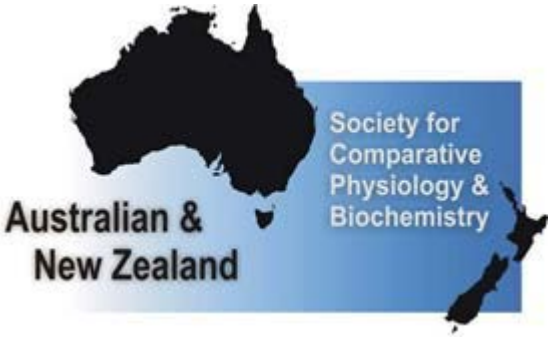

Australian and New Zealand Society for Comparative Physiology and Biochemistry

29th (Nearly the 30th!) Annual Meeting

School of Biological Sciences

University of Auckland

7th - 9th December 2012

ANZSCPB

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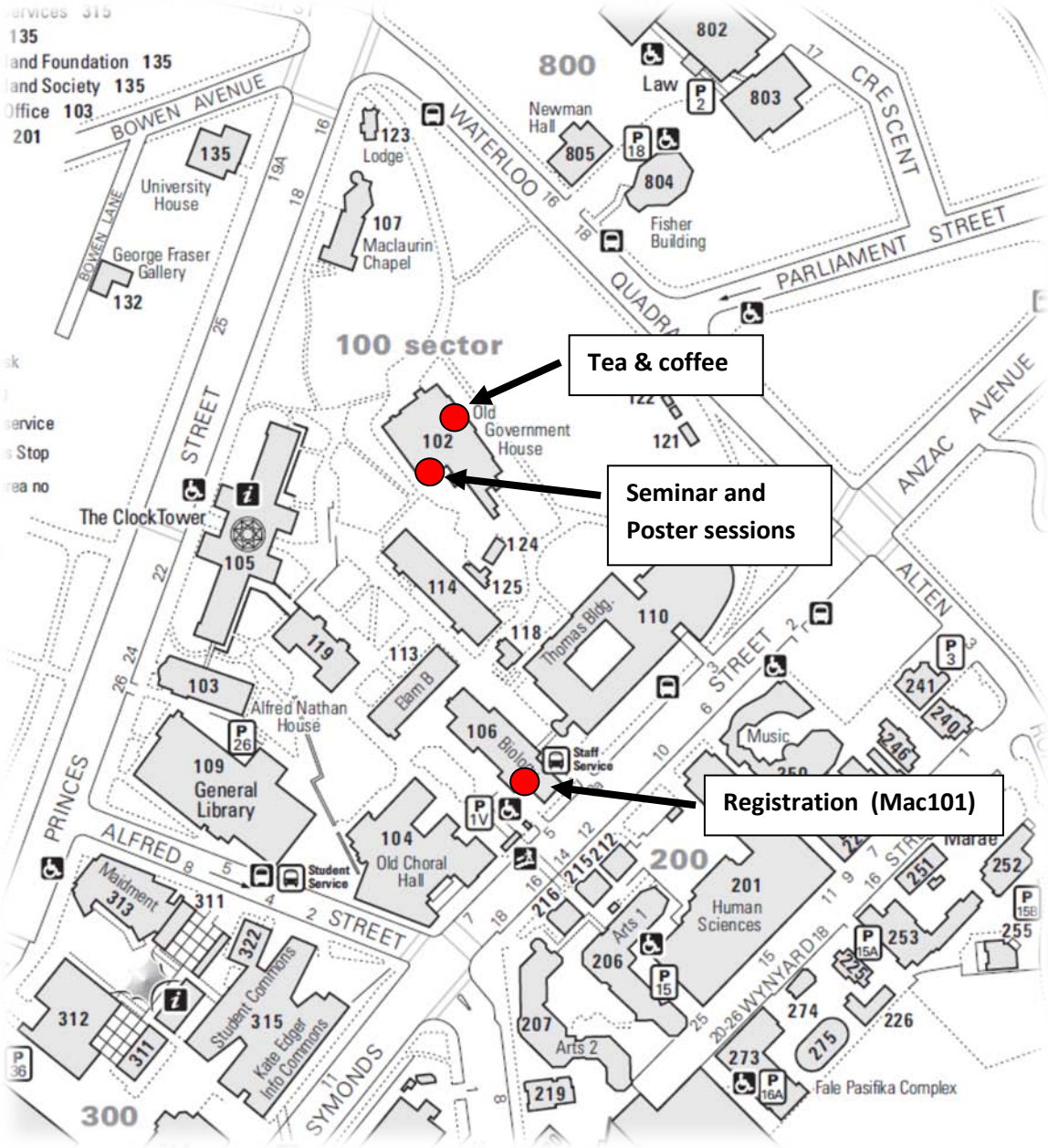
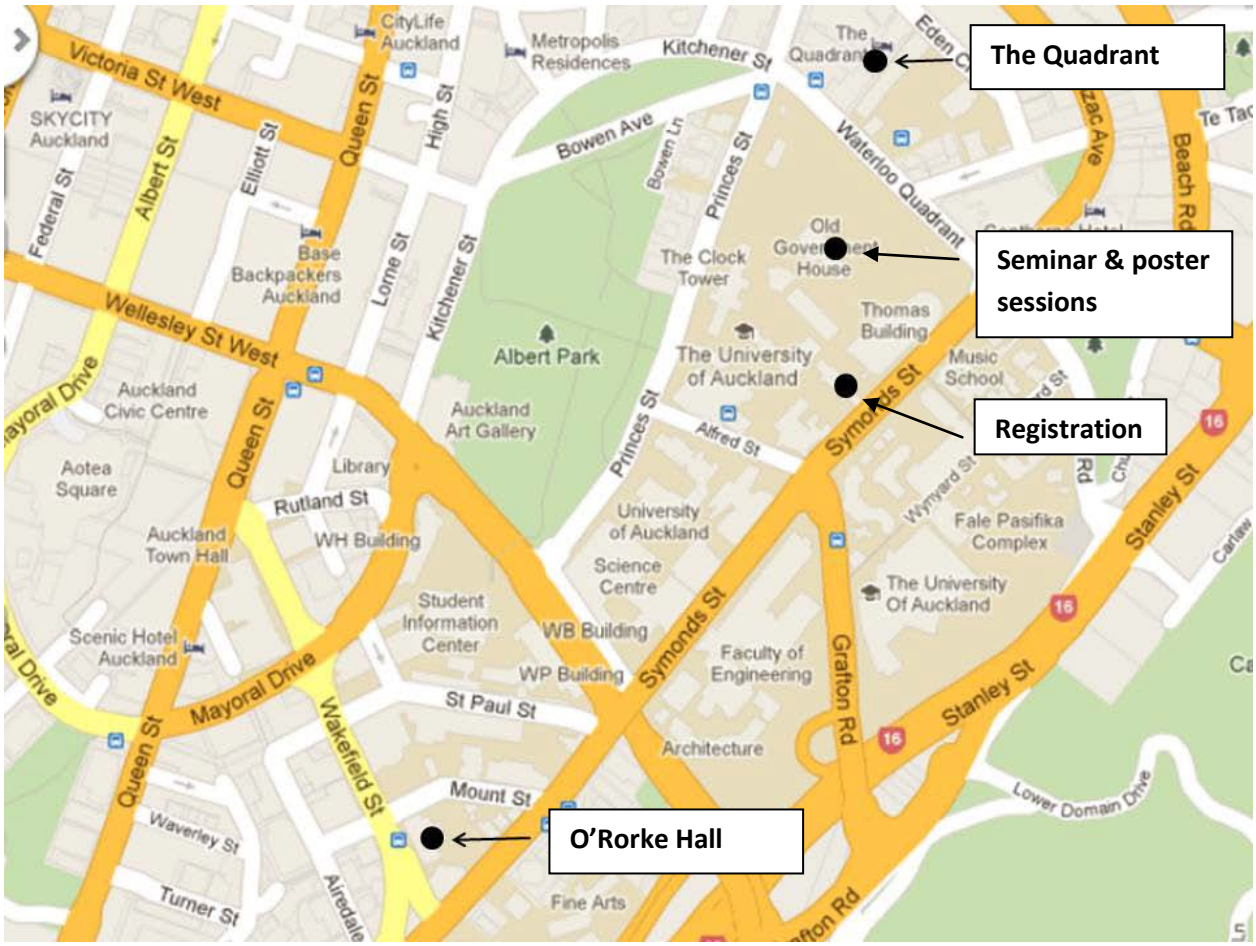
Conference Venues:



Accommodation Venues:



