may be found.

By direct measurement or by inference the Macropodidae and Peramelidae appear to resist the diabetogenic and nitrogen mobilising effects of glucocorticoid hormones, glucocorticoids exert a diabetogenic and nitrogen mobilising effect in the Petauridae and Phalangeridae, while in the Dasyuridae there is diabetogenic resistance but nitrogen mobilisation. Catecholamines in all groups exert the typical diabetogenic effect.

How may these different response patterns be related to marsupial phylogeny and what advantages does resistance to both a diabetogenic effect and nitrogen mobilisation confer upon an individual exposed to a physiologically stressful environment ?

**The role of thyroxine in the development of the brushtail possum,  
*Trichosurus vulpecula.***

*W. Buaboocha and R.T. Gemmell*

*Department of Anatomical Sciences, University of Queensland*

The brushtail possum, *Trichosurus vulpecula*, has a gestation period of 17.5 days and gives birth to one young weighing about 200mg. The young weighs approximately 100g at day 100 and about 1000g when weaned about day 180. The increase in body weight of the developing possum can be described by two regression lines with a change in growth rate at day 96 post partum. This change in growth rate may be initiated by activation of thyroid function in the young possum. Plasma concentrations of thyroxine ( $T_4$ ) in the young were less than 10 ng/ml prior to day 60, 12 ng/ml on day 60, rising to a peak of 45 ng/ml on day 120 post partum then diminishing to below 10 ng/ml by day 160 post partum. Thyroxine can be transferred from the mother to the young via the milk. Thus, the plasma concentration of thyroxine in the young is due mainly to this transfer early in pouch life and due to the activation of the young's thyroid during the latter third of lactation. To determine the role of the thyroid gland of the young possum on growth, the production of thyroid hormones were inhibited by administration of the drug, methimazole. The drug was administered via Alzet minipumps to five lactating possums during the first half of lactation and to five possums between day 100 and 144 post partum. The effects of this treatment on growth were monitored. The results indicated that methimazole had a clear effect on the development of the possum when administered to the young at 100 days post partum.

Blood gas, acid-base and ionic status of the amphibious crab *Leptograpsus variegatus*

Sandra L. Butler and Steve Morris

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

*Leptograpsus variegatus* inhabits the supralittoral of eastern Australian and New Zealand coasts and can be found in air or water. This study assessed changes in blood-gas, acid-base and ionic status associated with the transition between air and water.

*L. variegatus* maintained acid-base balance more effectively in water than air, since pH changes of only 0.05 pH units occur over a 12 hour immersion period whereas changes of 0.18 pH units occur over 12 hours of emersion. The constancy of haemolymph pH in immersed crabs appears to be correlated with greater osmoregulatory ability in water. Neither osmotic pressure nor Na and Cl concentrations in the haemolymph differed when crabs were forced to breathe water. However, all three of these variables increased when crabs were emersed which is probably due to evaporative water loss as the animals are removed from water. In contrast to the maintenance of acid-base and ionic status in water, *L. variegatus* appears better adapted to extract O<sub>2</sub> from an aerial environment since the content of O<sub>2</sub> in the arterial blood is much higher in crabs kept in air than in water.

Therefore, *L. variegatus* appears to be an obligate amphibious crustacean, utilising the aqueous environment for acid-base and ionic balance and the aerial environment for O<sub>2</sub> uptake. Further studies are required *in situ* to verify these findings and to more fully determine the effects of longer term emersion.

Scent glands and reproduction in the Brown Antechinus (*Antechinus stuartii*)

Nicholas Casey and Adrian J. Bradley

Department of Anatomical Sciences, The University of Queensland

This study investigated seasonal variation in plasma testosterone concentration and sternal (gular) scent gland morphology from May to September 1994 at Mt Glorious near Brisbane. It was predicted that scent gland activity would be correlated with plasma testosterone with the approach of the breeding season. This prediction was tested both in the field and in the laboratory. In the field, scent gland activity increased with the approach of the September breeding season, however the correlation between morphological indicators of gland activity and plasma testosterone was not as high as expected. Testosterone administration to captive castrate males for 14 days also failed to unequivocally stimulate the gular gland.

Using implanted mini-osmotic pumps releasing gonadotrophin releasing hormone GnRH for 14 days an attempt was made to reverse the failure of spermatogonial development which occurs in this species. While GnRH treatment failed to re-initiate normal spermatogenic activity, Leydig cell activity was clearly stimulated as indicated by a significant increase in nuclear diameter. Dorsal paracloacal gland size was also stimulated by GnRH treatment.

\*POSTER

Endocrine cells in the heart of the Australian lungfish,  
*Neoceratodus forsteri*.

Lisa K. Chopin<sup>1</sup> and Michael B. Bennett<sup>2</sup>

*Department of Anatomical Sciences, University of Queensland*

There are three cell types within the heart of the of the Australian lungfish that exhibit the ultrastructural features of endocrine cells. The internal surface of the heart is lined by a continuous thin layer of endothelial cells which regularly contain electron-dense, membrane-bound inclusion bodies. These cells could have an unrecognised endocrine function. Many atrial myocardiocytes contain large numbers of highly electron-dense membrane-bound granules 100-200 nm in diameter. These organelles appear to be identical to atrial dense granules, which store atrial natriuretic peptide (ANP) in mammals, birds, reptiles, amphibians and fish and it appears likely that these cells represent the storage sites for similar biologically active peptides in the lungfish. Atrial peptides in the lungfish might regulate salt and water balance as they do in other species and could be involved in the regulation of the cardiovascular system. Chromaffin cells, homologous to adrenal medullary cells in mammals, are present in large numbers within the atrium of *N. forsteri*. These cells contain large numbers of highly electron-dense, membrane-bound granules. The presence of catecholamines within these cells has been confirmed using catecholamine fluorescence studies and tyrosine hydroxylase immunohistochemistry. Catecholamines released from atrial chromaffin stores are likely to play an important role in the regulation of the cardiovascular system of *N. forsteri* as an adrenergic nervous system appears to be poorly developed in this species. The co-localisation of ANP-like peptides and catecholamine storing-cells within the heart might have a functional significance as ANP is known to inhibit the release of catecholamines from mammalian chromaffin cells. These two substances released from the heart could interact in their regulation of the cardiovascular system of the lungfish.

The secondary vessel system of the estuarine catfish.

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The recent application of vascular casting and scanning electron microscopy to the microvasculature of teleosts has allowed the three dimensional demonstration of the secondary vascular system. Secondary vessels arise from the primary arteries via a multitude of tightly coiled capillary-like origins. These origins anastomose to form larger straighter vessels, which run into the secondary arteries. These secondary arteries run in parallel with the primary artery. The function and control of this system remains obscure, but some information has been gained by studying the ultrastructure of this system in the estuarine catfish. The origins of the secondary vessels are guarded by specialised endothelial cells which project large tentacular microvilli into the lumen of the primary vessel. These microvilli might act to elicit plasma skimming and to prevent red blood cells from entering the secondary vessel system. They might also trap white blood cells and recruit them into the secondary vessels. The secondary origins are the most likely site for the control of blood flow into this system. As these origins are invested within the smooth muscle of the primary artery, the vessel tonus of the primary vessel might be the most important means of regulating flow into this system. The capillary-like origins of the secondary vessels appear to be adapted for exchange processes. Pericytes which envelope the endothelial cells of the secondary vessel origins might aid in the regulation of blood flow through this vessel system.

\*POSTER



## Metabolic Adaptations of Reptiles to Dry Season Conditions in Tropical Australia

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Measurements of field metabolic rates of reptiles in the wet-dry tropics indicate a variety of responses to dry season conditions ranging from no seasonal differences to 75% reductions in energy expenditure by aestivating animals. Seasonal differences can result from one or more of the following: seasonal changes in body temperature, changes in activity, changes in standard metabolic rate (SMR). All of these are involved, but this talk will emphasise the results of laboratory measurements of SMR. Although the degree of metabolic depression exhibited by aestivating animals is remarkable, perhaps the most interesting adaptation involves intermediate changes in SMR. Several species of lizards (including *Varanus scalaris*, *Chlamydosaurus kingii*, and *Lophognathus temporalis*) reduce their SMRs during the dry season by 40-65%. This is particularly interesting given that they remain active (although activity is reduced) throughout the year. Acclimatisation of this sort, in which the animals remain active and are not limited by the thermal environment, has not been previously documented.

### Osmoregulatory, respiratory and acid-base responses of *Leptograpus variegatus* to emersion and hypo-saline water

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The osmoregulatory, acid-base and respiratory status of *L. variegatus* was assessed during simulated hypo-saline immersion. *L. variegatus* was shown to be a good osmoregulator, maintaining blood osmotic pressure well above that of the external medium. The high permeability of the gills to both  $\text{Na}^+$  and  $\text{Cl}^-$  required active uptake from the water to maintain ion balance. Some ion uptake was facilitated by a 2-fold increase in gill  $\text{Na}^+/\text{K}^+$ -ATPase activity. However, the low affinity ( $K_m$ ) and uptake rate ( $J_{max}$ ) of the gill  $\text{Na}^+/\text{K}^+$ -ATPase for  $\text{Na}^+$  suggests that the gills are not the major organ in ion-regulation in *L. variegatus*.

