ANZSCPB2006

University of Queensland - AUSTRALIA

AUSTRALIAN AND NEW ZEALAND SOCIETY FOR COMPARATIVE PHYSIOLOGY AND BIOCHEMISTRY

8th - 10th December 2006

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8-10th December 2006

School of Integrative Biology

The University of Queensland, Brisbane, Australia

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Organising Committee:

Rebecca Cramp
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Information for presenters

Poster presentations

Please mount your posters in the Zoology Museum (level 4) of the Goddard Building on Friday 8 December before the sessions begin (between 8.00 - 8.30am), or during morning tea (10.30-11.00am). Please stand alongside and defend your posters on Saturday 10 December during the poster session between 3:30-5.00pm. Your posters will ideally be removed during morning tea on Sunday 11 December (10.45-11:15am) and no later than the end of the final session (1:00pm)

Oral presentations Speakers, please hand your talks on memory stick or CD to our assistant at least 30 min before the beginning of the session in which you are presenting. Our assistant will load the talk onto one of our laptops (pc or Mac). Do not make use of the laptops without the assistant's supervision. A laser pointer will be provided. When presenting, you will be told when 12 min have elapsed; ideally your talk will end about then to leave time for questions. If you are still talking at 15 min, you will be asked to end your presentation

Other useful information

For enquiries, please approach the registration desk or the following people:
Financial matters: Beth Symonds
Conference dinner: Lyn Beard
Friday Social: Gordon Grigg
Registration: Natalie Mathie
Oral presentations, Program: David Booth
Posters: Kirstin Pratt
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The majority of shops are located in the Union Complex (see Map), and are administered by the UQ Student Union.
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If you can't find what you need on campus, more shops, cafes and a supermarket are within a five minute walk from the campus. The St Lucia campus is close to two major

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Maps

Maps of the St Lucia campus area are provided in your registration pack

ANZSCPB2006 Programme Overview

Thursday 7th December 2006

4:00 – 7:00pm Registration Level 3 Foyer, Goddard Building (Building No. 8) University of Queensland St. Lucia Campus.
6:00 pm Welcome BBQ Roof of Goddard Building

Friday 8th December – Student Day

8:45 -10:30 Session 1 (Rm 388, Goddard building)
10:35 Morning Tea (Goddard level 3 Foyer)
11:00 - 1:00 Session 2 (Rm 388, Goddard building)
1:00 Lunch (Goddard cloisters)
1:45 – 3:30 Session 3 (Rm 388, Goddard building)
3:30 Afternoon tea (Goddard level 3 Foyer)
3:45 – 5:00 Session 4 (Rm 388, Goddard building)
5:00 Walk to UQ CityCat Stop for commute to Newfarm park for Friday Night Social
10:00 Return to UQ

Saturday 9th December

9:00 – 10:30 Session 5 (Rm 388, Goddard building)
10:30 Morning tea (Goddard level 3 Foyer)
11:00 – 1:00 Session 6 (Rm 388, Goddard building)
1:00 Lunch (Goddard cloisters)
1:45 – 3:30 Session 7 (Rm 388, Goddard building)
3:30 Afternoon tea and Poster session (Goddard level 3 Foyer and Goddard Museum, Level 4 Goddard Building)
6:00 Depart UQ for Conference Dinner at Indooroopilly Golf Club
11:00 Depart Indooroopilly Golf Club for UQ

Sunday 10th December

9:30 - 10:45 Session 8 (Rm 388, Goddard building)
10:45 Morning tea (Goddard level 3 Foyer)
11:15 - 11:45 Session 9 (Rm 388, Goddard building)
11:45 Presentation of Student Prizes and Close of ANZSCPB2006 conference
12:00 ANZSCPB business meeting
1:00 Lunch (Goddard cloisters)

ANZSCPB Program 2006

Friday 8th December – Student Day

8:45 Welcome & House keeping

Chair of Session 1: David Booth

9:00 Lisa Warnecke et al. Basking behaviour in small heterothermic arid zone marsupials
9:15 Gerard Wan et al. The role of muscular activity in thermal relations of the echidna, *Tachyglossus aculeatus*
9:30 Skye Cameron et al. Wintering thermal biology of the eastern water dragon (*Physignathus lesueurii*) in a sub-tropical environment
9:45 Scott van Barneveld et al. Nutrient-specific foraging is influenced by thermal environment in the lizard *Lampropholis delicata* (Scincidae: De Vis 1888)
10:00 Ryan Day The physiological and morphological basis of ontogenetic trophic shifts in the Hemiramphidae (Beloniformes): a model simple herbivore
10:15 Alexandra Schnell et al. Does Phenotypic Plasticity Facilitate the Geographic Expansion of the Fish, Oreochromis mossambicus?
10:30 Announcement by Prof. Ian Hume, Associate Editor JCP
10:35 Morning tea

Chair of Session 2: Robbie Wilson

11:00 Jeroen Brys & N. Ling. Comparative haemoglobin oxygen binding and habitat in four congeneric freshwater fishes: the endemic mudfish lineage
11:15 Philip Matthews. Compressible gas gills of diving insects – models vs. measurement
11:30 Sara Kayes & C Franklin. Diving bradycardia during voluntary dives in freshwater turtles: is it really beneficial?
11:45 Alecia Carter & R. Wilson. Improving sneaky-sex in a low oxygen environment: reproductive and physiological responses of male mosquito fish to chronic hypoxia
12:00 Vincent van Uitregt et al. Cooler temperatures increase sensitivity to UVb in embryos and larvae of the frog *Limnodynastes peronii*
12:15 Amanda C. Niehaus et al. Predicting complex phenotypes in complex environments: The ontogeny of Anurans in fluctuating temperatures
12:30 Magdalene Trzcionka et al. Proton flux and uncoupling proteins in mitochondria from the amphibian *Bufo marinus*
12:45 Beth Symonds et al. Getting the jump on skeletal muscle disuse atrophy: preservation of contractile performance in aestivating *Cyclorana alboguttata*
1:00 Lunch

Chair of Session 3: Bill Buttemer

1:45 Yvonne Eiby & D. Booth. Embryonic thermal tolerance and temperature variation in incubation mounds of the Australian brush-turkey (*Alectura lathami*)
2:00 Frank Seebacher (Honorary student) et al. Transition from ectothermy to endothermy: the development of metabolic capacity in a bird (*Gallus gallus*)
2:15 Isabel Walter & F. Seebacher. What impact has PGC-1α on the development and plasticity of metabolic capacity in birds?
2:30 Stewart Macdonald et al. Axial muscle activity in swimming crocodilians
2:45 Charlotte Kvennefors et al. Pattern Recognition Proteins in the Scleractinian Coral *Acropora millepora*
3:00 Timothy Green et al. Non-Self Recognition Proteins in Sydney Rock Oysters, *Saccostrea glomerata*
3:15 Natalie Mathie et al. Thermal plasticity of diving behaviour, aquatic respiration, and locomotor performance in the Mary River Turtle, *Elusor macrurus*
3:30 Afternoon tea

Chair of Session 4: Mike Thompson

3:45 Heather Hesterman & S. Jones. Captive management issues and the Tasmanian Devil Facial Tumour Disease (DFTD)
4:00 Joanna Biazik et al. Tight junctional protein, occludin is upregulated with evolution of viviparity
4:15 Ray Bartolo & J. Donald. Expression of renal aquaporins during water deprivation in the Spinifex hopping mouse
4:30 Sofie Trajanovska et al. Genomic analysis of chicken natriuretic peptide (NP) genes: new insights into the evolution of the NP system in tetrapods
4:45 Inga de Vries et al. Regulation of salt secretion and blood flow in the lingual salt glands of the estuarine crocodile, *Crocodylus porous*

Saturday 9th December

9:00 House keeping

Session 5: Student Chairperson

9:15 David Booth & C. Yan Yu. The influence of substrate water potential on the embryonic development of Brisbane river turtle eggs.
9:30 A.J. Hulbert , Laura Haddad and Louie Kelbert. Queens live longer than workers: are dietary fats responsible?
9:45 Fritz Geiser et al. The effect of photoperiod acclimation on daily torpor and tissue fatty acid composition in deer mice
10:00 Gerhard Körtner et al. Thermal biology, daily torpor and behavioral ecology

of an arid zone carnivorous marsupial, the brush-tailed mulgara
10:15 Stewart Nicol & N. Andersen. About a bout: what happens in a single hibernation cycle?
10:30 Morning tea

Session 6: Student Chairperson

11:00 Gordon Grigg. *Keynote Address*: The crocodile heart: unfinished business

11:45 Ashley Edwards & S. Jones. HPG axis activity in juvenile blotched blue-tongued skinks, *Tiliqua nigrolutea*
12:00 Susan Jones &A. Edwards. Does leptin provide a metabolic signal to the reproductive system in blue-tongued lizards, *Tiliqua nigrolutea*?
12:15 Robert Holland et al. The undiscovered hemoglobins of marsupials
12:30 Timothy Clark et al. Circulatory physiology and haematology of southern bluefin tuna (*Thunnus maccoyii*)
12:45 William Buttemer et al. Interactive effects of testosterone and immune challenge on aerobic performance in House Sparrows
1:00 Lunch

Session 7: Student Chairperson

1:45 Roger Seymour et al. Regulation of oxygen in the air-breathing organ of Pacific tarpon (*Megalops cyprinoides*) in response to aquatic hypoxia and exercise
2:00 Robbie Wilson et al. Honest and dishonest signals of strength in slender crayfish (*Cherax dispar*): are males cheating bastards?
2:15 Roger Lentle et al. Small gut motility in the brush tail possum: Do the longitudinal muscles of the small intestine of herbivores exhibit greater motility than those of other species?
2:30 Harry Battam et al. Thermogenic overshoot in albatrosses fed cold meals
2:45 Adam Munn & T. Dawson. A mechanistic explanation for the drought-related mortalities of juvenile kangaroos: implications for population biology
3:00 Tes Toop et al. Investigations into the effect of handling on the natriuretic peptide system of salmonids
3:15 Lyn Beard et al. Preliminary insights into the life of an echidna burrow young
3:30 Afternoon tea and Poster session
6:00 Depart UQ for Conference Dinner at Indooroopilly Golf Club

Sunday 10th December

9:30 House keeping

Session 8: Student Chairperson

9:45 Nicholas Hudson et al. Molecular control of vertebrate aestivation: potential

role for epigenetic modification

10:00 Scott Parker and R.M. Andrews. Evolution of Viviparity in Sceloporine Lizards: In Utero P_{O_2} as a Developmental Constraint during Egg Retention

10:15 Katayoon, Karimzadeh, A. Mostafaei & A. Zahmatkesh. Effects of ß-naphthoflavone on Liver Microsomal Mixed Function Oxidase Activities of *Huso huso*.

10:30 Morning tea

Session 9: Student Chairperson

11:00 Rebecca Cramp et al. Innervation of crocodilian salt glands: acclimatory responses of salt glands to hypersaline environments

11:15 Shaniko Shini. The effect of corticosterone on ultrastructural characteristics of chicken leukocytes

11:30 Craig E. Franklin and Frank Seebacher. The importance of changes in heart rate and blood flow during thermoregulation in the estuarine crocodile, *Crocodylus porosus*

11:45 Presentation of Student Prizes

12:00 ANZSCPB business meeting

1:00 Lunch

Oral Paper Abstracts

Basking behaviour in small heterothermic arid zone marsupials

Lisa Warnecke, James M. Turner and Fritz Geiser

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

The high energetic costs associated with endothermic rewarming from torpor are widely seen as a major disadvantage of torpor. We tested the hypothesis that small arid zone marsupials, which have limited access to energy in the form of food but ample access to solar radiation, employ basking to facilitate arousal from torpor and reduce the costs of rewarming. Therefore we investigated torpor patterns and sunbasking behaviour in free-ranging Fat-tailed dunnarts *Sminthopsis crassicaudata* and Giles' planigales *Planigale gilesi* using small temperature-sensitive transmitters. Animals entered torpor every night and basking was observed on 60% of the occasions. Torpid animals emerged from their resting sites in cracking soil at ~10:00h, with body temperatures as low as 14.6°C, and positioned themselves in the sun throughout the rewarming process as well as during much of the diurnal rest phase. The present study provides only the second direct evidence of basking in torpid mammals for reduction of energetic costs for arousal and behavioural thermoregulation. Our findings suggest that basking is widely distributed amongst heterothermic mammals and thus the energetic benefits from torpor use in wild animals may currently be underestimated.