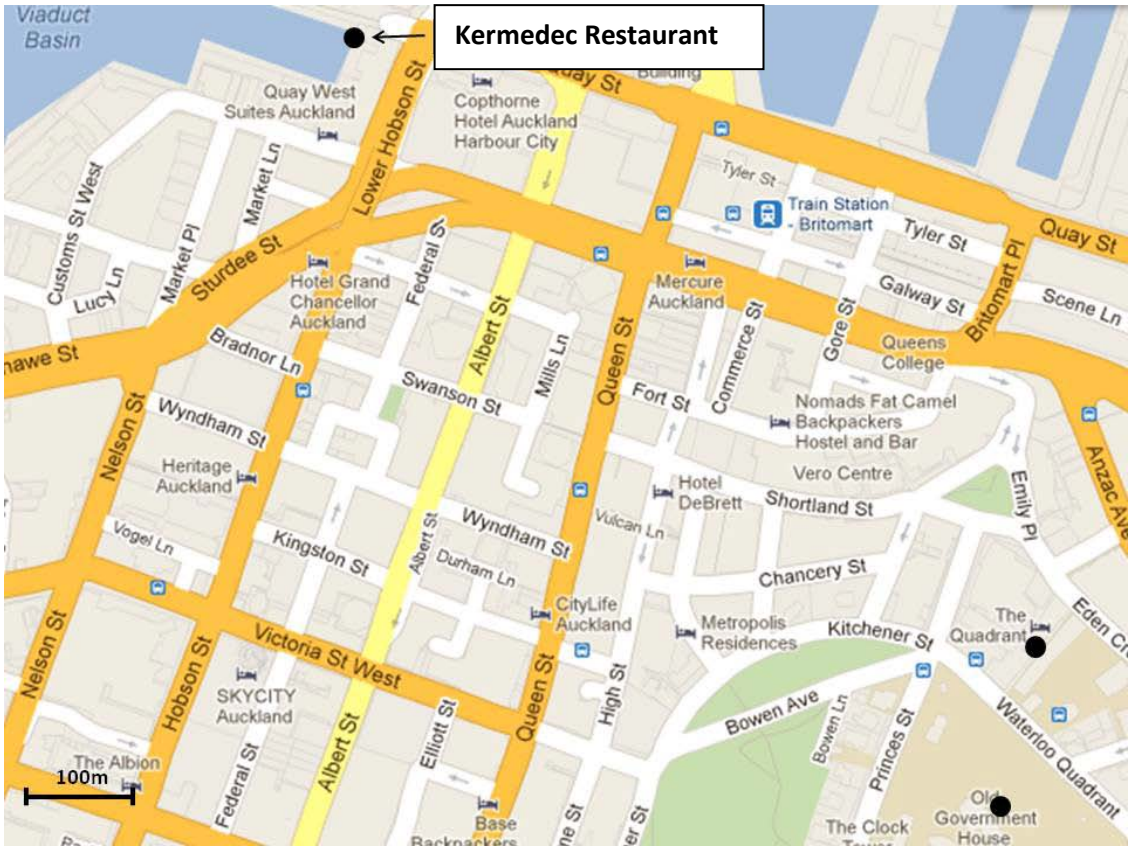
The Quadrant

10 Waterloo Quadrant, Auckland, **Phone:** (+64) 09 984 6000

O'Rorke Hall

16 Mount Street, Auckland, **Phone:** (+64) 09 302 0266

Dinner Venue:



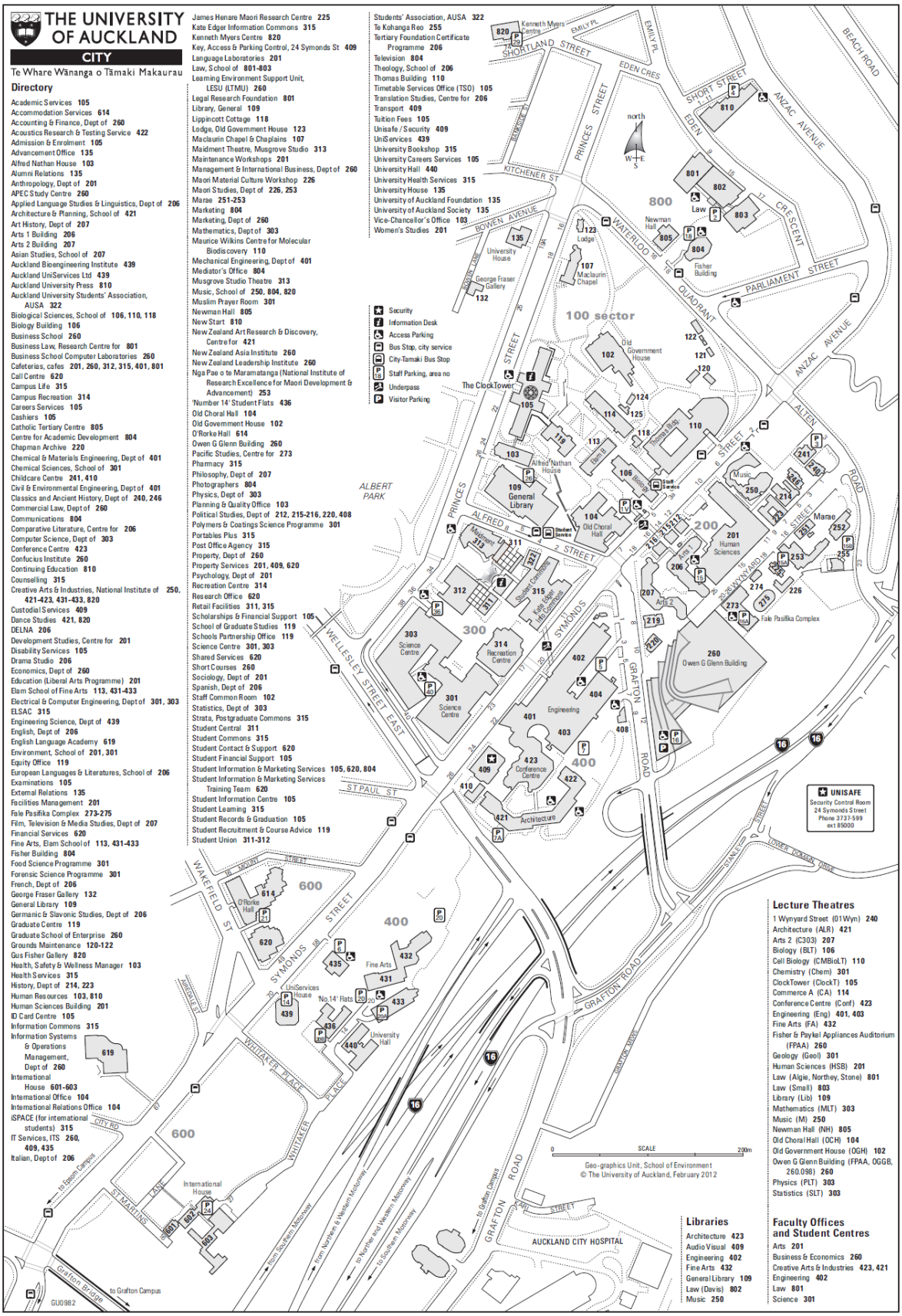
Kermadec Ocean Fresh Restaurant

Viaduct Harbour Level 1/204 Cnr Quay Street & Lower Hobson Street, Auckland
Phone: (+64) 09 304 0454

Distance: 2.2km from University campus (25min walk, 10min by taxi)

Detailed University Map:

[http://web.env.auckland.ac.nz/public/maps/city.pdf]



Housekeeping:

Breakfasts are supplied when staying at O'Rorke apartments and options are available on request for an additional charge when staying at the Quadrant. Alternatively, many cafes on campus and downtown offer breakfast menus, cabinet food, and coffee (such as Relax lounge, Slurp and others in the *Kate Edgar Student commons* – building 315 on map on page 2 & 5).

Conference Venue is the Old Government House, located in the North eastern corner of the campus, near the corner of Princes Street and Waterloo Quadrant (map on pg. 2, 3 & 5). The building was previously the seat of Government prior to 1865, whereupon the capital was moved to Wellington. For the next century the building served as Auckland's vice regal residence. The Queen has resided in the building on 6 occasions and it widely publicized that a brand new 'regal throne' or loo was installed specifically for each of her visits. The venue now serves as the University Staff Club, provides flats for visiting academics, rooms for the Federation of Graduate Women and a lecture theatre.

Dinner Venue, the conference dinner venue is Kermadec Ocean Fresh, located in the Viaduct Basin (Map on pg. 4). Dinner will start at 7.30pm on Saturday the 8th, ensure you collect your conference dinner tickets prior to the event. Drinks are included for the first hour and can be purchased independently afterwards. Aim to be at the venue by 7.00 pm. The restaurant is a 25 min downhill stroll through Auckland the Auckland CBD. Alternatively, taxis can be arranged on (09) 300 3000. Bus from Symonds street, take any bus going downhill towards Britomart bus terminus ($1.00). From Britomart it is a 5-10 minute walk.

General Dining is available from a wide variety of outlets, cafes, pubs, restaurants and food courts located in the city centre and surrounding streets, as well as neighbouring suburbs. Many different cuisines are available for any palate and every budget.

First aid facilities are available in all buildings, AFD's

Foreign Exchange and Banking facilities are available on campus with branches of the National Bank (soon to be renamed ANZ) on level one of the student commons (Building 315 on University map, pg 2 & 5). Numerous ATM's can also be found throughout the student commons.

Internet Access is available to conference attendees for the duration of the conference. Temporary usernames and passwords are provided here which will, enable access to the University of Auckland's Wireless facilities.

Username	**Password**
conf9525	1o2anti6
conf9526	guthasop

Morning Tea and Lunches will be served at times detailed in the Programme Overview.

Parking Options are fairly limited around the main campus. Princes Street is a good option for short term parking (15 - 90mins) at a cost of $4 per hour, payable to the electronic meters. Alternatively, longterm - undercover parking is available in the basement of the Owen Glenn building (Buiding 260 on University Map, pg 2 & 5).

Charges apply. Vehicle access is from Grafton Road at a light-controlled intersection. Beware of high charge rates at some other locations.

