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**Australian and New Zealand Society for
Comparative Physiology and Biochemistry**

22nd Annual Conference

9-11 December 2005

**University of Otago and the Otago Museum
Dunedin, New Zealand**



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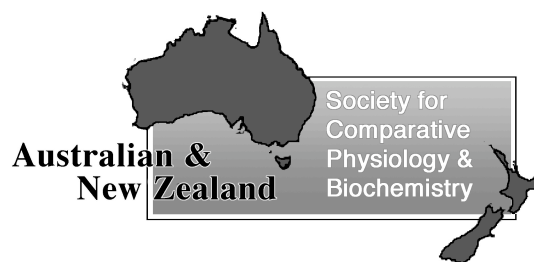
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ANZSCPB Proceedings
22nd Annual Meeting, 9-11 December 2005
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Acknowledgements

We thank the following organizations and individuals for their generous support of the 22nd annual conference.

For financial assistance and/or donations



Croxley Stationary Ltd, Peregrine Wines, Progressive Enterprises Ltd.

For student prizes

Best oral presentation by a postgraduate student:

A one-year on-line subscription to Journal of Experimental Biology, donated by the Company of Biologists Ltd, plus a \$100 book voucher donated by the University Book Shop, Dunedin.

Best use of statistics and/or graphics in an oral presentation:

A one-year subscription to Journal of Comparative Physiology – B, donated by Springer-Verlag, plus a copy of the statistics programme statistiXL, donated by Phil Withers, University of Western Australia (for a trial version, go to <http://www.statistixl.com/>).

Best oral presentation by an undergraduate student

A one-year subscription to Physiological Biochemistry and Zoology, donated by the Division of Comparative Physiology and Biochemistry of the Society for Integrative and Comparative Biology, and a \$50 book voucher, donated by Global Science.

Best poster (postgraduate or undergraduate)

A one-year subscription to Comparative Physiology and Biochemistry, donated by Elsevier, plus a copy of the book “Antarctica: an encyclopedia”, donated by NHNZ (Natural History New Zealand Ltd).

Runner-up best poster (postgraduate or undergraduate)

A one-year on-line subscription to Journal of Experimental Biology, donated by the Company of Biologists.

For administrative and graphical assistance

Leanne Bodmer, Bronwyn Carlisle, Bryony McNeill, Lynette Still and Claudine Tyrrell (administrative assistance); Helen Cavanagh (Cumberland Hall); Ken Miller (graphics and conference logo); Rob Wass and Nick Meek (website advice); Gordon Court [pigeon, penguin], Kathryn Hodgkinson [albatross], David Latham [crab], and Marcus Simons [fur seal, gecko, sea lion] (cover photos).

Information for presenters

Poster presentations

Please mount your posters in the Kakapo/Huia Room of the Otago Museum (enter through the Hutton Theatre entrance) on Friday 9 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 3:45-5.30pm. Your posters will ideally be removed during morning tea on Sunday 11 December (10.30-10.45am) and no later than the end of the final session (1:15pm).

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, conference dinner: Paul Donohoe

Accommodation, Thursday evening registration: Mark Lokman

Oral presentations, AV facilities, Sunday excursion: David Wharton

Posters: Peter Dearden and Mark Lokman

Anything else, including catering and Otago Museum liaison: Alison Cree

Some nearby shops and internet cafes

University Book Shop, 378 Great King Street (one-way going north, opposite the museum)

NZ Post Shop and KiwiBank (next to the University Book Shop)

Regent Night 'n' Day Foodstore, 2 Regent St (24 hour dairy, two blocks north and one west of the museum)

Centre City New World Supermarket, Centre City Mall, Great King St (two blocks south of the museum)

Countdown Foodmarket, 309 Cumberland St (about four blocks south of the museum)

A1 Internet Cafe, upstairs at 149 George St (main shopping street, southwest of the museum)

Khmer Satay Noodle House, 434 George St (internet, on main shopping street)

Some nearby banks

ANZ, cnr George & Hanover Sts; ASB Bank, 290 George St; BNZ, 58 Albany St; National Bank, 62 Albany St; Westpac, cnr George & Frederick Sts.

Some useful phone numbers

University Security: 479-5000

Emergency services (police, fire, ambulance): 111.

Taxis and airport shuttles: City taxis 4771-771; Dunedin Taxis 4777-777; Super-shuttle 0800-748-885.

Maps of the campus area and of the Dunedin city centre are provided in your registration pack.

Programme overview

Thursday 8 December

Time	Activity	Venue
4.00-8.00 pm	Registration, including drinks and nibbles	Dining Room, Cumberland Hall

Friday 9 December

Time	Activity	Venue
8.00-8.30 am	Registration for late arrivals Place posters on boards	Hutton Theatre and Kakapo/Huia Room, Otago Museum
8.30-8.45 am	Welcome	Hutton Theatre, O. Museum
8.45-10.30 am	Session 1: Morphology and Muscle	Hutton Theatre, O. Museum
10.30-11.00 am	Announcements and morning tea	Hutton Theatre, O. Museum
11.00-12.30 pm	Session 2: Thermoregulation	Hutton Theatre, O. Museum
12.30-1.45 pm	Lunch	ISB Link, U. Otago
1.45-3.30 pm	Session 3: Life history and physiology	Hutton Theatre, O. Museum
3.30-4.00 pm	Afternoon tea	Hutton Theatre, O. Museum
4.00-6.00 pm	Session 4: Osmoregulation, stress and cardiovascular physiology	Hutton Theatre, O. Museum
6.30-8.30 pm	Social, including light refreshments	ISB Link, U. Otago

Saturday 10 December

Time	Activity	Venue
8.20 am	Announcements	Hutton Theatre, O. Museum
8.30-10.30 am	Session 5: Physiology I	Hutton Theatre, O. Museum
10.30-11.00 am	Morning tea	Hutton Theatre, O. Museum
11.00-12.30 pm	Session 6: Hypoxia	Hutton Theatre, O. Museum
12.30-1.45 pm	Lunch	Staff Club, U. Otago
1.45-3.45 pm	Session 7: Lipids and metabolic rate	Hutton Theatre, O. Museum
3.45-5.30pm	Afternoon tea and Session 8: Global Science Poster Session	Hutton Theatre, O. Museum
6.45 pm	Departure for conference dinner	Buses leave from Otago Museum for Glenfalloch
10.30 and 11.00pm	Return from conference dinner	Buses return from Glenfalloch to Otago Museum/Cumberland Hall

Sunday 11 December

Time	Activity	Venue
8.35 am	Announcements	Hutton Theatre, O. Museum
8.45-10.30 am	Session 9: Metabolic rate	Hutton Theatre, O. Museum
10.30-11.15 am	Morning tea, prizes, business meeting	Hutton Theatre, O. Museum
11.15-1.15 pm	Session 10: Physiology II	Hutton Theatre, O. Museum
1.15-1.40 pm	Lunch (packed)	Hutton Theatre, O. Museum
1.40 pm	Departure for excursion to Otago Peninsula	Buses leave from Otago Museum
c. 7.00 pm	Return from excursion to wharf	Make own way on foot or by taxi to your accommodation

Notes

Order of presentations

*UG = Student, undergraduate work

*PG = Student, postgraduate work

Friday 9 December

Session 1: Morphology and Muscle 8.30-10.30

Chair: Malcolm Forster, University of Canterbury

Time	Speaker	Abstract	Title
8.30	Alison Cree		Welcome
8.45	Craig White	72	Kinematic and morphological correlates of the postural and net transport costs of pedestrian locomotion in birds
9.00	Craig Franklin	26	Phenotypic plasticity of the salt glands of estuarine crocodiles to environmental salinity
9.15	David Cannata	13	Differences in the contractile activation properties of fast- and slow-twitch muscles receiving different neural input *UG
9.30	Vera Schluessel	52	Functional morphology of olfactory structures in elasmobranchs *PG
9.45	Jan West	70	Longitudinal growth of long- and short-sarcomere fibres from the claw muscle of the yabby (<i>Cherax destructor</i>)
10.00	Mike Thompson	61	Morphological and functional changes to the uterus of lizards with different placental complexities
10.15	Shannon Simpson	55	Phase contrast imaging the neonatal marsupial lung using a synchrotron *PG

Session 2: Thermoregulation 11.00-12.30

Chair: Sue Jones, University of Tasmania

Time	Speaker	Abstract	Title
11.00	Adalto Bianchini	10	Heat shock proteins in key-species from the Atlantic southern coast of Brazil
11.15	Christine Cooper	15	Pronounced body temperature fluctuations in free-living laughing kookaburras (<i>Dacelo novaeguineae</i>)
11.30	Yvonne Ingen-Housz	37	The effects of incubation temperature on development in the Australian brush-turkey (<i>Alectura latham</i>) *PG
11.45	Kris Rogers	50	Thermal acclimation of metabolism in <i>Limnodynastes peronii</i> frogs *PG
12.00	Frank Seebacher	53	The significance of phenotypic flexibility in thermoregulating reptiles
12.15	Clare Stawski	57	Temperature and the blood respiratory properties of two reptiles, <i>Pogona barbata</i> and <i>Emydura signata</i> *UG

Session 3: Life history and physiology 1.45-3.30

Chair: Roger Seymour, University of Adelaide

Time	Speaker	Abstract	Title
1.45	Toni-Ann Alsop	3	Cyclic changes in epithelial Na ⁺ transport in the vaginal cul-de-sac of the brushtail possum, <i>Trichosurus vulpecula</i> *PG
2.00	Michael Usher	66	The effect of dietary alterations in the blowfly (<i>Calliphora stygia</i>) on membrane fatty acid composition and adult lifespan *PG
2.15	Sarah Bell	8	Steroid hormones and social dominance in the marsupial squirrel glider, <i>Petaurus norfolcensis</i> *UG
2.30	Phil Matthews	40	Backswimmers: diving bugs with buoyancy compensation devices *PG
2.45	Charlotte Minson	43	Kiwi caeca – fermentative function in an insectivore? *PG

Time	Speaker	Abstract	Title
3.00	Ashley Edwards	22	Regulation of the HPG axis in the blotched blue-tongued skink, <i>Tiliqua nigrolutea</i>
3.15	Sue Jones	38	Placental transfer of organic nutrients in viviparous skinks: a strategy for optimising neonatal fitness

Session 4: Osmoregulation, stress and cardiovascular physiology 4.00-6.00

Chair: Peter Frappell, La Trobe University

Time	Speaker	Abstract	Title
4.00	Tes Toop	63	Osmoregulatory strategy in the elephant fish, <i>Callorhynchus milii</i>
4.15	Sofie Trajanovska	64	A molecular study of reptilian natriuretic peptides *PG
4.30	Norman Ragg	60	Novel solutions to the challenges of gill perfusion and control in the abalone <i>Haliotis iris</i> *PG
4.45	Sarah Andrewartha	4	Incubation water potential affects morphology and physiology of the terrestrial embryos of the Victorian smooth froglet, <i>Geocrinia Victoriana</i> *UG
5.00	Kylie Wright	81	How stressed do seahorses get? A study into the stress response of the pot-bellied seahorse (<i>Hippocampus abdominalis</i>) *PG
5.15	Hamish Prosser	48	Cardiovascular effects of urotensin II on rat and salmon hearts *PG
5.30	Leonard Forgan	24	The gastrointestinal physiology of chinook salmon, <i>Oncorhynchus tshawytscha</i> (Walbaum) affected by gastric dilation air sacculitis (GDAS) *PG
5.45	Nicholas Tuckey	65	Effects of radical damage and antioxidants on tissue viability and storage properties in chinook salmon *PG

Saturday 10 December

Session 5: Physiology I 8.20-10.30

Chair: Craig Franklin, University of Queensland

Time	Speaker	Abstract	Title
8.20			Announcements
8.30	Kristy Demmers	17	The isolation and sequencing of the cystic fibrosis transmembrane conductance regulator (CFTR) of the brushtail possum, <i>Trichosurus vulpecula</i>
8.45	Natalie Harfoot	31	The distribution and expression of the cystic fibrosis transmembrane conductance regulator in the intestine of the possum, <i>Trichosurus vulpecula</i> *PG
9.00	John Donald	20	Nitric oxide regulation of vertebrate blood vessels
9.15	Ryan Naylor	45	Determining the suitability of a marsupial, <i>Antechinus stuartii</i> , as a model for Alzheimer's disease *UG
9.30	Timo Nevalainen	46	Phospholipase A2 in marine invertebrates
9.45	Flavia Zanotto	82	Calcium transport in epithelial cells of semi-terrestrial crabs
10.00	Grant Butt	11	The electrogenic secretory response of the ileum of the Australian brushtail possum, <i>Trichosurus vulpecula</i>
10.15	Sam Richardson	49	Cell division and apoptosis are differentially affected in the neural stem cell niche of adult transthyretin null mice

Session 6: Hypoxia 11.00-12.30

Chair: Grant Butt, University of Otago

Time	Speaker	Abstract	Title
11.00	Courtney Waugh	68	Respiration by echidnas, <i>Tachyglossus aculeatus</i> , while buried *UG
11.15	Max Gassmann	27	Erythropoietin regulates hypoxic ventilation in mice by interacting with brainstem and carotid bodies
11.30	Lyndal Horne	33	Factors influencing the hyperventilatory response in the Plains rat *UG
11.45	Roger Seymour	54	Non-invasive measurement of oxygen partial pressure, lateral diffusion and chorioallantoic blood flow under the avian eggshell
12.00	Jannie Sperling	56	Respiratory properties of the blood in the flatback turtle (<i>Natator depressus</i>) in comparison to the loggerhead turtle (<i>Caretta caretta</i>) *PG
12.15	Tony Hulbert	34	Membrane fatty acid composition and the determination of lifespan: recent insights from some special rodents

Session 7: Lipids and Metabolic Rate 1.45-3.45

Chair: Tony Hulbert, University of Wollongong

Time	Speaker	Abstract	Title
1.45	Nancy Berner	9	Seasonal fatty acid composition of skeletal muscle from the eastern red spotted newt (<i>Notophthalmus viridescens viridescens</i>) of North America
2.00	Bill Buttemer	12	Long-lived procellariiformes have peroxidation-resistant membranes
2.15	Sara Hiebert	32	Environmental regulation of dietary fatty acid preference in Siberian hamsters
2.30	Tamara Diesch	19	EEG in the developing tammar wallaby (<i>Macropus eugenii</i>) *PG

Time	Speaker	Abstract	Title
2.45	Joanne Avraam	5	Prenatal exposure to nicotine does not affect convective requirement at normal and elevated body temperature in mice at 2 or 7 days postpartum *PG
3.00	Claus Bech	7	Long-term repeatability of basal metabolic rate in the Zebra finch (<i>Taeniopygia guttata</i>)
3.15	Tim Clarke	14	Cardio-metabolic function in teleost fish *PG
3.30	Beth Symonds	58	Maintenance of muscle ultrastructure and metabolic capacity in aestivating green-striped burrowing frogs (<i>Cyclorana alboguttata</i>) *PG

Session 8: Global Science Poster Session 3.45-5.30

Time	Presenter	Abstract	Title
3.45-5.30	Claire Aland	2	Connectivity of olfactory regions in the brain of the marsupial mouse, <i>Antechinus subtropicus</i>
“	Yvonne Bazin	6	The overwintering strategy of a southern hemisphere frog, <i>Litoria ewingii</i> (brown tree frog) *PG
“	Sue Edwards	21	Evidence for NHE2 expression in the rectal gland of the spiny dogfish, <i>Squalus acanthias</i>
“	Paul Else	23	Membranes, temperature and sodium leak in endotherms and ectotherms
“	Jane Girling	28	Progesterone stimulates arteriogenesis in mouse endometrium
“	Maria Ikonomopoulou	36	Identification and properties of sex steroid binding proteins in nesting <i>Chelonia mydas</i> plasma *PG
	Harry Taylor	59	Tolerance of hyposaline exposure by intertidal crab embryos: osmoprotection or osmoregulation?
“	Lisa Warnecke	67	Body temperature variation in free-ranging and captive southern brown bandicoots <i>Isodon obesulus</i> (Marsupialia: Peramelidae) *UG

Time	Presenter	Abstract	Title
“	Jill Weakley	69	<i>In vivo</i> analysis of salinity related proton efflux in barramundi (<i>Lates calkifer</i>) *PG
“	David Wharton	71	Variation in the survival rates of intracellular freezing in <i>Panagrolaimus davidi</i> : a tool for investigating this adaptation to life in the Antarctic
“	Craig White	73	From the tropics to the poles: the basal metabolic rate of birds is associated with habitat temperature not productivity
“	Kerry Withers	76	Identifying reproductive state of the Julia Creek dunnart <i>Sminthopsis douglasi</i> by behavioural observations
“	Kerry Withers	77	The role of native rodents in seed dispersal and seed predation of the Bunya Pine (<i>Araucaria bidwillii</i>)
“	Kerry Withers	78	Use of pulp cavity/tooth width ratios to determine the age of adult wild dogs (<i>Canis lupus dingo</i> , <i>C. l. domesticus</i> and their hybrids)
“	Kerry Withers	79	Application of machine vision technology to identification of oestrus in the Julia Creek dunnart (<i>Sminthopsis douglasi</i>)
“	Kerry Withers	80	Skeletal muscle mitochondria as a source of non-shivering thermogenesis in the marsupial <i>Antechinus flavipes</i>

Sunday December 11

Session 9: Metabolic Rate 8.35-10.30

Chair: Ashley Edwards, University of Tasmania

Time	Speaker	Abstract	Title
8.35			Announcements
8.45	Sean Tomlinson	62	Comparative physiology of native Australian and introduced mice, <i>Pseudomys hermannsburgensis</i> and <i>Mus musculus</i> : facultative hypothermia and torpor in response to cold stress *UG
9.00	Terry Dawson	16	High metabolic potential of marsupials is independent of BMR: the story from muscles
9.15	Paul Fournier	25	Glycogen repletion without food following intense physical activities: are mammals different?
9.30	Kelly Hare	30	Low cost of locomotion in lizards that are active at low temperatures *PG
9.45	Lorae McArthur	41	Season influences the resting metabolism of three tropical species of Australian frogs (Anura: Hylidae) *PG
10.00	Andrew McKechnie	42	Phenotypic plasticity: a major contributor to avian metabolic diversity?
10.15	Craig Willis	74	Hibernation by pregnant North American hoary bats (<i>Lasiurus cinereus</i>)

Session 10: Physiology II 11.15-1.15

Chair: Tes Toop, Deakin University

Time	Speaker	Abstract	Title
11.15	Greg Ahearn	1	Zinc sequestration in crustacean hepatopancreatic lysosomes: role of anions in the detoxification event
11.30	Randy Rose	51	The effects of diet and habitat on milk composition and growth in young marsupials
11.45	James O'Shea	47	The autonomic nervous system and its possible function in the heart of the dragon lizard, <i>Ctenophorus nuchalis</i>
12.00	Ian Hume	35	Erythrocyte fragility in two species of brushtail possum exposed to dietary terpenes
12.15	Gordon Grigg	29	Comparative forage requirements of kangaroos and sheep: a reality check based on physiological data
12.30	Suzy Munns	44	Cardiorespiratory coupling during terrestrial locomotion in reptiles: consequences of intra-abdominal pressure and respiratory mechanics
12.45	Roger Lentle	39	Why herbivore poo should bounce: an exploration of the creep rheometry of possum digesta
1.00	Phil Withers	75	Physiology of the thylacine, based on allometric, phylogenetic and climate-corrected marsupial data

Notes

Abstracts

(in alphabetical order of the presenter*)

01

Zinc sequestration in crustacean hepatopancreatic lysosomes: role of anions in the detoxification event

Abearn, G.A.*, Mandal, P.K., Roggenbeck, B.