Notes:

The Role of Muscular Activity in Thermal Relations of the Echidna, *Tachyglossus aculeatus*

Wai Foong Gerard John Wan[1], Lyn Beard[1] and Gordon Grigg[1]
[1] *School of Integrative Biology, University of Queensland, Brisbane, Queensland*

Body temperature regulation is performed by many animals and all mammals. Regulation can have two forms homeothermy and heterothermy. Heterothermic endotherms have a more labile body temperature compared to homeotherms. Endothermy results from heat generated from different endogenous sources within the animal and also from shivering and nonshivering thermogenesis (NST). However, NST in the form of brown adipose tissue (BAT) is lacking in monotremes raising a puzzle about what equivalent mechanism/s they may have to explain their rapid arousals from torpor. Echidnas are an ideal study species because they undergo torpor and are known to have daily cyclical increases and decreases in T_b corresponding to activity and rest. Echidnas are also known to use shivering for thermogenesis even though they lack BAT. The primary purpose of the study was to identify whether shivering or locomotor activity or a combination of both provide an explanation for their arousals, or whether some other source of heat can be implicated and to quantify this in terms of oxygen consumption and heart rate. Activity monitoring was done using a video and a piezo electric sensor. Heart rates were recorded using a heart rate transmitter attached externally through the animals' hollow spines. Open flow respirometry was carried out to quantify oxygen consumption. During daily T_b cycles it was found that less intensive movements precede rises in T_b. There was an increase in heart rate and VO2 prior to rise in T_b during torpor and in daily T_b cycles. In an animal that went torpid no muscular activity was observed in the early stage of arousal, only when T_b reached 15°C was there any observed muscular activity, shivering started when T_b reached approximately 19°C. The results show that the rise in T_b is preceded by less intensive movements which do not qualify being called shivering, and this is evident at all ambient temperatures in all animals. These movements may be the precursor for more intensive muscular activity (shivering). The increase in VO_2 and heart rate was also found to be related to increases in muscular activity. Less intensive movements were found to precede arousals with shivering occurring at the latter stages. This is supported by the increases in heart rate and VO_2. The use of other heat sources is unknown and warrants further study.

Notes:

Wintering thermal biology of the eastern water dragon (*Physignathus lesueurii*) in a sub-tropical environment

Skye F. Cameron, Gordon C. Grigg and Robbie S. Wilson
School of Integrative Biology,
University of Queensland
Brisbane, Queensland

Few studies have examined the thermal biology of ectotherms during the cooler winter period, especially for organisms that inhabit sub-tropical environments that do not experience harsh ambient winter conditions. In this study, the thermal biology of the eastern water dragon (*Physignathus lesueurii*) was investigated during the winter period in a sub-tropical environment; specifically it was investigated whether *P. lesueurii* underwent periods of dormancy over winter and if they were due to environmental thermal constraints or facultative changes in behaviour. Periods of dormancy were observed for eastern water dragons over winter, ranging from 0 to 113 days. Although the total number of days individuals spent dormant was unrelated to measures of body condition or size, females (91.0 $\pm$ 22.9 days) spent approximately twice as many days dormant as males (42.7 $\pm$ 10.9 days). Periods of dormancy were broken by arousal events, where individuals raised body temperature (T_b) above maximum air temperature (T_a). As arousals were not associated with variation in T_a, solar radiation, rainfall or photoperiod, they may be driven by internal rather than external cues. Average deviation of T_b from the preferred body temperature T_p (*db*) varied significantly among months, and decreased across the study period. However, the effectiveness of thermoregulation (*E*) of *P. lesueurii* in the field was low throughout the study (e.g. *E*= 0.23 $\pm$ 0.07 in June), indicating these lizards were predominately thermoconforming to their environment. Given some individuals did not enter periods of dormancy and measures of operative temperature indicate high active body temperatures could be attained, it appears that *P. lesueurii* can adopt a flexible behavioural strategy to the cooler winter months.

Notes:

Nutrient-specific foraging is influenced by thermal environment in the lizard *Lampropholis delicata* (Scincidae: De Vis 1888)

Scott van Barneveld[1], Stephen J. Simpson[1] and Michael B. Thompson[1]
[1] *Department of Biological Sciences, University of Sydney, Sydney, Australia*

Foraging theory typically predicts that vertebrate predators select prey on the basis of size and availability not nutritional quality. We demonstrate that a reptilian predator, *Lampropholis delicata* (De Vis, 1888), a small widespread Australian scincid lizard, selects prey of particular nutritional quality according to a combination of its nutritional state, its past thermal experience and prevailing thermal conditions. Lizards were pre-treated on a diet of either protein- or lipid-rich crickets for eight days, then allowed to consume prey of either type *ad libitum*. Over the first 2 days, lizards responded to the pre-treatment by eating more of the alternative than of the pre-treatment crickets. Thermal environment was also manipulated, such that predators were acclimated to either 15 or 25 °C and then fed protein- or lipid-rich crickets at either 15^{o} or 25 ^{o}C. Prey consumption reflected an interaction between treatment diet, acclimation temperature and test temperature. . When acclimated to 25 °C, *L. delicata* consumed protein-rich prey; when temperature decreased to 15 °C they responded by switching to lipid-rich prey. Lizards at 15 °C consumed lipid-rich prey in response to increased temperatures (25 °C), and showed generalist foraging behaviour when in sustained cool conditions, eating both equal amounts of protein-rich and lipid-rich prey. Temperature directly influenced intake requirements: skinks ate more of both prey types in warm than in cool conditions, irrespective of thermal acclimation, but they gained weight in both cool and warm acclimation treatments.

Notes:

The physiological and morphological basis of ontogenetic trophic shifts in the Hemiramphidae (Beloniformes): a model simple herbivore

Ryan Day
Centre for Marine Studies, University of Queensland
Brisbane, Queensland 4068

Investigations on the development of the pharyngeal mill and digestive enzymes of hemiramphids were carried out using histology, scanning electron microscopy and digestive enzyme assays to examine the factors associated with trophic shifts in a simple fish herbivore. Compared to *H. regularis ardelio*, the medial dentigerous pharyngeals of *A. sclerolepis krefftii* were hypertrophied throughout ontogeny until growth reached an asymptote at 180 mm standard length. *H. regularis ardelio* reached homologous size at 200 mm standard length, at which point its growth reached an asymptote, leaving both species with similar pharyngeals. *H. regularis ardelio* had a longer gut length and relative gut length at small standard lengths (<100 mm) and a shorter gut length and relative gut length at larger standard lengths (>100 mm) than *A. sclerolepis krefftii*. Above 100 mm standard length, both fish had a Zihler Index of approximately 2.4, which predicts a carnivorous diet, as does to a relative gut length of approximately 0.5. Additionally the Zihler Index of smaller *H. regularis ardelio* (3.2) was significantly greater than small *A. sclerolepis krefftii* and larger specimens of both species. Images of the gut indicated there is a close interaction between mucus and the gut contents and it is likely that the mucus plays a role in digestion. Digestive enzyme assays indicate that *H. regularis ardelio* is capable of utilising both animal and plant material at any stage of development, with similar levels of α-amylase and lipase activity in small and large specimens (mean activity: small α -amylase = 9.56 ± 1.172 U, lipase = 0.77 ± 0.569 U; adult α -amylase = 4.06 ± 0.215 U, lipase = 1.41 ± 0.608 U). Considering the similarities in gut morphology and enzyme activity between small and large individuals it appears the pharyngeal jaw apparatus plays a critical role in facilitating hemiramphid herbivory.

Notes:

Does Phenotypic Plasticity Facilitate the Geographic Expansion of the Fish, *Oreochromis mossambicus*?

Alexandra Schnell[1], and Mike Thompson[1], Frank Seebacher[1]
School of Biological Sciences A08, University of Sydney, NSW 2006, Australia.

The performance of many organisms is determined by temperature due to the thermal dependence of biological functions. The thermal biology of an animal may therefore determine the ecology and distribution of species. In Australia, the introduced fish, *Oreochromis mossambicus*, has recently radiated into habitats that were previously thought to be thermally unsuitable. The aim of this project was to determine how plastic responses facilitate the geographic expansion of *O. mossambicus* across Australia. Juvenile fish were acclimated to 14, 17 and 22 ˚C. Sustained swimming (U_{crit}), resting and active rates of oxygen consumption, mitochondrial oxygen consumption, and metabolic enzyme activities of muscle and liver were measured at five temperatures (12, 14, 17, 22, 26 ˚C). Acclimation had a significant effect on U_{crit}, but not on whole animal oxygen consumption. Fish compensated for cold temperatures by up-regulating mitochondrial oxygen consumption and partially increasing enzyme activity. Lactate dehydrogenase activity was up-regulated in the liver at lower temperatures, but not in the tail muscle. Acclimation had no effect on cytochrome-c oxidase activity. The ability of *O. mossambicus* to acclimate at different physiological levels indicates that the fish could successfully expand its' range further south into New South Wales.

Notes:

Comparative haemoglobin oxygen binding and habitat in four congeneric freshwater fishes: the endemic mudfish lineage

Jeroen Brys[1] and Nicholas Ling[1]
[1] *Centre for Biodiversity and Ecology Research, Waikato University, Hamilton, New Zealand 3216*

New Zealand's five endemic mudfish (*Neochanna*) species have distributions that differ both geographically and by habitat type. Differences in habitat preferences between species have lead to the proposal of an evolutionary series within the group. A morphological cline may be observed from the galaxiform Chatham Island and Canterbury species inhabiting lakes and streams, respectively, to the anguilliform Northland and brown mudfishes of ephemeral wetlands. Morphological specialisations proposed for wetland dwelling include loss of pelvic fins, reduced eyes, enlarged nostrils, development of caudal flanges, and elongation of dorsal and anal fin bases to become almost confluent with the caudal fin. Ability to aestivate appears common to the group although this has not been investigated in the Chatham Island species. An expectation of adaptation to wetland dwelling is specializations in respiratory physiology to obtain oxygen from highly hypoxic or acidic waters. Fish living in such conditions typically possess haemoglobins with tight oxygen binding compared with the lower affinity haemoglobins of fish in normoxic habitats or air-breathing species. We have examined the four mainland *Neochanna* species to determine whether they show differences in haemoglobin oxygen binding correlating with differing habitat. Whole blood oxygen affinity was determined at differing pH (6.5, 7.0, 7.5 and 8.0) and temperature (10ºC, 15ºC and 20ºC) using a Hemox analyser. All four species showed high oxygen affinity (p50 = 6.5 to 9.5 mm Hg at pH 7.5 & 15ºC), and minor differences between species did not correlate with the evolutionary series proposed for specialization to dwelling in ephemeral wetlands.