Phones are available in the Old Government Building and in the tea room of the School of Biological Sciences (Building 110, pg 2 & 5; 75m downhill from OGH). Internal extensions can be dialed directly. For external local calls 1 must be dialed prior to dialing your number. Security can be reached by dialing ext 85000 from university telephones.

Poster Session will be held in the Old Government House, Posters can be fixed with velcro/drawing pins (as supplied) to the large notice boards provided. Posters will be introduced following the second session on Friday, with each presenter allowed 2 min and 1 power point slide to introduce themselves and their poster.

Registration will be from 5-7 pm in lecture theatre MAC101, on the main floor of the Biology Building (number 106 on University map, pg 2 & 5). Signs will assist location of this theatre. Registration packs, name badges, food and drinks will be provided. For late arrivals staying at O'Rorke, keys can be the afterhours accommodation services staff member on duty (see earlier email correspondence from Tony). Registration packs can also be collected from at the Old Government House on Friday morning, prior to the convening of the conference.

Taxi, call 09 300 3000 for Auckland Co-op Taxis who provide hybrids, standard car, and 'maxi van' options. Vehicles typically carry Eftpos payment facilities. Alternatively, call 09 373 0773 for Corporate (Flash) Taxis.

Toilets can be found towards the rear of the Old Government Buildings close to the seminar room, or on alternate floors in the stairwell in the Old Biology Building.

Troublemakers, if you are arrested by the police you should ask for a 'duty' solicitor immediately, even if you intend to admit to a crime or you know that a crime has nothing to do with you. Refusing to answer questions can later be held against you in court, so it is always best to have a solicitor present to give you advice on what to say. If you request a duty solicitor, the police must get one for you. You will be entitled to speak to your solicitor in private before the police interview you. The services of the duty solicitor are free while you are in the police station. You also have the right to have one phone call made on your behalf. You should use this opportunity to contact your own solicitor (if you have one), a friend or the conference organizer – providing the opportunity for ridicule to be widely distributed the following day.

University Security can be reached on 09 373 7599 or by extension 85000 when using an internal University telephone. Unisafe Officers constantly patrol the campus and can provide an immediate response to any emergency. All officers have first-aid training and are qualified to handle any security matter.

Emergency services (fire, ambulance, police) - dial **111** from any University phone or mobile.

Programme overview:

Registration will open from 5-8pm on the evening of Thrusday 6th December in the MacGregor Museum rooms in the Old Biology Building (see maps on pg. 2,3 & 5, building 106). Food and drink will be provided.

Friday 7th December		Saturday 8th December		Sunday 9th December	
09:15	Introduction				
09:30	Session 1	09:30	Session 4	09:30	Session 7
10:30	Coffee	10:30	Coffee	10:30	Coffee
11:00	Session 2	11:00	Session 5	11:00	Session 8
12:00	Poster talks	12:00	Lunch & poster session	12:30	Lunch & AGM
12:10	Lunch	14:00	Session 6		
14:00	Session 3	15:15	End of day discussion & coffee		
15:15	End of day discussion & coffee				
		7:00	Conference dinner at Kermedec Seafood Restaurant		

Friday 7th December

Time		Author		Title
9:15	Introduction			
9:30		Dennis	Alice	Comparing cold tolerance and transcriptional response among NZ stick insects
9:45		Doucette	Lisa	Prey availability affects daily torpor by a free-ranging bird
10:00		Lal	Swastika	Investigating the physiological and behavioural traits of personality in snapper (*Pagrus auratus*)
10:15		Mitchell	Duncan	Cheetah do not abandon hunts because they overheat
10:30	Coffee			
11:00		Urbina	Mauricio	A novel oxyconforming response in *Galaxias maculatus*: Physiological, metabolic and biochemical responses to hypoxia
11:15		Alqaisi	Kahlid	The expression of genes encoding yolk protein during the reproductive cycle of female sea star *Patiriella regularis*
11:30		Linton	Stuart	Sequencing of laminarinase (β-1,3-glucanase) gene from the midgut gland of the freshwater crayfish, *Cherax destructor*
11:45		Evans	Clive	Freeze avoidance in Antarctic fish
12:00	Poster talks			
12:10	Lunch			

Friday 7th December

Time		Author		Title
2:00		Narayan	Edward	Physiological stress response in captive koalas (*Phascolarctos cinereus*)
2:15		Vesterdorf	Kristine	Metaboilic programming *in utero* has a long-term impact on the metabolic response to diet in mink (*Neovison vison*)
2:30		Napier	Kathryn	The importance of paracellular absorption in mistletoe-feeding Australian birds.
2:45		Seymour	Roger	Scaling of standard metabolic rate in estuarine crocodiles Crocodylus porosus (0.19 – 389 kg) and a comparison of resting and maximum power output between crocodiles and mammals
3:00		Geiser	Fritz	Photoperiod acclimation affects torpor use and the composition of somatic fatty acids in the hamster, *Phodopus sungorus*
3:15	End of day discussion			

Saturday 8th December

Time	Author		Title
9:30	Ling	Nick	Oxygen binding properties of the diverse haemoglobins of ranbow trout
9:45	Pereira	Carla	Blood concentrations of lactate, glucose and corticosterone in dispersing hatchling sea turtles
10:00	Hilton	Zoë	Energy and nutrient budgets as tools to identify mechanisms of resource utilization & allocation in selective bred mussels (*Perna canaliculus*)
10:15	McLeod	Ian	Variable food supply and elevated temperatures influence performance of a larval coral reef fish
10:30	Coffee		
11:00	Grigaltchik	Veronica	The interaction of developmental plasticity and thermal acclimation in tadpoles of the striped marsh frog (*Limnodynastes peronii*)
11:15	McArley	Tristan	Reflex impairment as a tool for predicting discard mortality in sub-legal snapper (*Pagrus auratus*)
11:30	Lesku	John	You Snooze, You Lose: Adaptive sleep loss in polygynous pectoral sandpipers
11:45	Munns	Suzy	Pregnant lizards maintain lung diffusing capacity despite decreases in lung volume
12:00	Lunch		

Saturday 8th December

Time		Author		Title
2:00		Withers	Phil	Thermoregulation in sminthopsine marsupials; do they work like a PID?
2:15		Herbert	Neill	The whole blood oxygen binding properties of a large, and presumably sluggish polar elasmobranch, the Greenland shark *Somniosus microcephalus*
2:30		Khan	Javed	Optimum temperature for growth and feed conversion in cultured hapuku (*Polyprion oxygenios*) - Is there a link to aerobic metabolic scope and preferred temperature?
2:45		Booth	David	Mother and nest influence green turtle hatchling quality
3:00		Barker	Justine	Physiological adaptations of Western Australian Echidnas (*Tachyglossus aculeatus*)
3:15	End of Day discussion			
7:00	Dinner			

Sunday 9th December

Time	Author		Title
9:30	Cooper	Christine	Helox: beyond measurement of cold-induced maximal metabolic rate
9:45	Else	Paul	Dinosaur lactation
10:00	Pirtle	Elia	Climate change related extinction risk in *Sauromalus ater*
10:15	Falahati Marvast	Ali	Hormonal regulation of expression of growth differentiation factor-9 receptors (ALK5 and BMPRII) in the ovary of shortfinned eel, *Anguilla australis*
10:30	Coffee		
11:00	Baker	Daniel	Metabolic effects associated with early life history during rearing in high CO2 in the sea urchin, the kina
11:15	Maloney	Shane	The rhythm of core temperature predicts subsequent breeding intensity in wild rabbits
11:30	Snelling	Edward	Maximum metabolic rate, relative lift, wingbeat frequency, and stroke amplitude during tethered-flight in the adult locust Locusta migratoria
11:45	Douglas	Tegan	Thermoregulatory physiology of Australian birds
12:00	Hickey	Tony	Beating hypoxia: depressed free radical release from heart mitochondria of hypoxia-tolerant epaulette sharks
12:30	Lunch/AGM		

Comparing cold tolerance and transcriptional response among NZ stick insects

Dennis, A.[1,4], L. Dunning[1,2,4], R. Newcomb[1,2,3,4], B. Sinclair[5] and T. Buckley[1,2,4]

[1.] Landcare Research, 231 Morrin Rd, St Johns, Auckland 1072
[2.] School of Biological Sciences University of Auckland
[3.] The New Zealand Institute for Plant & Food Research, 120 Mt Albert Road, Mt Albert, Auckland
[4.] The Allan Wilson Centre for Molecular Ecology and Evolution
[5.] The University of Western Ontario, London, ON N6A 5B7, CANADA

The New Zealand alpine environment is relatively young, yet it contains representatives of all major insect lineages. This includes at least three New Zealand stick insect genera, including one alpine obligate. Living in these high altitude habitats means insects are exposed to a variety of environmental stresses, most notably more days where the temperature dips below freezing. To determine if there are differences among species in their tolerance of the cold, we have compared freeze tolerance and supercooling point among representatives of all major NZ stick insect genera. Not all alpine lineages are freeze tolerant, and this may be explained by microclimate differences among locales. We are now using Illumina sequencing to examine transcriptional response following a cold shock. Genes that are up-regulated in the cold will be compared between lowland, alpine, freeze tolerant, and non freeze tolerant species.