University of North Florida, Jacksonville, Florida 32224 USA

The crustacean hepatopancreas is a major organ system responsible for heavy metal detoxification. Published microprobe studies from a number of authors have shown that within this structure, lysosomes are centers for accumulation of both cationic metals and complexing anions. Little is known about the mechanisms by which the metals or their anion counterparts are transported into lysosomes or the processes responsible for stone formation once concretion constituents are present. In this study hepatopancreatic lysosomal membrane vesicles (LMV) from the American lobster (*Homarus americanus*) were prepared by standard centrifugation methods and $^{65}\text{Zn}^{2+}$, $^{36}\text{Cl}^-$, and $^{35}\text{SO}_4$ were used to characterize the processes responsible for transfer of these elements into the vesicles. $^{65}\text{Zn}^{2+}$ influx into LMV was a hyperbolic function of external $[\text{Zn}^{2+}]$ and followed the Michaelis-Menten carrier transport equation ($K_m = 32 \pm 10 \mu\text{M}$; $V_{\max} = 21 \pm 3 \text{ pmol/mg protein} \times \text{sec}$). This transport was stimulated by 1 mM ATP and abolished by the simultaneous addition of 1 mM ATP + 250 μM vanadate. Thapsigargin (10 μM) was also a significant inhibitor of zinc influx, but not as effective as vanadate. Cadmium and copper were competitive inhibitors of $^{65}\text{Zn}^{2+}$ influx ($\text{Cd}^{2+} K_i = 68 \pm 3 \mu\text{M}$; $\text{Cu}^{2+} K_i = 33 \pm 2 \mu\text{M}$). In the absence of ATP an outwardly-directed proton gradient stimulated $^{65}\text{Zn}^{2+}$ influx, while a proton gradient in the opposite direction inhibited metal uptake. Vesicles loaded with SO_4^{2-} or PO_4^{3-} led to a 3-fold greater steady-state accumulation of $^{65}\text{Zn}^{2+}$ than similar vesicles loaded with mannitol, Cl^- , or oxalate $^{2-}$. $^{35}\text{SO}_4$ influx was a hyperbolic function of external $[\text{Cl}^-]$ and $^{36}\text{Cl}^-$ influx appeared to occur only by diffusion in the absence of an antiport substrate. These results suggest that $^{65}\text{Zn}^{2+}$ influx occurred by a vanadate- and thapsigargin-sensitive ATPase transporter that was stimulated by an outwardly-directed proton gradient. Polyvalent inorganic anions, such as SO_4 and PO_4 , associate with the metal inside the vesicles and may result in concretion formation through precipitation at appropriate vesicular pH.

02

Connectivity of olfactory regions in the brain of the marsupial mouse, *Antechinus subtropicus*

Aland, R.C.*, Bradley, A.J.

The University of Queensland, Brisbane, Australia

Connectivity of central olfactory regions was investigated in the marsupial mouse, *Antechinus subtropicus*. *A. subtropicus* is a small dasyurid marsupial with an extreme life history. Leading up to the short, synchronised breeding period, the males display elevated blood levels of cortisol and testosterone. Following breeding is a complete male die-off at 11.5 months. At death, males suffer from various stress-related pathologies, such as gastric haemorrhage, renal dysfunction and selective hippocampal damage. Females live and may breed in subsequent years. Olfaction plays an important role in the life history of *A. subtropicus*, potentially mediating and exacerbating the stress response. The hippocampal formation appears involved in regulating the stress response via the hypothalamic-pituitary-adrenal axis. Connectivity was investigated using the carbocyanine tracers DiI and DiA. These tracers were applied to the main olfactory bulbs, olfactory cortex and the hippocampal formation in fixed brain tissue. The tissue was stored, allowing tracers to travel. The brains were sectioned and viewed using fluorescence. Connections of the main olfactory bulbs and olfactory cortex to various brain regions were noted, including the frontal cortex, anterior olfactory nucleus and thalamus. Injection of tracer into the hippocampal formation resulted in labelling in some olfactory regions and in areas connected with olfactory regions, suggesting that the hippocampal formation may receive olfactory input. These preliminary results suggest that the connections of the central olfactory regions in *A. subtropicus* are extensive. Particularly interesting is the demonstration of olfactory connectivity with the hippocampal formation, given the presumed involvement of the hippocampus and olfaction in the stress response.

Cyclic changes in epithelial Na⁺ transport in the vaginal cul-de-sac of the brushtail possum, *Trichosurus vulpecula*

Alsop, T.A.*¹, McLeod, B.J.², Butt, A.G.¹

¹Department of Physiology, University of Otago, Dunedin, New Zealand

²AgResearch Invermay, New Zealand

The female marsupial reproductive tract consists of a urogenital sinus leading to two lateral vaginal canals emptying into a single cul-de-sac from which two uteri protrude. In the brushtail possum the cul-de-sac increases in size leading up to ovulation then regresses through the remainder of the oestrous cycle. Associated with the increase in size of the cul-de-sac is a proliferation of the epithelial layer and the secretion of a mucus-rich fluid. As the composition of this fluid is determined by the epithelial lining of the cul-de-sac we have used the Ussing short circuit technique to determine whether cyclic changes in ion transport accompany the changes in the structure of cul-de-sac. The basal short circuit current (Isc) was <20 $\mu\text{A cm}^{-2}$ in anoestrus animals and increased to 60-80 $\mu\text{A cm}^{-2}$ in animals in the follicular or luteal phases of the oestrous cycle. In all cases the basal Isc was completely inhibited by serosal ouabain (1mM). Mucosal amiloride (10 μM) had no effect on the Isc in the anoestrus or follicular phases of the oestrous cycle, whereas it inhibited 35% of the Isc in the luteal phase. This amiloride-sensitive Isc was dependent on mucosal Na⁺ and had an IC₅₀ for amiloride of $\approx 1 \mu\text{M}$, consistent with electrogenic Na⁺ transport. These data demonstrate that cyclic changes in epithelial ion transport by the cul-de-sac occur during the oestrous cycle and that, during the latter stages of the cycle, fluid absorption is driven by electrogenic Na⁺ transport.

Incubation water potential affects morphology and physiology of the terrestrial embryos of the Victorian smooth froglet, *Geocrinia victoriana*

Andrewartha, S.J.*¹, Mitchell, N.J.^{1,2}, Frappell, P.B.¹

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²present address, School of Animal Biology, University of Western Australia, Perth, Australia

The maintenance of adequate water balance is critical to anuran eggs that develop in terrestrial situations. Wetter substrates have been shown to produce 'fitter' offspring and detrimental effects occur when embryos are incubated on substrates with low water potential. Terrestrial eggs of the Victorian smooth froglet (*Geocrinia victoriana*) were incubated at a range of vapour potentials determined by the isopiestic technique or with access to liquid water. Within the vapour potential treatments, a decrease in egg mass, perivitelline membrane diameter, capsule diameter, embryo dry mass, total embryo length, tail length and fin height was associated with a decrease in water potential. A decrease in water potential was not associated with a decline in the rate of oxygen consumption and hatchling success was only affected at a water potential below that for interstitial fluid ($-399 \pm 15 \text{ kPa}$). Embryos incubated on a wet substrate (access to liquid water) had a greater whole egg mass and dimensions, wet embryo mass, percentage tissue of total mass (i.e. tissue and gut), total length, snout-vent length and fin height when compared to control embryos (0 kPa above distilled water). This study has demonstrated that the form of water (vapour or liquid), in which terrestrial embryos develop has a dramatic influence on morphological and physiological traits.

Prenatal exposure to nicotine does not affect convective requirement at normal and elevated body temperature in mice at 2 or 7 days postpartum

Avraam, J.^{*}, Frappell, P.B.¹, Cohen, G.², Drago, J.³

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Nicotine absorbed by the mother passes into the fetal bloodstream, binding with nicotinic acetylcholine receptors. As areas involved in regulating breathing are rich in these receptors and interact with thermoregulatory control, we investigated the effect of prenatal nicotine exposure on newborn mice when thermal ability is limited. Mice were prenatally exposed to nicotine at 14 days (maternal dose 6 mg kg⁻¹ day⁻¹) and at postnatal day 2 and 7 following an initial rest period subjected to 30 min of intermittent asphyxia (2 min, 5%CO₂, 10%O₂ or air) at body temperatures (Tb) of 32°C or 35°C. At both ages and Tb, levels of ventilation (VE) and rates of oxygen consumption (VO₂) resulted in identical convective requirements (VE/VO₂ ≈ 23). Intermittent asphyxia resulted in similar levels of hyperventilation (VE/VO₂ ≈ 60), achieved primarily through hyperpnea. Nicotine resulted in an ~23% reduction in VO₂ with an associated parallel adjustment in VE thus maintaining VE/VO₂ at control levels in day 2 pups only, at either Tb. In all pups exposed to nicotine, asphyxia induced a hyperneic hyperventilation equal to that in control pups. These findings suggest that at birth the effect of nicotine appears to reduce metabolic rate and that the respiratory system appropriately matches VE to VO₂ and is capable of responding to asphyxia. The effect of Tb may compound this interpretation as recent experiments revealed that exposure to Tb = 35°C ablates the normal thermogenic response observed for pups of both ages.

The overwintering strategy of a southern hemisphere frog, *Litoria ewingii* (brown tree frog)

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The overwintering strategy of *Litoria ewingii* in Macraes Flat, Central Otago, New Zealand, was studied under laboratory and field conditions. Microhabitat temperature data collected from February to October 2005 at Macraes Flat has shown that frogs are routinely exposed to subzero conditions during the winter. Different criteria for both freeze tolerance and avoidance overwintering strategies were compared in the laboratory. *L. ewingii* demonstrated the ability to withstand freezing for periods up to 6 hours at -1°C and for the duration of their exotherm at -2°C. Furthermore, with the use of polystyrene insulation which increased the exotherm duration of the frozen frogs, they were able to tolerate 12 hours at -1°C. Supercooling experiments on wet and dry substrates indicated that *L. ewingii* can supercool to $-1.6 \pm 0.2^\circ\text{C}$ and $-1.2 \pm 0.1^\circ\text{C}$, respectively. These high supercooling points and the similarity between the two substrate groups suggest that *L. ewingii* has little ability to remain unfrozen in a subzero environment regardless of substrate type. The body ice content of frogs frozen for the duration of their exotherms at -2°C was determined calorimetrically and the blood metabolites of these frogs were then compared to control frogs as these are both determinants of freeze tolerance in anurans. Due to *L. ewingii*'s freeze tolerance capabilities, inability to supercool and exposure to subzero temperatures, we believe that *L. ewingii* must be sufficiently freeze tolerant to survive the subzero temperature encountered during winter in Central Otago.

07

Long-term repeatability of basal metabolic rate in the zebra finch (*Taeniopygia guttata*)

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The basal metabolic rate (BMR) is a physiological trait believed to show adaptational changes. However, few studies have tested whether BMR shows stable between-individual variations. Repeatability indicates that the trait might be heritable and therefore a possible target for natural selection. We tested whether BMR was repeatable over a considerable time of the lifespan of a small passerine bird: the zebra finch (*Taeniopygia guttata*). BMR was measured six times over a 2.5 years period in captive zebra finches. BMR residuals showed significant repeatabilities over a short (1.5 months) and over a long period (2.5 years) for each sex as well as for the pooled data. In contrast to earlier studies on metabolism, our calculated repeatability (R) did not change significantly from the short (R of 0.571) to the long period (R of 0.567). Our results show that there is consistent between individual variations in BMR on which natural selection can work upon provided that this trait is heritable.

08

Steroid hormones and social dominance in the marsupial squirrel glider, *Petaurus norfolcensis*

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The squirrel glider, *Petaurus norfolcensis* is a small nocturnal arboreal marsupial that belongs to the sub family Petauridae. In Australia there are 4 gliders that belong to this family, all of which are characterised by the gliding membrane (patagium), that extends from the wrist to the ankle. Dominance coalitions are formed in groups of related males to enhance their chance of reproductive success. Studies of the closely related *Petaurus breviceps* have shown that when several adult males are present in a group, dominance hierarchies are established. It has been suggested that *P. norfolcensis* may be more social than *P. breviceps* because they tolerate greater population densities and the average age at dispersal is higher. Steroid profiles provide indicators of dominance in males, shown in research on *P. breviceps* where dominant males had higher testosterone concentrations and lower cortisol concentrations than subordinate males. *P. norfolcensis* have a cutaneous frontal gland and a cutaneous sternal gland. The frontal gland has been suggested to be important in social cohesion, being used by the dominant male to mark other individuals in the group. The sternal gland however, is active in all males and is used to mark territorial borders. Presence of an active frontal gland may therefore be interpreted as an indication of social dominance. This study investigates the histological appearance of biopsies taken from the frontal and the sternal scent glands in wild *P. norfolcensis* and their relationship with steroid hormone profiles.

Seasonal fatty acid composition of skeletal muscle from the eastern red spotted newt (*Notophthalmus viridescens viridescens*) of North America

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Eastern red spotted newts are native to the eastern deciduous forests of North America, ranging from central Georgia, USA through Quebec, Canada. Adults of this species are aquatic, remaining in ponds where they are active year round, even during winter in ponds covered with ice. Prior research has shown that skeletal muscle activities of cytochrome c oxidase (CCO) and citrate synthase acclimatize seasonally in these newts. In particular, CCO activity is completely compensated in winter- over summer-captured newts. Such acclimatization is presumed to offset the Q₁₀ effect of the seasonal temperature on enzyme activity, allowing the newts to remain active at the seasonally colder temperatures. The present study was undertaken to determine if membrane remodelling may be a mechanism by which the CCO activity is increased in the skeletal muscle of winter-captured newts. Membrane fatty acid (FA) composition is highly correlated with metabolic rate and the activity of membrane bound proteins, presumably through changes in membrane physical properties. Thus, we are investigating the seasonal FA composition of skeletal muscle from these newts. If changes in FA composition are responsible for increasing CCO activity in winter, we expect to find a higher degree of polyunsaturation, more long chain FAs (particularly docosahexanoic acid 22:6 n-3), and a lower n-6/n-3 index for FAs from winter- than summer-captured newts. Ten newts were collected in winter and summer, 2005, in Tennessee, USA. An organic extraction of the lipids was performed on the trunk epaxial skeletal muscle dissected from each newt. These lipids were dried under nitrogen and shipped to the University of Wollongong, NSW, Australia for analysis by gas chromatography. Preliminary analyses show seasonal differences in their FA content, with increased polyunsaturation and 22:6 n-3 in tissue from winter-captured newts.