*L. variegatus* exhibited a marked acidosis due to both increased haemolymph  $\text{PCO}_2$  and metabolic acid production following immersion. The acidosis was partially compensated by *L. variegatus* immersed in dilute water, probably by increased pumping of  $\text{Na}^+$  into the blood (via increased basolateral  $\text{Na}^+/\text{K}^+$ -ATPase activity) coupled to the excretion of  $\text{H}^+$ . Thus ion regulation is linked with the maintenance of acid-base status. The increased oxygen consumption associated with increased  $\text{Na}^+/\text{K}^+$ -ATPase activity was met by increased blood perfusion and by increased ventilation and cardiac output.

Air-breathing *L. variegatus*, with only dilute drinking water available, decrease both the rate of drinking and the rate of urine excretion. Unlike some terrestrial species *L. variegatus* did not use the gills for absorption of ions from the urine. The inability of *L. variegatus* to completely maintain salt balance in air is likely a limiting factor in the adoption of a fully terrestrial existence.

Control of gut contraction by opioid agonists and RF-amide peptides  
in crickets and cockroaches

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The effect of the *kappa* opioid agonist U50488H on feeding of cockroaches and crickets was investigated, following a report that this drug stimulated feeding in cockroaches (Kavaliers *et al.* 1986). Injection of 1 mg/kg U50488H stimulated feeding in cockroaches, but no effect was found in crickets. To determine whether this increased feeding was a result of drug-induced foregut peristalsis, isolated foreguts of both species were exposed to different concentrations of the drug. Foregut contractions of cockroaches were slower and weaker than the contractions of the cricket in the absence of the drug. Addition of the *kappa* agonist to the bathing medium, increased the rate and strength of foregut contractions in both cockroaches and crickets, but only in the latter in a dose-dependent manner. Met-enkephalin (YGGFM) did stimulate the contraction of the cricket foregut, as did several FMRF-amide family peptides. N-extended FLRFamide peptides were inhibitory to cricket foregut contraction, but the tetrapeptide, FLRFamide, was stimulatory. The role of the opioid-type and the RF-amide peptides in control of foregut movement of insects requires further study, but our results suggest that crickets and cockroaches may differ in their responses to these chemicals.

Kavaliers, M., M.A. Guglick & M. Hirst (1987). *Life Sci* 40:665-672

**Myocardial fibre orientation in the ventricle of the rainbow trout**

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This study was undertaken to describe the patterns of myocardial fibre position and orientation within the wall of the ventricle. In all elasmobranchs and about 20% of teleosts the myocardium consists of an outer dense compact layer and an inner less-dense spongy layer.

Eight hearts were removed from anaesthetized trout and relaxed with cardioplegic solutions before being filling with buffered 10% formol saline and ligated. Most hearts were dissected under binocular microscope. Some were prepared for histological analyses.

Two distinct layers were found in the compact layer. Fibres from neither of these layers were continuous with fibres of the inner spongy layer. Patterns of fibre orientations were unique for individual fish but generalisations can be made. The superficial compact layer had on its lateral and caudal faces bundles of fibres running parallel to the all edges except the edge shared with the base. Fibres running from the lateral faces onto the base passed over the base toward the bulboventricular orifice where they inserted. Fibres from the caudal face that passed to the base run toward the atrioventricular orifice where they inserted.

The deeper compact layer consisted of highly ordered fibre bundles more closely packed than those of the superficial compact layer. Fibres of the deep and superficial compact layers were seen to run at angles to each other greater than 45°. Fibres of the deeper layer were arranged in circular patterns about the vertices and covered a smaller surface than fibres which encircle the apex.

The spongy layer had fibres which were not in any recognisable pattern. Bundles of fibres formed interconnecting networks throughout the tissue.

The role of compact layer in pressure generation has been suggested and the mechanisms by which this could be achieved will be explored. Changes in the size and geometry of the heart during contraction will be discussed.

The Physiological Dependency of the Christmas Island blue crab,  
*Cardisoma hirtipes*, on freshwater.

Jocelyn Dela - Cruz and Stephen Morris  
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On Christmas Is., *Cardisoma hirtipes* is found around freshwater seepages. Their physiological dependency on water was determined by examining gas exchange, acid-base balance, ion-water balance and nitrogenous waste excretion in both air and water.

When fully submerged the oxygen uptake rate of *C. hirtipes* decreases by at least 50%, compared to rates in air. The O<sub>2</sub> content and partial pressure in pulmonary and arterial blood decreased after 30 min submersion. Although O<sub>2</sub> delivery to the tissues was compromised, there was no increase in haemolymph lactate. The haemocyanin has a high affinity for O<sub>2</sub>, in water affinity is further increased by urate. In both air and water blue crabs maintained acid-base balance, with an average haemolymph pH of 7.6.

When held in air for 9 days, with only drinking water provided, *C. hirtipes* minimised water loss by complete cessation of drinking and "P" production. Ion balance was also maintained by urinary reprocessing, reabsorbing over 90% of Na<sup>+</sup> and Cl<sup>-</sup>.

Cessation of "P" production means that a potential avenue for nitrogenous waste excretion is lost. However, for blue crabs "P" was not a major pathway used to eliminate nitrogen. Similarly gaseous ammonia and faecal uric acid contributed negligible amounts to the crabs net nitrogen lost. However, when submerged the blue crabs excrete ammonia at a rate significantly higher than is excreted into air. Clearly, *C. hirtipes* is dependent on water to eliminate its nitrogenous wastes.

NATRIURETIC PEPTIDE RECEPTORS IN FISH GILLS

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The presence of natriuretic peptides (NP) in the heart and brain of cyclostome, elasmobranch, and teleost fish is well-established. If the biological role of NPs in fish is osmotic and ionic homeostasis, as it is in mammals, then the gills are a potentially critical target organ for the actions of NPs. The biological effects of NPs are mediated by cell surface receptors which have an intracellular guanylate cyclase (GC) domain and use cGMP as a second messenger. An additional function of the gills is uptake and metabolism of circulating substances. In mammals, the levels of circulating NPs are regulated in part by a specific type of NP receptor (NPR) called a 'clearance receptor', or C-receptor (NPR-C), which binds NPs and internalises the peptide for metabolism but is not linked to a guanylate cyclase. Since all blood must pass through the gills before distribution to the body the presence of NPR-C in the gills is potentially important in the regulation of plasma NP levels. In this study, the distribution and nature of NPR in the gills of hagfish, *Myxine glutinosa*; dogfish, *Squalus acanthias*; and toadfish, *Opsanus beta* was determined. In *Myxine*, tissue section autoradiography showed NP binding sites only on the lamellar epithelium, however, in *Squalus* binding occurred on the secondary lamellae, the filament epithelium, and on some blood vessels. In contrast, binding in the gills of *Opsanus* was restricted to the afferent and efferent filamental vasculature. Analysis of the binding sites using competition and GC assays showed that the gills of each species contained at least two types of NPR. The first receptor contains an intracellular GC domain and NP binding stimulates the production of cGMP. The second receptor binds C-ANF, a truncated NP that binds only to the mammalian NPR-C and not the GC receptors. In this study, C-ANF did not stimulate cGMP production and did not compete for all binding sites. These data indicate the presence of two types of NPR: a receptor with an intracellular GC domain and a receptor which may be similar in nature to mammalian NPR-C. Thus, the gills are an important effector tissue in the physiology of the NP system of fish. However, the variable distribution of binding sites in the gill tissues suggests that NPs have evolved a diversity of roles in regulating gill function. Supported by NSF DCB 8916413; NIHEHS-P30-ESO3828.

# Scaling of Respiratory Variables and the Breathing Pattern in Adult Marsupials

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Oxygen consumption ( $\dot{V}O_2$ ) and a number of components of the breathing pattern were measured in 14 species of non-fossorial marsupials ranging in mass from 0.008 to 30 kg. All the exponents of the allometric relationships for  $\dot{V}O_2$ , ventilation ( $\dot{V}_E$ ) and breathing pattern scaled as previously determined for eutherians. However, compared to eutherians, marsupials had significantly lower  $\dot{V}O_2$  and the breathing pattern was deeper (+23%) and slower (-31%). While  $\dot{V}_E$  was not significantly below that reported for eutherians it matched  $\dot{V}O_2$  such that  $\dot{V}_E/\dot{V}O_2$  remained mass-independent and at a level similar to that observed in the other infraclasses of mammals. Thus, it would appear that the increase in metabolic rate that occurred during the evolution of mammalian homeothermy was accompanied by parallel changes in  $\dot{V}_E$ . It is suggested that these changes in  $\dot{V}_E$  were mediated by a shortening of the respiratory cycle, facilitated by the eventual abolition of the end-expiratory pause ( $T_P$ ), and an increase in respiratory drive ( $V_T/T_I$ ).

In response to 5%  $CO_2$  all animals in this study increased their  $\dot{V}_E$  by increasing both tidal volume ( $V_T$ ) and frequency ( $f$ ), predominantly through the removal of  $T_P$ . The increase in  $\dot{V}_E$  was less than previously reported for eutherians, suggesting a reduction in chemosensitivity in marsupials. Furthermore, the similarity in slopes for  $\dot{V}_E$ ,  $V_T$  and  $f$  between air and 5%  $CO_2$  suggests that the gain of the respiratory system is independent of species size within marsupials.