Prey Availability Affects Daily Torpor by a Free-Ranging Bird

Lisa I Doucette[1,2], R. Mark Brigham[3], Chris R Pavey[4,5] and Fritz Geiser[1]

[1]Centre for Behavioural and Physiological Ecology, Zoology, University of New England, Armidale, NSW, Australia
[2]Present Address: Institute for Applied Ecology, University of Canberra, Canberra, ACT, Australia
[3]Department of Biology, University of Regina, Regina, SK, Canada
[4]Biodiversity Conversation, NRETAS, Alice Springs, NT, Australia
[5]Present Address: CSIRO, Alice Springs, NT, Australia

Food availability, ambient temperature (T_a), microclimate and prevailing weather conditions are presumed to influence the use of torpor, which is characterised by pronounced reductions in body temperature and energy expenditure. To a large extent, investigations on the determinants of torpor use have been based on measurements in the laboratory of animals placed on restricted diets and kept at low T_a. Information on the influences of torpor use by free-ranging animals, especially birds, is limited. We assessed winter torpor by insectivorous, free-ranging Australian owlet-nightjars (*Aegotheles cristatus*, 22 birds) over six winters (834 bird-days). Birds in three habitats were investigated to test whether torpor use is affected by annual T_a, rainfall, and arthropod abundance. Owlet-nightjars entered daily torpor regularly at all sites. Torpor frequency, depth and bout duration were greatest during two periods with lower arthropod abundance and moderate T_as, providing rare evidence of the link between food availability and torpor patterns of wild birds. Temporal organization of torpor was similar among sites, and nocturnal torpor was more frequent than previously reported. Our findings quantitatively demonstrate that reduced food resources affect torpor usage independently from T_a, and support the view that food availability is a primary ecological determinant of torpor use in the wild.

Investigating the physiological and behavioural traits of personality in snapper (*Pagrus auratus)*

Swastika Lal[1] and Neill A. Herbert[1]

[1]Leigh Marine Laboratory, The University of Auckland, Leigh, New Zealand

Animal personality, also known as behavioural syndromes, represents individual behavioural differences that are consistent, or largely maintained over time and/or across situations. Consistent individual differences (CIDs) in behavioural personality could potentially be linked with the large level of inter-individual variation in energy metabolism that physiologists commonly observe. For example, bold, aggressive personalities are thought to have higher metabolic rates than timid, subordinate personalities to support faster growth rates and increased fecundity. Since personality-related differences between individuals may influence future fitness in terms of food acquisition, growth and reproductive output, this study has taken the first step towards exploring the presence of CIDs in a commercially important finfish, the snapper, *Pagrus auratus.* Thirty uniformly sized snapper were screened individually for risk taking ability and then later screened for standard metabolic rate (SMR) to resolve whether risk-taking is indeed associated with maintenance metabolism. Marked consistent differences in behaviour and physiology were evident and results displayed significant correlations between behaviour and physiology. For example, individuals with high SMR (>145 mgO_2. kg^{-1} hr^{-1}) were the first to explore a novel environment and object and were generally more active in a novel, potentially dangerous environment. This study is important because it demonstrates that CIDs in behaviour not only exist in snapper but they are tightly coupled with metabolism . The implications of these observations will be discussed with respect to the fisheries management of snapper in New Zealand.

Cheetah do not abandon hunts because they overheat

Robyn S. Hetem[1], Brenda A. de Witt[1], Andrea Fuller[1], Linda G. Fick[1], Leith C.R. Meyer[2,1], Shane K. Maloney[3,1], Duncan Mitchell[1]

[1]Brain Function Research Group, School of Physiology, University of the Witwatersrand, Johannesburg, South Africa
[2] Department of Paraclinical Sciences, University of Pretoria Faculty of Veterinary Sciences, Pretoria, South Africa
[3]School of Anatomy, Physiology and Human Biology, University of Western Australia, Perth, Australia

Cheetah (*Acinonyx jubatus*) are the fastest terrestrial mammals, and use "stalk and chase" hunting. Anomalously, they abandon more than half of their chases when prey ought to be in range, and both successful and unsuccessful hunts are followed by periods of inactivity. The explanation that has become entrenched in the professional literature and lay media is that cheetah abandon hunts because they overheat. That explanation can be traced to a single study, in which Taylor and Rowntree (1973) ran two hand-reared cheetah on a treadmill, and concluded that cheetah stored metabolic heat to the extent that further exercise soon became impossibly thermally. They predicted that cheetah would not run if body temperature reached 40.5°C. We used implanted biologgers to measure core body temperature and locomotor activity of four cheetah, living in a conservancy in Namibia, where we observed both successful and unsuccessful hunts. We found that the cheetah abandoned many hunts but not because they overheated. Indeed, core body temperature did not rise significantly during a chase. It did rise gradually after the chase, for an average of 40 min for successful hunts. The temperature increase (1.3±0.2ºC vs. 0.6±0.1ºC) and the area under the temperature increase/time curve (78.3±19.1ºC.min vs. 8.9±8.6ºC.min) were higher after successful hunts than after unsuccessful hunts, despite there being no difference in activity level between successful and unsuccessful hunts. A similar increase in core body temperature was evident in cheetah participating in the feed after a successful hunt by siblings, but not directly involved in the hunt. We propose that the increased body temperature relates to sympathetic activation, greater following a successful than an unsuccessful hunt. Why cheetah abandon a chase when the prey ought to be in range therefore remains unknown.

A novel oxyconforming response in *Galaxias maculatus*: Physiological, metabolic and biochemical responses to hypoxia.

Mauricio A. Urbina[1]*, Malcolm E. Forster[1] and Chris N. Glover[1]

[1] School of Biological Sciences, University of Canterbury, Christchurch, New Zealand.
Corresponding author: mauricio.urbinafoneron@pg.canterbury.ac.nz

Near-coastal freshwaters in agriculturally-intense lowlands are increasingly prone to eutrophication, and consequently, hypoxia. Navigating through these waters as part of an amphidromous life cycle, the galaxiid fish, inanga (*Galaxias maculatus*) needs to have developed physiological and/or metabolic strategies to deal with hypoxia exposure. Surprisingly, investigation of inanga oxygen consumption as a function of environmental oxygen levels demonstrated that inanga oxyconform, a very rare characteristic among fish. Partitioned respirometry experiments showed that cutaneous gas exchange contributes significantly to total oxygen uptake. This is likely facilitated by the scaleless integument, and suggests that the skin may be a physiologically viable surface in this species. Studies examining the metabolic consequences of aquatic hypoxia exposure indicated that a series of rapid changes enhancing the haemoglobin oxygen-carrying capacity were triggered. As the length of hypoxic exposure increased, energy requirements were meet in part by anaerobic metabolism, evidenced by muscle lactate accumulation and a drop in extracellular pH. Decreases in glycogen and increases in glucose suggested that anaerobic metabolism were fuelled by glycogenolysis/glycolysis. Some evidence suggested that an energy saving strategy was enacted, hypothesised to occur via metabolic down-regulation of oxygen-consuming organs such as liver, and by decreasing the activity of the Na^+, K^+- ATPase pump. The latter led to a plasma ion imbalance. Calculations based on aerobic metabolic rate and the rate of lactate production indicated that inanga was able to depress it metabolism by nearly 50% during hypoxia. Considering the rapid depletion of muscle glycogen stores and that only 33% of the metabolism during hypoxia was fuelled by anaerobically, we propose that inanga is relatively poorly equipped to deal with aquatic hypoxia. Our findings indicate that increasing degradation of near-coastal waters may greatly impact the long-term sustainability of this economically-important and culturally-iconic fish species.

The expression of genes encoding yolk protein during the reproductive cycle of female sea star *Patiriella regularis*

Alqaisi K.M.[1], Lamare M.D.[2], Grattan D.R.[3] and Lokman P.M.[1]

[1]Department of Zoology, University of Otago, 340 Great King St., Dunedin, New Zealand. E-mail: alqkh943@student.otago.ac.nz
[2]Department of Marine Science, University of Otago, 310 Castle St, Dunedin, New Zealand.
[3]Department of Anatomy and Structural Biology, University of Otago, 270 Great King St, Dunedin, New Zealand.

Yolk protein is crucial for oviparous animal development as it provides nutrients for rapidly growing embryos. In most oviparous vertebrates and invertebrates, the yolk protein precursor, vitellogenin (Vtg), is a member of the large lipid transfer protein (LLTP) superfamily and accumulates in developing oocytes. In echinoderms, a yolk protein precursor homologous to transferrin-like protein was described in sea cucumber and sea urchin and termed major yolk protein (MYP). In sea star *Parvulastra exigua*, a Vtg belonging to the LTTP family was recently isolated, but details on Vtg synthesis and regulation remain open to question and require further investigations. This study therefore aimed to identify major yolk protein precursors in common New Zealand sea star *Patiriella regularis* and study their expression during oogenesis. Two cDNAs encoding Vtgs were isolated and designed as *Pr*Vtg1 and *Pr*Vtg2. Additionally, we isolated a partial cDNA encoding MYP precursor and designed it as *Pr*MYP. The deduced amino acid sequences of both Vtgs were corresponded to protein sequence analysis of prominent proteins isolated from ovulated eggs. The expression of *Pr*Vtgs and *Pr*MYP changed significantly in pyloric caeca during the reproductive cycle with highest expression in stage IV of reproductive cycle (partially spent ovary containing some primary oocytes). In the ovary, *Pr*Vtg1 mRNA levels changed significantly during the reproductive cycle, with highest expression at stage V (spent ovary in early gonadal recrudescence); there was no significant change in *Pr*Vtg2 and *Pr*MYP mRNA expression levels. These findings are novel and important in that they provide the first evidence showing that at least two Vtgs are corresponding to yolk protein in echinoderms. Moreover, it is the first time cDNA encoding MYP in asterinids has been isolated.