Heat shock proteins in key-species from the Atlantic southern coast of Brazil

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The objective of this study is to determine the pattern of variation of the heat shock proteins (HSP70) in blood cells from five key-species of the Atlantic southern coast of Brazil (S32°09.2'; W52°05.5'). Species studied are the bivalve mollusc *Mesodesma mactroides*, the crab *Chasmagnathus granulatus*, the fish *Micropogonias furnieri*, the dune lizard *Liolaemus occipitalis* and the dune mouse *Calomys laucha*. Results from this study will allow us to verify the possibility to employ these proteins as specific and sensitive indicators of thermal variations in the area studied. Thus, the specific objectives of this study are: (1) to standardize the methodology for determination of HSP70 in different animal species; (2) to determine in laboratory the pattern of HSP70 response to increased temperature in the five species in summer; (3) to determine in the field the basal levels of HSP70 expression in circulating immune cells of the five species along the year. Levels of HSP70 are being determined in circulating immune cells from the five species by flow cytometry. Purified cells are fixed and permeated using the Kit Cytofix/Cytoperm Plus (BD-Pharmigem). Immune reactions are being performed using a primary mouse anti-HSP70 antibody and a secondary rat-anti-mouse-FITC antibody (BD-Pharmigem). Previous results from winter samples indicated that HSP70 of all the five studied species were detectable with the antibodies employed. They also indicated a low level of variation within the same species, suggesting the reliability of the method employed for all species analyzed. Financial support: PROBIO – Ministry of Environment – Brazil.

The electrogenic secretory response of the ileum of the Australian brushtail possum, *Trichosurus vulpecula*

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In eutherian mammals the secretion of fluid by the intestinal epithelium is driven by the active secretion of Cl^- and is important as it assists in the digestion of the food material and ensures adequate hydration of the mucus secreted into the intestine. Despite this the hindgut of the possum, a metatherian mammal, does not respond to secretagogues. Therefore, we have investigated whether the small intestine of the possum is capable of secretion. The ileum of wild caught possums was removed, mounted as a flat sheet in a modified Ussing chambers and constantly short circuited to provide a measure of active electrogenic ion transport. The ileum responded to cAMP-dependent secretagogues with a sustained increase in short circuit current (Isc). However, the cAMP-dependent Isc was not affected by the removal of Cl^- from the bathing solution and bumetanide (100 μM serosal), a potent inhibitor of Cl^- secretion in eutherian mammals, did not inhibit the cAMP-dependent increase in Isc. In contrast, it was inhibited by the removal of Na^+ and HCO_3^- from the bathing solution and the serosal addition of 1 mM 4,4'-diisothiocyanostilbene-2,2'-disulfonic acid (DIDS), an inhibitor of HCO_3^- transporters, although acetazolamide (1 mM mucosal and serosal), an inhibitor of carbonic anhydrase, had no effect. These data indicate that the ileum of the possum is capable of a secretory response, but in the possum secretion is driven by electrogenic secretion of HCO_3^- rather than Cl^- .

Long-lived procellariiformes have peroxidation-resistant membranes

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Longevity in animals varies directly with body mass, but differs markedly between taxa. Birds, for example, live generally 3-times longer than similar-sized mammals, but variation in longevity between avian orders can be greater than this and is not related to differences in metabolic rate. The allometric relation between body mass and resting metabolic rate is indistinguishable between procellariiformes (petrels and albatrosses) and galliformes (quail, chooks, etc.), yet these seabirds live about 4.5 times longer than their gallinaceous counterparts. There are many physiological and morphological features associated with long-lived species, including a reduced susceptibility to oxidative damage. We characterised the fatty acids in phospholipids of flight and leg musculature and those of storage fats in procellariiformes ranging from 120 to 3200 g. Although muscle phospholipids and storage depots contained the same proportion of polyunsaturated fats (PUFAs), the phospholipid PUFAs were dominated by n-6 fatty acids whereas the storage fat PUFAs were dominated by n-3 fatty acids. This results in n-3/n-6 ratios of about 5 in storage fats versus less than 1 in muscle phospholipids. This demonstrates a high degree of selectivity in the types of fatty acids incorporated into these membranes and results in phospholipid peroxidation indices that are as low as those found in membranes of very long-lived mammals.

Differences in the contractile activation properties of fast- and slow-twitch muscles receiving different neural input

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The contractile properties of a muscle are determined by the neural input that the muscles receive. Skeletal muscles are innervated by cholinergic neurons (release the neurotransmitter acetylcholine) from the somatic nervous system. Acetylcholine (ACh) binds to specific receptors to transmit the electrical signal from nerve to nerve or from nerve to muscle. One mechanism of altering the neural input a muscle receives is by altering the structure of the ACh receptor. A knock-in mouse model has been developed where the binding site for ACh on the nicotinic acetylcholine receptor has been altered. In this study the Ca^{2+} and Sr^{2+} activation properties of single skinned (membrane removed) muscle fibres were examined in the fast twitch (extensor digitorum longus) and slow-twitch (soleus) muscle fibres from both wildtype and knock-in mouse models. Force-pCa ($-\log_{10}[\text{Ca}^{2+}]$) (pSr) relations were determined by activating the skinned preparations in EGTA buffered solutions containing different concentrations of Ca^{2+} (Sr^{2+}). Results showed that altering the ACh receptor decreased the sensitivity of the contractile apparatus to Ca^{2+} and Sr^{2+} in muscle fibres from both the EDL and soleus muscles from the knock-in animals from the female mice. In contrast, the Ca^{2+} - and Sr^{2+} activation profiles between the wildtype and knock-in mouse models for the male animals were not significantly different.

Cardio-metabolic function in teleost fish

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Historically, teleost fish were considered unusual among vertebrates in that they were thought to regulate cardiac output (\dot{V}_b) mainly via changes in cardiac stroke volume (V_s) as opposed to changes in heart rate (f_H). It is becoming evident, however, that many species of fish display a large scope in f_H such that variations in the frequency of pumping of the heart are of primary importance when regulating blood flow during activity. The Murray cod, a sedentary species, was found to maintain a 3.7-fold increase in oxygen consumption rate (\dot{V}_{O_2}) across a broad temperature range by utilising a 2.2-fold increase in f_H . More active fish species (e.g. kingfish, tuna) are thought to have a larger available scope in f_H to support their exceptionally high aerobic capacities, yet definitive data are limited for such species. Preliminary measurements using implantable data loggers in free-swimming 15 kg southern bluefin tuna have indicated that the scope in f_H ranges from at least 40 – 110 beats min^{-1} at a water temperature of 14°C (visceral temperature 17°C). This range is greater than has been reported for most other species of tuna, and is most likely due to the fact that our measurements are the first to be taken from untethered and free-swimming fish. If f_H proves to be the primary regulator of \dot{V}_b , and varies in a predictable manner with \dot{V}_{O_2} , then it should be possible to formulate a species-specific equation to predict energy expenditure of free-swimming fish when implanted with f_H data loggers.

Pronounced body temperature fluctuations in free-living laughing kookaburras (*Dacelo novaeguineae*)

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Laughing kookaburras (*Dacelo novaeguineae* order Coraciiformes) are the largest of the kingfishers (360g). In captivity, their body temperature (T_b) varies by $<2.5^\circ\text{C}$. However, their sedentary habit and seasonally variable diet puts free-living individuals under energetic stress during cold weather and may require a reduction in T_b for energy conservation. We measured daily T_b fluctuations of five free-living kookaburras during winter. All individuals reduced T_b at night; the mean decline in T_b was 3.6 to 6.8°C (individual maximum: 9.1°C). Four of the five kookaburras entered torpor ($T_b < 34^\circ\text{C}$). Mean torpor T_b ranged from 32.4 to 33.4°C and the lowest T_b recorded was 28.6°C . Torpor lasted 2.2 to 5.9 h, with arousal reaching $T_b = 34^\circ\text{C}$ occurring between 02:22 and 04:57 h, well before sunrise. Cooling rates ranged from 0.9°C h^{-1} to 2.2°C h^{-1} and were always slower than arousal rates (1.7 to 3.6°C h^{-1}). Kookaburras left the roost with a mean T_b of 37.1 to 39.0°C , 34 to 38 min before sunrise. Consequently kookaburras aroused by employing endogenous heat production and did not rely on passive warming to increase T_b . Our results suggest that wild kookaburras, presumably responding to a combination of thermal stress and limited food, show more pronounced T_b reductions at night than captive birds. Kookaburras are the second coraciiform species found to use torpor, providing further evidence that members of yet another avian order are heterothermic.

High metabolic potential of marsupials is independent of BMR: the story from muscles

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The low BMR of marsupials relative to that of eutherians has coloured our appreciation of their true metabolic capabilities. Recently, high aerobic capacities have been reported in marsupials. Kangaroos have an aerobic capacity comparable with athletic eutherians; they are not limited by their BMR. Does this situation apply for marsupials generally and, if so, how is it achieved? In placental mammals aerobic capacity is associated with the amount of mitochondria available to utilise oxygen in skeletal muscle tissue. We examined skeletal muscle mitochondria in 3 marsupials of differing size, *Macropus rufus*, *Bettongia penicillata* and *Sminthopsis crassicaudata*. Total muscle mass was obtained and a whole-body sampling procedure determined average muscle mitochondrial characteristics. Using transmission electron microscopy and stereological techniques, we measured surface density of the inner mitochondrial membranes, $S_v(\text{im,mt})$ and mitochondrial volume density, $V_v(\text{mt,f})$. $S_v(\text{im,mt})$ was consistent across muscles and species, as in eutherians, at $\sim 35 \text{ m}^2 \text{ ml}^{-1}$. Consequently, the $V_v(\text{mt,f})$ and muscle mass should provide a measure of the aerobic potential of species. We determined the relationship between total mitochondrial volume of skeletal muscle, $(V(\text{mt,m})/M_b)$ and maximum aerobic capacity ($\text{VO}_{2\text{max}}$) of the three marsupials. These data were compared with those from eutherians. The relationship between $\text{VO}_{2\text{max}}$ and $V(\text{mt,m})/M_b$ was identical across all mammals (except humans). In conclusion, $\text{VO}_{2\text{max}}$ is determined by total muscle mitochondria; the link with BMR is loose. Since fundamental aerobic features are identical in all mammals they probably evolved in the mammal-like reptiles, well back in the Mesozoic.

The isolation and sequencing of cystic fibrosis transmembrane conductance regulator (CFTR) of the brushtail possum, *Trichosurus vulpecula*

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In eutherian mammals, epithelial fluid secretion is driven by chloride transport and is critically dependent upon CFTR, an epithelial chloride channel. However, in the possum, electrophysiological studies have demonstrated that fluid secretion in the small intestine is dependent upon bicarbonate rather than chloride. Consequently, we have investigated whether CFTR is present in epithelia of the possum, as a modification of this ion channel may provide an explanation for the difference seen in the ion dependence of secretion. Possum CFTR (pCFTR) was amplified by RT-PCR from intestinal RNA using primers designed from conserved regions of other species. Full-length pCFTR cDNA was sequenced fully in both directions following long-distance and RACE-PCR. Tissue distribution of CFTR was defined by semi-quantitative PCR comparing the amplification of pCFTR with β -actin. Possum CFTR showed high similarity (84 – 86%) to other mammalian CFTR, and lower similarity to non-mammalian vertebrate CFTR. As seen in eutherian mammals, CFTR was present in a range of epithelia, with high levels of expression in lung, trachea, reproductive tract (ovary, epididymis) and intestine, lower levels in liver and pancreas, but was not detectable in kidney or adrenal gland. These results indicate that, as in eutherian mammals, CFTR is distributed in a variety of epithelia, consistent with a role in epithelial transport. However, the high homology demonstrated between possum and eutherian CFTR raises questions as to whether possum CFTR accounts for the secretion of bicarbonate in the intestine, and this will have to be investigated by electrophysiological studies.

EEG in the developing tammar wallaby (*Macropus eugenii*)

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In order to determine when, during development, neurologically immature animals are capable of suffering, we first have to determine when sensory inputs reach the higher centres of the brain, and secondly, when the animals become conscious, as there can be no suffering without consciousness. As the mammalian fetus is not easily accessible, conducting neurological research on them is technically demanding. In contrast, most marsupial development occurs postnatally in the mother's pouch and the joey is thus more accessible. We investigated the development of brain function and responsiveness of tammar wallabies using spectral electroencephalogram (EEG) analysis. Joeys of various ages were maintained on a light level of anaesthesia. After 5min of baseline EEG, their fourth phalange was squeezed firmly with artery forceps for 30sec and a further 5min of EEG were recorded. The EEG features examined include median frequency (F50), spectral edge frequency (F95) and total power (Ptot). Initial results will be presented and will be interpreted in terms of the development of sensory capabilities, and when, during pouch life, joeys may develop the capacity to suffer. This research may have relevance for situations where concern is raised about possible suffering in joeys during pest control operations or in the case of road accidents. In addition, knowing more about the development of brain function and responsiveness in the joey may allow us to determine whether the Tammar wallaby is a useful model for studying neurological development and for the treatment of neurological disorders of the fetus and neonate of eutherian mammals, including humans.

Nitric oxide regulation of vertebrate blood vessels

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In mammals, nitric oxide (NO) generated in vascular endothelial cells is a global regulator of blood pressure. NO is synthesised by the enzyme nitric oxide synthase (NOS), and diffuses from the endothelial cell to mediate relaxation of vascular smooth muscle cells via a soluble guanylyl cyclase. Initially, it was assumed that a similar endothelial signalling system was present in all vertebrates, but this point is controversial. We have found that endothelial NO signalling is absent in various species of teleost fish and amphibians, and that NO control of the large blood vessels is mediated by NO neurotransmission from nitrergic nerves. This physiological data was supported by histochemical studies that could not demonstrate NOS in the endothelium, but, in contrast, neural NOS could be shown in the perivascular nerves. Interestingly, we found that the majority of perivascular, nitrergic nerves in toad blood vessels are in fact adrenergic. Thus, the nerves have vasodilator and vasoconstrictor capabilities. In contrast to the findings in fish and amphibians, we found that the large arterial blood vessels of the saltwater crocodile are regulated by both endothelially- and neurally-derived NO. Furthermore, in crocodiles and other reptiles, anatomical studies have shown NOS in the endothelium and perivascular nerves. Thus, it is proposed that endothelial NO signalling first appeared in reptiles, and that NO control of blood vessels in amphibians and some bony fishes occurs only via nitrergic nerves.

Evidence for NHE2 expression in the rectal gland of the spiny dogfish, *Squalus acanthias*

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Electroneutral sodium/hydrogen exchange has been identified in virtually all cell types mediating the exchange of extracellular sodium for intracellular protons. It plays a vital role in the regulation of cell volume, transcellular reabsorption of Na⁺ and intracellular pH. The NHE2 isoform of the Na⁺/H⁺ exchanger (NHE) gene family has been localised to the apical membrane of epithelial cells in the distal tubule of the mammalian kidney and is highly sensitive to the NHE inhibitor HOE694. The function of the distal tubule NHE2 is thought to be the acidification of the distal tubule lumen, which in turn mediates bicarbonate reabsorption in response to acidosis. Recent physiological studies using isolated dogfish rectal glands, demonstrated the presence of sodium dependant bicarbonate reabsorption and proton excretion mechanisms in rectal gland tubules. The study found that the excretion of proton ions in the rectal gland tubules was strictly sodium dependant and that Na⁺/H⁺ exchange was inhibited in the presence of the HOE694. These findings strongly suggested the mechanism for proton excretion was via a HOE694 sensitive NHE isoform localised to epithelial cells in the rectal gland tubules. In this study, we have provided further evidence in supporting the presence of an NHE isoform in the rectal gland of the spiny dogfish, identifying a fragment of rectal gland cDNA homologous to dogfish gill NHE2 and describing the localisation of dogfish specific NHE2-immunoreactivity in rectal gland ductal epithelial cells.

Regulation of the HPG axis in the blotched blue-tongued skink, *Tiliqua nigrolutea*

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We have investigated seasonal and sex-related differences in the activity of the hypothalamic-pituitary-gonadal axis in the blotched blue-tongued lizard, *Tiliqua nigrolutea* as part of an investigation 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. We injected adult male and both reproductively active and non-reproductively active adult female lizards with GnRH at relevant times of year: males (Jan - quiescent; Mar - early spermatogenesis; Oct - late spermatogenesis/mating), females (Apr - past partum/quiescent; Oct - vitellogenesis/quiescent). Juveniles at ages 1, 6, 12 and 18 months of age have been injected with GnRH and had sex steroids measured in the same way, to examine the onset of HPG axis regulation and so, the onset sexual maturity. We plan to follow these juveniles over time until sexual maturity is reached. We have found clear seasonal and sex-related differences in the production of testosterone (T) and estradiol (E2) in each sex (as a measure of the activity of the HPG axis), and suggest that aromatase activity may be an important point of regulation of the multiennial cycle in females of this species. We are also using assay results to develop a model for sexing offspring via differences in the testosterone to estradiol ratio for future studies.