Notes:

Compressible gas gills of diving insects – Models vs. Measurement

Philip G. D. Matthews[1]
[1] *Environmental Biology, University of Adelaide, Adelaide, South Australia*

Compressible gas gills are bubbles of air carried by diving insects to facilitate oxygen uptake from the surrounding water. They function by allowing the development of a partial pressure gradient for the diffusion of oxygen into the bubble, but dissolve over time as nitrogen from the bubble diffuses out. While this appears to be a relatively simple system, the description of its behavior using differential non-linear equations has proved contentious. Since the accuracy of any model in entirely dependent on the values of the parameters used and other incorporated assumptions, this study aimed to fully characterize the function of a gas gill in a diving insect: the water boatman (*Agraptocorixa eurynome*). Oxygen uptake rate, bubble P_{O_2}, volume and surface area were measured, and these data were used to test the validity of the models. Here it is shown that gill area decreases in a manner not simulated by any current model, and, contrary to predictions, allows the establishment of a stable bubble P_{O_2}. The effect of ventilatory activity, previously ignored by models, is also important in reducing the thickness of the boundary layer around the bubble. Ventilation elevates bubble P_{O_2}, thereby increasing oxygen uptake, but it increases the oxygen demand and the rate of nitrogen loss and therefore gill lifetime.

Notes:

Diving bradycardia during voluntary dives in freshwater turtles: is it really beneficial?

Sara Kayes[1] and Craig E. Franklin[1]

[1] *The School of Integrative Biology, The University of Queensland, Brisbane, Queesnland 4072*

Upon submergence, diving reptiles, birds and mammals exhibit the 'dive response', a reduction in heart rate (bradycardia) and selective peripheral vasoconstriction. Thought to conserve oxygen stores for hypoxia intolerant organs thereby prolonging dive time, the significance of a diving bradycardia in extending voluntary dive durations however remains equivocal. Heart rate changes and behaviour during diving were examined in the Brisbane river turtle, *Emydura signata* before and after administration of cholinergic and adrenergic blockers. During control trials, *E. signata* displayed a significant bradycardia upon submergence, the extent of which was significantly decreased after administration of the cholinergic antagonist atropine. Administration of both a cholinergic and adrenergic antagonist almost completely abolished the development of a bradycardia during diving events. Despite this, no changes in mean or maximum dive duration were observed, suggesting that under the experimental conditions provided *E. signata* were not employing a diving bradycardia to extend dive duration.

Notes:

Improving sneaky-sex in a low oxygen environment: reproductive and physiological responses of male mosquito fish to chronic hypoxia

Alecia J. Carter[1] and Robbie S. Wilson[1]
[1] *School of Integrative Biology, University of Queensland, St Lucia, Queensland*

Few studies have examined the adaptive significance of reversible acclimation responses. The aerobic performance and mating behaviour of the sexually coercive male eastern mosquito fish (*Gambusia holbrooki*) offers an excellent model system for testing the benefits of reversible acclimation responses to mating success. We exposed male mosquito fish to normoxic or hypoxic conditions for four weeks and tested their maximum sustained swimming performance and their ability to obtain coercive matings under both normoxic and hypoxic conditions. We predicted that hypoxia-acclimated males would possess greater swimming and mating performance in hypoxic conditions than normoxic-acclimated males, and vice versa when tested in normoxia. Supporting our predictions, we found the sustained swimming performance of male mosquito fish was greater in a hypoxic environment following long-term exposure to low partial pressures of oxygen. However, the benefits of acclimation responses to mating performance were dependent on whether they were tested in the presence or absence of male-male competition. In a non-competitive environment, male mosquito fish acclimated to hypoxic conditions spent a greater amount of time following females and obtained more copulations than normoxic-acclimated males when tested in low partial pressures of oxygen. When males were competed against each other for copulations, we found no influence of long-term exposure to different partial pressures of oxygen on mating behaviour. Thus, despite improvements in the aerobic capacity of male mosquito fish following long-term acclimation to hypoxic conditions, these benefits did not always manifest themselves in improved mating performance. This study represents one of the first experimental tests of the benefits of reversible acclimation responses, and indicates that the ecological significance of physiological plasticity may be more complicated than previously imagined.

Notes:

Cooler temperatures increase sensitivity to UVb in embryos and larvae of the frog *Limnodynastes peronii*

Vincent O. van Uitregt[1], Robbie S. Wilson[1] and Craig E. Franklin[1]
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Recent studies suggest that complex interacting processes are driving global amphibian declines. Increased ultraviolet B radiation in the solar spectrum due to ozone depletion has been implicated in declines and evidence suggests that the effects of UVb radiation on amphibians can be exacerbated at low temperatures. We tested the thermal sensitivity of UVb effects on amphibians in a controlled factorial experiment using the striped marsh frog, *Limnodynastes peronii* as a model species. We compared survival, growth and locomotor performance of embryonic and larval *L. peronii* reared under low and high UVb exposures at both 20 and 30ºC. Embryonic and larval *L. peronii* proved extremely sensitive to UVb damage and exhibited greater sensitivity at 20ºC compared to 30ºC. Embryonic survival to Gosner stage 25 was unaffected by UVb exposure at 30ºC, but at 20ºC survival was reduced to 52% under high UVb. Larval survival exhibited a similar trend. At 20ºC, all tadpoles survived under low UVb, whereas under high UVb there was 100% mortality. At 30ºC, 86% survived under low UVb, but only 46% survived under high UVb. Embryonic malformation and reduced growth induced under high UVb, showed similar trends of exacerbation at 20ºC. We also found a UVb induced reduction in locomotor performance of larval *L. peronii* which may have significant impact on survival due to its importance in predator avoidance. Again, UVb effects on locomotor performance were exacerbated at 20ºC. Our results strongly indicate that UVb damage in amphibians is markedly increased at lower temperatures. Thus, populations of UVb sensitive species occurring at cold climates may be at greater risk of declines due to increased solar UVb radiation.

Notes:

Predicting Complex Phenotypes in Complex Environments: The Ontogeny of Anurans in Fluctuating Temperatures

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Although diurnal temperature changes are widespread in nature, many lab studies only consider plasticity among stable conditions. Do these studies provide information that can be generalised to a real world that's much more complex? To address this question, we used an anuran model (*Limnodynastes peronii*) to examine the predictability of ontogeny in thermally-fluctuating environments. We recorded the growth and developmental patterns of tadpoles in a series of stable temperatures (18, 22, 26, 30, and 34°C) at various stages between embryogenesis and metamorphosis. Using model selection, we determined the best-fitting curve for each pattern and integrated across a daily cycle to predict growth and development for tadpoles raised in two diurnally-fluctuating regimes (18-28°C and 18-34°C), which approximated more realistic natural conditions. Tadpoles in the 18-34°C treatment spent 4 h per day at the chronically-lethal temperature of 34°C, during which time we assumed development and growth would be zero. Overall, developmental rates could not be predicted for either fluctuating treatment. In the 18-28°C fluctuating treatment, which fell entirely within the range of the stable temperature groups, growth was predictable for almost all stages. In contrast, growth in the 18-34°C fluctuating treatment was never accurately predicted. Clearly, predicting phenotypic outcomes at maturity can be a difficult task, but to make sure that we understand patterns of phenotypic plasticity in nature it will be necessary to design ecologically-relevant experiments that encompass the entire developmental period.

Notes:

Proton flux and uncoupling proteins in mitochondria from the amphibian *Bufo marinus*

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Physiological adaptations of mitochondrial bioenergetics have been rarely studied in the past. Investigations in hibernating frogs suggest dramatic changes in respiration rate and membrane potential (St Pierre *et al.* 2000). These data suggest that respiration rate and proton leakage are decreased in periods of limited energy supply, ensuring survival of the organism under hypometabolic conditions. In order to investigate mitochondrial adaptations due to nutritional state and acclimation temperature in amphibians, we exposed male cane toads (*Bufo marinus*) to either 10°C or 30°C. At each acclimation temperature, cane toads were divided into two groups, one group was fasted for nine days and the other group fed for nine days. Proton leak kinetics of isolated mitochondria from liver and skeletal muscle was measured at 25°C. Notably, we observed five times higher respiration rates in skeletal muscle compared to liver mitochondria in all toads.

In skeletal muscle, proton leakage was higher in cold acclimated toads. However, fasting increased proton conductance under cold and warm conditions. Pertaining to the liver, ambient temperature had minor effects on proton leak kinetics. Interestingly, fasting decreased liver membrane potential across the inner mitochondrial membrane of cane toads acclimated to 10°C as expected, but increased liver membrane potential in cane toads acclimated to 30°C. The molecular nature of the changes in proton conductance in amphibians is not known. Therefore, we searched for uncoupling activity based on the recent identification of UCP1 in liver of fish and preliminary data on the identification of gene expression in the liver of *Xenopus*. Whereas the UCP-activator palmitate increased state 4 respiration under all conditions, the inhibitor GDP failed to diminish these effects as it does in mammals. Temperature and nutritional state have minor effects on proton motive force in isolated liver and skeletal muscle mitochondria of mammals. Here, we demonstrate the importance of mitochondrial adaptations of ectotherm vertebrates by dramatic changes in proton conductance and respiration in the cane toad.

Notes:

Getting the jump on skeletal muscle disuse atrophy: preservation of contractile performance in aestivating *Cyclorana alboguttata*

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Prolonged immobilisation or unloading of skeletal muscle causes muscle disuse atrophy, which is characterised by a reduction in muscle cross-sectional area and compromised locomotory function. Animals that enter seasonal dormancy, such as hibernators and aestivators, provide an interesting model for investigating atrophy associated with disuse. Previous research on the amphibian aestivator *Cyclorana alboguttata* (Günther, 1867) demonstrated an absence of muscle disuse atrophy after three months aestivation, as measured by gastrocnemius muscle contractile properties and locomotor performance. In this study, we aimed to investigate the effect of aestivation on iliofibularis and sartorius muscle morphology and contractile function of *C. alboguttata* over a longer, more ecologically relevant time-frame of nine months. We found that whole muscle mass, muscle cross-sectional area, fibre number and proportions of fibre types remained unchanged after prolonged disuse. There was a significant reduction in iliofibularis fibre cross-sectional area (declined by 36% for oxidative fibre area and 39% for glycolytic fibre area) and sartorius fibre density (declined by 44%). Prolonged aestivation had little effect on the isometric properties of the skeletal muscle of *C. alboguttata*. There was a significant reduction in the isometric contraction times of the relatively slow-twitch iliofibularis muscle suggesting that the muscle was becoming slower after nine-months of aestivation (time to peak twitch increased by 25%, time from peak twitch to half relaxation increased by 34% and time from last stimulus to half tetanus relation increased by 20%) . However, the results of the work loop analysis clearly demonstrate that despite changes to muscle morphology and isometric kinetics, the overall contractile performance and power output levels of muscles from nine-month aestivating *C. alboguttata* are maintained at control levels.

Notes:

Embryonic thermal tolerance and temperature variation in incubation mounds of the Australian brush-turkey (*Alectura lathami*)

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The Australian brush-turkey (*Alectura lathami*) is a megapode bird that constructs incubation mounds and relies on the heat produced by respiring microorganisms in these mounds to incubate its eggs. Previous investigations posited stable mound temperatures however, this study challenges this assertion. A new method for monitoring mound and egg temperature was used to determine both mound thermal variability and the thermal tolerance of embryos. From the day of laying, the temperature of eggshells and surrounding mound material were monitored continuously throughout incubation. All mounds exhibited greater temperature fluctuations than previously reported or predicted by modeling. Furthermore, brush-turkey embryos were tolerant of prolonged exposure to suboptimal incubation temperatures, developing successfully despite experiencing temperatures 5.5^{o}C above or 9^{o}C below the optimum for 12h. This is the first evidence of bird embryos enduring long term exposure to suboptimal temperatures during development. Remarkably, natural incubation periods were not correlated with mean incubation temperature and were 3-6d shorter than previously reported for this species. In reptiles, incubation temperature has been shown to have profound affects on embryonic development and hatchling phenotypes. Given this, further research is needed to determine if differences in incubation temperature affect ecologically important traits in brush-turkey chicks.