## Effects of dietary fats, photoperiod, temperature, and season on morphological variables, torpor, and BAT fatty acid composition of hamsters, *Phodopus sungorus*

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We investigated how dietary fats and oils of different fatty acid composition influence the seasonal change of body mass, fur colour, testes size, torpor and brown adipose tissue (BAT) fatty acid composition of Djungarian hamsters, *Phodopus sungorus*, which were maintained from September to January (autumn and winter) under different photoperiods and temperature regimes. Dietary fatty acids influenced the occurrence of torpor in hamsters maintained at 18°C under natural and artificial short photoperiods. Torpor was most pronounced in individuals on a diet containing 10% safflower oil (rich in polyunsaturated fatty acids), intermediate in individuals on a diet containing 10% olive oil (rich in monounsaturated fatty acids) and least pronounced in individuals on a diet containing 10% coconut fat (rich in saturated fatty acids). Torpor in hamsters on chow containing no added fat or oil was intermediate between those on coconut fat and olive oil. The BAT fatty acid composition from hamsters maintained at 18°C and under natural photoperiod strongly reflected that of the dietary fatty acids. Dietary fatty acids had little effect on torpor in animals maintained within the thermoneutral zone at 23°C. Hamsters maintained under long photoperiod were never observed in torpor irrespective of their diet. Body mass, fur colour and testes size were also little affected by dietary fatty acids. Our study suggests that the seasonal change of body mass, fur colour and testes size of *P. sungorus* are not strongly influenced by dietary fatty acids. However, dietary fats influence the occurrence and depth of torpor in individuals maintained at low temperatures and which have been photoperiodically primed for the display of torpor.

\* POSTER

The role of the fetal thyroid gland on growth and organ development in two eutherians, the rat and sheep, and two marsupials, the possum and bandicoot.

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Eutherians have a relatively long gestation period and a short lactation period whereas marsupials have a short pregnancy and a long lactation period. Eutherians are born at different stages of development. The rat has a gestation of 22 days and gives birth to an altricial litter, each young weighing approximately 5 grms is hairless, blind and resides in a nest. The sheep has a gestation of 147 days and gives birth to a precocial lamb and has a body weight of approximately 4000 grms has hair, sight and can walk. Marsupials give birth to altricial young, less developed than any eutherian young. Birth is an event that occurs during the development of all young and requires the maturation of certain organ systems for the young to survive. All four of the above species have a functional anterior pituitary and adrenal gland axis at birth. All possess an olfactory system, a utricule, functional lungs and limited motor skills. The obvious differences in development at parturition may be due to the initiation of thyroid activity in the developing young. In this paper a comparison will be made between the role of the thyroid gland in the development of the eutherian (rat and sheep) and marsupial (possum and bandicoot). The timing of the activation of the thyroid gland in both groups of mammals will be examined to determine the advantages of the various modes of reproduction.

Short term changes in physiological indicators of stress and reproductive function following odour presentation in the Brush-tail Possum (*Trichosurus vulpecula*)

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Several physiological parameters were monitored in both male and female *T. vulpecula* fitted with chronically indwelling jugular catheters to allow serial blood samples to be taken during presentation of odours from the sternal (gular) and oil secreting paracloacal glands. The physiological parameters measured included heart rate, blood glucose and plasma free cortisol concentration. To assess effects on the sympatho-medullary axis plasma noradrenalin and adrenalin were measured using HPLC-electrochemical detection. To assess effects on the pituitary-gonadal axis, plasma testosterone and oestradiol concentrations were measured in males and females respectively.

After establishing a stable control baseline for heart rate and blood glucose concentration the presentation of an unfamiliar odour caused increases in heart rate, plasma adrenalin and noradrenalin, and plasma free cortisol concentrations. Generally, the plasma adrenalin concentration was higher than the noradrenalin concentration. With a longer time lag, increases occurred in plasma glucose concentration. Plasma testosterone and oestradiol concentrations were also affected by the odour presented indicating an effect upon the pituitary-gonadal function.

\*POSTER

Dietary assimilation in a herbivorous terrestrial crab, *Gecarcoidea natalis*.

Peter Greenaway & S.M. Linton  
School of Biological Science, UNSW

*Gecarcoidea natalis* feeds chiefly on litter falling from rainforest canopy; predominantly leaves but also flowers, fruits bark etc. The ability of *Gecarcoidea natalis* to assimilate salts, nitrogen, energy and fibre components from fallen leaves of *Ficus macrophylla* and *Erythrina variegata* will be discussed. Dry matter and energy assimilation coefficients were closely similar (41-46%). Assimilation of nitrogen was 39-49.2% respectively for the two diets while N retentions were 1.5 and 4.2 mmol.kg<sup>-1</sup>.d<sup>-1</sup>. Neutral detergent soluble materials were the main components of dry matter assimilation on both diets (assimilation coefficients 42.7±12.6 and 58.3±9.3%) but hemicellulose was also well assimilated from leaves of *Ficus* (49.2%) and was a major component of assimilated dry matter. A substantial ability to digest cellulose in both leaf types was evident (42.6% and 37.7%). Lignin assimilation was very low on leaves of *Erythrina* (8.7%) and the high assimilation of lignin from leaves of *Ficus* (39.1%) may have represented tannins. Food retention time measured on a diet of *Erythrina* was quite short (mean retention time 11.6±5.42h) indicating a fairly rapid passage of digesta.

Oxygen carriage in embryonic blood of marsupial and eutherian mammals.

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In the earliest stages of mammalian development there are special embryonic hemoglobins whose  $\alpha$ -like and  $\beta$ -like globin chains differ considerably from the  $\alpha$  and  $\beta$ -like chains of adult and fetal Hb. In addition, the embryonic red cells are large and nucleated. However because of the difficulty in getting enough blood for study, there has been relatively little done on oxygen carriage by embryonic blood. Now, using a thin-film method (modified Hemoscan) an oxygen equilibrium curve (OEC) can be done on as little as 1-2  $\mu$ l blood. OECs have been determined and a Hill plot made using the logarithmic form of the Hill equation.

$$\log \left\{ \frac{\text{saturation}}{1 - \text{saturation}} \right\} = nH (\log PO_2 - \log P_{50})$$

The slope of the plot,  $nH$ , is an index of cooperativity indicating how many O<sub>2</sub> molecules combine or dissociate together. The OECs of rabbits and pigs were left-shifted compared with maternal OECs, but less so than the fetal OECs. In the tammar wallaby and possum the OECs were to the right of the maternal. In the fat-tailed dunnart the rather right-shifted embryonic OEC was to the left of the very right-shifted maternal OEC. In all five species studied, the Hill plot was bent about the middle with  $nH$  in upper part being greater than 4. This indicates aggregation of hemoglobin tetramers. In all species the CO<sub>2</sub>-O<sub>2</sub> interaction (Bohr effect) was decreased, this almost certainly being caused by blocking of the N-terminal  $\alpha$ -amino group in embryonic  $\alpha$ -like globin chains.

Many colleagues co-operated in this work and will be acknowledged in the presentation. Support was from the Australian Research Council.

## How Does the Fish Do It? Problems of Fish Eye Growth

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The fish is faced with the problem of maintaining appropriate eye structure during growth. Narrow structural tolerances must be held to allow sharp image formation. A detailed computer model of the optics of an eye based on anatomical measurements allows insight into its design, optical function and growth. Contact with reality is maintained by detailed measurements of actual optical function.

The fish eye offers a relatively simple starting point for understanding the structure and function of a general vertebrate lens and eye. The cornea, with fluid on both sides, is optically ineffective, abandoning the task of forming an image to the lens. Unlike a glass or crystal ball, the nearly spherical fish lens has a strong gradient of refractive index, increasing from the cortex to a much higher value at the core. This gradient causes rays inside the lens to follow curved paths, adding power to the lens, and allowing light to come to a much sharper focus than would be possible for a homogeneous glass lens.

Biological lens material, unlike glass, has irregular cell structure similar in size to the wavelength of light. This structure interacts with light, interfering with optical function.

Another problem is that the refractive index of lens material and hence lens power varies with wavelength. Although this is in principle correctable, as in modern manufactured optics, it is not corrected in an eye, resulting in a multicoloured image.

The most formidable problem is maintenance of an appropriate index gradient shape during lens growth. Precise control of the gradient form is required while the lens grows, in order to maintain a sharp image. Certainly a very sophisticated feedback control mechanism is in play.

### Breeding Australian lungfish in captivity

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In 1992, Macquarie University began developing a facility for lungfish research. It now comprises 2 large earthen ponds (15X50m each), 5 large holding tanks, a well-equipped laboratory in close proximity to the holding tanks and a soon-to-be-constructed aquarium room for rearing young lungfish. The ponds have a shallow (0.5m depth) end planted with *Vallisneria* and a deep (2.5m depth) end. To maintain physical conditions appropriate for keeping fish, floating islands of *Eichornia* and paddle wheel aerators were introduced. The ponds were completed in September, 1992 and one of these was stocked progressively over the ensuing 12 months with 9 adult lungfish, obtained from several sources and representing fish originally derived from the Brisbane, Mary and Burnett Rivers.

In December 1993, the lungfish began exhibiting spawning behaviour and soon after eggs were collected and incubated in sterile pond water. They hatched early in January and, although many have been used experimentally, the remainder are growing well in small aquaria. The majority of the eggs were left in the pond and periodically sampled to compare growth rates with those in the aquaria. Unfortunately, the ponds became attractive to several fishing birds in mid-1994 and many of the young lungfish were lost before the ponds could be completely enclosed in bird-proof netting.

This year spawning began again in October so that it now appears likely that these lungfish will breed naturally in captivity on an annual basis, thus providing large numbers of larval, juvenile and later adult lungfish for ontogenetic and physiological studies.