Membranes, temperature and sodium leak in endotherms and ectotherms

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The rate at which ions such as sodium and potassium move across the cell membrane of endotherms (mammals and birds) is generally greater than that found in ectotherms (reptiles, amphibians and fish) when measured at endothermic body temperature. Increased membrane ion permeability in endotherms is commonly termed leaky membranes and is associated with the increased ionic movement inherently linked with the increased ionic traffic coupled to higher rates of metabolism. To understand the phenomenon of leaky membranes the thermal dependency of ion leak across membranes was investigated in isolated liver cells with the temperature profile of sodium entry into cells of endotherms and ectotherms determined. The endotherms investigated showed distinct transition points in their sodium entry whereas ectotherms did not (within their physiological temperature ranges). Investigations of potential sources of sodium ion entry targeting the Na^+/H^+ , $\text{Na}^+/\text{Ca}^{2+}$, $\text{Na}^+/\text{K}^+/\text{Cl}^-$ transporters and voltage-sensitive sodium channels in liver cells of an endo- and ectotherm (note: sodium-coupled amino acid and glucose transporters were not examined as they were absent in the incubations) suggests that only the Na^+/H^+ cotransporter is a significant contribution (~30% of total sodium influx) to sodium entry in both species. Therefore the largest component of sodium entry in cells of endo- and ectotherms is yet to be clearly identified.

The gastrointestinal physiology of chinook salmon, *Oncorhynchus tshawytscha* (Walbaum) affected by Gastric Dilation Air Sacculitis (GDAS)

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A syndrome known as Gastric Dilation Air Sacculitis (GDAS) has recently been described by Lumsden *et al.* (2002) for chinook salmon (*Oncorhynchus tshawytscha*, Walbaum), in seawater culture in New Zealand. This syndrome has a significant financial cost for the New Zealand salmon farming industry. The syndrome is characterised by distended abdomens, gastric dilation and air sacculitis (GDAS), increased feed conversion ratios (FCR) and mortality (Lumsden, 2002). Consequently, financial returns on affected stocks are greatly reduced. A study into the epidemiology of the syndrome was initiated. The process of GDAS development in *O. tshawytscha* is characterised by a loss of smooth muscle tone of the stomach as it distends. The application of Laplace's law ($P = 2T/r$) predicts that unless muscle mass increases, muscular tone will be lost and consequently a loss of gastrointestinal (GI) motor function will result. Therefore, GI smooth muscle contractility, osmoregulatory ability and the control of the GI system in pathologically affected and unaffected smolt were investigated. GDAS was experimentally induced in saltwater alone by feeding a commercially manufactured pelleted feed previously associated with a high incidence of GDAS, thus implicating osmoregulatory stress and feed integrity in GDAS development. Affected fish showed GI smooth muscle and osmoregulatory dysfunction. The 'Intestinal-brake' hypothesis for GDAS onset is presented and argued for. In addition, potential humoral mediators of the 'intestinal-brake' were investigated.

Glycogen repletion without food following intense physical activities: are mammals different?

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Glycogen plays an important role in supporting the energy demands of skeletal muscles during intense physical activities associated with “fight or flight” responses. Despite its importance, so little glycogen is stored in muscles that most of it could be depleted in response to a single bout of intense activity and fall to levels that could compromise an animal’s ability to “fight or fly”. What if food is not available to replenish those glycogen stores during recovery? We and others have shown that, even without food, skeletal muscles in fish, amphibians and reptiles have the capacity to replenish completely their glycogen mainly at the expense of lactate. In contrast, until recently, mammals have been reported to replenish only partially their glycogen stores due to increased lactate oxidation post-exercise. Our past and current work in rodents and humans challenges this latter view as our results show not only that lactate availability does not affect the levels of glycogen attained during recovery, but also that mammals are not different from other species in that there are conditions where they can replenish completely their muscle glycogen stores without food. The partial replenishment of muscle glycogen reported by others in mammals is explained on the basis that skeletal muscles protect set levels of glycogen against sustained depletion and the extent of glycogen repletion post-exercise is determined primarily by the amount of glycogen required to attain those protected levels, with only partial replenishment taking place if pre-exercise glycogen concentrations are above those levels.

Phenotypic plasticity of the salt glands of estuarine crocodiles to environmental salinity

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Across their geographic range the estuarine crocodile (*Crocodylus porosus*) can be found inhabiting freshwater, estuarine, marine and hypersaline environments and appear to move freely between these habitats. In saline environments, hyperosmotic to their body fluids, *C. porosus* utilises lingual salt glands to eliminate excess sodium and chloride ions and to maintain homeostasis. These extra-renal salt glands are located in the tongue of *C. porosus* and secrete a concentrated NaCl solution via a series of pores opening onto the surface of the tongue. The aim of this study was to examine the phenotypic plasticity of the lingual salt glands of *C. porosus* acclimated to freshwater and salt water environments and in animals fed a high salt diet. The gross morphology, ultrastructure, and distribution of key neuropeptides (substance P, vasoactive intestinal peptide, pituitary adenylate cyclase activating peptide) in the secretory tissues were examined, together with measurements of the functional capacity of the salt glands. The lingual salt glands of *C. porosus* demonstrated a high degree of structural and functional plasticity, up-regulating the secretory capacity of the glands when acclimated to salt water and a high salt diet.

Erythropoietin regulates hypoxic ventilation in mice by interacting with brainstem and carotid bodies

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Apart from its role in elevating red blood cell number, erythropoietin (Epo) exerts protective functions in brain, retina and heart upon ischemic injury. However, physiological non-erythroid functions of Epo remain unclear. Here we use a transgenic mouse line (Tg21) constitutively overexpressing human Epo in brain to investigate Epo's impact on ventilation upon hypoxic exposure. Tg21 mice showed improved ventilatory response to severe acute hypoxia and moreover improved ventilatory acclimatization to chronic hypoxic exposure. Furthermore, following bilateral transection of carotid sinus nerves that uncouples the brain from the carotid body, Tg21 mice adapted their ventilation to acute severe hypoxia while chemodenervated wild type (WT) animals developed a life-threatening apnea. These results imply that Epo in brain modulates ventilation. Additional analysis revealed that Epo receptor (EpoR) is expressed in the main brainstem respiratory centers and suggested that Epo stimulates breathing control by alteration of catecholaminergic metabolism in brainstem. The modulation of hypoxic pattern of ventilation after i.v. injection of recombinant human Epo in WT mice and the dense EpoR immunosignal observed in carotid bodies showed that these chemoreceptors are sensitive to plasma levels of Epo. In summary, our results suggest that Epo controls ventilation at central (brainstem) and peripheral (carotid body) levels. The physiological relevance of these novel findings are useful to better understand respiratory disorders including those occurring at high altitude.

Progesterone stimulates arteriogenesis in mouse endometrium

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Specialised 'spiral arterioles' in the human endometrium coil and thicken when progesterone concentrations increase during the secretory phase of the menstrual cycle. However, there is no evidence that progesterone is responsible for the observed arteriogenesis (process whereby vessels acquire a coat of vascular smooth muscle cells [VSMC]). In this study, we aimed to determine whether progesterone stimulates arteriogenesis in mouse endometrium by quantifying changes in the proportion of vessels covered by α -smooth muscle actin (α -SMA: mural cell marker staining both VSMC and pericytes) in hormone-treated ovariectomised mice. Ovariectomised mice were given three consecutive daily injections of progesterone (1 mg) or vehicle. Before dissection, mice were injected with BrdU enabling proliferating mural cells to be quantified with α -SMA/BrdU double-immunohistochemistry. The percentage of vessel profiles with no, minimal, extensive or complete α -SMA coverage were quantified. There was a significant decrease in the percentage of vessel profiles with no α -SMA coverage following progesterone treatment ($20 \pm 4.3\%$ [mean \pm SE] vs. $57 \pm 4.6\%$, $p < 0.001$), and a significant increase in the percentage of vessels with minimal or extensive α -SMA coverage ($44 \pm 3.4\%$ vs. $27 \pm 3.7\%$, $p = 0.019$ and $27 \pm 4.3\%$ vs. $5 \pm 0.5\%$, $p = 0.001$, respectively), in comparison to vehicle-treated mice. The percentage of vessels with complete α -SMA coverage did not change significantly in comparison to vehicle-treated mice ($8 \pm 2.3\%$ vs. $10 \pm 1.2\%$). Proliferating mural cells were only observed in progesterone treated animals (median = 7 [range 4–27]). These results show that progesterone stimulates arteriogenesis in the mouse endometrium and are consistent with a role for progesterone in the development of spiral arterioles in the human endometrium.

Comparative forage requirements of kangaroos and sheep: a reality check based on physiological data

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The carrying capacity of a sheep property is often expressed in terms of the forage requirements of a 45 kg merino wether, the unit being a 'dry sheep equivalent', DSE. Although studies of kangaroo diet have shown that their requirements overlap with sheep mainly during drought, kangaroos are regarded as serious competitors for sheep by most wool growers in Australia's sheep rangelands. This opinion is reinforced by the long accepted value of 0.7 DSE for the forage requirement of a kangaroo. However, this value is much too high. The use of 0.7 comes from the resting metabolic rate of marsupials being about 70% that of eutherian mammals, and its application to comparative forage requirements assumes that the ratio of their field metabolic rates (FMR), also, is similar. Further, it assumes that sheep and kangaroos have similar body weights. Neither of these assumptions is correct. Average body mass of kangaroos is much less than 45kg, and two studies, using completely different approaches, have reported that kangaroo-sized marsupials have substantially lower FMR than eutherians. Appropriate DSE values for kangaroos are much more likely to be in the range of 0.15 - 0.20. Of course, total grazing pressure (TGP) depends on the number of individuals in a population as well as their individual impact, but the recognition that kangaroo DSE is very much less than the 'traditional' value has implications for rangeland management; woolgrowers hoping for substantial increases in wool production as a result of lowering kangaroo numbers are likely to be disappointed.

Low cost of locomotion in lizards that are active at low temperatures

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The physiology and activity of ectothermic taxa such as reptiles are dependent on temperature. A low energetic cost of locomotion (C_{\min}) has evolved in nocturnal geckos, which increases maximum aerobic speed and partially offsets the decrease in maximum oxygen consumption caused by activity at low nocturnal temperatures. This is termed the nocturnality hypothesis. We test the generality of the nocturnality hypothesis by comparing the C_{\min} values of four lizard species (two nocturnal and two diurnal), including a nocturnal scincid lizard. C_{\min} is calculated as the energy required to move a gram of body mass over a kilometre and is measured during steady exercise on a treadmill respirometer. We accept the hypothesis that nocturnal lizards in general have a low C_{\min} . Evidence is also provided that low C_{\min} is present in high latitude diurnal lizards that experience low temperatures during their activity periods. The C_{\min} values of the four lizard species measured in this study (range = 0.21-2.00 ml O₂ g⁻¹ km⁻¹) are lower than diurnal lizards from elsewhere, and within or below the 95% confidence limits of C_{\min} values of nocturnal geckos. A low C_{\min} increases the range of locomotor speeds possible at low temperatures and provides an advantage to species that are active at these temperatures. We conclude that there may be a reduced C_{\min} in all nocturnal lizards as well as lizards from high latitudes. The low C_{\min} in lizards living at high latitudes may enable extension of their latitudinal range into thermally sub-optimal habitats.

The distribution and expression of the cystic fibrosis transmembrane conductance regulator in the intestine of the possum, *Trichosurus vulpecula*

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In the small intestine and colon of eutherian mammals fluid secretion is driven by the active transport of Cl⁻ and is dependent on the cystic fibrosis transmembrane conductance regulator (CFTR), a cAMP activated Cl⁻ channel. In contrast, fluid secretion in the possum small intestine is dependent on HCO₃⁻ and the colon does not exhibit a secretory response. Despite this, possum CFTR transcript has been detected by PCR in both tissues. We have investigated CFTR further by using *in situ* hybridisation to determine the distribution of transcript and Western blots to test for the presence of mature protein in possum intestinal tissue. In the small intestine of the possum, CFTR was localised to the crypts and base of the villi. However in the colon, CFTR had a punctate distribution with high levels of transcript in individual cells or small groups of cells throughout the epithelium. Mature CFTR protein was present in both the small intestine and colon of the possum. These data demonstrate a distribution of CFTR in the small intestine comparable to that in eutherian mammals and the presence of mature protein suggests it is involved in the secretory response. However, the presence of CFTR in the colon, in spite of the lack of a secretory response, suggests that possum CFTR may have other functions unrelated to secretion, as observed for CFTR in eutherian mammals.

Environmental regulation of dietary fatty acid preference in Siberian hamsters

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Previous experiments by Geiser, Florant and others have demonstrated that the saturation of dietary fatty acids affects thermoregulation and energetics in seasonal and daily heterotherms. To determine whether a daily heterotherm, the hamster *Phodopus sungorus*, could choose the saturation level of dietary fatty acids appropriate to its thermal environment, hamsters were presented a choice between a diet rich in saturated fatty acids and a diet rich in unsaturated fatty acids under a variety of environmental conditions. Two hypotheses, the Homeoviscous Adaptation Hypothesis and the Membrane Pacemaker Hypothesis, independently predict that animals exposed to cold should increase the incorporation of unsaturated fatty acids into cellular and/or mitochondrial membranes. We therefore predicted that exposure to cold, or to environmental signals associated with future cold exposure, should increase the preference of hamsters for a diet rich in unsaturated fatty acids, which could serve as a source of fatty acids to be incorporated into membranes. Hamsters increased their preference for a diet rich in unsaturated fatty acids 1) in response to decreased ambient temperature, even when they were not displaying daily torpor, 2) in response to short-day exposure, a predictor of approaching winter, and 3) in response to melatonin treatments that mimic the photoperiod-dependent hormonal milieu that results from short-day exposure. This finding demonstrates that diet choice is a possible mechanism by which mammals could regulate the fatty acid composition of somatic tissues, and that environmental conditions can regulate the expression of dietary lipid choice.

Factors influencing the hyperventilatory response in the Plains rat

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Differences in lifestyle, gender, ambient temperature and circadian pattern are known to affect the hypoxic ventilatory response. We studied the hyperventilatory response of adult Plains rats (*Pseudomys australis*), a native semi-fossorial rodent, to both acute (20 min) exposure to hypoxia (10% O₂) at a range of ambient temperatures (Ta) and to chronic (2 days) exposure at room temperature (Ta = 22°C). During acute exposure, hyperventilation at any Ta was achieved primarily by hypometabolism with no significant change in ventilation. At all Ta the hyperventilation was appropriate for the level of inspired O₂. A decline in body temperature was also observed in hypoxia, becoming more marked at low Ta. While such a response is typical of small mammals, the hypoxia-induced alterations in breathing pattern were not as expected. Hypoxia normally causes an increase in respiratory drive and breathing rate. Such a response was evident following chronic but not acute exposure to hypoxia, suggesting that *P. australis* may have a delayed hyperpnea on initial exposure to hypoxia. In addition, females showed a reduced Tb on acute exposure to hypoxia that disappeared after ~24 hr exposure. We conclude that while *P. australis* does not show an attenuated hyperventilatory response to acute hypoxia, as shown in other burrowing species, the pronounced hypometabolism to hypoxia may be advantageous to cope with acute exposures. Long term suppression of metabolic rate is detrimental and a hyperpneic response is required with chronic exposure to enable metabolic rate to return to normal values.

Membrane fatty acid composition and the determination of lifespan: recent insights from some special rodents

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The oxidative stress theory, the most accepted explanation of aging, proposes that reactive oxygen species (ROS) are produced from aerobic metabolism and consequently damage cellular nucleic acids, proteins and fats. Fatty acids vary dramatically in their susceptibility to ROS attack; those that are highly polyunsaturated being most vulnerable to peroxidation. The membrane pacemaker theory highlights the fatty acid composition of membrane phospholipids and its effects on lipid peroxidation in aging. A peroxidation index (PI) can be calculated for membranes by combining the peroxidation-susceptibilities of individual fatty acids with the fatty acid composition. In mammals and birds a 19-24% decrease in PI is associated with a doubling of maximum lifespan. We have tested the membrane pacemaker theory by examining the fatty acid composition of tissue phospholipids in (i) the longest-living rodent species, and (ii) strains of wild-derived *Mus* that show extended longevity. The naked mole rat (*Heterocephalus glaber*) has a maximum lifespan of ~28 years. Its tissue phospholipids have a very low docosahexaenoic acid (22:6 n-3) content which results in peroxidation-resistant membranes. Two strains of mice (Idaho & Majuro), both derived from wild-trapped progenitors, show extended lifespans in captivity when compared to a genetically heterogeneous laboratory strain of mice (DC). Muscle and liver phospholipids from these long-living mice have a reduced 22:6 n-3 content compared to DC mice. The relationship between maximum longevity and membrane PI is similar for these mice as previously observed for mammals in general. Peroxidation-resistant membranes are likely an important component of extended longevity.

Erythrocyte fragility in two species of brushtail possum exposed to dietary terpenes

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The common brushtail possum (*Trichosurus vulpecula*) and the short-eared possum (*T. caninus*) are closely related marsupial herbivores that differ in several aspects of their ecology, including diet; *T. vulpecula* consumes significant amounts of *Eucalyptus* spp. foliage, while *T. caninus* feeds mainly on *Acacia* spp. Eucalypt foliage is protected against herbivory by several plant secondary metabolites (PSMs), including terpenes, tannins and formylated phloroglucinol compounds (FPCs), while acacia foliage is protected mainly by tannins. The addition of sesquiterpenes to a PSM-free basal diet resulted in significant decreases in fragility of the erythrocytes of both possum species when exposed *in vitro* to either monoterpenes or sesquiterpenes extracted from eucalypt foliage. The decrease was significantly greater in *T. vulpecula* than *T. caninus* on the monoterpene treatment, but similar for both species on the sesquiterpene treatment. The molecular mechanism for the protection of the membranes of erythrocytes from terpenes *in vitro* when sesquiterpenes were added to the basal diet is unknown. Plasma concentrations of bilirubin (a powerful antioxidant) were 4-6 times higher in *T. vulpecula* on both the basal and the sesquiterpene-supplemented diets. Urinary excretion of glucuronic acid increased 3-fold in *T. vulpecula* in response to dietary supplementation with sesquiterpenes, but there was no increase in *T. caninus*. These results suggest that *T. vulpecula* is better equipped to deal with oxidative stress and thus able to feed on a wider range of plant species and occupy a wider range of habitats than *T. caninus*.