Notes:

Transition from ectothermy to endothermy: the development of metabolic capacity in a bird (*Gallus gallus*)

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The evolution of endothermy is one of the most significant events in vertebrate evolution. Adult mammals and birds are delineated from their early ontogenetic stages, as well as from other vertebrates, by high resting metabolic rates and consequent internal heat production. We used the embryonic development of a bird (*Gallus gallus*) as a model to investigate the metabolic transition between ectothermy and endothermy. Increases in aerobic capacity occur at two functional levels that are regulated independently from each other: (i) upregulation of gene expression; and (ii) significant increases in the catalytic activity of the main oxidative control enzymes. Anaerobic capacity, measured as lactate dehydrogenase activity, is extremely high during early development, but diminishes at the same time as aerobic capacity increases. Changes in lactate dehydrogenase activity are independent from its gene expression. The regulatory mechanisms that lead to endothermic metabolic capacity are similar to those of ectotherms in their response to environmental change. We suggest that the phylogenetic occurrence of endothermy is restricted by its limited selective advantages rather than by evolutionary innovation.

Notes:

What impact has PGC-1α on the development and plasticity of metabolic capacity in birds?

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Birds are endotherms and incur energetic costs to maintain a stable body temperature, especially in a cold environment. Consequently, the tight regulation of metabolic capacity is crucial for maintenance of a constant body temperature. In mammals, aerobic metabolic capacity is controlled by several nuclear hormone receptors, such as peroxisome proliferator activated receptors (PPARα, γ and δ) and, most importantly, their coactivators. The coactivator PGC-1α is referred to be the major metabolic regulator in mammals. Despite its importance in mammals, a function of PGC-1α as metabolic regulator in birds is unknown. This prompted us to investigate if PGC-1α acts as key metabolic regulator during development in chicken (*Gallus gallus*). Therefore we analyzed PGC-1α and PPAR γ gene expression in skeletal muscle and liver. Furthermore we compared the influence of slightly colder incubation temperature on PGC-1 α signaling during embryogenesis, and its potential consequences for the postnatal plasticity of metabolic capacity. PGC1α and PPAR γ gene expression are elevated during embryogenesis in skeletal muscle and liver in comparison to adults. After hatching gene expression decreased. Colder incubation temperature (35°C instead of 38°C) leads to a delayed response in PGC1 α and PPAR γ expression.

Notes:

Axial muscle activity in swimming crocodilians

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The pattern of muscle activity in the axial musculature of swimming saltwater crocodiles (*Crocodylus porosus*) was assessed using videography and synchronised electromyography (EMG). Saltwater crocodiles exhibit unilateral, uniphasic contractions in the epaxial muscles on the side of the body that is in flexion. A travelling wave of EMG activity is closely followed by a travelling wave of lateral undulation. Maximum lateral displacement is seen on the contralateral side near the end of muscle activity. Preliminary studies also show that at least some hypaxial muscles are involved in swimming, but further study is needed to quantify this. These observations support the hypothesis that the epaxial muscles and at least some hypaxial muscles function to produce lateral undulation of trunk and tail during swimming. These observations also fit within a model of the crocodilian bracing system that allows the initiation of movement and the bracing of mechanical loads during locomotion.

Notes:

Pattern Recognition Proteins in the Scleractinian Coral Acropora millepora

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Scleractinian corals are sessile basal invertebrates that form the framework of coral reefs, which are the most diverse marine ecosystems on the planet. Reefs form the basis of billion dollar industries and provide subsistence living for more than 100 million people globally. Coral reef ecosystems are under immense pressure from a multitude of threats, including increasing frequencies of bleaching and disease events, which have been linked to human activities, and a multitude of natural and anthropogenically-influenced environmental stressors. Despite these threats, knowledge of coral immune responses and host-pathogen interactions is surprisingly sparse. This study is part of a larger project that aims to identify the major components and pathways of the coral immune system, which provides the foundation for further studies into the effect of environmental stressors on basal invertebrate immunity functions. We have isolated two pattern recognition proteins (PRPs) from the coral *Acropora millepora*. PPRs are receptors that bind to pathogens and other non-self molecules and often initiate an immune response. The proteins were isolated using affinity chromatography, and the N-terminal protein sequence was obtained using Edman degradation. The cDNA sequence for one of the proteins has been obtained and shows homology to mannose binding lectins involved in immunity in a wide range of organisms, including vertebrates. The identified protein contains a C-type lectin domain and includes binding sites for mannose and calcium. Biotinylation studies indicate binding to G+ bacteria and potential function. Real-time PCR and immuno-chemistry studies are underway and will reveal detailed information on the function of the identified proteins. The presented work represents the first step in examining the response of the coral immune system to environmental stress and disease. Additionally, the findings provide useful information for immunity research in corals, as well as marine invertebrates in general, and for elucidating the evolution of the innate immune system.

Notes:

Non-Self Recognition Proteins in Sydney Rock Oysters, *Saccostrea glomerata*

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The Sydney rock oyster industry is currently worth $33.2 million to Regional NSW and Southern Queensland. The most significant threat to the industry is QX disease caused by the protozoan parasite *Marteilia sydneyi*, which infects oysters in late summer and autumn (Wesche *et al.,* 1999). The majority of oysters die (up to 98%) 6 to 8 weeks later due to the large number of parasites directly blocking the digestive gland and effectively starving the oyster to death (Wolf, 1972). There is no treatment for the disease and current disease management and prevention practices are failing. The long term commercial viability of the industry relies on the development of disease resistant stock. Identification of an immunological marker that can be used to assess the level of resistance of Sydney rock oysters to QX disease would rapidly speed up the process of producing resistant oysters (Berthe et al., 2003)

The small percentage of Sydney rock oysters that survive infection from QX must have some means of distinguishing *M. sydneyi* as being foreign for either the destruction or eventual removal of the parasite from the host. The role of carbohydrate-binding proteins (lectins) in non-self recognition/defence mechanisms in bivalves is well documented (Vasta, 1996). Oysters that possess lectins which are capable of recognising *M. sydneyi* would be considered good candidates for a selective breeding program. A mannose/galactose binding lectin was isolated from the hemolymph of Sydney rock oysters using affinity chromatography. Analysis by SDS-page under reducing and non-reducing conditions revealed a multimeric structure composed of 16 000 Da subunits. Binding experiments using biotinylated lectin to several oyster pathogens including *Vibrio* bacteria and *M. sydneyi* will be presented.

Notes:

Thermal plasticity of diving behaviour, aquatic respiration, and locomotor performance in the Mary River Turtle, *Elusor macrurus*

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Locomotion is a common measure of performance used in studies of thermal acclimation due to its correlation with predator escape and prey capture. For sedentary animals like freshwater turtles, we propose that diving behaviour is a more ecologically relevant measure of performance. Increasing dive duration in hatchling turtles reduces predator exposure and therefore functions as a performance benefit. Diving behaviour is known to be thermally dependent and is also influence by aquatic respiration. In this study we examined the influence of thermal acclimation on diving behaviour, aquatic respiration, and locomotor performance in the endangered Mary River Turtle, *Elusor macrurus*. Diving behaviour was found to partially acclimate at 17ºC with those being acclimated to a cold environment (17ºC) having a significantly longer dive duration than those acclimated to a warm environment (28ºC). This increase in dive duration at 17ºC was not a result of physiological alterations in metabolic rate but was due to increased aquatic respiration. Increasing aquatic oxygen consumption permitted one cold acclimated hatchling to remain submerged underwater for over 2.5 days. Warm acclimated turtles did not thermally acclimate as predicted. When burst swimming speed was used as the measure of performance thermal acclimation was not detected. Overall, *E. macrurus* demonstrated little ability to acclimate to changes in environmental temperature.

Notes:

Captive management issues and the Tasmanian Devil Facial Tumour Disease (DFTD)

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Devil Facial Tumour Disease (DFTD) is a newly emerged, contagious and fatal disease that is decimating wild populations throughout Tasmania. DFTD has now also spread to devils in one of the state's wildlife parks, placing emphasis on the need for improved biosecurity. A priority role for DFTD management is to try to ensure the health and genetic diversity of captive populations of devils for the future, as a backup strategy against continuing loss of the species in the wild. The only existing captive populations that remain are housed at institutes in Tasmania and on mainland Australia. In the past, the ability to maintain a 'self-sustaining' captive devil population has been constrained by limited reproductive success, which is exacerbated by a previous lack of knowledge of the species' breeding biology, and their characteristically short reproductive lifespan of only three years. As DFTD continues to spread throughout wild populations, the need to protect and effectively maintain genetically and demographically viable captive populations for the future is a vital conservation issue. In response to this, in early 2005 the Tasmanian government established an "insurance population" of devils from the wild to be retained in-state pending further information about the disease and its' mode of transmission. Because DFTD usually manifests in adult animals, as a risk-reduction measure the founders were retained in purpose-built quarantine facilities until they became sexually mature. Captive management of the founder devils included application of our knowledge of the species' reproductive physiology (Hesterman and Jones, submitted papers), and successful breeding of insurance devils was achieved. The quarantine period is complete with all insurance devils remaining in good health, and exports to mainland zoos are intended to occur mid-Nov to mid-Dec 2006.

Notes:

Tight junctional protein, occludin is upregulated with evolution of viviparity.

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Evolution of viviparity (live-bearing) is associated with changes in the functional role of the uterine epithelium. In oviparous amniote vertebrates, the uterine epithelium protects the developing egg and is more or less just a passive container, whereas in viviparous species the uterine epithelium serves to nourish the developing embryo. Tight junctions between adjacent cells constitute the 'barrier' to the passage of ions and molecules through the paracellular pathway and functions as a 'fence' within the plasma membrane to create and maintain apical and basolateral membrane domains. We studied the tight junction and essentially the upregulation of occludin, a key tight junctional protein, in uterine epithelial cells of lizards. The study included bimodally reproductive *Saiphos equalis* and *Lerista bougainvilli* and *Pseudemoia,* a genus with a complex reptilian placenta. Transmission electron microscopy (TEM), immunohistochemistry, and western blot analysis of occludin, an integral protein that exclusively labels tight junctional strands was carried out to determine whether changes in occludin expression occurs between these different reproductive modes. The tight junction, was for the first time identified in oviparous and viviparous lizards ultrastructurally, however occludin was only present in the uterine epithelium of viviparous *P. entrecasteauxii* and *P. spenceri,* both species with a complex reptilian placenta. Occludin upregulation in viviparous species and not in oviparous species corresponds with the tight junction becoming more impermeable or 'tighter' during the evolution of viviparity in lizards. Additionally this may suggest that different mechanisms may be involved in fluid and ion transport which relate to differences in the uterus between oviparous and viviparous species.

Notes:

Expression of renal aquaporins during water deprivation in the Spinifex hopping mouse

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Aquaporins (AQPs) are a family of transmembrane water channel proteins that maintain the osmotic pressure and volume of cells, and are critical in the reabsorption of water in the kidney. The proximal tubule and descending limb of the loop of Henle reabsorb up to two-thirds of filtered water and solutes, and play a crucial role in the production of the cortico-medullary osmotic gradient, and are the site of AQP1 expression. Aquaporins 2, 3 and 4 are expressed in the collecting duct (CD) of the mammalian nephron. The CDs are the final segment from which water can be reabsorbed from the filtrate, and are the site that vasopressin elicits its physiological response. AQP2 is stored in intracellular vesicles and inserted into the apical membrane of CD epithelial cells when vasopressin binds to V2 receptors on CD cells. AQP3 and –4 are located in the basolateral membrane of CD cells, thus forming the exit pathway for water from CDs. The Spinifex hopping mouse, *Notomys alexis*, is able to produce highly concentrated urine; thus the movement of water across the epithelium of the renal tubules must be stringently regulated. Aquaporins 1, 2, 3 and 4 were cloned and the mRNA expression was determined during different periods of water deprivation. After each period, there was an increase in the mRNA expression of AQP1 and -3, but AQP2 mRNA increased after three and seven days only, while AQP4 mRNA decreased after three and seven days of water deprivation. In addition, the cellular localisation of AQP2 immunoreactivity was shown to change considerably during water deprivation and was almost exclusively expressed on the apical membrane of CD cells after 14 days of water deprivation. Furthermore, AQP3 immunoreactivity appeared to increase, while AQP4 immunoreactivity decreased in the collecting ducts in the kidney of *N. alexis* during water deprivation.