Thyroid function, reproduction and physical condition during life history  
in the Fawn-footed Melomys (*Melomys cervinipes*)

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This study investigated seasonal variation in thyroid hormone concentration in a field population of *Melomys cervinipes* from May to September 1994 at Mt Glorious near Brisbane. While there was no significant seasonal variation in thyroid hormone concentration, mean monthly  $T_4$  concentrations increased markedly from June through to September. Plasma testosterone concentrations in male *M. cervinipes* decreased consistently from May through to August with a significant rise in testosterone occurring between August and September.

Based upon morphological measurements a physical condition index was calculated for individuals within the population and this was related to both thyroid function and reproduction.

Body temperature rhythms and activity in reproductive male *Antechinus stuartii*  
(Marsupialia)

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To determine whether locomotor activity and body temperature ( $T_b$ ) rhythms change during the mating season of male *Antechinus stuartii* and whether that could contribute to post-mating die-off, we measured both variables in individuals kept in the laboratory under natural photoperiod. Activity was measured with passive infrared detectors and  $T_b$  was measured with implanted temperature-sensitive transmitters. Before the mating season *Antechinus* were mainly nocturnal. However, during the mating period (end of August) males showed a markedly elevated activity level and some animals extended their activity period well into the light phase. After about two weeks, normal activity patterns were restored. Activity was always accompanied by elevated  $T_b$ s, whereas resting phases were characterized by low  $T_b$ s. During the mating season the average daily  $T_b$  was increased because animals maintained high  $T_b$ s for longer time periods and because the resting  $T_b$  was also elevated.

The rise of both,  $T_b$  and locomotor activity during the mating season indicate that energy expenditure is increased even when agonistic encounters between males and mating are excluded. However, under laboratory conditions with food *ad libitum* and without severe cold stress and social interaction, male *Antechinus stuartii* were able to avert post-mating die-off.



## The effect of exogenous aldosterone on renal function in chickens

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The effect of aldosterone on renal function in chickens was investigated. Two groups of birds were used, a control group and a group which was infused with aldosterone. Birds were anaesthetised with DIAL, one wing vein cannulated for the infusion of renal function markers and aldosterone, a carotid artery cannulated for collection of blood samples and the hindgut tied off to allow collection of urine uncontaminated with faeces. In the control group, birds were infused with isotonic sodium chloride solution containing 200 mg/100 ml inulin and 200 mg/100 ml PAH for a total of 8.5 hours. Urine samples were collected after the first 30 minutes and then hourly. Blood samples were collected before and after the urine collections. In the aldosterone-infused group, the same infusion solution as for the control group was used. However, after the first 30 minute collection, aldosterone was added to the infusate ( $12.5 \mu\text{g}\cdot\text{ml}^{-1}$ ) following a bolus injection of 125  $\mu\text{g}$  of aldosterone. Blood samples were centrifuged and the plasma reserved to allow for assays for the hormones aldosterone and prolactin, chemical analyses for the renal function markers and analyses for sodium, chloride, potassium and osmolality. Urine samples were analysed for electrolytes and renal function markers.

The infusion of the exogenous aldosterone produced plasma levels in excess of 1  $\text{ng}\cdot\text{ml}^{-1}$  and these high aldosterone levels had some effects on renal function. Sodium and chloride excretion were lower in the aldosterone-infused group 150-210 minutes into the experiment. However, by 330-390 minutes, the values were similar in the two groups. The clearance of PAH was consistently higher in the aldosterone-infused group. The infusion of aldosterone had no effect on plasma prolactin levels which remained low throughout the experiments in both groups.

## OSMOTIC AND IONIC REGULATION IN DEVELOPING EGGS OF AN INTERTIDAL CRAB

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### ABSTRACT

The osmolality and the total volume of developing eggs of *Heterozius rotundifrons* were measured after 24 hours exposure to different salinities at 15°C. The solvent volume was also estimated by a dilution method, using a vapor pressure osmometer. The developing eggs osmoregulated and also regulated their volumes in hyposaline and hypersaline media. Almost 100% of eggs survived exposure to 50% seawater for 96 hours exhibiting hyperosmotic regulation in this media.

The overall cation concentrations in developing eggs were measured by AAS after 24 hours exposure to different salinities at 15°C. Sodium and potassium were the main cation in the eggs. Sodium concentration in eggs at the early stage acclimated to 100% seawater were much lower than in the medium, whereas potassium concentrations were three times higher. Magnesium concentrations were very low at the early stage eggs, but increased strongly with their development. The calcium concentration at the beginning of development was lower than in the medium. All cation concentrations increased during development and all decreased with exposure to lower salinities. The proportional change was greater for sodium and magnesium and least for potassium and calcium.

Nitrogenous excretion in the amphibious crab *Holthuisana transversa*

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*Holthuisana transversa* is an amphibious crab from the arid and semi arid regions of Australia. In this habitat it tolerates extreme conditions of drought, flooding and heavy rain. During drought the adult lives in a dry burrow and emerges to forage after flooding and heavy rain.

Nitrogenous excretion was examined under wet and dry conditions. Crabs were kept in metabolism chambers with and without access to water and with and without food. The main nitrogenous excretory product was ammonia. This was excreted across the gills into the water. Faeces, excretory fluid released and ammonia gas volatilisation were minor routes of excretion. Negligible amounts of nitrogen were excreted under simulated drought conditions (starved with no access to water). Any nitrogen produced during this time may have been stored.

Primary and secondary stress responses to line capture in the marine teleost, Blue Maomao (*Scorpius violaceus*)

Timothy E. Lowe and Rufus M.G. Wells

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Stress responses in fish are characterised by a surge of catecholamines. The effects of catecholamines on blood oxygen transport in fish can be very dramatic, and are thought to have adaptive significance during exercise (Thomas and Perry, *J. Exp. Zool.* 263: 160, 1992). To date, the action of catecholamines on blood oxygen transport has been limited mainly to salmonids and laboratory acclimated fish.

Wild fish were played on a rod and reel from 20 sec to 30 minutes and compared with laboratory acclimated fish either at rest or exercised for up to 5 minutes. Adrenaline levels ranged from 13-315 nmol L<sup>-1</sup> in wild fish, while an increase from 1.5 to only 30.5 nmol L<sup>-1</sup> was measured in laboratory fish. Erythrocyte swelling, an indicator of an adrenergic response, was clearly observed in wild fish, but not in exercised laboratory acclimated fish. Exercise did not result in changes to hemoglobin nucleotides (modifiers of oxygen affinity) in either wild or laboratory acclimated fish, but levels of GTP and ATP were significantly lower in wild fish. However, the total erythrocyte nucleotide pool was similar in both groups and is explained by high levels of di and mono-phosphates in the wild fish.

In conclusion, laboratory acclimated fish appear unable to manifest the stress response observed in wild fish. Furthermore, low levels of routine activity in captive fish may result in lower hemoglobin oxygen affinity, than that in spontaneously more active wild fish.

**Effects of cortisol and testosterone administration on renal structure and function in *Antechinus stuartii*.**

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Renal structure and function change seasonally in *Antechinus stuartii*. These changes are coincident with the hormonal changes observed in *A. stuartii*. Therefore we determined the hormonal effects on renal physiology, of depot injections of synthetic, long-acting cortisol ("Depredil", 75 mg/kg/dy), testosterone ("Durateston", 150 mg/kg/dy), testosterone and cortisol, in comparison to saline on male *A. stuartii* in May, when native hormone levels are normally low.

Glomerular filtration rate was determined prior to hormone injections and then four weeks after the initial injection. Body weight, paired kidney weight, kidney size, paired adrenal weight, prostate weight, bulbourethral gland weight and testes weight were measured after sacrifice. Glomerular filtration rate was somewhat reduced in males treated with cortisol and testosterone, although this was not significant. The kidney weights of cortisol treated males were larger than all other groups. The kidney sizes of testosterone treated males were significantly smaller than saline and cortisol treated groups. Body weights of testosterone treated males were significantly larger than all other groups. The weights of bulbourethral and prostate glands of testosterone treated males were larger than all other groups and those of the cortisol and testosterone treated males were larger than those of the saline or cortisol treated males. The paired adrenal glands of the testosterone and cortisol treated males were smaller than the saline treated males. Many of these changes mimic those of wild *Antechinus stuartii* in August (when the males die), although some results, such as decrease in renal function, were less pronounced.

**Vasoconstriction in response to galanin in three species of elasmobranchs**

***Clare McManus, Elaine Preston, Ann-Cathrine Jonsson<sup>1</sup> and Gillian Courtice***

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The neuropeptide, galanin, is found in perivascular sympathetic neurons in a wide range of vertebrate species. Although in eutherian mammals, galanin has either no effect on blood pressure (BP), or has weak depressor effects, it has been shown to be a potent pressor agent in 2 species of marsupial and 1 amphibian, and causes contraction in isolated teleost vascular strips. To investigate the vasoconstrictor effects of galanin in another phylogenetic group, we tested the BP response to intravenous porcine galanin (Peninsula, 20µg/kg) in 3 species of anaesthetised elasmobranchs, the Port Jackson shark *Heterodontus portusjacksoni*, the Epaulette shark *Hemiscyllium ocellatum* and the Giant Shovelnose ray *Rhinobatos typus*. Contraction of the isolated pancreatico-mesenteric artery in an organ bath was measured also in response to increasing doses of galanin (to 10<sup>-6</sup>M). Galanin caused a significant rise in mean caudal arterial BP in *H. portusjacksoni* (P<0.001, n=6) and *H. ocellatum* (P<0.01, n=7), but no change in *R. typus*. Galanin (10<sup>-6</sup>M) caused 30-40% of the maximum K<sup>+</sup> induced contraction in the isolated gut artery in all species. In *R. typus*, isolated efferent branchial arteries were tested also, but galanin caused no response. Parallel immunohistochemical studies showed that galanin was located in perivascular nerves surrounding the pancreatico-mesenteric artery but not the efferent arteries of all 3 species. In conclusion, galanin, or a galanin-like peptide is present in nerve fibres surrounding some blood vessels of these elasmobranchs and it causes differential vasoconstriction in various vascular beds. These data extend the number of vertebrate groups in which galanin has been shown to be a vasoconstrictor peptide.