Sequencing of a laminarinase (β-1,3-glucanase) gene from the midgut gland of the freshwater crayfish, *Cherax destructor*

Stuart M. Linton**,** Melissa S. Cameron, Michael Gray, and John A. Donald

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

Laminarinase (β-1,3-glucanase) is a digestive enzyme which is present within the digestive juice of decapod crustaceans. It hydrolyses β-1,3-glycosidic bonds to digest hemicelluloses such as laminarin and callose. Although a laminarinase has been purified from the midgut gland of the crayfish, *Cherax destructor* and the Christmas Island red crab, *Gecarcoidea natalis,* the gene responsible for its production, and thus endogenous production, has yet to be determined. The laminarinase gene was successfully amplified and sequenced from cDNA synthesised from RNA isolated from the midgut gland of the crayfish, *C. destructor*. This was achieved by utilising degenerate primers, PCR and rapid amplification of the 3' and 5' ends. This generated a 1503 bp sequence which encodes for a laminarinase, 364 amino acids long with an estimated molecular mass of 41.5 kDa. This molecular mass matches that of a previously purified laminarinase. The sequence also contains the correct amino acids to be catalytically active, and as hypothesised, belongs to glycosyl hydrolase family 16. The amino acid sequence aligned with that of β-glucan binding proteins (an immune protein) of other crustaceans. However, this sequence is unlikely to be that of a β-glucan binding protein given it is expressed in a digestive tissue, the midgut gland and the amino acid sequence is highly conserved to that of short peptides, determined by orbitrap mass spectroscopy, of a laminarinase from *G. natalis*. It is however likely that one gene, has evolved to encode two proteins, one a digestive enzyme and another, an immune protein.

Freeze-avoidance in Antarctic fish

Clive W Evans

School of Biological Sciences, University of Auckland, New Zealand

Antarctic notothenioid fishes thrive in the freezing waters of the Southern Ocean, one of the most inhospitable marine environments on earth. Their success in this environment is largely attributable to the synthesis of antifreeze glycoproteins (AFGPs), which protect against the life-threatening potential of internalized ice. Although AFGPs were first identified and characterized over forty years ago, we still remain ignorant of many of the details of how they function in conferring freeze-avoidance and exactly how fishes cope with internal ice. Our recent work provides answers to these outstanding problems.

Physiological stress response in captive Koalas (*Phascolarctos cinereus*)

Edward J. Narayan[1], Jean-Marc Hero[1], Koa Webster[3], Vere Nicolson[2] and Al Mucci[2]

[1] Environmental Futures Centre, School of Environment, Griffith University, Gold Coast Campus, 4222 Queensland, Australia
[2] Dreamworld, Coomera, 4209 Queensland, Australia
[3] Department of Biological Sciences, Faculty of Science, Macquarie University 2109 NSW, Australia

Koalas (*Phascolarctos cinereus*) are the only extant representatives of Australia's unique marsupial family Phascolarctidae, and were listed as Vulnerable nationally in 2012. Causes of mortality are diverse, although disease (chlamydia), dog attack, collisions with cars, and loss of habitat represent the principal reasons for the continued decline of the species. The Dreamworld Koala Breeding Facility in Queensland, Australia was established to educate the public about Koala conservation. Koalas at Dreamworld were used to develop non-invasive tools for monitoring stress hormone levels in scats. While
blood glucocorticoid concentrations have been used as indices of stress, their usefulness in long-term studies with intractable wildlife species is limited due to the circadian rhythm and the pulsatile nature of glucocorticoid secretion, and the possible induction of a stress response during sampling procedures. Conversely, the excretion of metabolized blood steroids in faeces permits the monitoring of physiological functions without disturbance to animals. Here, we present new data on faecal cortisol concentrations in Koalas. We describe the biological (adrenocorticotropic hormonal challenge) and laboratory validation (parallelism of Koala faecal cortisol metabolite enzyme-immunoassay). We also compared faecal cortisol metabolite levels in adult male and female Koalas in response to handling. Individuals varied in their cortisol levels, suggesting territorial activity and enclosure conditions influence stress levels in captive male koalas. Surprisingly, there was no significant difference between the handled and non-handled groups in faecal cortisol metabolites. These data suggest handling activity associated with human – photograph interactions has little influence on koala stress hormone levels. Overall, the results highlight the usefulness of non-invasive stress hormone metabolite monitoring for understanding the physiological responses of the Koala. In future, this tool can be used to evaluate the physiological effect of management activities (including caging), nutritional status, behaviours and disease.

Supported by the Dreamworld Conservation Funding to E.J.N and J-M. H.

'Metabolic programming *in utero* has a long-term impact on the metabolic response to diet in mink (*Neovison vison*)'

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It is well established that metabolic programming *in utero* has long-lasting consequences for human health, however, the consequences for affected mink kits and their ability to adjust post-natally remains unknown. This study addressed the issue of protein restriction *in utero* on the liver proteome of mink kits at 20, 25 and 50 weeks of age *cf* kits fed an adequate diet *in utero*. Furthermore, the potential restorative effects of an adequate diet from weaning to 50 weeks of age, were investigated. Four dietary treatment groups were established as a combination of fetally low (FL) or adequate protein (FA) provision, followed post-weaning by either low (LP) or adequate protein (AP) provision. Protein restriction *in utero* did not affect liver- or body composition, however, the liver proteome was affected. As such, changes in the abundance of proteins involved in the glycolysis, fat metabolism, and oxidative status, as well as proteins, which have been linked to the onset of various non-communicable diseases, including Type-2 diabetes and cardiovascular disease, were observed in FL mink. Low protein provision after weaning induced a higher hepatic fat content in LP kits *cf* controls as well as an elevated abundance of fatty acid binding protein. In conclusion, this study demonstrates a clear effect of *in utero* protein restriction on the abundance of proteins involved in key metabolic processes and the potential development of non-communicable disease in adult life, and identifies a number of proteins that may be of interest for future mink and human metabolic programming research.

The importance of paracellular absorption in mistletoe-feeding Australian birds.

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Small birds **face strong selection pressure to digest food rapidly and thereby reduce** digesta mass carried during flight. One way they may do this is by rapidly absorbing a high prop**ortion of glucose via the paracellular (non-mediated) pathway[1].** Nectarivorous birds show extensive absorption of L-glucose, which increases with diet sugar concentration, indicative of significant non-mediated glucose uptake[2]. D-xylose is a pentose sugar that is a major component of some nectars and mistletoe fruit. The absorption mechanisms of xylose are currently unknown in small birds. We investigated the apparent assimilation efficiency (AE) of D-xylose and D-glucose and the bioavailability of radiolabelled L-glucose and D-xylose in three Australian bird species, the mistletoebird *Dicaeum hirundinaceum*, silvereye *Zosterops lateralis***,** and singing honeyeater *Lichenostomus virescens.* We also assessed the effects of food energy density and intake rate on the bioavailability of L-glucose and D-xylose at two *sugar* concentrations (250 and 1000 mmol/L hexose). D-glucose AE was extremely high in all three species (>99%) and D-xylose AE was significantly lower (ranging from 56 to 78%). Mistletoebirds assimilated significantly less D-xylose than silvereyes and singing honeyeaters. Bioavailability was significantly greater for both L-glucose and D-xylose for silvereyes and singing honeyeaters when feeding on the more concentrated diet; in contrast, diet concentration did not have a significant effect on bioavailability for mistletoebirds. Silvereyes had the highest L-glucose (81%) and D-xylose (89%) bioavailability while L-glucose bioavailability was fairly low in the mistletoebird (33%). The higher bioavailability of D-xylose on the more concentrated diet in silvereyes and singing honeyeaters suggests that D-xylose may be absorbed by both paracellular and mediated mechanisms, possibly in the same manner as D-glucose. It also appears that mistletoebirds may not rely as extensively on the paracellular pathway as the more generalist feeders in this study

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Scaling of standard metabolic rate in estuarine crocodiles *Crocodylus porosus* (0.19 – 389 kg) and a comparison of resting and maximum power output between crocodiles and mammals

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There are three controversies in comparative physiology that this study addresses: (1) the universality of quarter-power scaling, (2) the relationship between standard metabolic rate and maximal metabolic rate and (3) the question of whether the dinosaurs were endotherms or ectotherms.
(1) Standard metabolic rate (SMR, ml O_2 min^{-1}) of captive *Crocodylus porosus* at 30°C scales with body mass (kg) according to the equation, SMR = 1.01 $M^{0.829}$. The exponent is significantly higher than 0.75, so does not conform to quarter power scaling theory, but rather is likely an emergent property with no single explanation. SMR at 1 kg body mass is similar to the literature for *C. porosus* and for alligators, but this study expands the mass range for crocodiles to 3.3 orders of magnitude. The high exponent is not related to feeding, growth, or obesity of captive animals.
(2) Maximal power output for exercising wild *C. porosus* of similar mass range is calculated from published data on maximum rates of lactate formation, oxygen consumption and estimates of creatine phosphate content of crocodilian muscle. The factorial metabolic scopes between SMR and maximal power production are similar in crocodiles and mammals, suggesting that SMR represents the cost of maintaining the machine in proportion to its maximal power output.
(3) A 1 kg crocodile is expected to produce 21 Watts from aerobic and anaerobic energy sources during the first burst of exhaustive activity, which is 60% of that expected for a totally aerobic mammal. A 200 kg crocodile would produce 1060 Watts, or only 30% of that for an aerobic mammal. The anaerobic component of mammalian energy production would increase their short-term power even higher. If dinosaurs had similar exercise physiology as crocodiles, then it seems unlikely that they would have been so successful for 180 million years while coexisting with mammals.