Notes:

Genomic analysis of chicken natriuretic peptide (NP) genes: new insights into the evolution of the NP system in tetrapods

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Natriuretic peptides (NPs) are key regulators of fluid and ion homeostasis and the cardiovascular system. The NP family consists of multiple subtypes in teleost fish, including atrial, B-type, ventricular, and C-type NPs (ANP, BNP, VNP, CNP-1 to -4, respectively), but only ANP, BNP, CNP-3, and CNP-4 have been identified in tetrapods. To trace the molecular evolution of NPs in the tetrapod lineage, we identified NP genes in the chicken genome. Previously only BNP and CNP-3 have been identified in birds, but we isolated two new chicken NP genes that were characterized by cDNA cloning, chromosomal synteny, and phylogenetic analyses. One gene is an orthologue of CNP-1, which has only ever been reported in teleostei and the primitive bichir. The second gene encodes an NP that could not be assigned to a particular subtype due to high sequence divergence. However, we named this novel peptide renal NP (RNP) because it was abundantly expressed in the adult chicken kidney. In chicken, the CNP-3, RNP and BNP genes are tandemly located on chromosome 21, while CNP-1 is present on chromosome 1. The locations of the NP genes and surrounding gene loci were also mapped in the human and pufferfish genomes to identify orthologous regions. From conserved chromosomal synteny, we propose that the CNP-4 and ANP genes have been lost in chicken and that the RNP gene is possibly an orthologue of fish VNP. This study demonstrates that CNP-1 was retained in the tetrapod lineage and that chickens possess a unique renal NP. Future studies will examine the physiological role of RNP in chickens, and determine if the peptide is present in reptiles. RNP may also have future applications in human health, specifically the treatment of hypertension.

Notes:

Regulation of salt secretion and blood flow in the lingual salt glands of the estuarine crocodile, *Crocodylus porosus*

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The estuarine crocodile, *Crocodylus porosu*s can maintain osmotic and ionic homeostasis over a wide range of salinities from freshwater to hypersaline environments. In environments hyperosmotic to their body fluids, *C. porosus* utilise lingual salt glands, located on the tongue, to eliminate excess Na^+ and Cl^- ions. These extra-renal salt glands appear as a series of pores on the surface of the tongue and are richly innervated and vascularised. The glands which number between 20-40 produce a highly concentrated NaCl solution. The aim of this study was to examine the regulation of salt secretion from the lingual salt glands of *C. porosus* and to determine the relationship between secretion rate and blood perfusion. Blood flow in the salt glands was recorded using laser-Doppler flowmetry and recordings from restrained animals showed spontaneous changes in flow to the glands. We investigated the effect of methacholine chloride (MeCh), brain natriuretic peptide (BNP), vasoactive intestinal peptide (VIP), and angiotensin II (ANGII) on secretion rate and blood flow. Intravenous administration of MeCh, BNP and VIP all resulted in an increase in salt secretion from the lingual salt glands. Our findings suggest that while MeCh appears to be acting directly on the secretory tissue to stimulate salt secretion, the peptides appear to be influencing the glands both by acting on the epithelial tissue and by increasing blood flow.

Notes:

The influence of substrate water potential on the embryonic development of Brisbane river turtle eggs.

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Rigid-shelled eggs of the Brisbane river turtle were incubated in substrates of three water potentials, -100kPa, -350KPa, and -850kPa at 30^{o}C. Repeat measures ANCOVA with initial egg mass as the covariate indicated that clutch ($DF=3$, $F=19.721$, $P<0.001$) and water potential ($DF=2$, $F=126.66$, $P<0.001$) affected the pattern of egg mass change during incubation. Two weeks after incubation began eggs incubated at -100 kPa began to gain mass and this process continued until pipping. The mass of eggs incubated at -350 kPa remained relatively constant throughout incubation, while eggs incubated at -850 kPa began to lose mass after 4 weeks. Incubation period was not influenced by incubation water potential or clutch. Hatchling mass and size were not influenced by incubation water potential, and once adjusted for differences in initial egg mass, clutch of origin also did not influence these variables. Hatchling carcass dry mass was influenced by clutch of origin, but unaffected by incubation water potential, while residual yolk dry mass was influenced by both clutch of origin and incubation water potential. Residual yolk dry mass was greatest as a fraction of hatchling dry mass in hatchlings incubated at a water potential of -850 kPa and smallest in hatchlings incubated at -100 kPa.

Notes:

Queens live longer than workers: are dietary fats responsible?

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In the honey bee (*Apis mellifera*) females can be either workers or queens depending on their upbringing. Although they are genetically identical, the maximum life span of queens is an order-of-magnitude longer than workers. As a test of the membrane pacemaker theory of aging, we have measured and compared the fatty acid composition of phospholipids (and consequently membranes) of queen and worker honey bees. The cell membranes of both young and old honey bee queens are highly monounsaturated with a very low content of polyunsaturates. They are thus very resistant to lipid peroxidation. Newly-emerged workers have a similar membrane fatty acid composition to queens but within the first week of hive life they increase the polyunsaturate content and decrease the monounsaturated content of their membranes, likely as a result of consumption of pollen. This means their membranes become much more susceptible to lipid peroxidation. The 3-fold difference in peroxidation index of phospholipids is enough to explain the order-of-magnitude difference in longevity of queens and workers. The results support the membrane pacemaker theory of aging and furthermore, suggest that it is what is not in royal jelly (rather than what is in it) that is an important determinant of this longevity difference in honey bees.

Notes:

The effect of photoperiod acclimation on daily torpor and tissue fatty acid composition in deer mice

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Photoperiod and dietary lipids both influence thermal physiology and the pattern of torpor of heterothermic mammals. The aim of the present study was to test the hypothesis that photoperiod-induced physiological changes are linked to differences in tissue fatty acid composition of deer mice, *Peromyscus maniculatus* (~18 g body mass). Deer mice were acclimated for >8 weeks to one of three photoperiods: LD 8:16 (short photoperiod), LD 12:12 (equinox photoperiod), and LD 16:8 (long photoperiod). Deer mice under short and equinox photoperiods showed a greater occurrence of torpor than those under long photoperiods (71%, 70%, 14%, respectively). The duration of torpor bouts was longest in deer mice under short photoperiod (9.3±2.6 h), intermediate under equinox photoperiod (5.1 ± 0.3 h), and shortest under long photoperiod (3.7 ± 0.6 h). Physiological differences in torpor use were associated with significant alterations of fatty acid composition in ~50% of the major fatty acids from leg muscle total lipids, whereas white adipose tissue fatty acid composition showed fewer changes. Our results provide the first evidence that physiological changes due to photoperiod exposure do result in changes in lipid composition in muscle tissue of deer mice and suggest that these may play a role in survival of low body temperature and metabolic rate during torpor, thus enhancing favourable energy balance over the course of the winter.

Notes:

Thermal biology, daily torpor and behavioral ecology of an arid zone carnivorous marsupial, the brush-tailed mulgara

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The thermal biology of free-ranging mulgaras (*Dasycercus blythi*, 6M:3F) was investigated in hummock grassland in Uluru Kata Tjuta NP between June and August 2006, coinciding with the beginning of the breeding season. Mulgaras were implanted with temperature- sensitive transmitters and monitored between 6 and 55 days. Ambient temperature (T_a) showed pronounced daily fluctuations (~20°C) and dropped regularly <0°C during the night, whereas burrow T_a was more stable and remained >10°C. Mulgaras moved among burrows frequently and some burrow sharing was observed, apparently primarily associated with mating. Males and females displayed torpor, but patterns differed remarkably between sexes. In June, males entered brief, shallow torpor only occasionally with body temperatures (T_b) remaining >25°C. In contrast, females entered long torpor bouts (~10 hours) almost daily with T_b <15°C. Later, in July/August, males also displayed long and deep torpor similar to that of females. One of the 3 females gave birth in mid-August and at that time remained normothermic with T_b ~ 36°C. Most torpor entries occurred either shortly after sunrise or much earlier, at the beginning of the activity phase. Occasionally animals exhibited 2 torpor bouts, the first during the night and the second in the morning. On most days, arousals occurred near midday and activity commenced later around sunset. T_a had no discernable influence on torpor frequency and depth probably because the burrows' thermal inertia. In conclusion, torpor appears to be an energy-saving mechanism, which is employed frequently by mulgaras during winter, however, at different times for both males and females, torpor appears to be suppressed by reproduction.

Notes:

About a bout: what happens in a single hibernation cycle?

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Over the last 10 years we have used implanted temperature loggers to study hibernation in free-ranging echidnas. As in other hibernators, the echidna hibernation season is characterised by repeated cycles of cooling, hibernation, and rewarming to euthermic temperatures, or hibernation bouts. An individual echidna may show 10 - 25 such bouts during a season, and we have over 39 echidna years of data from 14 animals, providing a huge data set. Detailed analyses of Tb during these bouts have provided important insights into the physiology of hibernation in echidnas.

During each entry into hibernation, Tb follows a Newtonian cooling curve until it is in equilibrium with the substrate temperature. Conductances calculated from cooling curves are identical to those observed in cold-exposed euthermic echidnas. During hibernation bouts Tb closely tracks substrate temperature, but over the hibernation season echidnas showed behavioural thermoregulation by changing hibernacula. Early in the season echidnas preferred to hibernate in cool areas, but during the coldest months they moved to warmer hibernacula. Thermal buffering against excessive variation in Tb seems to be as important as maintaining a low Tb.

During rewarming, Tb follows a sigmoid curve, with the maximum rewarming rate at the midpoint. The average maximum rewarming rate was 5.0 ± 0.8 ºC h^{-1}, (64 arousals from 6 echidnas), and the average highest rate measured from each echidna was 6.6 ± 1.1 ºC h^{-1} (n=6) equivalent to a peak oxygen consumption rate of 1.3 ± 0.2 ml O_2 g h^{-1}, approximately 9 times the basal metabolic rate. This is approximately half the rewarming rate of an eutherian hibernator of equivalent mass.

Notes:

HPG axis activity in juvenile blotched blue-tongued skinks, *Tiliqua nigrolutea*

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We are investigating seasonal sex- and age-related differences in the activity of the hypothalamic-pituitary-gonadal axis in the blotched blue-tongued lizard, *Tiliqua nigrolutea* as part of an overall study of the onset of sexual maturation and its subsequent annual regulation in a seasonally-breeding, viviparous reptile. Males of this species breed annually, while females exhibit a multiennial cycle; age at maturity is unknown. Juveniles at ages 1, 6, 12, 18, 24 and 30 months of age were injected with GnRH and we measured the resulting plasma sex steroid (testosterone (T) and oestradiol (E2)) concentrations, to examine the age of onset of HPG axis regulation: we plan to follow these juveniles over time until sexual maturity is reached. We found clear sex- and age-related differences in the production of T and E2 (as a measure of the activity of the HPG axis) from a relatively early age. We have used a number of techniques to confirm sex in these juveniles, and have correlated this with changes in T:E2 at ages 24 and 30 months. This longitudinal study of juveniles of (now) known sexes has allowed us to examine characteristics such as head width and body size (growth) for the onset of the sexual dimorphisms observed in adults. We have correlated these to endocrine profiles and other aspects of life history.

Notes:

Does Leptin Provide a Metabolic Signal to the Reproductive System in Blue-tongued lizards, *Tiliqua nigrolutea*?