Identification and properties of sex steroid binding proteins in nesting *Chelonia mydas* plasma

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The presence of a sex steroid binding protein in the plasma of sea turtles has not previously been studied. The information presented on steroid-protein interactions in this study provides an insight into the reproductive status of sea turtles. A sex high-affinity, low-capacity sex hormone steroid binding protein was identified in nesting *Chelonia mydas* and its thermal profile was established. At 4°C testosterone and oestradiol bind in nesting *Chelonia mydas* with high affinity ($K_d = 1.39 \times 10^{-8}$ M; 1.89×10^{-7} M) and low binding capacity ($B_{max} = 4.08 \times 10^{-5}$ M; 3.90×10^{-5} M), respectively. At 23°C the binding affinity and capacity of testosterone and oestradiol were similar with those determined at 4°C ($K_d = 1.8 \times 10^{-8}$ M; 1.9×10^{-7} M) and ($B_{max} = 1.9 \times 10^{-5}$ M; 8.3×10^{-5} M), respectively. At 36°C testosterone maintained its binding affinity and capacity with those of at 4°C and 23°C ($K_d = 1.2 \times 10^{-8}$ M) and ($B_{max} = 4.9 \times 10^{-5}$ M), respectively however, oestradiol showed no binding activity at 36°C. The results of this study indicate that temperature has minimal effect on the high affinity binding of testosterone to sex steroid binding protein but the high affinity binding of oestradiol to sex steroid binding protein is abolished at a hypothetical high (36°C) body temperature. This suggests that at high core body temperatures most of the oestradiol would become biologically available to the tissues rather than a large percentage of the total hormone remaining inactive in the plasma.

The effects of incubation temperature on development in the Australian brush-turkey (*Alectura lathami*)

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The Australian brush-turkey (*Alectura lathami*) belongs to a unique group of birds, the megapodes, which use environmental heat to incubate their eggs and provide no parental care to their offspring. The effect of incubation temperature on hatchling quality, such as differences in chick and residual yolk size and composition were investigated at constant temperatures of 32, 34 and 36°C. A surprising level of variation was observed in the lipid concentration both within and between yolks. Samples from a single dry yolk often varied by 10%, once as much as 17%, in their proportion of lipid. Following homogenization, the variation in yolk lipid concentration ranged from 41 to 81% of dry mass, a high and variable concentration compared to a predictable 33% in domestic chickens. The proportion of lipid in the dry yolk-free hatchlings ranged from 17 to 34%, which is probably due to differing stores of fat. Energy reserves are important to hatchlings which must dig their own way out of the incubation mound and secure their own food resources so, these differences are likely to affect survival. As the energy content of hatchlings is indirectly proportional to the energetic cost of development, some results on the energetics of embryonic development at different incubation temperatures will also be presented.

Placental transfer of organic nutrients in viviparous skinks: a strategy for optimising neonatal fitness

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Viviparous squamates exhibit an extraordinary range of placental types. Similarly, the degree of dependence on yolk versus placental transfer of nutrients ranges from primarily lecithotrophic to microlecithal species in which the placenta supplies almost all of the materials needed to support embryonic development. Placental supply of nutrients may be facultative (= a “bonus” supplement to already adequate yolk supplies) or obligate (= necessary for successful embryonic development). We suggest that placentotrophy first evolved as a facultative mechanism enabling mothers to supplement an adequate yolk supply and thus enhance neonatal fitness if environmental conditions are good during gestation. We have assessed placental transport of organic nutrients in three closely related species of viviparous skinks: *Niveoscincus metallicus* (primarily lecithotrophic), *N. ocellatus* (moderately placentotrophic) and *N. microlepidotus* (a lecithotrophic alpine species in which gestation is greatly extended). In all species tested, we have demonstrated placental transfer of organic precursors for protein (leucine) and lipid (oleic acid) into embryonic compartments. Transfer of leucine indicates that placental nutrition is important to embryonic development but the relative contribution varies between species. Oleic acid is also transferred across the placenta, and is incorporated into the embryonic fat bodies. This supports our hypothesis that facultative placentotrophy enhances neonatal fitness through providing fat reserves that may be utilised *in utero* if birth is deferred, or during early neonatal life.

Why herbivore poo should bounce: an exploration of the creep rheometry of possum digesta

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We explored the creep and recovery rheometry of the colonic digesta of the brushtail possum (*Trichosurus vulpecula*) and examined the time bases of rheometric components with respect to known peristaltic and segmentation frequencies. The results help explain the differences in the transit times of the liquid and the solid phases of digesta and how caecal sequestration of particulate matter may be linked to contraction frequency.

Backswimmers: diving bugs with buoyancy compensation devices

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Unlike all other diving insects, backswimmer bugs of the genus *Asinops* exploit the pelagic zone by temporarily achieving near-neutral buoyancy while submerged. Bugs begin a dive positively buoyant because of the large volume of air carried in their ventral air stores. During the dive the air store's volume shrinks due to respiration and the loss of CO₂ and N₂ to the surrounding water. However, instead of experiencing a steady drop in buoyancy, *Asinops* enter a protracted phase of near-neutral buoyancy before becoming negatively buoyant. This dive profile is attributable to the haemoglobin present in large tracheated cells in the abdomen that communicate through spiracles with the bug's ventral air store. The P_{50} of *Anisops deanei* haemoglobin is around 4 kPa, allowing it to supply the air store with O₂ once the PO_2 in the bubble has dropped by around 17 kPa. A drop in PO_2 from 21.2 kPa to 4 kPa reduces the gas volume by ~17 %, a value very close to the % volume decrease required to achieve neutral buoyancy from measured initial air store volumes. Thus, once the air store has decreased to a size where near-neutral buoyancy is conferred, oxygen released from the haemoglobin temporarily stabilises the bubble's volume. It also extends the dive time some 50 % longer than the air store itself would allow. This study used CO to elucidate the effect of haemoglobin on the PO_2 and buoyancy of *A. deanei*'s air store during diving. Changes in buoyancy were measured using tethered backswimmers.

Season influences the resting metabolism of three tropical species of Australian frogs (Anura: Hylidae)

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The wet and dry seasons of tropical northern Australia provide a challenging environment for amphibians, yet a large number of species live in this region of extremely seasonal water availability. Cocoon forming species depress metabolism between 50 and 80% to conserve energy during seasonally adverse dry conditions. A large proportion of species living in the wet-dry tropics are tree frogs belonging to the non-cocoon forming genus *Litoria*, and little is known about how non-cocoon forming species respond to seasonally dry conditions. In this study, we compared the seasonal resting metabolism, using respiratory gas analysis, of the cocoon forming giant frog, *Cyclorana australis* and two non-cocoon forming frogs, the green tree frog, *Litoria caerulea* and the Dahl's aquatic frog, *Litoria dahliei*. All three frogs are similar in size (20 – 45 g) and belong to the family Hylidae. *C. australis* depress metabolism about 70% during dormancy in the laboratory, which is comparable to xeric-dwelling species, and they remain dormant for approximately six months in the field. In contrast, *L. caerulea* and *L. dahliei* depress metabolism about 50% during simulated dry conditions, which suggests that during extreme adverse conditions, *L. caerulea* and *L. dahliei* have the ability to remain inactive within refuges and moderately depress metabolic rate. However, metabolic depression was not evident in field-caught individuals of these two species during the dry season, suggesting that conditions and selecting active frogs in the field was not a good indicator of metabolic response.

Phenotypic plasticity: a major contributor to avian metabolic diversity?

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There is increasing evidence that avian basal metabolic rate (BMR) is not a static physiological trait, but varies through time within individuals in response to changes in energy supply and/or demand. For instance, long-distance migrants often show significant seasonal variation in BMR, and many species adjust BMR in response to thermal acclimation/acclimatization. Despite numerous studies showing short-term BMR adjustments, the contribution of phenotypic plasticity to avian metabolic diversity in large, interspecific data sets has not been examined. We addressed this question by taking advantage of the fact that the available avian BMR data comprise estimates from a combination of wild-caught and captive-raised populations. Compared to populations in natural environments, birds raised in captivity can be viewed as belonging to experimental populations maintained under conditions of reduced thermal variability, greater quantitative and qualitative homogeneity of energy, nutrient and water resources, and reduced levels of activity. We obtained BMR and body mass (M_b) data for 231 species, of which 58% were obtained from wild-caught populations and the remainder from populations raised in captivity. The scaling exponents relating BMR to M_b differed between wild-caught (0.74) and captive-raised birds (0.67), with captive-raised birds exhibiting a significantly shallower BMR- M_b slope once phylogenetic relatedness was controlled for. These differences persisted when we controlled for migratory tendency and habitat aridity. Our results reveal that avian BMR scaling is partly determined by environmental factors, suggesting that hypotheses concerning the BMR of wild-caught birds should not be tested using data from captive-raised populations, or *vice versa*.

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Kiwi caeca – fermentative function in an insectivore?

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If the kiwi is to thrive and reproduce on an artificial diet, it is important to understand their digestive strategy including the extent that relatively indigestible substrates such as chitin may be broken down by fermentative digestion. Whilst it is known that the caeca are a site of fermentative digestion in herbivorous birds, no information is available regarding their role in insectivorous species. Morphometric studies of caeca are likely to be of value in ratite species, as their digestive strategy is not constrained by the need for flight. Thus the comparative morphology of the kiwi gut is more likely to reflect digestive function than any need to reduce overall mass. This study examined caecal morphology in kiwi carcasses along with the relative distributions of particle sizes in various segments of their gastrointestinal tract in order to cast light on caecal digestive function. We also examined the distribution of incombustible residues in the various particle size fractions of digesta from each gut segment in order to determine whether there was any particle sorting on a basis of relative density.

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Cardiorespiratory coupling during terrestrial locomotion in reptiles: consequences of intra-abdominal pressure and respiratory mechanics

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Exercise places increasing demands on both the cardiovascular and respiratory systems. However the mechanical act of locomotion can act to limit cardiovascular and respiratory performance in reptiles and thus limit the capacity for exercise. The aim of this study was to investigate the effects of locomotion on interaction of ventilation, venous return and intra-abdominal pressure (IAP). Cardiorespiratory coupling during treadmill locomotion was investigated in the Savannah monitor lizard, *Varanus exanthematicus*, and the American alligator, *Alligator mississippiensis*. In varanids, costal ventilation was reduced at high speeds due to the conflicting demands of ventilation and locomotion on the hypaxial musculature (axial constraint). Axial constraint was the likely cause of an increased IAP during exercise in varanids, and has important consequences for hemodynamics. Venous return is a major determinant of cardiac output and is affected by alterations in both ventilation and IAP. Ventilation, specifically expiration, can aid venous return. The reduction in costal ventilation during locomotion in varanids reduces the supplementary effect of the respiratory pump on venous return. Elevated IAP collapses the major veins in the abdomen and decreases venous return. Alligators differ from varanids in their respiratory mechanics (hepatic-piston pump) and cardiovascular anatomy, both having important consequences for cardiorespiratory coupling during exercise. The respiratory pump is not compromised during terrestrial locomotion in alligators, and the small increase in IAP has no significant effects on venous return. Thus, cardiorespiratory coupling is compromised during terrestrial locomotion in varanids due to increasing IAP and axial constraint, but not in alligators.

Determining the suitability of a marsupial, *Antechinus stuartii*, as a model for Alzheimer's disease

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Alzheimer's disease is a chronic neurodegenerative disease characterised by the development of neurofibrillary tangles, neuronal death, oxidative damage and extracellular deposition of beta-amyloid peptides. There is controversy as to the pathogenic process of the disease. One hypothesis purports the overproduction of beta-amyloid as the ultimate cause; another attributes the neuropathology to concentration changes in circulating stress hormones. Beta-amyloid is produced from the amyloid precursor protein (APP) by proteases known as secretases. *Antechinus stuartii* is a marsupial that exhibits a highly synchronised seasonal biology, culminating in total male mortality at the end of the breeding season. Male mortality is related to increased circulating testosterone and cortisol and subsequent effects of these hormones on organ systems. We examined glucocorticoid receptors in kidney, and beta-amyloid production in *A. stuartii* to explore their suitability as a model for Alzheimer's disease progression. Glucocorticoid receptors from the cytosol of kidneys from adult female *A. stuartii* were found to have a specific binding of [³H]-dexamethasone of 44 fmol/mg, approximately twice that of mouse glucocorticoid receptor specific binding. Preliminary immunohistochemical data indicated beta-amyloid immunoreactivity in the brains of post-breeding males and 2–3 year old post-breeding females. However, Western analysis of brain homogenates failed to detect beta-amyloid in adult marsupials of either sex, despite the presence of APP mRNA and γ -secretase. The beta-amyloid region of *A. stuartii* APP was cloned and sequenced. The derived amino acid sequence revealed four residue differences compared to beta-amyloid from eutherian species. We speculate these differences may affect APP processing in marsupials.

Phospholipase A2 in marine invertebrates

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Hydrolysis of cell membrane phospholipids by phospholipase A2 (PLA2) results in the release of fatty acids including arachidonic acid for the synthesis of prostanoids such as prostaglandins, leukotrienes and the platelet activating factor. PLA2s are involved in cellular signal transduction, digestion and inflammation. In addition, PLA2s have antibacterial and toxic properties and are important components of snake and other venoms. We measured the catalytic activity of PLA2 in tissue extracts of primitive marine invertebrates of the phyla Porifera (sponges) and Cnidaria (corals, sea anemones and jellyfish). Poriferan species with high PLA2 activity levels included *Cymbastela coralliophila*, *Acanthella cavernosa*, *Spirastrella vagabunda* and *Theonella swinhoei*. High PLA2 activity levels were measured in the stony coral *Pocillopora damicornis* and the hydrozoan fire coral *Millepora* sp. that cause skin irritation upon contact. High levels of PLA2 activity were also found in the acontia of the sea anemone *Adamsia cariniopados*. Acontia are long threads containing nematocysts that are used in defense and aggression by the animal. There are thousands of nematocysts in the tentacles of the scyphozoan (jellyfish) and cubozoan (box jellyfish) species. High PLA2 activity levels were measured in jellyfish tentacles including those of the life-threatening multi-tentacled box jellyfish *Chironex fleckeri*. The functions of poriferan and cnidarian PLA2 may include roles in eicosanoid metabolism, capture and digestion of prey and antimicrobial and toxic defense of the animal. The current observations support the idea that cnidarian PLA2 may participate in the sting site irritation and systemic envenomation syndrome resulting from contact with cnidarians.

The autonomic nervous system and its possible function in the heart of the dragon lizard, *Ctenophorus nuchalis*

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The occurrence and influence of the two major antagonistic branches of the autonomic nervous to the heart was investigated on rate and force changes over a range of temperatures in the isolated cardiac preparations of the dragon lizard, *Ctenophorus nuchalis*. The effect(s) of the stimulation of the intramural nerves to the various cardiac chambers was established and the identity of the nerves influencing the force and rate changes was identified by alternatively mimicking the responses with known neurotransmitters and also by blocking both the nerve-mediated responses and those produced by applied neurotransmitter with known receptor blocking agents. The atria were found to have both sympathetic and parasympathetic nerves mediating force and rate changes. In contrast to the reports for other squamates, where parasympathetic nerves mediating force decreases have not been reported, they were found in the dragon ventricle. The magnitude and influence of the parasympathetic or inhibitory innervation to the ventricle was investigated. These nerves to the ventricle are capable of producing or preventing substantial changes in the contractile force of the muscle. The activity and influence of the two branches of the nervous system do not appear to be greatly compromised at a range of temperatures between 15°C and 35°C. The innervation pattern demonstrated in these lizards is similar to the pattern that we have previously described in mammalian heterotherms. The “novelty” or otherwise of the “anomalous innervation pattern” to these mammalian heterotherms may simply reflect the retention/re-establishment of an earlier pattern.

Cardiovascular effects of urotensin II on rat and salmon hearts

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Urotensin II (UII) is a peptide first discovered in the urophyses of goby fish and later in humans. UII has been shown to have significant effects on cardiovascular homeostasis from fish to humans. Research to date is very inconsistent as UII is very species and tissue specific. The aim of this study is to ascertain the direct effects of UII on the heart of salmon and rats using both native and non-native forms. Rat hearts were perfused via the Langendorff isolated heart system. Myography was employed to investigate the effect of UII on salmon coronary arteries measuring changes in tension in response to UII. Native UII caused potent and sustained, dose-dependent dilation of the coronary arteries in the rat. Non-native UII (human and trout UII) showed severely reduced vasodilation compared to the native rat UII (rUII). Urotensin related peptide (URP) dilated rat coronary arteries with reduced potency compared to rUII. In salmon, native trout UII (tUII) caused sustained dilation of the coronary arteries, while non-native hUII and URP caused significant constriction. Therefore, the N-terminus is vital for correct receptor activation in both salmon and rats. In sum, UII is very species specific, and thus is imperative to use the native peptide when trying to elucidate the true biological effects of UII on the cardiovascular system from fish to mammals.