Photoperiod acclimation affects torpor use and the composition of somatic fatty acids in the hamster, *Phodopus sungorus*

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Seasonal changes in thermal physiology and torpor expression of many heterothermic mammals is controlled by photoperiod. As function at low body temperatures during torpor requires changes of tissue lipid composition, we examined whether and how fatty acids are affected by photoperiod acclimation in hamsters, *Phodopus sungorus*, and how this is related to changes in morphology and thermal biology. Hamsters in short photoperiod had smaller reproductive organs and most had a reduced body mass in comparison to those in long photoperiod. Fur colour of hamsters under short photoperiod was almost white while that of long photoperiod hamsters was brown. Short photoperiod acclimation resulted in regular (28% of days) torpor use by all individuals, whereas all hamsters in long photoperiod remained normothermic. The composition of total fatty acids differed between acclimation groups for brown adipose tissue (56% of fatty acids), heart muscle (50% of fatty acids) and leg muscle (25% of fatty acids) and the percent composition of fatty acid was correlated with skin temperature. While some of the compositional changes of fatty acids were consistent with a homeoviscous response, this was not the case for all fatty acids, and the sums of saturated and unsaturated fatty acids did not differ between acclimation groups. Our study confirms that the seasonal change in morphology and thermal biology of the species is largely controlled by photoperiod and suggests that some of the functional changes are linked to the composition of tissue and organ fatty acids.

OXYGEN BINDING PROPERTIES OF THE DIVERSE HAEMOGLOBINS OF RAINBOW TROUT

Ling, N., Tempero, G.W.

Many fish express multiple haemoglobins and the relative proportions of haemoglobin isomorphs has been shown to vary with environmental variables such as temperature and hypoxia, implying adaptive plasticity in haemoglobin oxygen transport. Rainbow trout have one of the most diverse haemoglobin systems which can be separated into anywhere from 4 to 14 haemoglobin isomorphs depending on the analytical method used. We used cellulose acetate electrophoresis to identify 14 distinct haemoglobins consisting of 7 electrophoretic dimers (3 cathodal and 4 anodal) that could be purified by FPLC into 7 discrete fractions. These fractions were then analysed for their functional oxygen binding properties. Cathodal haemoglobins I-III were insensitive to changes in temperature, pH and adenosine triphosphate (ATP) concentration and mildly sensitive to chloride, whereas the anodal haemoglobins (IV – VII) were all found to be sensitive to temperature, pH and ATP. The apparent diversity of rainbow trout haemoglobins is therefore misleading in that only two functional groups (anodal and cathodal) exist. It is therefore presumed that the observed diversity is merely the result of gene duplication rather than the result of any selective adaptation to the diversity of habitats associated with the diadromous life history of trout.

Blood concentrations of lactate, glucose and corticosterone in dispersing hatchling sea turtles

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Natal dispersal of sea turtles is an energetically demanding activity that is fuelled primarily by aerobic metabolism. However, during intense exercise reptiles can use anaerobic metabolism to supplement their energy requirements. We assessed anaerobic metabolism in dispersing hatchling loggerhead and flatback turtles by measuring the concentrations of blood lactate during crawling and at different times during the first four hours of their frenzy swim. We also measured concentrations of blood glucose and corticosterone. Blood lactate (12.13 to 2.03 mmol/L), glucose (6.25 to 3.8 mmol/L) and corticosterone (8.13 to 2.01 ng/mL) concentrations decreased significantly over time in both loggerhead and flatback hatchlings and no significant differences were found between the species. These results indicate that anaerobic metabolism makes a significant contribution to the dispersal phase of hatchling sea turtles during the beach crawl and the first few hours of the frenzy swim.

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Energy and nutrient budgets as tools to identify mechanisms of resource utilisation & allocation in selectively bred mussels (*Perna canaliculus*).

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The endemic New Zealand green-lipped mussel *Perna canaliculus* forms the major proportion of New Zealand's aquaculture production, yet it remains a relatively under-studied species. The Cawthron Institute has a well-established selective breeding programme with the goal of providing mussels with a range of desirable phenotypes for aquaculture production. One aspect of this programme has focused upon understanding the detailed biology of 8 target families bred from parental families known to exhibit a broad range of growth rates. This study aimed to construct energy and nutrient budgets for these 8 family lines to examine the mechanisms underlying differential growth, resource utilisation and allocation under a range of food conditions. Juvenile mussels were maintained for 12 months in a controlled environment with flow-through seawater under fixed temperature (18ºC) and food input (a 1:1 mixture of two cultured microalgal species: *Isochrysis galbana* clone T-Iso and *Chaetoceros calcitrans* forma *pumilum*). Four treatment groups were given different food rations, corresponding to environmentally relevant chlorophyll a levels (0.15, 0.5, 1.0 & 4.0 µg Chl-a L^{-1}). Growth rate was measured over the full time-course of the trial, while metabolic and biochemical parameters including ingestion and egestion rates, oxygen consumption (MO_2), organic and calorific content, total lipid, carbohydrate and protein in shell, tissue and faeces were measured on individuals at the time of initial stocking and 4 months later. In addition, a sub-sample of individuals was progressively starved in 1µm-filtered seawater for up to 7 months, and in this group, MO_2 and biochemical parameters were determined in sacrificial samples at 2 – 4 week intervals. Differences in specific growth rates were observed between families, and clear differences were seen between the different food ration treatments with animals exhibiting highly significant differences in growth rate, ingestion, metabolic rate, and hence calculated scope for growth. The putative drivers of differential growth amongst individuals and between genetic groups will be considered in this presentation.

Variable food supply and elevated temperatures influence performance of a larval coral reef fish

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Climate change models predict that tropical sea surface temperatures will increase by 2-3°C this century. Warmer ocean temperatures can directly affect metabolism and energy requirements of marine fish. Warmer temperatures can also alter plankton communities that are food for many marine fish larvae. However, little is known about the potential interacting effects of ocean warming and changes to food supply on the performance of larval reef fish. We raised coral reef anemonefish (*Amphiprion percula*) larvae in an orthogonal experiment comprising 3 temperatures and 3 feeding schedules. Temperatures were chosen to represent current-day summer averages (29.2°C) and end-of-century climate change projections, +1.5°C (30.7°C) and +3°C (32.2°C). Feeding schedule (daily, every 2 days, or every 3 days) was chosen to represent increased variability in food availability. Overall, larvae took longer to settle under high temperatures and less frequent feeding. The time to settlement increased from 10.3± 0.5 days at 29.2°C and high food supply to 15.0±1 days at 32.2°C and low food supply. Fish from the lower feeding regimes had a lower condition and decreased survivorship. Routine oxygen consumption rates ($\dot{M}O_{2Routine}$) were highest (1610 ±100 mg O_2 kg^{-1} h^{-1}) for fish raised at 32.2°C and fed every third day and lowest (1210 ± 130 mg O_2 kg^{-1} h^{-1}) at 29.2°C and fed daily. Elevated $\dot{M}O_{2Routine}$ and therefore energy use at higher temperatures may leave less energy available for growth, resulting in the longer time to settlement and lower body condition. Increased larval duration and reduced body condition could reduce survivorship during the larval phase and post-settlement, with consequences for population dynamics. This eco-physiological approach has allowed us to highlight important physiological mechanisms that could be impacted in larval fishes under climate change scenarios.

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The interaction of developmental plasticity and thermal acclimation in tadpoles of the striped marsh frog (*Limnodynastes peronii*)

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An organism can experience a variety of environmental conditions within its lifetime. Plastic responses during development, generally through epigenetic modifications[1-2], can also determine the ability to reversibly acclimate at later stages in life[3]. In this study, we looked at the effect of empbryonic developmental (incubation) temperature on thermal acclimation in striped marsh frog (*Limnodynastes peronii*) tadpoles. Field collected, freshly-laid nests were incubated at either warm (25^{o}C) or cold (15^{o}C) temperatures and acclimated to the same temperature as incubation or changed to the other temperature. After six weeks of exposure to the acclimation temperature, we measured burst swimming performance, resting oxygen consumption, and enzyme activities in tail muscle to determine the interaction between developmental temperature and acclimation capacity. We also determined growth in each treatment through weekly sampling. We found that acclimation to the warm temperature caused an increase in growth. Simultaneously, resting oxygen consumption was lower with warm acclimation. Embryonic development affected the ability to acclimate acceleration during burst swimming performance and both anaerobic metabolism (lactate dehydrogenase activity) and aerobic metabolism (citrate synthase and cytochrome *c* oxidase activities). Our findings show that thermal conditions during embryonic development affect acclimation capacity later on in life. This may be particularly important as cold acclimation appears to incur a cost (i.e. reduced growth) in this species. In this way, developmental plasticity can be beneficial for organisms responding to environmental variation and may have consequences for future survival.