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Animals require adequate energy reserves to fuel successful reproduction: there must, therefore, be a physiological signal that informs the reproductive axis about the body's nutritional state. We aimed to test the hypothesis that leptin provides a metabolic signal to the reproductive system in blue-tongued lizards, a species in which energy intake is constrained by hibernation, and in which the females, but not the males, exhibit a multiennial reproductive cycle. We compared the annual cycles of plasma leptin, and corticosterone, as a second major metabolic hormone, in male and female blue-tongued lizards. In males, plasma corticosterone is high during the spring mating period, lowest during summer, and rises to a significant peak during late hibernation. In both reproductive and non-reproductive females, plasma corticosterone is minimal in spring. In pregnant females corticosterone peaks during late gestation, falling sharply around the time of birth: this pattern is not apparent in non-pregnant females. Plasma leptin concentrations vary between males and females but again, there was no significant difference between the patterns of plasma leptin in reproductive and non-reproductive females. These results suggest that that other factors, such as thyroid hormones, may contribute to determining an individual female's decision to breed in any one year.

Notes:

The Undiscovered Hemoglobins of Marsupials

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Marsupials are born at a very early stage; blood is all embryonic with multiple hemoglobins. One of these is found in low concentration and only for a few days. Its beta-type globin is similar to bird beta-globins; its gene forms part of the alpha cluster. Investigation of the alpha-gene family in marsupials has shown, not only the genes for the two embryonic alpha-type globins found in the blood of the Tammar Wallaby, but also a gene for the alpha-type theta globin, and another gene similar to the alpha-D-globin found in birds and some reptiles.

The theta and alpha-D genes found in marsupials appear to have no features that would prevent their translation into functioning globins. Genetic analysis, with particular reference to comparison of divergence rates of synonymous bases (silent sites) with divergence rates of non-synonymous (amino-acid changing) bases, indicated that the theta and alpha-D globins were not evolving at neutral rates but were under stabilizing selection. This indicates that each gene codes for a functioning molecule, at least during some stage of development, although the protein products are not as yet identified. The most likely stage for the appearance of such proteins is pre-natal.

Notes:

Circulatory physiology and haematology of southern bluefin tuna (*Thunnus maccoyii*)

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Haemoglobins (Hb) of several species of tuna are reported to display unusual O_2 binding properties when faced with changes in blood temperature. Some species reportedly have temperature insensitive Hb-O_2 binding, whereas others display a 'reversed' temperature effect such that an increase in temperature causes an increase in Hb-O_2 affinity. We studied the haematological properties of a hitherto unstudied species, the southern bluefin tuna (*Thunnus maccoyii*), to examine the prevalence of this phenomenon amongst tuna species. Haematocrit and Hb concentrations were 49 ± 2% and 153 ± 8 g dl^{-1}, respectively, which are comparable to values for mammals. At 0.5% CO_2, the oxygen partial pressure at which Hb was 50% saturated (P_{50}) was 2.89 kPa at 10°C, 1.74 kPa at 23°C and 1.95 kPa at 36°C, indicating a reversed temperature effect between 10°C and 23°C ($P<0.05$), and temperature insensitivity between 23°C and 36°C ($P>0.05$). At 23°C, the Hb displayed a marked Root effect (10% decrease in O_2 content from 7.8 to 7.0 mmol l^{-1} as CO_2 increased from 0.5% to 1.5%) and had a large Bohr factor ($\Delta\log P_{50}/\Delta pH = -1.55$). In another experiment, a newly designed implantable data logger has enabled us to obtain the first simultaneous measurements of heart rate (f_H) and body temperature (Tb) in untethered tuna. In 18°C water, Tb and f_H remained within the ranges of 19.1 – 23.3°C and 62 – 116 beats min^{-1}, respectively. These values were at their lowest when the tuna were fasted, and at their highest during feeding and postprandial periods. This technology should help to revolutionize our understanding of physiology and ecology of high performance fish in their natural environment.

Notes:

Interactive effects of testosterone and immune challenge on aerobic performance in House Sparrows

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There is a common perception that rises in circulating testosterone result in immunosuppression in breeding male vertebrates. Because immunity is believed to come at a cost, immune challenge is more likely to result in reduced peak aerobic performance in pre-breeding than in breeding males. We examined this question by measuring basal and peak metabolic rates in male and female sparrows under four different treatments: 1) testosterone treated/immune challenged, 2) testosterone treated/sham injected, 3) empty implants/immune challenged, and 4) empty implants/sham injected. The immune challenges consisted of three consecutive intraperitoneal injections of sheep red blood cells along with an intramuscular injection of keyhole limpet haemocyanin (KLH). Peak metabolic rate (during flight) and basal metabolic rate were measured before and after each of these treatments. Testosterone significantly reduced specific immunity in males, but had no significant effect on antibody formation to KLH in females or on constitutive immunity in either gender. Neither peak metabolic rate nor basal metabolic rate were affected by testosterone or by immune challenge. We therefore conclude that testosterone-induced immunosuppression is very limited in male sparrows and has little consequence on energetically costly activities.

Notes:

Regulation of oxygen in the air-breathing organ of Pacific tarpon (*Megalops cyprinoides*) in response to aquatic hypoxia and exercise

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Pacific tarpon are elopomorph teleost fish with an air-breathing organ (ABO) derived from a physostomous gas bladder. Oxygen partial pressure (P_{O_2}) in the ABO was measured on juveniles (238 g) with fiber-optic sensors during exposure to selected aquatic P_{O_2} and swimming speeds. At slow speed (0.65 BL s^{-1}), progressive aquatic hypoxia triggered the first breath at a mean P_{O_2} of 8.3 kPa. Below this, opercular movements declined sharply and visibly ceased in most fish below 6 kPa. At aquatic P_{O_2} of 6.1 kPa and swimming slowly, mean air-breathing frequency was 0.73 min^{-1}, ABO P_{O_2} was 10.9 kPa, breath volume was 23.8 ml kg^{-1}, rate of oxygen uptake from the ABO was 1.19 ml kg^{-1} min^{-1}, and oxygen uptake per breath was 2.32 ml kg^{-1}. At maximum speed (2.4 BL s^{-1}) at 6.1 kPa, ABO oxygen uptake increased to 1.90 ml kg^{-1} min^{-1}, through a variable combination of breathing frequency and oxygen uptake per breath. In normoxic water, tarpon rarely breathed air and apparently closed down ABO perfusion, indicated by a drop in ABO oxygen uptake rate to less than 1% of that in hypoxic water. This occurred at a wide range of ABO P_{O_2} (1.7 – 26.4 kPa), suggesting that oxygen level in the ABO was not regulated by intrinsic receptors.

Notes:

Honest and dishonest signals of strength in slender crayfish (*Cherax dispar*): are males cheating bastards?

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Intraspecific combat is routinely employed by animals to resolve territorial disputes and gain access to mates. Many animals resolve disputes without combat by signaling their own fighting potential via threat displays and comparing this against the potential of their opponent. Current theory predicts signals of potential strength should be honest, while dishonest displays should only occur at very low frequencies in nature. We tested this prediction by investigating the relationship between morphology, performance and social dominance in males and females of the slender crayfish, *Cherax dispar*. Crayfish routinely use their enlarged front claws (chelae) for both intimidation and fighting, making them an ideal system to examine the honesty of signals of fighting capacity. We evaluated five competing models relating morphological and physiological traits (body length, body condition, claw size, and claw strength) to dominance during paired competitive bouts. In an honest signaling system, claw size and strength will be good predictors of dominance during competitive interactions. We found females that possessed large chelae were more likely to possess stronger chelae and those individuals with stronger chelae were more likely to win competitive bouts, thus supporting current theory. In contrast, we found chelae strength of individual male *C. dispar* had no bearing on their dominance ability, indicating that displays of claw size were dishonest signals and the enlarged claws of males function more for intimidation than actual strength. Given the extent of bluffing among male *C. dispar*, it appears current theory underestimates the potential importance of dishonest signals in intraspecific animal communication.

Notes:

Small gut motility in the brush tail possum: Do the longitudinal muscles of the small intestine of herbivores exhibit greater motility than those of other species?

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We have developed a high resolution spatiotemporal mapping system that allows high fidelity resolution of longitudinal muscle movements in the isolated small intestine.

Mapping of wall movements in the isolated ileum of the brushtail possum (*Trichosurus vulpecua*) using this system shows the presence of alternating longitudinal waves of contraction that may reflect greater responsiveness to 'slow wave' activity. These contractions continue during and between peristaltic events. Longitudinal contractions of this frequency have not been observed in the ileum of non herbivore species and may represent an adaptation to the high apparent viscosity of herbivore digesta.

The phasic relationship of longitudinal with circumferential contractions varies during peristalsis in a manner that may modulate the apparent viscosity of the contents to promote mixing by vorticeal flow. Longitudinal movements between peristaltic events may also serve to promote circulation of the fluid phase between adjacent villi.

Notes:

Thermogenic overshoot in albatrosses fed cold meals

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We investigated the thermogenic response of albatrosses to cold meals and estimated, using measured physical properties of food used, that the energy cost required to heat a meal at 0ºC was 4.8% of assimilated energy (AE). We found that the energy cost was over 50% higher than our estimate, or some 7.5% of AE. We attribute this effect, which has been reported for other endotherms, to the time required for an endotherm to restore elevated post prandial metabolic rate to resting rate following the thermal equilibration of a cold meal with body core.

Notes:

A mechanistic explanation for the drought-related mortalities of juvenile kangaroos: implications for population biology

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Red kangaroos (*Macropus rufus* Desmarest) are one of the largest (>20 kg) extant marsupial herbivores and inhabit much of arid and semi-arid inland Australia. The population dynamics of red kangaroos are intimately associated with rainfall, mediated largely through juvenile survival. A crucial stage is the young-at-foot (YAF) stage, when kangaroos have permanently left the mother's pouch but continue to take some milk. Along with recently weaned kangaroos, YAF have the highest drought-related mortalities of any age cohort and forage quality has been implicated as the major factor affecting their survival. We compared the digestive capabilities of juvenile kangaroos with those of mature, non-lactating females on high-quality, low-fibre (neutral-detergent fibre; NDF) forage (chopped lucerne *Medicago sativa* hay; NDF 43 ± 1%) and poorer quality, high-fibre forage (chopped oaten *Avena sativa* hay; NDF 64 ± 1%). On chopped lucerne apparent dry matter (DM) digestibility by the YAF, weaned and mature female kangaroos were similar (55 - 59%). On chopped oaten hay apparent DM digestibility was lower in the YAF (35.9 ± 2.3%) followed by weaned (43.4 ± 2.8%) and mature females (44.6 ± 1%). The digestion of NDF and its components (mainly cellulose and hemicellulose) was lowest among the YAF followed by weaned and then mature females. The YAF and weaned kangaroos could not sustain growth on the poor-quality diet, and appeared to be at or near maximal gut fill on both forages. In contrast, mature females were not significantly affected by diet, maintaining DM intakes by increasing gut fill from 264 ± 24 g DM on chopped lucerne to 427 ± 26 g d DM on chopped oaten hay. Our results provide the first mechanistic explanation linking the physiological constraints faced by juvenile red kangaroos in relation to their drought-related mortalities, rainfall and forage quality, the three principal factors affecting recruitment and overall population dynamics.