Cell division and apoptosis are differentially affected in the neural stem cell niche of adult transthyretin null mice

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Thyroid hormones are fundamentally involved in the regulation of growth and development, particularly in the brain. Thyroid hormones are required for full proliferative activity of neural stem cells in the subventricular zone of adult mouse brains, and thyroid hormones also affect the normal fate of progenitor cells, including apoptosis. Transthyretin (TTR) is a thyroid hormone distributor protein in the blood and cerebrospinal fluid. TTR synthesis and secretion by the choroid plexus (the blood-cerebrospinal fluid barrier, part of the blood-brain barrier) is involved in the transport of thyroid hormones across the blood-brain barrier into the cerebrospinal fluid. Given the role of TTR in CNS thyroid hormone homeostasis, we investigated the regulation of cell cycle of neuronal stem cells in the subventricular zone of adult TTR null mice. Markers for neural stem cell mitosis, the levels of which are reduced during hypothyroidism, did not differ between genotypes. However, in “euthyroid” TTR null mice the level of apoptosis, the normal fate of most progenitor cells, was as low as that in brains of hypothyroid wild type mice. Regulation of neural stem cell proliferation and apoptosis in the subventricular zone can be taken as physiological indicators of consequences of lack of TTR synthesis and secretion by the choroid plexus. Lack of TTR results in reduced thyroid hormone transport into the cerebrospinal fluid via the choroid plexus. We show that neural stem cell proliferation and apoptosis in the subventricular zone are differentially affected by the lack of TTR synthesis in the choroid plexus.

Thermal acclimation of metabolism in *Limnodynastes peronii* frogs

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We investigated how adult brown-striped marsh frogs (*Limnodynastes peronii*) acclimate their metabolism to lowered temperature. We took an integrative approach, measuring metabolism at the whole animal, cellular, and biochemical levels in frogs acclimated to 15 and 25°C. This species is distributed over a wide latitudinal gradient, and is capable of breeding during early spring and late autumn. We were specifically interested in finding whether the metabolic capacity of organs essential for maintaining reproductive processes in cold temperature underwent thermal acclimation. We found no evidence of acclimation of resting metabolic rate, but there were changes between the two acclimation groups in mitochondrial respiration and enzyme activity. The respiratory control ratio (RCR) is a ratio between basal Mt oxygen consumption and maximal oxygen consumption during oxidative phosphorylation. Cold acclimated frogs maintained the RCR of liver and calling muscle at their acclimation temperature. This appears due to the up-regulation of the capacity of the mitochondria during oxidative phosphorylation. Thermal acclimation in the activity of enzymes depended both on the type of tissue and enzyme, with aerobic pathways unregulated in calling muscle and heart. These two tissues are essential for males to call to maintain territories, and attract mates.

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The effects of diet and habitat on milk composition and growth in young marsupials

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It is well-known that marsupials milk changes dramatically during the lactation period from dilute high carbohydrate/low fat to thicker milk higher in fat. Little is known of the similarity of milk of related species. Much less is known of the effects of captivity 'v' wild diets. In this talk we will illustrate differences in milk composition and pouch young growth Tasmanian bettong, *Bettongia gaimardi* and the Tasmanian pademelon, *Thylogale billardieri*. The bettong is a mycophagous species the diet of which cannot be replicated easily in captivity and the pademelon is a browsing/grazing herbivore. Growth rates and body condition were generally and not surprisingly better in captive than in wild animals but there were variations in milk composition and growth that were related to diet and habitat. Although hypogeous fungi are purportedly poor in protein, the milk of wild feeding bettongs was rich in protein. Captive pademelons had better quality milk and growth but this difference decreased towards late lactation.

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Functional morphology of olfactory structures in elasmobranchs

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Elasmobranchs have a complex sensory ecology and use an array of different sensory systems. While several of these systems have already been studied extensively, information on the olfactory system is scarce and most often limited to external nasal morphology. In this study, nasal morphology and ultrastructure of the olfactory organs of 11 southern hemisphere species were described and compared to known northern hemisphere species. The surface area of the olfactory epithelium was calculated using lamellae counts and morphometric measurements. The surface structure of the olfactory epithelium was analyzed using light and scanning electron microscopy, and the size of the olfactory rosette, olfactory bulbs and telencephalon was compared between different species. Olfactory organs in elasmobranchs vary considerably in size and shape among different taxa, suggesting olfactory adaptations to a specific environment or lifestyle. All elasmobranchs examined were found to possess cilia as well as microvilli covering their olfactory epithelia. However, while microvilli were found covering the nonsensory epithelium and the surface of receptor cells in the sensory epithelium, cilia were only observed in the sensory epithelium on the surface of supporting cells, but not on any receptor cells. These results support previous findings, indicating that elasmobranchs may differ in this respect from teleosts, which generally bear cilia and microvilli on the apical surface of their olfactory receptor cells (sensory epithelium). On average, sensory epithelial area, olfactory bulb size and size of telencephalon appear to be greater in free swimming species, which might therefore rely more heavily on olfaction than benthic species.

The significance of phenotypic flexibility in thermoregulating reptiles

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Animals may maintain their fitness in fluctuating thermal environments either by regulating their body temperature to maintain a constant internal environment, or by altering the thermal sensitivity of physiological and biochemical rate functions to compensate for thermodynamically induced variation. We tested the hypothesis that both compensation and behavioural thermoregulation act in concert to maintain fitness even in a tropical reptile (*Crocodylus porosus*). Animals were exposed to simulated summer and winter environments within which they were able to thermoregulate behaviourally. Crocodiles changed their thermoregulatory behaviour and regulated their body temperature to significantly lower levels after 30 days of exposure to the winter environment. Concomitant to changes in behaviour, crocodiles compensated for lower body temperatures at all levels of organisation: protein, cellular, and whole animal. Hence, sustained swimming performance, mitochondrial O₂ consumption, and the activities of metabolic enzymes were altered so that performance was maintained despite significantly lower body temperatures. Experimental designs in research on reptilian thermal physiology should incorporate the capacity for phenotypic flexibility as a null-hypothesis, because the significance of differential body temperature–performance relationships (thermal reaction norms) between individuals, populations, or species cannot be assessed without testing that null-hypothesis.

Non-invasive measurement of oxygen partial pressure, lateral diffusion and chorioallantoic blood flow under the avian eggshell

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A modified technique has been developed to measure PO₂ under the shell of bird eggs indirectly. A small glass tube is glued to the shell, creating a 0.05 ml gas space behind a mercury seal. The space equilibrates with the sub-shell gas space and PO₂ is measured with a microelectrode. This technique requires a smaller area of contact with the shell and a shorter equilibration period than more established techniques, and it allows measurements at several locations simultaneously and over a long period of time without endangering the embryo. PO₂ under the shell of chicken eggs decreased to 14.3 kPa on the day before hatching (day 19). There was no significant difference in PO₂ between the aircell end of the egg and the equator. By sealing the pores in the eggshell around the sampling tube with wax and measuring the effect on PO₂, we estimated Krogh's coefficient for lateral oxygen diffusion in the shell membranes at 1.1 mmol cm⁻¹ d⁻¹ kPa⁻¹, a value about a third of a previous estimate. Indirect measurements of PO₂ under the shell in the allantoic region of the egg therefore need to account for the effect of restricted lateral diffusion in the shell membranes. Sampling of gas under sufficiently large regions of waxed shell can indirectly measure chorioallantoic venous PO₂, which is 3.8 kPa on day 19. Assuming 14.3 represents arterialized blood leaving the chorioallantois, it is possible to calculate the effective chorioallantoic blood flow rate of 3.5 ml min⁻¹.

Phase contrast imaging the neonatal marsupial lung using a synchrotron

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Marsupials provide a good model for elucidating lung development in mammals. They are born in a highly underdeveloped state, after a short gestation, with structurally immature lungs. We have applied high-resolution x-ray refraction enhanced imaging (REI) to investigate aerated neonatal marsupial lungs at the SPRING-8 synchrotron facility, Japan. Beamline BL20XU, at 14 keV and a sample detector distance of 31 cm, was employed to image 13 tammar wallabies (*Macropus eugenii*) and 33 fat-tailed dunnarts (*Sminthopsis crassicaudata*) at various developmental stages ranging from the time of birth to 20 days after birth. The results clearly show the immaturity of the marsupial lungs at birth, particularly in the dunnart, and the rapid growth and progression of branching with increasing age. Our results also support observations in the literature suggesting that newborn dunnarts breathe through their skin, and do not commence lung respiration until several days after birth. The images obtained from the synchrotron will be compared and contrasted to those using conventional techniques (such as scanning electron microscopy) that, together with gene expression profiles, will improve our understanding of the ontogenetic changes occurring in the marsupial lung.

Respiratory properties of the blood in the flatback turtle (*Natator depressus*) in comparison to the loggerhead turtle (*Caretta caretta*)

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Oxygen equilibrium curves and other respiratory-related variables were determined on blood from the flatback turtle (*Natator depressus*) and, for comparison, on some samples of the loggerhead turtle (*Caretta caretta*). Oxygen carrying capacity of 11.0 - 19.4 ml/dl ($n = 49$) in the flatback turtle is at the high end of the range in diving reptiles. The flatback has an oxygen affinity, P_{50} , of 37 - 55 mmHg (43 ± 5.3 SD, 25°C, pH 7.17), only slightly higher than the 43 - 49 mmHg in loggerheads (46 mmHg ± 2.0 SD) at comparable pH and temperature. The oxygen equilibrium curves, though, differed in sigmoidicity with a Hill n coefficient of 2.8 and 1.9 in flatbacks and loggerheads, respectively. The flatback is not known to dive deep, and it is suggested that the respiratory physiology of flatbacks is particularly suited to sustain prolonged dives using a strategy that differs somewhat from other shallow diving sea turtles. The results showed high lactate levels which might be a result of sampling during activity associated with their hauling out to nest. A low Bohr-effect was consistent with previous studies on marine turtles.

Temperature and the blood respiratory properties of two reptiles, *Pogona barbata* and *Emydura signata*

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The capacity of two reptiles, an agamid lizard *Pogona barbata* and a chelid turtle *Emydura signata*, to compensate for the effect of temperature on their blood respiratory properties was investigated. This was accomplished by measuring the P_{50} (at 10°C, 20°C and 30°C), hematocrit (Hct), haemoglobin concentration ([Hb]) and mean cell haemoglobin concentration (MCHC) in field acclimatised and laboratory acclimated individuals. The acute effect of temperature on P_{50} in *P. barbata*, expressed as heat of oxygenation (ΔH), ranged from -16.8 ± 1.84 to -28.5 ± 2.73 kJ/mole. P_{50} of field acclimatised *P. barbata* increased significantly from early spring to summer at the test temperatures of 20°C (43.1 ± 1.16 to 48.8 ± 2.07 mmHg) and 30°C (54.7 ± 1.18 to 65.2 ± 2.25 mmHg), but showed no acclimation under laboratory conditions. For *E. signata*, ΔH ranged from -31.1 ± 6.32 to -48.2 ± 3.59 kJ/mole. Field acclimatisation and laboratory acclimation of P_{50} did not occur. However, in *E. signata*, there was a significant increase in [Hb] and MCHC from early spring to summer in turtles collected from the wild (1.0 ± 0.09 to 1.7 ± 0.21 mmol/l and 4.0 ± 0.31 to 6.7 ± 0.72 mmol/l, respectively).

Maintenance of muscle ultrastructure and metabolic capacity in aestivating green-striped burrowing frogs (*Cyclorana alboguttata*)

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We have previously found that prolonged aestivation (9 months) had no effect on wet muscle mass or jumping performance of several hindlimb muscles of the green-striped burrowing frog. The aim of this study was to examine the effect of prolonged aestivation on the ultrastructure and metabolic capacity of hindlimb muscles of the green-striped burrowing frogs (*Cyclorana alboguttata*). Whole muscle area, fibre type, fibre number and fibre cross-sectional areas were recorded for both active (control) and aestivating frogs. Additionally, the activity levels of ATP synthase and cytochrome *c* oxidase were measured from mitochondria extracted from the hindlimb muscles. Overall, aestivation had no significant effect on the muscle ultrastructural characteristics measured, though some changes to fibre type were observed. Aestivation had no significant effect on the activity levels of mitochondrial ATPase and CCO. Preliminary results suggest that the green-striped burrowing frog has the ability to inhibit the effects of muscle disuse atrophy during inactivity without compromising the metabolic capabilities of its muscles.

Tolerance of hyposaline exposure by intertidal crab embryos: osmoprotection or osmoregulation?

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Crab embryos are enclosed within egg membranes that are attached ventrally to the abdominal pleopods of ovigerous females and, in euryhaline species, may be exposed acutely or chronically to dilute sea water. Postgastrula embryos of the New Zealand shore crabs *Hemigrapsus edwardsii*, *H. crenulatus*, *Cyclograpsus lavauxi* (Grapsidae), and *Heterozius rotundifrons* (Bellidae) survived acute external dilution for several days without mortality or impaired development. Gastrulation also coincided with the acquisition of the ability to maintain the embryonic fluids strongly hyperosmotic to the external environment. Studies on other euryhaline decapods have concluded that a capacity for osmoregulation is absent during early embryogenesis. Apparent hyperregulation of the body fluids or periembryonic fluid has been explained in terms of osmoprotection by impermeable egg envelope which restrict diffusive and osmotic exchanges with the medium. Silver-staining of *Hemigrapsus* spp. eggs demonstrate the appearance at gastrulation of a novel chloride-permeable patch (putatively the embryonic dorsal organ). Turnover times of less than a few hours for radiolabeled water and sodium ions in the eggs of *H. edwardsii* and *H. rotundifrons*, and the absence of significant internal pressure in *H. rotundifrons* eggs, indicate that the anisotonic condition is not the result of osmotic isolation but reflects a dynamic steady state involving active uptake of salts and excretion of water.

Novel solutions to the challenges of gill perfusion and control in the abalone *Haliotis iris*

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Haliotis iris retains the ancestral gastropod arrangement of paired bipectinate gills, providing an opportunity to study respiratory and cardiovascular function in a living evolutionary precursor. The gills receive venous haemolymph from the basibranchial sinus, which is believed to drain blood primarily from the right kidney. The right renal vasculature, in turn, provides a renal portal system for most of the systemic venous haemolymph. Despite this serial vascular arrangement a distinct pressure pulse was observed in the basibranchial sinus, in phase with the aortic pulse. Conversely, the post-branchial pressure wave was in perfect anti-phase, thus maximising peaks in the pressure gradient across the gills. These haemodynamic observations are discussed in relation to the discovery of an extensive system of gut sinuses and a large 'renal bypass vessel' that appears to facilitate the direct delivery of haemolymph from these sinuses to the gills. During periods of high oxygen demand, haemolymph flow was partitioned nearly equally between left and right gills (mean 13.3 and 10.8 mL.kg⁻¹.min⁻¹, respectively) and their efferent haemolymph oxygen pressures (P_{aO_2}) were similar (means 81.9 Torr and 87.3 Torr, respectively). In settled animals, branchial haemolymph flow decreased to 9.1 mL.kg⁻¹.min⁻¹, primarily resulting from the virtual shut-down of the left gill, which contributed only 4.6% of total flow. Right gill P_{aO_2} (85.5 Torr) was essentially unchanged while P_{aO_2} of the poorly perfused left gill rose to 105.3 Torr, close to that of exhalant seawater (104.5 Torr). This appears to be the first observation of unilateral perfusion of paired gas exchange organs to accommodate reduced oxygen demand.

Morphological and functional changes to the uterus of lizards with different placental complexities

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Morphological changes during pregnancy in viviparous lizards have been well described at the light microscope level in a range of species, but the functions of the structures are not known. We have quantified the net uptake of nutrients across the placenta, and studied the ultrastructure of the uterus using scanning and transmission electron microscopy in a number of species. Here we compare *Eulamprus tympanum*, which has little net nutrient uptake and morphologically simple placentae, and *Pseudemoia entrecasteauxii*, which has substantial net nutrient uptake and morphologically complex placentae. In both species, there is net uptake of inorganic ions, but only *P. entrecasteauxii* has a detectable net uptake of organic matter. The omphalloplacenta and the placentome of the chorioallantoic placenta of *P. entrecasteauxii* develop into complex transport structures, with apparent apocrine secretion by the omphalloplacenta and some non-apocrine mechanism in the placentome, resulting in histotrophic nutrient uptake by the embryo. The paraplacentome develops into a gas exchange surface termed a cyto-epitheliochorial placenta. The chorioallantoic placenta of *E. tympanum* is not differentiated as in *P. entrecasteauxii*, and both the chorioallantoic and omphalloplacentae remain simpler than in *P. entrecasteauxii*. The chorioallantoic placenta develops into a gas exchange surface, but the blood-uterine lumen distances are about 10 times greater than in *P. entrecasteauxii*. Interestingly, the omphalloplacenta of *E. tympanum* shows features of a nutrient transport structure, which presumably helps to provide the inorganic ions (and water), and probably some organic nutrients that could not be detected by the methods used in the net nutrient studies.