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Reflex impairment as a tool for predicting discard mortality in sub-legal snapper (*Pagrus auratus*)

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Snapper (*Pagrus auratus*) is harvested extensively by recreational anglers throughout New Zealand but knowledge of discard mortality is essential for sustainable management. Reflex impairment (RI) is a method which uses the presence or absence of reflexes in captured animals to produce a RI score. RI is thought to correlate with delayed mortality so could potentially be used as a simple in field tool to predict the fate of discards. A RI index for snapper was developed under laboratory conditions at 15°C in order to investigate its use in predicting discard mortality. RI was measured in sub-legal snapper (<27cm FL) exposed to simulated angling involving graded levels of chasing stress and air exposure. Delayed mortality was then monitored over 15 days. A second group of fish exposed to the same angling stress treatments were sampled for blood and tissue to investigate physiological stress status. Reflex impairment was greatest in the most extreme angling stress treatment (5 minutes chasing stress and 3 minutes air exposure) and lowest in the least extreme treatment (0.5 minutes chasing stress). Surprisingly, no correlation between RI and delayed mortality was observed as no fish died. Simulated angling caused physiological stress including altered blood physiology and metabolic acidosis. This suggests the winter discard mortality rate is potentially low yet physiological disturbance resulting from up to 5 minutes of chasing and 3 minutes of air exposure is sub-lethal. The results are promising for snapper fishermen but should be interpreted cautiously until depth and hooking location are also examined in future field studies.

You Snooze, You Lose: Adaptive Sleep Loss in Polygynous Pectoral Sandpipers

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Sleep is thought to perform restorative processes that sustain adaptive waking brain function. Accordingly, sleep restriction and fragmentation can diminish neurobehavioural performance in animals, including humans. However, here we provide the first evidence that sleep loss can be beneficial. Pectoral sandpipers (*Calidris melanotos*) migrate annually from the southern hemisphere to Alaska to breed in the summer under continuous daylight. Males are polygynous and engage in intense male-male competition in order to copulate with fertile females during a 3-week period. We measured activity levels in mature males and the number of male-female interactions. The parentage of virtually all chicks on the study site was determined genetically. In a separate year, we implanted electrodes using standard techniques to measure electroencephalogram (EEG) and neck electromyogram (EMG) activity in free-roaming birds on the tundra. Activity levels varied considerably across males[1]. The most extreme male was active > 95% of the time for 19 d. EEG-defined wakefulness and sleep were associated with high and low EMG activity, respectively, and birds rapidly transitioned from active wakefulness to sleep, such that activity is a good proxy for wakefulness in these animals. The total time males spent sleeping correlated positively with the number, and mean and maximum duration of sleep episodes. Despite having more fragmented sleep, males that slept the least showed the greatest slow wave activity during sleep – a measure of sleep intensity – suggesting that they compensated, at least partially, for sleep loss. Most importantly, males that slept the least interacted with more females and sired the most offspring[1]. Thus, males that slept less performed better on the most important measure of performance from an evolutionary perspective. Thus, reduced neurobehavioural performance is not a universal outcome of sleep restriction and fragmentation. As such, these results challenge notions on the adaptive value of sleep.

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Pregnant lizards maintain lung diffusing capacity despite decreases in lung volume

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Tiliqua nigrolutea, the blotched blue tongue, is a large viviparous skink. Pregnancies in this species typically have high gestational loads, with clutch masses at birth representing 20-50% of maternal body mass. The large embryos have the potential to compromise the function of numerous organ systems, particularly those susceptible to compressive forces such as the respiratory system. Previous studies have shown that breathing patterns are altered and the energetic cost of breathing increased threefold during pregnancy in sleepy lizards (*Tiliqua rugosa*). In this study we measured the changes in lung diffusion capacity and total lung volume during pregnancy in *Tiliqua nigrolutea*. Lung diffusion capacity is a measure of the ability of gases to diffuse across the alveolar membranes and provides a measure the efficiency of the compressed lung during pregnancy. Small light weight breathing masks were attached to the lizards (pregnant n=6; non pregnant n=8) and lung volume and lung diffusing capacity were determined by giving the lizards low concentration of carbon monoxide (0.3%) and helium (13%) to breathe. Lung volume significantly decreased in pregnant lizards during the last 12 weeks of pregnancy. However, despite this reduction in lung volume pregnant lizards were able to maintain lung diffusion capacity and the rate of oxygen consumption. These results suggest that pulmonary perfusion maybe increased to non compressed areas of the lung during pregnancy. Exercise capacity was significantly reduced in gravid lizards due to limitations on exercise induced changes in tidal volume, rate of inspiration, breathing frequency and oxygen consumption.

Thermoregulation in sminthopsine marsupials; do they work like a PID?

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Body temperature regulation was examined for sminthopsine marsupials over a range of ambient temperatures from cold to thermoneutrality, with respect to a generic proportional-integral-derivative (PID) feedback control system. A model for thermoregulation by *Sminthopsis psammophila* (sandhill dunnart; 44g) suggested that a simple proportional (P) feedback system reduced body temperature at low air temperatures (thermolability), with the magnitude of body temperature decline dependent on the proportional gain. Increased gain resulted in less thermolability at slightly increased metabolic cost. There appears to be a considerable scope before increased gain results in thermal oscillation, reflecting limited heat production capacity relative to heat capacity. Thermolability can also be reduced by including an integral control term (PI) at equivalent metabolic cost. Although body temperature would overshoot at high integral gain, requiring damping by including a derivative control term (PID), a proportional-integral (PI) control seems adequate for describing observed patterns of variability in thermolability by sminthopsine species. Variation in integral control may be the mechanism for observed adaptive variation in thermal responses of various sminthopsines in response to habitat and environmental variables.

The whole blood oxygen binding properties of a large and presumably sluggish polar elasmobranch, the Greenland shark *Somniosus microcephalus*

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Only a few species of elasmobranch live in cold polar waters and the Greenland shark (*Somniosus microcephalus*) is the most notable example. These extremely large and long-lived sharks are thought to be sluggish but their active feeding lifestyle has recently been questioned by the finding of mobile prey species in their stomach (i.e. squid, fish and seal). The whole blood oxygen binding property of *S. microcephalus* was therefore examined on a recent research cruise to Greenland to provide clues on the aerobic potential of this species and to gauge the O_2 carrying capacity of its blood during acute thermal change. The blood of *S. microcephalus* at 2^{o}C revealed a high O_2 binding affinity (P_{50} = 11.7 mmHg at a PCO_2 level of 2.3 mmHg) and a surprisingly low sensitivity of binding to pH (the Bohr factor, $\phi = \Delta \log P_{50}/\Delta pH$ = -0.22). Following an acute increase in temperature to 7^{o}C, there was a slight loss of Hb-O_2 affinity (P_{50} = 16.8 mmHg at a PCO_2 level of 2.3 mmHg. $P<0.05$) but no significant increase in the Bohr factor (ϕ = -0.35. $P>0.05$). The blood-oxygen transport system of *S. microcephalus* does not therefore appear compatible with an active lifestyle and is not overly sensitive to an acute rise in temperature. The implications of these observations will be discussed with respect to the known feeding ecology and depth distribution of the species.

Optimum temperature for growth and feed conversion in cultured hapuku (*Polyprion oxygeneios*) – Is there a link to aerobic metabolic scope and preferred temperature?

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Understanding how basic environmental variables such as temperature affect the growth and production efficiency of finfish species is core to the optimisation of culture conditions, especially for new candidate species that often lack basic information at start-up. Temperature-based growth trials are lengthy, expensive and laborious to run so juvenile hapuku (*Polyprion oxygeneios*) were used to assess whether respirometry tests and behavioural preference methods could be used to resolve the optimum temperature for growth and feed conversion ratios (FCR). On the basis that the energetic costs of rapid growth are substantial and need to be accommodated physiologically, it was hypothesised that aerobic metabolic scope (AMS) would be maximised at a temperature that also leads to maximal rates of growth and FCR. It was further hypothesised that *P. oxygeneios* would behaviourally self-select temperatures that lead to the greatest level of AMS, growth and FCR performance. Acclimating *P. oxygeneios* juveniles to 12, 15, 18, 21 and 24°C for 4 weeks resulted in a peak in specific growth rate (SGR) across 18 - 21°C with slower growth at the lower and higher temperatures. FCR, however, was inversely related to temperature so the most and least efficient rates of conversion occurred at 12°C and 24°C respectively. AMS showed a peak at 18-21°C and was therefore tightly coupled with SGR but there was no such coupling of AMS with FCR. The behavioural thermal preference (T_{pref}) range of *P. oxygeneios* also fell within the optimum range for growth but T_{pref} was unrelated to FCR. The conclusions of the study are three-fold: 1) *P. oxygeneios* has the capacity to self-select temperatures that optimise both AMS and growth. 2) AMS and T_{pref} appear tightly linked with SGR and could possibly be used to predict the optimum temperature for growth in novel species such as *P. oxygeneios*. 3) AMS and T_{pref} have no utility in predicting the optimum temperature for FCR but standard metabolic rate (SMR), a component of AMS, did show a link with FCR so may be the most revealing variable in terms of FCR optimisation.