Notes:

Investigations into the effect of handling on the natriuretic peptide system of salmonids

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The natriuretic peptide (NP) system is a regulator of teleost osmotic and cardiovascular homeostasis. Recently, we investigated whether the NP system is also involved in the stress response of salmonids. In an initial study, mRNA expression of cardiac NPs increased as did the response of the gills to NPs, as measured by guanylyl cyclase activity, in seawater salmon subjected to handling and tank transfer. We also investigated the mRNA expression of the guanylyl cyclase receptors, NPR-A and NPR-B, in saltwater and freshwater rainbow trout gills subjected to handling and crowding for 4 hours (handled). Traditional indicators of stress: plasma osmolality, glucose, lactate and cortisol were also measured immediately following the handling (0 hours) and at 12 and 48 hours post-handling. NPR-A mRNA expression decreased in saltwater, handled fish at 12 h compared with controls and pretreatment, and at 45 hours compared with pre-treatment. Control values were also depressed at this time. There was no difference in NPR-A expression between freshwater, handled fish and controls. NPR-B mRNA expression increased in saltwater, handled fish at 12 hours and at 0 hours in the freshwater, handled group. Plasma cortisol was elevated in all groups at 0 hours but remained elevated in stressed fish at 48 hours. Plasma osmolality was elevated in saltwater, handled fish by 12 h and remained so; while in freshwater, handled fish the osmolality decreased at 0 hours but had returned to control values by 48 hours. Plasma glucose was elevated in both groups of freshwater fish at 0 hours and remained elevated throughout the experiment. In saltwater, handled fish, glucose increased at 0 hours and 12 hours, but had regained control values by 48 hours. Plasma lactate was variable and no differences were observed. Our initial findings support a role of the natriuretic peptide system in the stress response of salmonids exposed to typical stressors encountered in aquaculture settings.

Notes:

Preliminary Insights into the Life of an Echidna Burrow Young

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A recent, limited, opportunity to monitor a female echidna and her young has afforded some interesting preliminary insights into the thermoregulatory abilities and feeding behaviour of young echidnas after they leave the mother's pouch for "burrow life".

Notes:

Molecular control of vertebrate aestivation: potential role for epigenetic modification

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Adult green-striped burrowing frogs, *Cyclorana alboguttata*, survive droughts by entering a deep metabolic depression called aestivation. Metabolic depression in *C. alboguttata* is manifest, in part, by silencing of skeletal muscle bioenergetic genes, such as NADH ubiquinone oxidoreductase 1 and ATP synthase. In this study we tested the hypothesis that muscle gene silencing is associated with chromatin remodelling. We assessed mRNA transcript abundance of seven candidate genes, with known roles in epigenetically-mediated gene silencing in other vertebrates: transcriptional co-repressor SIN3A, DNA (cytosine-5-) methyltransferase, methyl CpG binding protein 2, chromodomain helicase, chromatin assembly factor 1, histone deacetylase 1 and silencing mediator for retinoid and thyroid hormone receptors. All of these seven genes showed a modest (1.1-3.5 fold) up-regulation in the aestivating muscle. This reached statistical significance for SIN3A and methyltransferase in standard pair-wise comparisons ($p<0.05$), and the candidates as a whole when analysed by Fishers's combined probability test ($p<0.01$). These data lend support to the hypothesis that bioenergetic gene silencing during aestivation is controlled by activation of several key genes, such as SIN3A, that mediate chromatin remodelling.

Notes:

Evolution of Viviparity in Sceloporine Lizards: In Utero PO_2 as a Developmental Constraint during Egg Retention

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Reptilian viviparity evolves through selection for increasingly prolonged egg retention in the oviduct. In the majority of sceloporine lizard species, however, egg retention past the normal time of oviposition results in retarded or arrested embryonic development. The present study was designed to test the hypothesis that O_2 availability is the proximate factor that determines the rate and degree of development when eggs are retained in utero. The four species of sceloporine lizards we used are characterized by developmental arrest (*Urosaurus ornatus* and *Sceloporus undulatus*) retarded development (*Sceloporus virgatus*), and normal development (*Sceloporus scalaris*) when eggs are retained. Eggs of these species were incubated for 10 days at 5.3, 7.9, 13.8, and 18.5 kPa partial pressure oxygen (Po_2) starting at the normal time of oviposition. These data were used to establish a standard curve for the relationship between Po_2 and the amount of development. The standard curve was then used to predict the Po_2 associated with the observed rate of development of embryos retained in utero. The results of this study showed that Po_2 in utero is directly related to the amount of development that occurs in eggs that are retained beyond the time of normal oviposition, ie., Po_2 increased in the order of *U. ornatus, S. undulatus*, *S. virgatus*, and *S. scalaris*. The results indicate that selection for extended egg retention is associated with incremental increases in the availability of oxygen to embryos.

Notes:

Effects of ß- naphthoflavone on Liver Microsomal Mixed Function Oxidase Activities of *Huso huso*.

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Cytochrome P4501A1 is a major isoenzyme in fish monooxygenase system which is induced by polycyclic aromatic hydrocarbons (PAHs) compounds. In this research, the inducing effect of β-naphtoflavone was studied in *Huso huso* liver and some catalytic properties of this isoenzyme were determined. Fish received an i.p. injection of β-naphthoflavone at three different doses. The enzyme activity was measured with deethylation of ethoxyresorufin reaction (EROD) by fluorometry method and relative amount of induced proteins were determined with polyacrylamide gel electrophoresis (SDS-PAGE). The results showed that EROD activity in the treated fish microsomal fraction was 15-26 folds to the control group. Optimum activity of this enzyme were observed at 20-25 º C. The maximum of enzyme activity was seen in precence of 180 microgrms of microsomal protein and 1.53µM of 7-ethoxyresorufin. SDS-PAGE of microsomal protein pattern in the treated fish showed that the relative amount the protein with molecular mass 58±1 KDa which is cytochrome P4501A. So the induction effect ofβ-naphtoflovone in fish liver could be induced cytochrome P4501A gene and is increased the enzyme biosenthysis, which is raised the enzyme activity in EROD reaction.

Notes:

Innervation of crocodilian salt glands: acclimatory responses of salt glands to hypersaline environments

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Most avian and reptilian salt glands display marked phenotypic plasticity when animals are exposed to hyperosmotic conditions. In addition, the activity of most salt glands is under considerable control by the nervous system and nerves containing cholinergic, adrenergic and peptidergic neurotransmitters have been identified in avian and reptilian salt gland tissues. The present study sought to determine whether the salt glands of the Estuarine crocodile, *Crocodylus porosus* contain the peptidergic neurotransmitters SP, CGRP, VIP, and PACAP and the gaseous neurotransmitter, NO. In addition, we sought to determine whether there was any evidence for the adaptation of the *C. porosus* salt gland nervous system to hyperosmotic conditions. Salt glands from fresh- and salt-water acclimated *C. porosus* hatchlings were sectioned and examined immunohistochemically for neurotransmitters within the tissue. Neurons containing SP, CGRP, VIP, PACAP and NO synthase were identified within *C. porosus* salt glands. There was no difference in the overall number (density) of neurons within SW-acclimated tissues when compared with FW-acclimated animals. However, there was a significant reduction in density of neurons containing SP and PACAP in SW-acclimated animals. *C. porosus* salt glands display phenotypic plasticity following exposure to hyperosmotic conditions. In addition to cholinergic and adrenergic neurons, they contain a variety of peptidergic neurotransmitters and the gaseous neurotransmitter NO. Moreover, there appears to be some evidence of acclimation of the nervous system of *C. porosus* to hypersaline conditions, although the functional significance of these changes remains to be determined.

Notes:

The effect of corticosterone on ultrastructural characteristics of chicken leukocytes

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Although the knowledge of the mechanisms of the immune system is being generated at a much faster rate in mammals, avian immunology has continued to make great steps forward in recent years. Among the avians, the immune system of the chicken has been studied most extensively. The immune system of birds, as of mammals, comprises a number of cells and soluble factors that work in concert to produce a protective immune response. Immune cells or leukocytes are essential for the maintenance of an effective immunity and their numbers and proportions in the blood provide an important representation of the state of activation of the immune system. Moreover, morphological and cellular identification of leukocytes is an important tool in understanding the response of lymphoid and nonlymphoid tissues to microbial and nonmicrobial challenges. In birds, it has been indicated that a variety of stressors, including exposure to corticosterone (CORT) challenges the leukocyte response and affects the heterophil to lymphocyte (H/L) ratio. In fact, the H/L ratio itself represents a mathematical calculation of limited clinical value. It has been also documented that heterophilia (a counterpart to neutrophilia) predominates mainly in the acute inflammatory response and bacterial protection. In order to better understand the role played by CORT in the regulation of the leukocyte cell response (number, morphology and distribution) this study aimed to examine ultrastructural alterations of leukocytes separated from peripheral blood and bone marrow of stressed chickens. So far, results show distinct alterations of the ultrastructure of circulating leukocytes from CORT-treated birds. Changes were observed in heterophil shape, and density and size of granules. An increased number of promyelocytes and myeolocytes, mainly heterophil myeolocytes were detected in the bone marrow. This increase was associated with the presence of band heterophils in the blood stream, indicating the recruitment of immature heterophils from the bone marrow due to CORT. Morphological changes were examined also in lymphocytes and thrombocytes. It was demonstrated that CORT stimulates an increased release of heterophils from bone marrow and the marginated pool into the circulation. The mechanism behind this response which involves the activation of the cytokine network and the neuroendocrine system is likely to be similar to that of mammals. Variations may exist due to the differences in cytokine and chemokine regulation and inflammatory responses to which heterophils contribute.

Notes:

The importance of changes in heart rate and blood flow during thermoregulation in the estuarine crocodile, *Crocodylus porosus*

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In reptiles, rates of heat transfer between the animal and its environment have been shown to be controlled by the regulation of blood flow between the core and periphery. A faster heart rate during heating compared to cooling is thought to facilitate heat transfer, allowing the reptile to attain a higher body temperature more rapidly, thereby decreasing the behavioural cost of thermoregulation. In this study we tested the hypothesis that regional differences in blood flow during heating and cooling occur independently from changes in heart rate (cardiac output). We measured heart rate, blood pressure, and surface flow by Laser Doppler flowmetry, and we used coloured microspheres to track blood flow patterns to different parts of the body of the estuarine crocodile, *Crocodylus porosus* during heating and cooling with and without a blockade of cholinergic and β-adrenergic receptors. Heart rates during heating were significantly faster than during cooling in the control, but not when autonomic receptors were blocked. There were no significant differences in blood flow distribution between control and autonomic blockade treatments. Rates of heating and cooling were unaffected by the blockade of autonomic receptors and we conclude that animals partially compensated for lack of differential heart rates during heating and cooling by maintaining some control over peripheral flow.

Notes:

Poster Paper Abstracts

Isolation and purification of novel cellulase and hemicellulase enzymes in *Cherax destructor*

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The digestion of cellulose and hemicellulose in invertebrate species has traditionally been believed to be a result of cellulase enzymes produced by symbiotic microorgansims within the gut. Recent discoveries have indicated that some invertebrates are capable of endogenous cellulase production. However it is not understood how these enzymes work together to achieve efficient cellulose and hemicellulose hydrolysis. The first step in this characterisation is the purification of the major cellulase and hemicellulase enzymes. Cellulase enzymes, endo-β-1,4-glucanse and β-1,4-glucosidase and the hemicellulase enzyme, laminarinase were purified from the midgut gland of the crayfish, Cherax destructor using liquid chromatography. Together endo-β-1,4-glucanase and β-1,4-glucosidase may be capable of hydrolysing cellulose to glucose. Endo-β-1,4-glucanase randomly cleaves β-1,4-glycosidic bonds within the polysaccaride to produce shortened polymers and cellobiose, while β-1,4-glucosidase hydrolyses cellobiose into component glucose molecules. Laminarinase is able to hydrolyse β-1,3-glycosidic bonds, the other main glycosidic bond found in structural carbohydrates. An endo-β-1,4-glucanase of approximately 120 kDa in size was purified using firstly anion exchange (Macro prep DEAE) chromatography and secondly gel filtration (Bio-Gel P60 gel) chromatography. Three proteins of 80, 100 and 160 kDa are possible candidates for laminarinase. These proteins were isolated using DEAE anion exchange chromatography and then hydrophobic interaction (Macro-prep methyl HIC support) chromatography. Work is in progress to purify β-1,4-glucosidase using a combination of anion exchange chromatography, hydrophobic interaction chromatography and gel filtration chromatography.

Notes:

Comparing apples with apples, how to compare arousal rates in different species.