Comparative physiology of native Australian and introduced mice, *Pseudomys hermannsburgensis* and *Mus musculus*: facultative hypothermia and torpor in response to cold stress

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A laboratory-based investigation was undertaken into the thermal biology of the Australian endemic mouse *Pseudomys hermannsburgensis* (Rodentia: Muridae) and the ubiquitous *Mus musculus* (Rodentia: Muridae) using flow-through respirometry. The hypothesis was that *M. musculus* would readily enter torpor in response to food deprivation and low temperature stresses, while *P. hermannsburgensis* would be unable to do so. The introduced species had a mass-corrected BMR of almost exactly that predicted by allometric regression of body mass (2.29 mL O₂·g⁻¹·h⁻¹ at 30.16 °C) and readily underwent torpor. Body temperatures of normothermic *M. musculus* were close to 35 °C across all ambient temperatures, but averaged 23.8 °C in torpid mice at T_a of 15 °C. The native mouse had a reduced BMR from that predicted by allometric regression (60%; 1.45 mL O₂·g⁻¹·h⁻¹ at 29.81 °C). Also, normothermic T_{bs} approximated 35 °C across all ambient temperatures but they did display hypothermia (T_b averaging 17.3 °C at T_a = 15 °C). Although *P. hermannsburgensis* readily displayed metabolic reduction in response to thermal challenge, the mice did not spontaneously arouse, did not rouse based upon circadian rhythm, and could not rouse even when violently disturbed in the metabolic chamber. In conclusion, they could undergo metabolic reduction in the face of low temperature which did not constitute torpor, and may reflect a unique strategy. Torpor in *M. musculus* is thought to represent an energy conservation strategy, whereas hypothermia in *P. hermannsburgensis* is theorised to be a survival mechanism for individuals when their social structures fail temporarily.

Osmoregulatory strategy in the elephant fish, *Callorhynchus milii*

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To overcome hyperosmotic stress in the marine environment, elasmobranchs maintain an isoosmotic or slightly hyperosmotic plasma compared with seawater (SW) primarily through the retention of urea. The holocephalans are a primitive group of cartilaginous fishes and previous reports indicate that plasma ion concentrations of chimaeras (a group in holocephalan fishes) are higher while urea levels are lower than those of elasmobranchs. We chose to examine some aspects of osmoregulation in another holocephalan exemplar, the elephant fish, *Callorhynchus milii*, which, unlike chimaeras, migrates inshore to breed. In control fish, plasma Na and urea concentrations were approximately 300mM and 450mM, respectively. These values are equivalent to those of elasmobranchs, but the plasma urea concentration of elephant fish was considerably higher than that reported for chimaeras. In elephant fish, a discrete rectal gland, which is a specialized salt-secreting organ in elasmobranchs, was absent. Instead, approximately 10 tubular structures were located in the post-valvular intestine wall. Each tubular structure was composed of a putative salt-secreting component consisting of a single-layered columnar epithelium, which stained positively with anti-Na⁺/K⁺-ATPase serum. After transfer of fish to a hyperosmotic environment (115% SW), plasma ions increased quickly 1 day after transfer and sustained the levels for 3 days. Plasma osmolality also increased on day 1, but continuously increased on day 2 in parallel with urea until plasma became isoosmotic to the surrounding SW. Na⁺/K⁺-ATPase activity in the rectal gland-like tissue was increased after transfer to the hyperosmotic environment, supporting the idea that the tubular structure functions in osmoregulation.

A molecular study of reptilian natriuretic peptides

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The natriuretic peptide (NP) system comprises a group of structurally related hormones that have an essential role in the regulation of cardiovascular and body fluid homeostasis. The cardiac NPs have been cloned and sequenced from the heart of mammals, birds, amphibians and fishes, but no members of the NP family have been identified in the heart of reptiles; this represents a considerable gap in our understanding of the NP system. We report the isolation and cloning of full-length brain NP (BNP) cDNAs from the atria of representative species of each reptilian order. Sequence analyses of the reptilian BNP precursors revealed a remarkable degree of homology to chicken BNP, particularly in the putative C-terminal mature peptide; such sequence similarity is not seen when comparing NP sequences of other vertebrate classes. In addition to BNP, another member of the NP family, atrial NP (ANP) was cloned from the heart of tortoise. Interestingly, ANP could not be amplified from the heart of any of the other reptiles examined, which suggests that ANP has been lost in the evolution of diapsid reptiles and birds. These findings contribute new information to our understanding of the molecular structure of NPs across different phylogenetic groups, and suggest that the physiological role of NPs has diverged during the evolution of reptiles and birds.

Effects of radical damage and antioxidants on tissue viability and storage properties in chinook salmon

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Free radical damage and antioxidant depletion may affect short term tissue viability and long term storage properties in chinook salmon muscle tissue. We have looked at markers of free radical damage such as protein carbonyls and TBARS, and also at antioxidant changes in the tissue by measuring vitamin C and E concentrations. Perfusion of a salmon tail at 15°C over 5 or 10 hours with oxygen saturated Ringer introduced significant free radical damage into the red muscle tissue, but not the white muscle. The introduction of vitamin C and urate into the Ringer did not prevent the damage in the red muscle tissue despite significantly increasing their respective concentrations in the tissue. This indicates the difficulties associated with attempting to extend tissue viability and also highlights the difference in susceptibility of the two muscle types to free radical damage. In fillets of white muscle tissue aged at 15°C over a number of days, protein carbonyls began to accumulate steadily following a protection period of approximately 24 hours, but well before the onset of rancidity (defined by the presence of a distinct foul smell). TBARS concentrations only began to rise significantly following the onset of rancidity, suggesting that much of the smell was due to lipid oxidation. Vitamin C concentrations rose during the protection period before dropping exponentially. Vitamin E concentrations did not change, even with the onset of the TBARS rise and rancidity.

The effect of dietary alterations in the blowfly (*Calliphora stygia*) on membrane fatty acid composition and adult lifespan

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The recently developed “membrane pacemaker” theory of ageing proposes that membrane composition will influence ageing and lifespan of animals. Specifically, that polyunsaturated membranes will be more susceptible to oxidative damage and result in a shorter lifespan. This hypothesis suggests that an intervention which alters membrane susceptibility to peroxidation has the potential to alter lifespan. A good model to explore this theory is the blowfly, *Calliphora stygia*. This species has a short adult lifespan, is a manageable size and has dietary habits that make it a useful model. The aim of the present study was to determine if altering fatty acids in the diet of blowfly larvae could alter membrane fatty acid composition of adults, and consequently affect adult lifespan. Blowfly larvae were fed seven diets that differed in fat composition. The influence of these diets on membrane lipids was determined. In a separate group of blowflies fed the same larval diets, adults were fed only sucrose and water, with individual lifespans measured. Diets rich in n-6 polyunsaturated fatty acids increased 18:2 n-6 and decreased 18:1 n-9 membrane content ($p < 0.0001$). Alterations in larval diet were also found to influence adult survival, in particular the elevation of n-6 fatty acids produced a significant decline in average adult lifespan ($p < 0.05$). These results demonstrate that membrane composition of *C. stygia* can be manipulated by dietary alterations, and that such dietary alterations can influence adult lifespan.

**Body temperature variation in free-ranging and captive southern brown bandicoots
Isodon obesulus (Marsupialia: Peramelidae)**

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To investigate patterns of thermoregulation in free-ranging and captive southern brown bandicoots *Isodon obesulus*, we measured abdominal body temperature (T_b) of five free-ranging bandicoots over 42 days using implanted data loggers and T_b of three captive bandicoots using implanted calibrated temperature-sensitive radio transmitters over three months during autumn/winter 2004. Free-ranging bandicoots had a mean T_b of 36.5 ± 1.0 °C (range 33.4 °C - 39.8 °C) but showed a pronounced nycthemeral pattern with two distinct temperature phases (36.0 ± 0.8 °C and 37.3 ± 0.8 °C). A T_b increase occurred at 1330 ± 2.6 h each day and T_b remained warm for 10.65 ± 2.07 h. Mean T_b of captive bandicoots was 36.7 ± 1.1 °C (range 33.0 - 39.8 °C) with a less pronounced daily cycle. In contrast to free-ranging bandicoots, in captivity T_b increased at about sunset and declined at dawn. Our study suggests that southern brown bandicoots rely on daily T_b variation to save energy in the wild and it further supports previous work demonstrating differences in thermoregulation between captive and free-ranging mammals.

Respiration by echidnas, *Tachyglossus aculeatus*, while buried

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Short-beaked echidnas have an impressive ability to submerge completely into soil or sand and remain there, cryptic, for long periods. This poses questions about how they manage their respiration, cut off from a free flow of gases and threatened by the risk of inhaling particles of soil. We measured the gradient in oxygen partial pressure (PO_2) away from the snouts of buried echidnas and oxygen consumption (VO_2) in the same individuals under similar conditions, in three substrates with different air filled porosities (f_a). A theoretical diffusion model indicated that diffusion alone was insufficient to account for the flux of oxygen (O_2) required to meet measured rates of VO_2 . However it was noticed that echidnas often showed periodic movements of the anterior part of the body, as if such movements were a deliberate effort to flush tidal the tidal air space surrounding their nostrils. These “flushing movements” were subsequently found to temporarily increase the levels of interstitial oxygen in the soil around the head region. Flushing movements were more frequent just after digging in when VO_2 was higher and also in substrate with lower f_a . We conclude that oxygen supply to buried echidnas is maintained by diffusion through the soil augmented by periodic flushing movements which ventilate the tidal airspace that surrounds the nostrils.

***In vivo* analysis of salinity related proton efflux in barramundi (*Lates calclifer*)**

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While internal buffering systems allow fishes to lessen the impact of acid/base fluctuations, limitations on buffering mean that final compensation for alterations in pH is accomplished by the transepithelial excretion of the excess acid or base (e.g. H^+ , HCO_3^- , NH_3) from the fish to the surrounding water. Potential sites for acid-base exchanges include the skin, urinary system and intestinal tract, although it is well documented that the branchial epithelium account for 90-99% of all acid transfers in most species studied. The current hypothesis for the maintenance of systemic acid-base regulation in fish involves V-type H^+ -ATPase and/ or members of the Na^+/H^+ exchanger family located on the apical surface of branchial epithelial cells and preferential expression of these transmembrane proteins is thought to be altered by environmental salinity concentrations. Previous studies, used molecular techniques to clone partial cDNA fragments demonstrating a high homology to 2 members of the NHE gene family, NHE1 (677bp) and NHE3 (611bp) and in addition a V-type H^+ -ATPase (752bp) from the gills of the euryhaline Barramundi (*Lates calclifer*). The Barramundi is an ideal model to study the impact of environmental salinity on proton excretion because of its ability to tolerate a wide range of salinities. Fish acclimated to freshwater, brackish water (15ppt) and seawater (30ppt) where subjected to acidosis by induction of ambient hypercapnia (1% CO_2 in air), which resulted in a increase in net H^+ excretion from the fish to the water in all 3 salinities. The net increase in H^+ effux is indicative of the Barramundi's ability to respond to acidosis by the excretion of acid/base relevant ions across the branchial epithelium possbily via an apically located branchial V-type H^+ ATPase or NHE.

Longitudinal growth of long- and short-sarcomere fibres from the claw muscle of the yabby (*Cherax destructor*)

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Decapod crustaceans grow continuously throughout their life and must periodically shed their hard exoskeleton to grow. Muscle fibres within the exoskeleton must also elongate to remain attached to the new, larger exoskeleton. Crustacean muscle fibres can be categorised into two main types. Short-sarcomere fibres have sarcomeres $\leq 4 \mu m$. Long-sarcomere fibres have sarcomeres $\geq 6 \mu m$. These fibre types differ in their sarcomere length, ultrastructure and contractile properties. During developmental stages crustaceans are thought to lengthen their muscle fibres by increasing the length of individual sarcomeres. Fibres from post-larval animals are thought to maintain a constant sarcomere length and increase the length of the fibres by the addition of "new" sarcomeres. This study examined the sarcomere lengths of muscle fibres from chelae ranging in size from 1 – 70 mm. Major findings were (1) sarcomeres increased in length in both short- and long-sarcomere fibre types as chelae increased in size; (2) the proportion of short-sarcomere fibres decreased as chelae increased in size; (3) the total number of muscle fibres in the chela increased as chelae increased in size; (4) An additional population of fibres with ultralong sarcomeres, i.e. $\geq 11.0 \mu m$, was present in the chelae of medium and large sized animals.

Variation in the survival rates of intracellular freezing in *Panagrolaimus davidi*: a tool for investigating this adaptation to life in the Antarctic

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Panagrolaimus davidi, a nematode species endemic to continental Antarctica, remains the only animal known to survive intracellular freezing since this was first shown by Wharton and Ferns in 1995. In addition to this unique adaptation, more recent work has found *P. davidi* to employ a range of survival strategies (including the cryoprotective dehydration mechanism) to survive low temperatures. However, there has been a great deal of variation reported in the literature in the survival rates of this nematode after freezing. This has cast doubt on the reality of the survival of intracellular ice formation in this species. Here evidence supporting this ability is presented. Several variables involved in the culture procedure used to grow *P. davidi* are shown to be important predictors of the variation in survival of intracellular freezing. Through manipulation of the culture procedure, nematode samples with either high or low abilities to survive intracellular freezing have been generated, which have then been used for comparative experiments. Cryo Field Emission Scanning Electron Microscopy, cold-stage light microscopy and the use of fluorescent vital dyes have enabled the comparison of the patterns of ice formation, thawing, tissue damage and the size of the ice crystals within the nematodes, providing further insight into how the survival of intracellular freezing is possible in *P. davidi*.

Kinematic and morphological correlates of the postural and net transport costs of pedestrian locomotion in birds

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In birds, rate of oxygen consumption (\dot{V}_{O_2}) during pedestrian locomotion is linearly related to speed, and net cost of transport (NCOT) is represented by the slope of the line. The extrapolated \dot{V}_{O_2} at zero speed is typically higher than that observed at rest (i.e. the y-intercept is elevated above resting \dot{V}_{O_2}). Originally thought to represent the energetic cost of maintaining the body in a locomotory posture ('postural' cost of transport, PCOT), this difference could instead be an artefact arising from several possible experimental stresses. NCOT relates to body mass but considerable variation is unaccounted for. In the case of penguins, which have high mass independent NCOT, this can be explained by their high rates of force generation ($1/t_c$, where t_c is foot contact time, s) due to their short strides. This study examines the origin of the elevated y-intercept and the relationship between NCOT and $1/t_c$ using new data combined with data gleaned from the literature. We filmed cormorants *Phalacrocorax carbo* and barnacle geese *Branta leucopsis* during treadmill exercise and also measured \dot{V}_{O_2} of the cormorants during this exercise. From the film footage we determined t_c . We found that, across bird species, $1/t_c$ is positively associated with NCOT and hip height is negatively associated with the elevated y-intercept. This suggests that the elevated y-intercept does indeed represent PCOT, and that the high metabolic rate of waddling species is associated with short strides necessitating high rates of force generation.

From the tropics to the poles: the basal metabolic rate of birds is associated with habitat temperature, not productivity

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A classic example of ecophysiological adaptation is the observation that animals from arid environments have lower basal metabolic rates (BMR, ml O₂ min⁻¹) than those from non-arid ones. However, the term 'arid' conceals within it a multitude of characteristics including extreme ambient temperatures (T_a, °C) and low annual net primary productivities (NPP, g C m⁻²), both of which have been shown to correlate with BMR. To assess the relationship between environmental characteristics and metabolic rate, we collated BMR measurements for wild-caught individuals of 74 species of bird, and examined the relationships between BMR and precipitation, T_a, and NPP. Using a phylogenetic generalised least-squares approach, we found no support for a relationship between BMR and NPP, despite including species captured throughout the world in environments spanning a 35-fold range in NPP, and little support for a relationship between BMR and precipitation. Instead, BMR was negatively associated with T_a: species from warmer environments have lower BMRs. This observation potentially provides a tool for predicting the response of birds to climate change.

Hibernation by pregnant North American hoary bats (*Lasiurus cinereus*)

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Many mammals save energy during food shortage or harsh weather using controlled reductions in body temperature and metabolism known as torpor. However, torpor slows offspring growth so reproductive individuals are thought to avoid it because of reduced fitness resulting from delayed offspring development. Therefore, we were surprised to record deep, multi-day torpor bouts by pregnant female hoary bats (*Lasiurus cinereus*) in southern Canada. Torpor bouts lasted 4.3 ± 1.3 days with daily skin temperature minima of 7.6 ± 3.9 °C ($n = 3$ of 4 radiotagged bats). Prolonged torpor occurred during spring storms with heavy rain and snow, and when conditions improved females aroused and gave birth within several days. Parturition occurred 3.1 ± 1.3 days after final arousal. The fourth bat, which did not use prolonged torpor, was never observed with pups so she either gave birth and abandoned her young or resorbed the embryos. These are the first direct observations of multi-day torpor in any reproductive mammal and they imply a fitness advantage of torpor in addition to energy conservation. Reduced foetal growth rate could delay parturition until conditions are more favourable for lactation and neonatal survival.