**Which part of the galanin molecule is responsible for its vasoconstrictor activity in toads?**

David Mahns and Gillian Courtice

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Galanin is a 29 or 30 amino acid neuropeptide which has a widespread distribution in both the central and peripheral nervous systems. In particular it is found in perivascular sympathetic neurons in a wide range of vertebrate species. Although in eutherian mammals, galanin has either no effect on blood pressure (BP), or weak depressor effects, it is potent at raising BP in the Cane toad, as well as in a number of other vertebrate species. Various fragments of the galanin molecule were tested for their vasoconstrictor activity in the Cane toad, *Bufo marinus*, with a view to isolating segments of the molecule responsible for its vasoconstrictor action. In response to galanin or a fragment, changes in BP were measured in anaesthetised toads, and changes in tension of isolated segments of mesenteric artery were measured in an organ bath. Results from a series of fragments representing regions throughout the molecule showed that the order of potency of the BP response was 1-18=1-16>1-15>5-20>10-25=15-30=21-29, the last 3 showing no activity. Thus, vasoconstrictor activity was dependent on the N terminal region of the molecule being intact. In a 2nd series of experiments, the galanin fragment 1-18 was tested in whole animals with each amino acid in turn replaced with an alanine residue. Vasoconstrictor activity was abolished when either the 2nd or the 9th amino acid was replaced with alanine. In conclusion, the N-terminal region of the galanin molecule is necessary for vasoconstrictor activity and the 2nd and 9th amino acid represent areas of the molecule critical for its biological activity.

Sensory processing in the fish brain: cerebellar circuits and synaptic plasticity  
discriminate signals from self-generated noise.

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In both the electrosensory system of elasmobranch fishes, and the mechanosensory lateral line of all fishes, sensory reafference generated by the animal's own movement is a significant source of unwanted noise. Behavioural, anatomical and physiological processes operating at the level of the sense organs minimise, but cannot eliminate the problem, so central mechanisms exist to discriminate between real signals and self-generated noise. Secondary sensory neurons have excitatory and inhibitory receptive fields which have the effect of reducing reafference which is common to receptors. This "common-mode" suppression is likely to be particularly effective in reducing electrosensory reafference generated by ventilation, since this form of reafference is very similar in all receptors. More complex forms of reafference are generated by fin movements, and in the mechanosense by ventilation movements. This reafference is removed in the hindbrain by a cerebellar-like circuit which uses information the animal has about its own movements (efference copy signals, proprioception, and other sensory input) to generate a cancellation signal which nulls the unwanted sensory reafference. In both electrosense and mechanosense, secondary sensory neurons learn to cancel any stimulus which is coupled to the animal's own movement. This adaptive capability can be attributed to synaptic plasticity in the cerebellar-like part of the hindbrain nuclei which process electro and mechano-sensory inputs.

**Aquatic hypoxia as an impetus for breathing air: The Australian Yabby  
*Cherax destructor* as a case study.**

Stephen Morris and Jonathan Callaghan

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The yabby, *Cherax destructor*, has a wide distribution that seems dependent on colonising isolated and temporary water bodies. Thus the ability to breathe air may be important when habitat conditions deteriorate. The respiratory and metabolic responses of *C. destructor* were assessed with respect to aquatic hypoxia and air breathing.

*Cherax destructor* increases ventilation rate to compensate for decreased  $O_2$  ( $P_{wO_2}$ ) and possesses a haemocyanin with a high  $O_2$  affinity to maximise  $O_2$  extraction from the water but is not an oxyregulator. *C. destructor* remains aerobic only by entering a hypometabolic state with lowered  $O_2$  demand. A  $P_{wO_2}$  of 20 torr was insufficient to support respiration and *Cherax* increasingly supplements production by a progressive reliance on anaerobiosis. *C. destructor* survives prolonged hypoxia but is limited, by meagre glycogen stores and by unusually high rates of anaerobiosis and hence fuel utilisation, to  $\approx 5$  hours.

When in hypoxic water *C. destructor* clearly prefers to breathe air, since when immersed at very low  $P_{wO_2}$  it spent more than 60% of the time out of the water. The ability of *C. destructor* to breathe air was the most advanced of any crayfish species examined to date. The movement from hypoxic water to air promoted an increase in haemolymph  $O_2$  levels, although there was no concomitant increase in the  $O_2$  affinity of the respiratory protein, haemocyanin. *C. destructor* is well adapted to breathe air and will leave the water, an ability that may extend the distribution range away from permanent water bodies and provide an insight as to impetus and conditions encouraging the evolution of obligate air breathing in the crustacea.

The importance of touch in the Northern Quoll (*Dasyurus hallucatus*)  
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Northern Quolls with normal senses as well as quolls with the vibrissae cut or hands anaesthetised were videorecorded while they were reaching through a hole to obtain food pellets, and while they were catching crickets. Analyses of these recordings revealed that the wrist (ulnar carpal) vibrissae and the vibrissae on the upper lip provided information to guide the reach, while the hand provided information on the form and timing of the grasp. The results indicate that touch rather than vision is the sense used by the quolls for reaching out and grasping objects close to the head.

Control of respiration in hibernating echidnas  
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Resting, euthermic echidnas have very slow respiratory rates, with frequencies as low as 6 breaths/minute. During hibernation echidnas show not only a very great reduction in average respiratory frequency (0.2 breaths/min at  $T_b = 10^\circ\text{C}$ ), but also a change in respiratory pattern. Instead of the steady, continuous respiration shown by euthermic animals hibernating echidnas show periodic respiration, with bursts of about 30 breaths. Average time between bursts is more than 2 hours, and the interburst periods are punctuated by occasional single breaths or small bursts. Changes in ambient temperature, body temperature and inspired gas composition can alter this pattern. Moderate hypercapnia increases the number of breaths in a burst, while higher levels totally abolish the breaks between bursts. Hypoxia appears to stimulate the interburst breaths and abolish the bursts themselves. Hyperoxia abolishes the interburst breaths, and dramatically increases the time between bursts.

The Respiratory and acid base response of the Southern Rock Lobster *Jasus edwardsii* to low temperature and air exposure: an application to the shipping methods in the live seafood market.

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The Southern rock lobster *Jasus edwardsii* is an important component of the Tasmanian and New Zealand fisheries. Lobsters are transported live in air for up to 30 h where their survival and subsequent market value is often threatened by sudden changes in temperature and handling.

Lobsters exposed to air exhibited a hyperventilation and a tachycardia. Oxygen consumption was depressed, confirmed by the reduction in blood  $P_{O_2}$  and  $CO_2$ . There was a large respiratory and metabolic acidosis with anaerobic metabolism contributing the major supply of energy. *Jasus edwardsii* subjected to the common practice of "chilling" or the cold-shock treatment prior to shipment were not heavily reliant on anaerobic metabolism for their energy needs and  $O_2$  transport in the blood was enhanced through the chilling process. Levels of IMP remained low in emersed animals, maintaining the fresh seafood taste.

By subjecting live lobsters to the cold-shock treatment prior to shipment, animals avoid a large blood acidosis and a significant dependence on anaerobiosis. Taste can be maintained for up to 30 h and the lobsters reach their destination in an active state, without any irreversible physiological effects.

A lactic acid "ceiling" arrests profound acidosis in the haemolymph of an ion-conforming crab, (*Ranina ranina*), during emersion at 25°C

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The gas, acid-base balance and the dynamics of lactic acid accumulation were studied in the haemolymph of the spanner crab *Ranina ranina* during emersion at 25°C. This crab is marketed live and is typically stored out of water after harvest for up to 8 hours or more. Spanner crabs could not regulate their haemolymph pH during emersion, showing an uncompensated respiratory and metabolic acidosis during the first couple of hours out of water. In this respect they resembled other crabs that are ionic conformers. After 3 hours in air, the rate of acidosis slowed. Haemolymph calcium concentration did not rise at this point and there was no evidence of a large rise in total carbon dioxide concentration in the haemolymph which normally accompanies compensation responses. Instead, the fall in haemolymph pH mirrored the rise in lactic acid concentration in the haemolymph. Both symptoms remained relatively constant after 3 h out of water. Apparently, the spanner crab's only means of surviving for long periods in air is to stall the entry of  $CO_2$  and lactic acid into the haemolymph, perhaps by reducing its overall demand for oxygen. Storing spanner crabs in air for any period causes unacceptable amounts of stress unless steps are taken to reduce the animal's metabolic rate by lowering the temperature and thereby curtail the build up of harmful wastes in the crab.