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In all hibernating mammals the hibernation season is characterised by repeated cycles of cooling, hibernation, and rewarming to euthermic temperatures, or hibernation bouts. In woodchucks (*Marmota monax*) these rewarmings have been estimated to account for approximately 70% of the mass lost during hibernation (Zervanos & Salsbury, 2003). Rewarming rates provide insights into the energetics of arousal, and peak rewarming rate is a measure of thermogenic capacity. A number of measures, such as total rewarming time, or time between two arbitrary Tb values, have been used to estimate rewarming rates, but these have generally failed to allow for the fact that rewarming rates are not uniform (Lyman, 1948).

In analysing rewarming data from echidnas we have found that rewarmings from all Tbs can be closely modelled by a four parameter sigmoid curve (mean adjusted r^2 value for 24 arousals from four echidnas = 0.9988). The same model also closely fitted data from hibernating alpine marmots (*Marmota marmota*, mean adjusted r^2 value for 35 arousals from six marmots = 0.9982). Maximum rewarming rates can be easily calculated from the first derivative of the fitted curve.

We will present data from several different taxa to demonstrate the utility of this approach.

Notes:

Effects of chronic exposure of amphibian embryos and larvae to low pH environments

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The long-term effects of low pH conditions on amphibians have not been well documented. Short-term studies have found that acidic water conditions can have detrimental effects on amphibian embryos and larvae, which include decreased hatching success and disruption of body ion content. In addition, water from naturally occurring acidic environments, such as sphagnum bogs and peats, can have detrimental effects on larval amphibians beyond that expected from equivalent low pH conditions. The waters of the wallum environment of south-east Queensland have high concentrations of dissolved organic acids and represent naturally acidic and soft-water environments. In this study, I will examine the long-term effects of acidic conditions on the survival, growth, development and performance of striped marsh frog (*Limnodynastes peronii*) tadpoles, a species that is not commonly found in the wallum environment. Performance measures will include burst swimming performance of larvae and jumping performance of metamorphs. In addition, I will determine if long-term exposure to wallum water exacerbates the effects of a stressful acidic environment. I predict that embryos will display decreased hatching success, and increased time to hatching in acidic treatments. I expect that larvae and metamorphs will have a decreased growth rate and show evidence of decreased swimming and jumping performance. I also predict that long-term exposure to wallum water will further; decrease hatching success, increase time to hatching and decrease the performance of the striped marsh frog larvae and metamorphs.

Notes:

Chemical coding of nitrergic nerves in the cane toad, *Bufo marinus* and the saltwater crocodile, *Crocodylus porosus*.

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In mammals, nitric oxide (NO) and its role in the regulation of vascular tone is well-understood. Nitric oxide is produced by the enzyme nitric oxide synthase (NOS), and in mammals, both neural NOS (nNOS) and endothelial NOS (eNOS) are involved in vascular regulation. However, whether eNOS is present in amphibians remains controversial, since there is evidence for the absence of vascular eNOS in the toad, *Bufo marinus*. Our laboratory has provided compelling evidence that NO control of toad blood vessels is provided by a nNOS system. We have also demonstrated the colocalisation of nNOS and tyrosine hydroxylase (TH) in perivascular nerves of the large blood vessels of *B. marinus*; TH is a marker for adrenergic nerves. These findings indicate the presence of nNOS in sympathetic adrenergic nerves, which is unusual given that the neurotransmitters have opposite functions. Using immunohistochemistry and confocal microscopy, we investigated the colocalisation of nNOS and TH in distributing arteries and small resistance blood vessels in the toad. We found that the majority of blood vessels in peripheral tissues contained nerve fibres that exhibited nNOS/TH colocalisation. In addition, triple labelling immunohistochemistry found that the majority of nNOS/TH nerves in toad large blood vessels also contained neuropeptide Y. We then compared the distribution of nNOS/TH nerves in toad to that of the saltwater crocodile, *Crocodylus porosus*, which has been shown to have eNOS- and nNOS-mediated NO control of the vasculature. The percentage of nNOS/TH perivascular nerves in small resistance blood vessels in *C. porosus* was markedly decreased when compared to that of *B. marinus*. In birds and mammals there is essentially no colocalisation of nNOS and TH in perivascular nerves. Thus, there appears to be a correlation between the presence of nNOS/TH nerves in toad blood vessels and the absence of endothelial NO control.

Notes:

Honest and dishonest signals of strength in the slender crayfish (*Cherax dispar*): Morphological, physiological and behavioural correlates.

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Intra-specific displays are commonly used by organisms during courtship and aggressive encounters to portray attractiveness to mates and demonstrate strength capabilities. Current theory predicts displays of potential strength should be honest, while dishonest displays should only occur at very low frequencies in nature. However, a recent study has reported that males of the slender crayfish (*Cherax dispar*) routinely utilise dishonest displays of strength during aggressive interactions, while females employ honest signalling displays. In this study, we investigated whether the different signalling systems used by male and female *C. dispar* were associated with differences in morphology, physiology and fighting behaviour. I predicted that males would have stronger and larger claws than females and they would possess greater variation in strength for a given claw or body size. I also predicted that females would respond to seasonal variation in environmental temperature by acclimation of their claw strength because force is ecologically important for their dominance interactions, but males would not exhibit an acclimation response. I also expected that the type of signalling system would be reflected in their fighting behaviour. We found male *C. dispar* possessed greater variation in both claw size and strength for a given body length than females, indicating males would not be able to accurately assess the strength of competitors from claw size alone. In contrast, no differences were detected between male and female *C. dispar* in their ability to respond to long-term changes in the thermal environment by acclimation. In addition, no differences in fighting behaviour were detected between males and females.

Notes:

An investigation of the molecular, functional and adaptive basis of reversible phenotypic plasticity in the zebrafish (*Danio rerio*).

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Reversible phenotypic plasticity is the capacity to reversibly and repeatedly alter the phenotypic expression of a trait after exposure to a new environment. The functional advantages of reversible plastic responses are well documented but whether reversible plasticity provides an adaptive advantage is largely unknown. In this project, I will utilize an integrative multiple-trait and multiple-environment approach to examine the molecular, functional and adaptive basis of seasonally-induced reversible phenotypic plasticity in the zebrafish (*Danio rerio*). I will examine the functional advantages and the molecular mechanisms that underpin seasonal changes in whole-animal performance. I will then examine the morphological, functional and adaptive significance of reversible seasonal plasticity on zebrafish gametes. To test whether reversible plasticity provides a fitness benefit, I will compete sperm from male zebrafish maintained in treatments replicating cool winter and warm summer environments to fertilize a group of eggs in both seasonal conditions. This experiment will represent the first test of the adaptive benefit of reversible phenotypic plasticity in any organism. I will then connect the studies of the functional and adaptive significance of seasonal plasticity of sperm phenotype with an analysis of the molecular basis of spermatogenesis in different seasonal environments.

Notes:

The effect of incubation temperature on locomotor performance of hatchling loggerhead turtles from Mon Repos, Queensland.

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Incubation temperature is known to influence sea turtle incubation duration, sex determination and the possibly the fitness of hatchlings. However, knowledge about the impact of incubation temperature on the locomotor performance of hatchlings, especially loggerhead turtles in the first few hours after hatching is limited. In the 2005-2006 nesting season at Mon Repos rookery, Eastern Australia, 24 loggerhead sea turtle nests had their temperature monitored, and 290 hatchlings from these nests had their running speed measured, and 60 hatchlings had their swimming performance measured over a 4 hour period immediately after their emergence from natural nests. The average incubation temperature varied between 30^{o}C to 33^{o}C, which is above the pivotal temperature of this nesting population (28.6^{o}C). The emergence success was lower than in 2003-2004 nesting season (69% and 78% respectively). The average running speed of hatchlings was 0.07 m/s and running speed negatively correlated with mean incubation temperature and positively correlated with hatchling mass. Swimming ability of hatchlings was neither correlated with their running speed nor incubation temperature, but decreased as hours of swimming increased. The swimming force was also weakly correlated with carapace length and influenced by clutch of origin. These results are important because they indicate that increased beach temperatures may influence hatchling performance and recent modeling has shown that the air temperatures for the southern Great Barrier Reef (including Mon Repos) are likely to rise by 2^{o}C by 2050.

Notes:

Endogenous induction triggers for aestivation in the frog, *Cyclorana alboguttata.*

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Aestivation is a survival strategy for dealing with potentially desiccating situations, such as a lack of food or water, hypersalinty and high temperatures. During aestivation, metabolic rate is depressed to 5-20% of the resting metabolic rate, allowing animals to survive months to years buried underground without access to food or water. To achieve this metabolic depression, a complex set of physiological and biochemical changes need to occur in a coordinated fashion, yet the endogenous mechanisms involved in initiation and maintenance of metabolic depression during aestivation remain poorly understood. The aim of my study is to identify the endogenous trigger responsible for the initiation of metabolic depression in the green striped burrowing frog, *Cyclorana alboguttata*.

Notes:

Why not to swim after lunch: the conflict of digestion and diving

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Many vertebrates dive for extended periods of time – anywhere from minutes to days. This may be to avoid predators, increase foraging success or to reduce energetic costs. The length of time an animal can dive for depends on oxygen stores and the rate at which oxygen is consumed. Any activity that increases oxygen consumption, for example locomotion, has the potential to affect diving behaviour. Digestion is an extremely metabolically demanding and enduring process, particularly for infrequently feeding animals like snakes. Through manipulating meal size consumed by the aquatic Arafura file snake (*Acrochordus arafurae*), the effects on diving performance can be determined. Preliminary studies have shown dive duration was significantly reduced as meal size increased. Fasted animals dived for approximately 22 min, however, after meal sizes of 3%, 14% and 19% of body mass were ingested, average dive time reduced to 11, 7 and 3 min respectively. More work will be directed at determining the relationship between meal size and diving behaviour, incorporating rates of oxygen consumption with various meal sizes.

Notes:

Dispersal and predation of bunya pine (*Araucaria bidwillii*) seeds by native rodents and possums

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The bunya pine (*Araucaria bidwillii*) is the only extant species of the Section Bunya, which was widespread throughout the world during the Jurassic. Distribution of this ancient tree within Australia is now very limited, possibly because of poor seed dispersal. There were previously no documented dispersal agents for *A. bidwillii* seeds. However, possums and rodents have been observed consuming the seeds. The aim of this research is to document dispersal and predation of *A. bidwillii* seeds by native fauna. Groups of tagged *A. bidwillii* seeds were placed at a small stand of *A. bidwillii* trees at Mt Mee and a larger stand at the Bunya Mountains, Qld. Some seeds were covered by wire mesh to exclude predators & permit germination. Seeds were also placed near Queen Mary Falls in areas of similar habitat, but devoid of *A. bidwillii* to assess the generalist nature of the predators. Activity of fauna at some sites was monitored using CCD cameras. Six short eared possums (*Trichosurus caninus*) and six fawn footed melomys (*Melomys cervinipes*) were captured in the Bunya Mountains National Park and fed bunya seeds for several days, to enable seeds eaten by rodents to be distinguished from seeds eaten by possums. The ragged edge of bunya seeds eaten by captive *T. caninus* is readily distinguished from the neatly trimmed edge of seeds eaten by captive *M. cervinipes*. This enables seeds in the wild to be identified as having been consumed by rodents or by possums. Many tagged seeds were moved from their original locations by *T. caninus*, but none by rodents. *T. caninus* ate more seeds than *M. cervinipes*. However, enough seeds escaped predation to produce seedlings. Some seeds were dispersed more than 8m, providing them with an opportunity to develop to maturity beyond the canopy of the parent tree. Fauna spread germinated seeds a greater distance at the Bunya Mountains than other areas. Seed dispersal by the short-eared possum (*T caninus*) may help to maintain this ancient tree species. The methodologies developed for this research are a significant component of a larger study of the ecology of *A. bidwillii*.

Notes:

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