Physiology of the thylacine, based on allometric, phylogenetic and climate-corrected marsupial data

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The thylacine (*Thylacinus cyanocephalus*) is a large (25 kg) marsupial that at the time of European settlement was the only extant representative of the marsupial family Thylacinidae. It was once present on the Australian mainland in prehistoric times but was restricted to Tasmania in historic times, and is now almost certainly extinct. We have estimated some basic physiological parameters for the thylacine based on an allometric, phylogenetic, diet and climate-corrected analysis of physiological variables for extant marsupials. For marsupials, body mass explains nearly all of the variability in basal metabolic rate (98 % of variability), thermal conductance (93 %) and evaporative water loss (95 %), and some of the variability in body temperature (16 %). Phylogenetic history, diet and climate account for some of the remaining variability. Because marsupials are a physiologically conservative taxon, we have a high level of confidence in predicting body temperature, basal metabolic rate, wet thermal conductance and evaporative water loss for the thylacine.

Identifying reproductive state of the Julia Creek dunnart *Sminthopsis douglasi* by behavioural observations

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The Julia Creek dunnart (*Sminthopsis douglasi*) is an endangered carnivorous marsupial. A recovery plan for the species initiated by Queensland Parks and Wildlife Service includes captive breeding. An important factor in breeding *S. douglasi* is identification of oestrous. The current method of identifying oestrous consists of examination of urine for the presence of cornified cells. To collect urine, the animals have to be removed from their cages and restrained. This method may be stressful for the animals, as well as time consuming. The aim of this study is to identify specific behaviour of *S. douglasi* associated with oestrous that can be readily observed without the need for handling the animal. This would provide a non-invasive way of identifying oestrous, which would facilitate breeding the species in captivity and designing conservation programs. Behaviour of *S. douglasi* was observed throughout their active period by video recording. The most striking result was the increase in activity of the female when she was in oestrous compared to non-oestrous. The most informative behaviour to observe was entry of the female into the nest box and the frequency with which she enters the area closest to the wall (zone 3). A Discriminant Function Analysis generated an equation which had an Over-all Predictive Power (OPP, the probability of making an accurate identification of reproductive state) of 89%. This result was gained by watching two random five-minute epochs of activity. The equation was tested and verified with new individuals and attained a similar level of OPP.

The role of native rodents in seed dispersal and seed predation of the Bunya Pine (*Araucaria bidwillii*)

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The bunya pine (*A. bidwillii*) has a very limited distribution within Australia. Part of the reason for this is likely to be its poor seed dispersal. Macropods, possums and rodents are known predators of both the seeds and tubers. However, there are no reported dispersal agents for *A. bidwillii* seeds. The aim of this research is to determine the extent to which *A. bidwillii* seeds are destroyed or dispersed by native rodents. Predation and dispersal of *A. bidwillii* seeds are being examined in southern Queensland in a small stand of *A. bidwillii* at Mt Mee, and in a larger stand at the Bunya Mountains and in northern New South Wales in a similar habitat devoid of *A. bidwillii* near Queen Mary Falls. At each site, groups of 100 seeds are placed on the soil; 25 seeds are covered by wire mesh to exclude predators and 75 are left uncovered. Each seed is marked by a pink tag to assist in locating it and tracking its movement. Activity of rodents is monitored using CCD cameras connected to video-recorders. Hair tubes are also utilized for identification of rodents. Preliminary results indicate that a small but significant amount of seeds are eaten. However, enough seeds escape predation to produce seedlings. Germination is rapid under field conditions. Seeds were dispersed more than 16 m from their original locations, permitting some to germinate in open sites away from the bunya pine canopy, where they are more likely to survive.

Use of pulp cavity/tooth width ratios to determine the age of adult wild dogs (*Canis lupus dingo*, *C. l. domesticus* and their hybrids)

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Determining the age of wild dogs destroyed in control programs is useful management practice. We compared the pulp cavity: tooth width ratio measurement and ratio calculation of the Thomson and Rose (TR) method to that of the Knowlton and Whittemore (KW) method on upper and lower canine teeth from a CSIRO collection of 68 skulls. The skulls were from mixed gender, known-age wild dogs ranging in age from 9 months to 13 years and reared at either Alice Springs or Canberra. The relationships between age and pulp cavity ratios for both TR and KW methods were significant ($p=0.0001$). The KW ratios exhibited stable error variance and normal distribution, whereas the TR ratios exhibited heteroscedasity in error variance. Significant differences in KW pulp cavity ratios were detected between upper and lower jaw teeth ($p<0.0001$) and gender ($p=0.01$) but not geographic origin. The KW method of calculating pulp cavity as a proportion of tooth width measured 15 mm from the root tip and averaged over both upper canines, is the more accurate of the two methods for estimating the age of adult wild dogs.

Application of machine vision technology to identification of oestrous in the Julia Creek dunnart (*Sminthopsis douglasi*)

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Identification of oestrous is an important step in the captive breeding of the Julia Creek dunnart (*Sminthopsis douglasi*) at David Fleay Wildlife Park (Qld). This is necessary in order to prevent injury to the male and/or the female of this endangered carnivorous marsupial. Oestrous in *S. douglasi* is currently identified by the presence of cornified cells in the urine. However, oestrous has recently been identified in *S. douglasi* by behavioural observations. This research aims to identify and quantify reproductive behaviour through the application of machine vision technology, thus reducing the amount of human observation required in the identification of oestrous in this and similar species. Images from two adjoining cages containing a male and a female *S. douglasi* were captured on video via a CCD camera. The cages were illuminated by an array of infrared light-emitting diodes. Contrast between the animals and the background in successive frames of the video stream enables the location of the dunnart in each image to be defined. This location is recorded in terms of vertical and horizontal position based on a local coordinate system. This stream of coordinate data can be transformed into behavioural information. Behaviours useful in identifying oestrous have been validated in a previous study.

Skeletal muscle mitochondria as a source of non-shivering thermogenesis in the marsupial *Antechinus flavipes*

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The source of non-shivering thermogenesis (NST) in non-placental mammals is not known as they apparently lack brown adipose tissue (BAT) and uncoupling protein 1 (UCP1). In placental mammals, UCP1 mediates uncoupled respiration in BAT to generate heat. A thermogenic role of UCP3 and the adenine nucleotide transporter (ANT) has been debated for bird skeletal muscle. It has been established that 4-hydroxy-2-trans-nonenal (HNE) induces uncoupling activity by UCPs and/or ANT. Whether UCP or ANT is activated can be distinguished by GDP-inhibition of UCPs or carboxyatractyloside (CAT)-inhibition of the ANT. Here, we investigate the role of proton conductance, UCP3 and ANT in skeletal muscle mitochondria during cold exposure of marsupials. We acclimated the yellow-footed Antechinus (*A. flavipes*) to either 10°C or 24°C for 3-4 weeks and measured proton leakage in isolated skeletal muscle mitochondria. Whereas basal proton conductance was unchanged between different acclimation temperatures, a higher inducible proton conductance by HNE was observed in the cold acclimated group. Inhibition by CAT indicated uncoupling activity by the ANT. Pertaining to UCP3, GDP-inhibition could not be observed and might have been missed by applying assay conditions routinely used for rodents. Regarding the dominant contribution of skeletal muscle to body mass, we demonstrate a potential role of their mitochondria in adaptive thermogenesis. The capacity of proton leakage is increased in the cold and can be regulated by an ANT-dependent mechanism. The present study provides no evidence for the involvement of UCP3 in adaptive thermogenesis of marsupials.

How stressed do seahorses get?

A study into the stress response of the pot-bellied seahorse (*Hippocampus abdominalis*)

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Seahorses have been exploited for many years for traditional Chinese medicine, curios and more recently the aquarium trade. Wild stocks cannot sustain this level of exploitation. Recently, in Australasia, the pot-bellied seahorse has been farmed in order to reduce the fishing pressure on wild stocks. Many of these fish are transported live to markets. Little is known about the stress response to transportation in seahorses. This study aimed to characterize the stress response in the pot-bellied seahorse, particularly related to transportation. It also aimed at establishing the location of the head kidney, an essential organ in the stress response. The head kidney was tentatively located in the region of divergence in the anterior kidney, which is posterior to both the brain and gills and anterior to the heart. It appeared that pot-bellied seahorses do not have an acute stress response. This research also indicated that both containment and transportation initiated a chronic stress response, with transportation initiating a greater stress response than containment alone. At this stage it is unclear as to why the acute stress response was lacking. It is proposed that the sedentary life history of the seahorse has resulted in the acute stress response becoming redundant.

Calcium transport in epithelial cells of semi-terrestrial crabs

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Ca²⁺ influx was measured in different types of crustacean cells in suspension using the fluorescent dye, Fluo-3. The aim of this study was to characterize the simultaneous contribution of both apical and basolateral calcium transporters in intact cells suspended in media containing a variety of calcium concentrations. Tissues from hepatopancreas, antennal glands and gills of *Sesarma rectum* and *Chasmagnathus granulata*, were separated into cell suspensions. The dye Fluo-3 was equilibrated with the cells in a saline solution without Ca²⁺. After this equilibration period, known concentrations of Ca²⁺, as well as Ca²⁺ inhibitors, were added to the incubation media and Ca²⁺-induced changes in fluorescence were measured. Ca²⁺ influx by hepatopancreatic cells was a biphasic function of Ca²⁺ concentration, suggesting a Michaelis-Menten transporter combined with an apparent diffusional process. Amiloride and verapamil partially reduced the contribution of the carrier process, mainly by lowering the V_{max}. Similar biphasic uptake functions, sensitive to both amiloride and verapamil, were found for cell suspensions of antennal gland. For gills, influx kinetics showed a sigmoidal pattern, suggesting that the transport in these tissues was cooperative. Verapamil and amiloride did not affect the sigmoidal kinetics consistently. Hill coefficients were high for the control treatment as well as for verapamil and amiloride conditions. The results suggest that Ca²⁺ transport in different tissues and different crabs follow distinct patterns of uptake, commensurate with their respective postulated functions in osmoregulation and Ca homeostasis. The results also validate the methodology of using tissues from single animals for later use in measurements of Ca transport in molting animals.

Notes

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Late abstracts - ANZSCP 2005

83 (oral, Friday 9 December 11am, replaces abstract 10 by Bianchini)

Bioenergetic consequence of apparent specific dynamic action in southern bluefin tuna (*Thunnus maccoyii*)

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Bluefin tuna are among the most valuable fish in the oceans. They represent 5% of the world's fishery catch and in 2001, a single bluefin tuna sold on the Japanese market for over US\$175, 000. Though the obvious financial significance of tuna species, their size and difficulty of handling has meant that our understanding of their physiology lags far behind that of other teleost. This lack of physiological understanding has become more critical due to the greatly expanding tuna farming practice around the world. In an attempt to overcome some of these logistics of tuna metabolic physiology research, we have designed built a large flexible static respirometer that can be installed into small marine farm out at sea. With this respirometer, we have been successful in making the first recording of metabolic rates of bluefin tuna, with the individuals being the largest teleost examine in fish physiology research. We have also been able to make the first recording of apparent specific dynamic action in a tuna species. These recording have significant consequences on our understanding bioenergetics in tuna, which will be discussed.

84 (poster)

Body temperature and metabolism of the feral cat (*Felis catus*) in Western Australia

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To investigate the energy consumption of feral cats (*Felis catus*) we measured the metabolic rate of 6 feral cats from arid regions of Western Australia. The basal metabolic rate (BMR) ($5.7 \pm 0.42 \text{ Jg}^{-1}\text{h}^{-1}$) is 21% below prediction from the allometric equation of cats. We also compared these data to a previous study on the energy consumption of domestic cats. These individuals ($3.9 \pm 0.92 \text{ Jg}^{-1}\text{h}^{-1}$, $n = 5$) have metabolic levels 32% below the BMR of feral cats with no apparent diurnal cycle. To discover the pattern of thermoregulation in free-ranging and captive feral cats, we measured abdominal body temperature (T_b), by using implanted data loggers, of 5 free-ranging feral cats in the north-eastern Goldfields of Western Australia over a period of 4 months (March - July 2005) and T_b of 4 captive feral cats over a period of 8 months (November 2004 - July 2005). Free-ranging cats had a mean T_b of 38.7 ± 0.83 °C (range 35.5°C – 41.9 °C) and showed a pronounced daily cycle, with higher temperatures during their active phase at night. Mean T_b of captive cats was 38.1 ± 0.58 °C (range 34.4 – 40.1 °C) with no pronounced daily cycle. Our study indicates that captive conditions have a strong impact on the natural circadian rhythms of physiological parameters in endotherms. We therefore suggest that physiological studies should focus on work with undisturbed free ranging animals to ensure collection of biologically relevant data.

85 (poster)

Is avian moult an inherently costly and inefficient process?

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Most bird species carefully schedule moult, avoiding overlap with other energetically demanding events. This is assumed to be a physiological adaptation arising from assumed trade-offs between current reproduction, survival, and future reproduction. Empirical studies reveal a close relationship between moult costs (C_f) and mass-specific basal metabolic rate (BMR_m) such that the smallest birds experience the highest moult costs per gram of feathers. However, many small passerines, including white-plumed honeyeaters (WPHE; *Lichenostomus penicillatus*), may breed throughout the year and do so without interrupting moult. This raises the question: is moult less costly in species that display moult/breeding overlap than in those that segregate these events? The energetic cost of moult in WPHE was estimated from increases in BMR during their natural moult period in 7 wild-caught birds. Birds were held in captivity for the duration of moult, with moult progression and BMR measured weekly. Total cost of feather production over the moult period in captive WPHE (497 ± 68 kJ g dry feathers⁻¹; n=7) was very similar to costs reported for similar-sized birds from highly seasonal north-temperate environments. Thus, regardless of life history pattern, there appears to be a strong relationship between moult cost (C_f) and mass-specific metabolic rate in all species studied to date ($C_f = 309 \cdot (BMR_m)^{1.11}$; $r^2=0.967$, $p<0.0001$). However, the duration of moult in WPHE was more than twice that expected for their BMR_m . Consequently, their daily moult costs were 40% of predicted values. Thus, moult rate reduction rather than reduced total moult cost appears to be the main difference between WPHE and species with moult/breeding segregation.

86 (poster)

Developmental plasticity of early-larval anurans in diurnally-fluctuating thermal environments

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To cope with environmental unpredictability, many organisms retain plasticity in behaviour, morphology and physiology throughout development, and responses to environmental parameters such as temperature can have long-term phenotypic effects. Temperature strongly influences ectothermic ontogeny and may vary widely on a diurnal or seasonal basis in many habitats. In diurnally-fluctuating thermal conditions, the physiological rates responsible for ontogeny also fluctuate, but the overall effect of these daily rate changes on the ontogeny of many ectotherms, including larval anurans, is unknown. In this study, we raised striped marsh frog (*Limnodynastes peronii*) tadpoles in stable (18, 22, 26, 30, and 34°C) and fluctuating (18-28°C and 18-34°C) thermal regimes to determine the effects of thermal instability on early-ontogenetic phenotype. In the stable groups, tadpoles hatched earliest and largest at the hottest viable temperatures (26 and 30°C) and subsequent growth and development was most rapid in these conditions. Although the fluctuating groups hatched at similar, intermediate timing, body lengths at hatch and post-hatch developmental rates of the hotter-fluctuating group were among the greatest observed among any of the treatments. The two hottest groups (30°C and 18-34°C) also showed the greatest size-corrected swimming performance a few days after hatch. Further work will elucidate the mechanistic and behavioural properties underlying the developmental plasticity shown here and in later developmental stages. Our results emphasize the need to place studies of thermal biology in an ecologically-relevant context, including fluctuating thermal regimes.

87 (poster)

Is it the squirrel glider, *Petaurus norfolcensis* or the sugar glider, *Petaurus breviceps* that inhabits South Stradbroke Island in south-east Queensland?

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This study determined the marsupial glider species inhabiting South Stradbroke Island, based on morphological criteria. Following species identification, aspects of glider morphology, physiology and ecology were analysed and, where appropriate, compared with other mainland populations of the same species. From observations of gliders on South Stradbroke Island it has been suggested that the species existing there is the sugar glider, *Petaurus breviceps*. From morphological analysis in this study and comparison with a previous study, it appears that it is squirrel glider, *P. norfolcensis*, which lives on South Stradbroke Island. As in mainland populations, head and left pes length and head length/head width ratio were the principal features allowing distinction between sugar and squirrel gliders. Body weight was also another significant factor in suggesting that *P. norfolcensis* inhabits South Stradbroke Island, as *P. norfolcensis* is larger than *P. breviceps*. In the three sites trapped in this study, all gliders displayed similar morphology, suggesting that *P. norfolcensis* exists throughout the island. When compared to other studied mainland populations of squirrel gliders, South Stradbroke Island gliders differed in certain aspects of morphology, physiology and ecology. Results from this study indicate that *P. norfolcensis* is the species inhabiting South Stradbroke Island. Despite this, around 10000 years of separation from any of its nearest relatives could be sufficient enough time for certain aspects of its life history to change when compared to squirrel gliders on the mainland. There is a need for further studies into the biology and ecology of South Stradbroke Island *P. norfolcensis*, including genetic studies to elucidate this species relatedness to its mainland relatives.