**Comparative vascular responses in elasmobranchs to different structures of Neuropeptide Y (NPY) & Peptide YY (PYY).**

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NPY and PYY are peptides belonging to the pancreatic polypeptide family, both causing vasoconstriction in mammals. NPY has been well conserved during evolution with a 92% homology seen between shark and human NPY. NPY and PYY share 86% homology in the shark, but only 60-70% in mammals. Experiments were carried out in 3 species of elasmobranchs, the Port Jackson shark, *Heterodontus portusjacksoni*, the Epaulette shark, *Hemiscyllium ocellatum* and the Giant Shovelnose ray, *Rhinobatos typus*. Blood pressure was measured in anaesthetised fish, and contraction of isolated mesenteric arteries were recorded in an organ bath to test 1. whether the conservation of NPY structure is reflected in the vascular response to shark, frog or human NPY, 2. whether there are differences in the vascular response to NPY and PYY and 3. what type of receptor mediates the vascular response. In all 3 species, all types of NPY caused a similar vasopressor effect except human NPY, which lowered BP in Portjackson sharks. NPY and PYY both contracted isolated gut arteries and were equipotent at raising BP in 2 species, but PYY was more effective than NPY in the Epaulette shark. The response is likely to be mediated via a Y1-receptor, since Y1 specific agonists were usually more potent than Y2 agonists in whole animals and isolated vessels. Parallel immunohistochemical studies in the 3 species show that NPY is located in perivascular nerves surrounding the pancreatico-mesenteric artery but generally not the efferent arteries. In conclusion, NPY is present in nerve fibres surrounding some blood vessels in the 3 elasmobranchs and causes a vasopressor response which may be mediated via a Y1-like receptor. Thus, the vascular function of NPY and PYY appear to be conserved also during evolution.

Social organisation, stress and population regulation  
in the Fawn-footed Melomys (*Melomys cervinipes*)

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Evidence from a limited number of studies indicates that the abundance of *Melomys cervinipes* remains relatively stable in natural populations. This study examined the space-use and stress physiology of individuals from a population of *Melomys cervinipes* in a rainforest habitat. It was hypothesised that territoriality limits the population size by excluding a proportion of individuals from the breeding population. It was predicted that individuals from this so-called "surplus" population experience reproductive inhibition via the actions of stress, invoked by aggressive encounters with territorial individuals.

Territorial males and females exhibited lower free corticosterone concentrations than individuals which were believed to constitute the surplus population. Territorial males were found to have higher testosterone concentrations than non-territorial males, though the difference was not great enough to infer that the latter were reproductively inhibited. Corticosterone concentrations were highest in the month that corresponded with the highest rate of recruitment of juveniles into the population.

\*POSTER



## Cellular lipid bilayers are more polyunsaturated but less monounsaturated in a mammal compared to reptiles: a basis for the evolution of endothermy ?

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It has been suggested that the fatty acid composition of cellular membranes is a determinant of the difference in energy metabolism between endotherms and ectotherms. However there is a little systematic information to support this suggestion.

Specifically, I have sought to answer the following questions: are the differences in phospholipid fatty acid composition previously reported for liver and kidney from an endotherm and an ectotherm also found in other tissues ? Do subcellular membranes show the same differences observed in total tissue phospholipids ?

I have determined by gas chromatography the fatty acid composition of phospholipids extracted from brain, heart, kidney, liver, skeletal muscle and testes of a typical endotherm, the rat (*Rattus norvegicus*) and two species of lizards (*Pogona vitticeps* & *Trachydosaurus rugosus*). There were consistent differences in fatty acid composition of tissue phospholipids between the two groups. In the tissues from the endotherm there were more polyunsaturated fatty acids whilst those from the ectotherms contained more mono-unsaturated fatty acids. Fractionation of subcellular membranes by differential centrifugation have produced a plasma membrane fraction (not pure), and fatty acid analysis of this fraction suggests that the difference found in the total tissue phospholipids also exists in plasma membranes. The physiological implication of this difference will be discussed.

### Regulation of salt gland function in the green sea turtle *Chelonia mydas*.

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The secretory rate and sodium concentration of secretions from the lachrymal salt gland of hatchling green sea turtles *Chelonia mydas* were measured under a number of experimental conditions. Possible peptidergic and cholinergic influences on salt gland activity were examined. Hatchlings responded rapidly to a salt load of 2700  $\mu\text{moles Na}^+$ .(100g bodyweight)<sup>-1</sup> injected into the thoracic cavity, with tear secretion from the salt gland commencing 5-15 minutes after salt loading. Injection of the same volume of isotonic saline did not produce any secretory response. The concentration of tears produced ranged from 1600 to 2000 mosmol.kg<sup>-1</sup>, approximately 97% of which consisted of Na<sup>+</sup> and Cl<sup>-</sup> with the balance being mainly K<sup>+</sup>. Total Na<sup>+</sup> secretion rates from a single gland following salt loading averaged 381  $\mu\text{moles Na}^+$ .hr<sup>-1</sup>.(100g bodyweight)<sup>-1</sup> in the first hour of secretion with approximately one third of the injected salt load being secreted during this time.

The compounds tested for salt gland regulatory activity were Arginine Vasotocin 30 ng.kg bodyweight<sup>-1</sup> (AVT), Vasoactive Intestinal Peptide 3.5-300 ng.kg<sup>-1</sup> (VIP), Atrial Natriuretic Peptide 30-60 ng.kg<sup>-1</sup> (ANP) and a cholinergic agonist Methacholine (methylcholine chloride) 2-20 mg.kg<sup>-1</sup>. None of these substances elicited a secretory response from the salt glands in the absence of a salt load. VIP and ANP did not change the characteristics of secretion when injected either simultaneously with a salt load or 20 minutes after secretion had been initiated by a salt load. When AVT was injected under this latter condition, it appeared to slightly reduce the secretory rate, although further work is needed to establish the significance of this. The injection of methacholine simultaneous with a salt load delayed the onset of the secretory response by 35-45 minutes. When injected 20 minutes after secretion had been initiated by a salt load, methacholine abolished secretion within 2 minutes of application. Secretion did not resume during the subsequent 40 minutes of the experimental period.

The results indicate that activity of the sea turtle salt gland is inhibited by a cholinergic agonist. The osmoregulatory peptides examined did not appear to directly stimulate or inhibit the secretory response, although AVT may have some slight suppressive activity.

## Autoregulation by the avian kidney

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Modified right kidneys (consisting of the caudal division with all major blood vessels intact and accessible) were produced in roosters. Birds were divided into two groups according to whether they gained or lost weight during saline challenges at 3 and 5 months of age. Autoregulation experiments were conducted at 8-9 months of age. Anaesthetised animals had cannulae placed in the left brachial vein, one carotid artery and the right ischiadic artery. Snares were placed around the proximal and distal regions of the right ischiadic artery and a Transonic perivascular flow probe was placed around the proximal ischiadic artery. Renal function experiments were conducted at ambient right renal arterial perfusion pressure and when the pressure in the right kidney was reduced, stepwise, to 100, 90, 80, 70, 60, 50 mm Hg.

The right renal arterial plasma flow decreased when the snare was tightened and remained relatively constant across the range of pressures. Plasma flow was lower in the "lost weight" group. Autoregulation of glomerular filtration rate occurred at pressures above 70-80 mm Hg and there was no difference between the two groups for autoregulatory range. True filtration fraction of the right kidney increased during reduced arterial pressure and tended to be higher in the "gained weight" group. The clearance of PAH tended to be higher in the "lost weight" group.

## The Effect of Hypoxia and Temperature on the Relationship between Metabolism and Ventilation during Development in the Rat

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Oxygen consumption ( $\dot{V}O_2$ ) and ventilation ( $\dot{V}_E$ ) were measured in rats from birth to adult to determine the overall pattern of metabolism and ventilation. A number of age groups were selected based on the  $\dot{V}O_2$  pattern observed. These were exposed to differing levels of hypoxia (21, 17.5, 15, 12.5 and 10%  $O_2$ ) at both 30°C (adult thermoneutrality) and 20°C. At 30°C and in normoxia (21%  $O_2$ )  $\dot{V}O_2$  displayed a marked overshoot from values predicted for adult eutherians that commenced at a mass of 35 g, peaked at 85 g and slowly declined to predicted values by adult mass. Under these conditions  $\dot{V}_E/\dot{V}O_2$  remained mass independent with an average value of 36; this is the value reported for adult mammals of any infraclass. Exposure to an ambient temperature of 20°C resulted in a marked increase in  $\dot{V}O_2$  in certain age groups (presumably due to thermogenesis) that was accompanied by a proportional change in  $\dot{V}_E$  such that  $\dot{V}_E/\dot{V}O_2$  remained mass independent and unchanged from the 30°C value. In general, hypoxia at 30°C induced a hyperventilatory response (*i.e.* an increase in  $\dot{V}_E/\dot{V}O_2$ ) that increased with increasing hypoxia. The age of the animal decided how the hyperventilatory response was mediated; young animals opted for a marked decrease in  $\dot{V}O_2$  and little increase in  $\dot{V}_E$  whereas older animals opted predominantly for an increase in  $\dot{V}_E$ . At 20°C the effect of hypoxia (10%  $O_2$ ) on  $\dot{V}O_2$  was more marked in animals that had elevated  $\dot{V}O_2$  above the value found for 30°C. Nevertheless whether a drop in  $\dot{V}O_2$  or an increase in  $\dot{V}_E$  was the predominant response to hypoxia,  $\dot{V}_E/\dot{V}O_2$  remained mass independent and increased to the same extent as at 30°C and 10%  $O_2$ ; average value 72. The results show that  $\dot{V}_E/\dot{V}O_2$  is predetermined regardless of the level of metabolism and is only altered with changing levels of oxygen.

\* POSTER

### **The evolution of thyroxine distributor proteins.**

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Binding to protein prevents thyroxine from disappearing from the bloodstream by partitioning into lipid cell membranes. Thyroxine-binding plasma proteins in the human are albumin, transthyretin (TTR), and thyroxine-binding globulin (TBG). Albumin is the phylogenetically oldest, TBG the most recent thyroxine-binding plasma protein. The genes for albumin and TBG are only expressed in the liver. Expression of the TTR gene first evolved in the choroid plexus of reptiles. TTR gene expression in the liver evolved later and independently in eutherians, Australian diprotodont marsupials, two lineages of American polyprotodont marsupials and birds.

Similarity in amino acid sequence suggests that a common ancestor protein existed for albumin, vitamin D-binding protein and  $\alpha$ -fetoprotein before the separation of bony fish and Amphibia. TBG structures are only known for humans, rats and sheep, precluding a more general evolutionary analysis. The evolution of TTR gene structure is correlated with that of the tissue pattern of expression. The amino acids forming the binding site for thyroxine in a central tunnel in the tetrameric TTR are identical in reptiles, birds and mammals. However, a change in RNA splicing between exon 1 and intron 1 led to a change in character of the N-terminus of TTR from hydrophobic to hydrophilic in eutherians, possibly influencing access of thyroxine to the binding site. We suggest that the requirement for appropriate distribution of thyroxine is the selection pressure for evolution of both tissue pattern of expression and structure of the transthyretin gene.

### **Oxygen uptake by amphibian egg masses**

***Roger S. Seymour***

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Respiration by amphibian embryos is aerobic and depends on the availability of oxygen from the environment. Embryos deposited in egg masses potentially have difficulties in obtaining sufficient oxygen by diffusion through the jelly. Oxygen availability depends on the distance between the embryo and the free environment, and the rates of oxygen uptake by other embryos in the mass. Embryos embedded in jelly become "black holes" with respect to oxygen; all oxygen coming close to the embryo is consumed by it. Diffusion pathways through the jelly are non-linear, with the possibility of oxygen diffusing around the side of an egg, reversing direction, and entering the egg from the side closest to the center of the mass. It is therefore impossible for oxygen to be moved by convection across the perivitelline space and released to supply embryos deeper in the mass. Although the jelly is a barrier to gas exchange, it has the vital respiratory function to separate the eggs, which reduces the "respiratory density" (volume-specific oxygen consumption rate) and consequently permits a larger number of embryos to occupy the mass. Nevertheless, the size of globular gelatinous egg masses is severely limited in aquatic and terrestrial environments. Limits to egg mass size can be extended by (1) spreading the eggs to reduce the diffusion distance, either at oviposition or during development, (2) perforating the egg mass with channels in which water is convected, (3) depositing the eggs in foam, which supplies oxygen directly from the bubbles and augments the coefficient of oxygen diffusion, and (4) incorporating symbiotic algae into the mass.

## The Cerebral Vasculature of the Emu

J.D. Smith and T.J. Dawson

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The emu (*Dromaius novaehollandiae*) faces high temperatures in arid Australia. In these conditions emus use evaporative water loss (EWL) from the respiratory tract to dissipate heat. In extreme thermal conditions body temperatures may increase markedly to offset extensive water loss and delay dehydration. Arterial blood destined for the brain may be cooled via an ophthalmic rete to protect the brain against over heating. This network of fine arteries and veins creates a body-to-brain temperature gradient that can be increased during times of heat stress. This study describes the circulatory system in the head of the emu, particularly focusing on areas involved in EHL and cooling of cerebral blood.

The vascular anatomy was examined from resin casts, latex injections and dissection. Histological investigations were made of the ophthalmic rete and nasal turbinals. Arterial supply to the cerebral tissues from the ophthalmic rete includes the internal ophthalmic artery and the cerebroethmoid artery. The venous system supplying the ophthalmic rete originates in the nasal turbinals and ocular tissues, sites where heat loss can occur by EWL. Veins to the rete include the nasal and ethmoid veins, the ophthalmic veins and the maxillary and lingual veins.

The ophthalmic rete of the emu is long (15mm) and provides a large surface area for counter-current heat flow from the arteries to the veins. A high surface area is available in the turbinals for evaporative heat loss, especially via the maxillary turbinal. The turbinals exhibit a complex folding pattern. Histologically, they feature multi-cellular mucous secreting glands, ciliated epithelium and a high density of lymphocytes. The turbinals contained many arterio-venous anastomoses (AVA's) which allow for the shunting of blood to areas where heat can be rapidly lost.

### Reduction of metabolic rate and thermoregulation during daily torpor

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The physiological mechanisms causing the reduction of metabolic rate (MR) during torpor in endotherms are controversial. The original view that MR is reduced because the lowered body temperature ( $T_b$ ) reduces tissue metabolism has been challenged by a recent hypothesis which claims that MR during torpor is actively downregulated and is a function of the temperature difference between the animal and its environment ( $\Delta T$ ) rather than  $T_b$ .

In the present study, both the steady-state MR and  $T_b$  of torpid *Sminthopsis macroura* showed two clearly different phases in response to change of air temperature ( $T_a$ ). At  $T_a$ s between 14 and 30°C, MR and  $T_b$  decreased with  $T_a$  and MR showed an exponential relationship with  $T_b$  ( $R^2 = 0.74$ ). The  $Q_{10}$ s for MR were between 2 and 3 over the  $T_b$  range of 16 to 32°C. The  $\Delta T$  over this  $T_a$  range did not change significantly, and MR was not related to  $\Delta T$  ( $P = 0.35$ ). However, the overall conductance decreased with  $T_a$ .

At  $T_a$ s below 14°C, MR increased linearly with the decrease of  $T_a$  ( $R^2 = 0.58$ ) and  $T_b$  was maintained in the range of 16 to 28°C, largely independent of  $T_a$ . In this  $T_a$  range, MR was positively correlated with  $\Delta T$  ( $R^2 = 0.61$ ). Nevertheless, the  $Q_{10}$  for MR between normothermic and torpid thermoregulating animals at the same  $T_a$  was also in the range of 2 to 3.

These results suggest that in the  $T_a$  range in which  $T_b$  of *S. macroura* was not metabolically defended, MR was largely a function of  $T_b$ . At  $T_a$ s below 14°C, at which the animals showed an increase of MR to regulate  $T_b$  during torpor, the negative correlation of MR with  $T_a$  was a function of  $\Delta T$  as during normothermia. However, even in thermoregulating animals, the reduction of MR from normothermia to torpor at a given  $T_a$  can also be explained by  $Q_{10}$  effects.

### Function of transthyretin in thyroid hormone transport in blood and brain.

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Thyroid hormones are essential for normal development of the brain. Thyroid hormones are distributed in blood bound to specific binding proteins. There is a barrier to the movement of proteins from blood to brain. In the last few years, we have found a special mechanism for the transport of thyroid hormones from the blood to the central nervous system. Transthyretin (TTR) is the major thyroid hormone-binding protein in the brain. TTR is synthesized and secreted by the choroid plexus, the organ which produces most of the cerebrospinal fluid (CSF) and which forms the blood-CSF barrier. *In vivo* and *in vitro* studies suggest that this protein is involved in transporting thyroxine into the brain and in the distribution of hormone within the central nervous system (Endoc. 133:2116, 1993). Synthesis and secretion of TTR by the choroid plexus may determine the concentration of thyroxine in the CSF and allow independent T4 homeostasis in the brain relative to the blood. TTR gene expression is regulated independently in brain and liver permitting independent control of thyroid hormone distribution in the body and brain. A surprising recent finding is that TTR is synthesised in the choroid plexus of most vertebrate groups (except amphibia) but in the liver only in more recently evolved groups, suggesting that this protein evolved in the brain before the liver (Am J Physiol 266:R1359, 1994). Thus requirements for the distribution of thyroid hormone in the brain may have provided the selective pressure for the evolution of specific thyroid hormone-binding proteins.

### Live transport of the Western rock lobster. A comparison of the effect of chilling procedures on the energy metabolism of *Panulirus cygnus*..

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The Western rock lobster industry is a major export industry in Australia in which the emphasis in marketed products is shifting from frozen and cooked lobsters to export of live animals. The methods and techniques currently used by the industry have been developed empirically by fishermen and processors over the last 20 years.

More specifically, the handling procedures currently used by processing companies to optimise health and survival of live products during dry transport are aiming at inducing metabolic rate depression by chilling the animals prior to packaging.

The response of the Western rock lobster (*P.cygnus*) to chilling procedures and subsequent hypoxia induced by aerial exposure has not yet been investigated with reference to high-energy adenylate nucleotides titre and to the predominant anaerobic metabolites and/or end-products formed. The present paper reports on studies of chilling regimes and of aerial exposure of *P.cygnus*.

\*POSTER

## Vasomotion in crab gills

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The neural and endocrine control of decapod circulation, at the level of the heart and cardio-arterial valves is well-established. However, it is generally believed that vasomotor mechanisms are lacking in the peripheral and branchial circulations.

Ultrastructural studies demonstrate that haemolymph flows across each lamella in the gills of crabs within a flat sinus, spanned by pillar cells, and also in the marginal canal. In several crabs it has been shown that the exit routes into the efferent vessels are narrowed by sphincter-like partial septa (the so-called efferent valves). In principle, contractility of either the pillar cells or the valves potentially could modulate branchial resistance and/or redirect flow within the lamella.

This study demonstrates that cardioactive hormones (5HT,  $F_1$  peptide, octopamine), produced in the pericardial organs of crabs, modulate the flow resistance of isolated perfused crab gills (*Ovalipes catharus*, *Callinectes sapidus*, *Cancer magister*). Investigations using a micropressure system indicate that the response to 5HT is located within, or close to, the efferent valves.

## Energetic Cost of Embryonic Development in Lizards

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Recent models of energy consumption by avian embryos during development divide the total energetic cost into two components: biosynthesis and maintenance of tissue once it is synthesised. These models can be tested in two ways by using interspecific comparisons: 1. measuring the total mass-specific cost of development of two species with different hatchling mass but similar incubation period, or 2. measuring total mass-specific cost of development in two species of similar mass, but with different incubation periods. We made both comparisons using four species of skinks, two in the genus *Morethia* (fresh eggs masses of 170 and 220 mg; incubation periods = 58 days) and two *Eumeces* (egg masses of 290 and 400 mg; incubation periods = 27 and 25 days). Theory predicts that there would be no mass-specific difference in the metabolic cost of the two *Morethia* species or of the two *Eumeces* species, but that *Eumeces*, with its shorter incubation period, would expend considerably less energy during development. There was no significant difference in cost of development between the two species of *Morethia* (*M. boulengeri* =  $18.2 \pm 2.0$  kJ/g; *M. adelaidensis* =  $19.9 \pm 2.4$  kJ/g;  $p = 0.112$ ) but the *E. fasciatus*, which has the shorter incubation period, was significantly less than *E. anthracinus* (*E. fasciatus* =  $12.4 \pm 0.5$  kJ/g; *E. anthracinus* =  $13.6 \pm 0.7$  kJ/g;  $p = 0.0018$ ), although there was considerable overlap in the data (12.48-14.22 kJ/g; 11.98-13.54 kJ/g). As predicted, comparison between the largest eggs of *Morethia* and the smallest *Eumeces* showed that the short incubation period *Eumeces* incurred a significantly smaller energetic cost than longer incubation *Morethia* ( $p < 0.001$ ). Thus, data for skinks is consistent with the avian model that splits the cost of embryonic development into a components for biosynthesis and maintenance.

The effects of heat stress on egg shell quality in laying hens  
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Commercial laying hens were subjected to heat stress to investigate the effects on the quality of the egg shells. Two groups of birds were used and maintained in constant temperature rooms. Initially, both were maintained at 20°C, after which the temperature of one room was increased to 30°C for a period of 4 weeks. Eggs were collected daily and feed intake was monitored. Eggs were weighed, the length and width measured and the specific gravity determined. Egg-shell breaking strength was assessed by quasi-static compression and egg shell weight and thickness measured.

The heat stress resulted in significant decreases in feed intake, egg weight, egg width, shell weight and shell thickness. However, there was no significant effect on egg shell breaking strength. Previous studies in our laboratory have shown that the thinner egg shells laid by hens exposed to heat stress were of superior ultrastructure. The results of the present study suggest that this improved ultrastructure is able to compensate for the reduced amount of shell present to produce egg shells which are just as strong as those laid by hens maintained at 20°C.

**11-ketotestosterone increases heart size and reduces adrenergic sensitivity of rainbow trout (*Oncorhynchus mykiss*).**

*Helgi Thorarensen and Peter S. Davie*  
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The relative ventricle mass (RVM: ventricle mass / body mass) of mature male rainbow trout is more than double that of mature females and immature fish. Preliminary studies in this laboratory suggested that the greater heart size of the males was caused by androgens, most likely 11-ketotestosterone (11-KT) which is the principal androgen of teleost fish. To test this hypothesis, silastic pellets containing 11-KT, testosterone (T), or blank pellets (as controls) were implanted in rainbow trout of both sexes. Moreover, the contractile properties of the atria of the fish were compared to examine whether contractility and adrenergic sensitivity of the hearts changed with heart size or as a result of the androgen treatment. At the end of the seven week androgen treatment, the RVM of the fish treated with 11-KT and T was 63% greater than that of control fish. Fish of both sexes responded to the androgen treatment with increased RVM. These results are consistent with the hypothesis that the greater heart size of mature males is caused by higher plasma titres of 11-KT. The sensitivity of paced atrial strips to the  $\beta$ -agonist isoprenaline was lower in 11-KT treated fish ( $EC_{50} = -7.4$ ) than in control fish ( $EC_{50} = -8.2$ ), while the maximum twitch force of the strips was not affected by androgen treatment. These results suggest that 11-KT may modulate the sensitivity of the atria to catecholamines.

**The effect of exercise training on internal oxygen convection ( $\dot{V}O_2$ ), oxygen consumption ( $\dot{V}O_2$ ), and swimming performance of chinook salmon (*Oncorhynchus tshawytscha*)**

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The  $\dot{V}O_2$  is determined by cardiac output ( $\dot{Q}$ ), the oxygen content of arterial blood ( $C_aO_2$ ), and the fractional extraction of oxygen from blood as it passes through the capillaries ( $EO_2$ ;  $\dot{V}O_2 = \dot{Q} \cdot C_aO_2 \cdot EO_2$ ). To examine the effect of exercise training on  $\dot{V}O_2$ , a group of chinook salmon (T) was subjected to a training regime whereby, on alternate days for 2-4 months, swimming velocity was increased in steps until all fish had fatigued. Otherwise, the fish maintained a swimming velocity of 0.5 body-lengths $\cdot$ s $^{-1}$  (bls $^{-1}$ ). Control fish (C) swam continuously against a current of 0.5 bls $^{-1}$  for the same period of time.

The  $\dot{V}O_{2max}$  of the T fish was 50% higher than that of the C fish. The  $EO_{2max}$  was 62% and 92% in the C and T groups respectively, while neither  $\dot{Q}_{max}$  nor  $C_aO_2$  were significantly different in the two groups. Therefore, the higher  $\dot{V}O_{2max}$  of the trained fish was supported by greater extraction of oxygen from the blood as it passes through tissues.

Although the  $\dot{V}O_{2max}$  of the T fish was significantly higher than that of the C fish, the maximum swimming speed, which could be maintained for at least 30 minutes, was not different in the two groups of fish. It is suggested, that the higher  $\dot{V}O_{2max}$  of the T fish allowed them to simultaneously support various functions, other than locomotion, while they swam maximally.



Nitrogen metabolism in the terrestrial crab *Geograpsus grayi* (Brachyura: Grapsidae).

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Haemolymph amino acid profiles of *Geograpsus* were measured by reverse phase HPLC and the activities of enzymes involved in the formation and degradation of glutamate and glutamine were assessed in gill, muscle and hepatopancreas to identify the role of amino acids in nitrogen transport and as precursors in ammoniogenesis. In *Geograpsus*, ammonia has previously been shown to be excreted by ion exchange across the gill epithelium and volatilisation from the branchial fluid in periodic bouts of excretion.

The most abundant amino acid found in the haemolymph of excreting crabs was glutamine (1.28 mmol.L<sup>-1</sup>) followed by alanine (0.41 mmol.L<sup>-1</sup>) and proline (0.38 mmol.L<sup>-1</sup>), while glutamate levels remained low during both excretory and non-excretory periods (0.014 and 0.015 mmol.L<sup>-1</sup> respectively).

Glutamate dehydrogenase activity in total gill tissue measured in the forward direction, that is ammonia production, was sufficient to account for 32% (69.9  $\mu$ mol.kg.h<sup>-1</sup>) of gaseous ammonia excretion measured during excretory bouts. The activity was significantly lower (38.2  $\mu$ mol.kg.h<sup>-1</sup>, p<.05) and slightly exceeded excretion rates observed in crabs during intervening periods of low ammonia excretion.

Phosphate dependent glutaminase was not detected in gill and this terrestrial crab does not utilise the same pathways for nitrogen transport in the haemolymph and ammoniogenesis in the gill found in aquatic brachyurans. The role of other amino acids in nitrogen metabolism is discussed.

Plasma Triglyceride and  $\beta$ -Hydroxybutyric Acid Levels in Red-sided Garter Snakes (*Thamnophis sirtalis parietalis*)  
at Emergence from Hibernation

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Measurement of plasma levels of triglycerides and  $\beta$ -hydroxybutyric acid in females and males of the red-sided garter snake (*Thamnophis sirtalis parietalis*) suggests that the former may provide a useful physiological marker of condition. Levels of triglycerides at emergence from hibernation in females were significantly greater than males and she-males, a subset of the male population that mimics females. Higher levels of triglycerides in the females may be attributed to their greater body mass per unit length, which was correlated with the level of triglycerides. Plasma triglyceride levels declined in females within one month of emergence, and were unrelated to mating. These findings suggest that triglyceride levels are not affected by changes in circulating prostaglandin and oestradiol that occur after mating.

$\beta$ -hydroxybutyric acid levels were uniformly low and did not change during the sampling period. We conclude that this metabolite of fatty acid oxidation does not accumulate in the blood when fat reserves are used for sustenance during the annual cycle.

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