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Mother and nest influence green turtle hatchling quality

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We used a split clutch design to experimentally separate nest effects from clutch effects on hatchling morphology and locomotor performance in field nests of green turtles (*Chelonia mydas*). We found both clutch of origin and nest to influence hatchling morphology and locomotor performance in some but not all field nests. Using egg mass as a surrogate for clutch and nest temperature as a surrogate for nest in multiple regression followed by partial correlation analysis, we found clutch of origin had a greater influence than nest on the morphological attributes of hatchling mass and carapace size, but nest had a greater influence than clutch on the performance attributes of self-righting time, self-righting propensity, swim thrust during the first 30 minutes of swimming, and power-stroke rate during the first 30 minutes of swimming.

Physiological adaptations of Western Australian Echidnas (*Tachyglossus aculeatus*e)

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Echidnas are the only Australian mammal known to inhabit all major terrestrial environments (1), yet there have been few studies investigating adaptations of the regional sub-species. Here we investigate the physiology of Western Australian echidnas over a range of ambient temperatures. Most physiological parameters of echidnas follow the pattern of a typical endotherm, except body temperature. Metabolic rate is highest at low ambient temperatures (10°C), decreasing to the lowest point at thermoneutrality (25-30°C) and increasing above their upper temperature limit (32.5°C). Evaporative water loss remains relatively stable at all temperatures below their thermoneutral zone, with a marked increase above this, as does both wet and dry thermal conductance. Body temperature, however, is not strictly maintained within a very small range as is expected for an endotherm; echidnas are very thermolabile and body temperature varies with ambient temperature. Their normothermic body temperature ranges from 24°C (T_a = 10°C) to 32°C (T_a = 32.5°C). When compared to the published data for echidnas in other geographic locations (2, 3), basal metabolic rate was lower than those from Queensland but similar to those from New South Wales and body temperature was lower than both. This may be due to a range of reasons including geographic variation and measurement protocol. There is currently no water loss data available for this species

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Helox: beyond measurement of cold-induced maximal metabolic rate

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Helox is a mixture of oxygen and helium gasses, commonly used to simulate exposure to low ambient temperatures by increasing thermal conductivity. Although the metabolic response of endotherms to helox exposure at low ambient temperatures is well understood, the effects at higher ambient temperatures and impact of evaporative water loss are relatively unknown. Here we examine the effect of helox on various physiological variables including evaporative water loss, at a range of ambient temperatures, for a small native endotherm, the ash grey mouse (*Pseudomys albocinnereus*). As expected for a predominately homeothermic endotherm, body temperature was independent of ambient temperature and was not influenced by helox exposure. Thermal conductance was significantly higher for mice exposed to helox. As a consequence of increase heat loss, metabolic rate was significantly higher for mice exposed to helox, although there was a significant interaction between ambient temperature and helox exposure, with the difference between metabolic rate in normal air and in helox greater at lower ambient temperatures. Metabolic rate in normal air and helox was indistinguishable at thermoneutrality. Evaporative water loss was significantly higher in helox than in normal air at low ambient temperature, but was equivalent at thermoneutrality. However, this observed increase in evaporative water loss in helox was not as great as predicted from physical experiments of evaporation rates. Understanding the effect of helox on physiological variables over a range of ambient temperatures suggests that helox may be used to manipulate physiological responses independent of ambient temperature, for example to non-invasively partition evaporative water loss into respiratory and cutaneous components.

Dinosaur Lactation

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Lactation is a process associated with mammals, yet secretions are used by a number of animals in order to facilitate the growth of their young. This study examines the possibility that some dinosaurs used secretory feeding to increase the rate of growth of their young estimated to be similar to that of present day birds and mammals. Dinosaur 'lactation' could also have facilitated immune responses as well as extend parental protection due to feeding newly hatched young in nest environments. While the arguments for dinosaur lactation are somewhat generic, a case study for lactation in herbivorous site-nesting dinosaurs will be made. It is proposes that secretory feeding could have been used to bridge the gap between hatching and establishment of the normal diet in some dinosaurs.

Climate change related extinction risk in *Sauromalus ater*

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Climate is expected to change significantly in the next hundred years, and numerous recent publications have predicted that lizard species may be especially vulnerable to extinction as they are forced into suboptimal thermal environments [1,2,3]. In fact, local extinctions of lizard species have been predicted to reach 40% worldwide by 2080 [1]. However, these predictions are generally based upon assumptions of inflexible physiology and behavior and may overlook mechanisms by which populations can avoid extinction. Those mechanisms form our testable hypotheses. To minimize or avoid the effects of climate change, a species might: (1) move to a new location, (2) adjust behaviour and activity times, (3) adjust physiology to increase performance, and/or (4) evolve to become better suited to new climatic and thermal conditions. This study seeks to determine whether populations of *Sauromalus ater*, a desert-dwelling lizard already experiencing extreme summer temperatures [2], could avoid extirpation under circumstances of climate change by means of two of these mechanisms: adjustment of behaviour and physiology. We compared several measures of physiology as a function of body temperature between two lizard populations of differing elevations across two points in the active season, as well as recording operative temperature and a sample of field body temperatures of male lizards at both elevational sites. Preliminary analyses indicate that there is no significant difference at the low elevation site in any measure of physiology between the spring and summer seasons. However, at the high elevation site, metabolic rate was significantly lower during the summer than the spring, regardless of body temperature, while panting temperature appears higher during the summer than spring, suggesting some populations of *S. ater* have the ability to alter physiology, perhaps as a means of improving performance seasonally.

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Hormonal regulation of expression of growth differentiation factor-9 receptors (ALK5 and BMPRII) in the ovary of shortfinned eel, *Anguilla australis*

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Growth differentiation factor-9 (GDF9), a member of the transforming growth factor-β superfamily, regulates ovarian folliculogenesis by driving granulosa cell proliferation and differentiation in mammals. GDF9 exerts its effects via two receptors: activin receptor-like kinase 5 (ALK5) and bone morphogenetic protein type II receptor (BMPRII). The temporal change in expression of each receptor in the ovarian follicle is likely to be indicative of a functional role for its ligand at a particular stage of follicular development. There is a lack of information on the expression and localization of these receptors in the ovary of teleosts. Similarly, there is no information about how the expression of these genes is controlled during early oogenesis in fish. In this study, we localized ALK5 and BMPRII in the ovarian follicles of shortfinned eel (*Anguilla australis*) using immunohistochemistry and *in situ* hybridization and examined mRNA expression patterns of these receptors during early oogenesis by quantitative real-time PCR assay. Furthermore, ovarian fragments from previtellogenic and early vitellogenic fish were cultured with different doses (0, 1, 10 and 100 ng/ml or nM) of follicle-stimulating hormone (FSH), luteinizing hormone (LH), growth hormone (GH), epidermal growth factor (EGF), insulin-like growth factor I (IGF-I) or 17β-estradiol (E2) to examine their effects on ALK5 and BMPRII mRNA levels using quantitative real-time PCR. Shortfinned eel ALK5 and BMPRII transcript levels increased significantly from the perinucleolar to the early vitellogenic stage and were localized in the somatic cells of the ovarian follicles. In contrast, GDF9 was expressed in the oocyte. Lastly, we found the expression of these genes in shortfinned eel to be stage-specific and responsive to FSH, LH, GH, EGF, IGF-I and E2. We conclude that trends in expression and localization of the receptors that mediate GDF9 actions in shortfinned eel largely resemble trends in mammals.

Metabolic consequences and mitochondrial dysfunction following acute chronic exposure to high CO_2 in larval echinoderms.

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The Intergovernmental Panel of Climate Change has predicted that oceanic CO_2 levels will increase to as high as 1000ppm in the next century and 1800ppm by 2300. Echinoderms are known to be highly sensitive to ocean acidification, possibly due to the high magnesium content of their shells. Larvae in particular are believed to be at risk, although various hypotheses exist about why this might be true. We proposed that rearing echinoderm larvae under acidic conditions may result in metabolic inefficiency associated with direct effects of low pH on mitochondrial function, and tested this hypothesis on larvae from summer, winter and seasonally indiscriminant spawning echinoderms. We used novel protocols and cutting edge technology to measure oxygen consumption and mitochondrial function *in vivo* (high resolution respirometry and OROBOROS™ Oxygraphs). Elevated CO_2 induced changes in larval MO_2, and the pattern of these changes was dependent on temporal and genetic aspects. Overall, larval echinoderms acutely exposed to high CO_2 exhibited elevated MO_2, but those reared in elevated CO_2 exhibited a decrease instead. In the kina, *Evechinus chloroticus*, rearing under only moderate CO_2 conditions was associated with a loss of mitochondrial capacity. These metabolic modifications could translate into significant reductions in echinoderm recruitment, but in addition the results from this research provide a mechanism with which ocean acidification may exert its deleterious effects (i.e., reduction in routine MO_2 and mitochondrial capacity), and so provide a tool for assessing sensitivity among larval species.

The rhythm of core temperature predicts subsequent breeding intensity in wild rabbits

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The maintenance of a constant body temperature above ambient temperature requires energy. The cost of existence in mammal and birds is about 10 times that in reptiles, a large fraction of which is related to the energy cost of heat production for thermoregulation. Many small mammals and birds abandon homeothermy and enter torpor, a state where body temperature is regulated below the normal level. Energy is saved during torpor because energy is not expended defending the normal temperature gradient to the environment, and resting metabolism decreases due to the Q_{10} effect.

Mammals larger than 10 kg do not enter torpor, but we have observed heterothermy associated with a reduced minimum daily body temperature in many species, including kangaroos, rabbits, sheep, oryx, and alpacas. A central question is whether this heterothermy, like torpor, saves energy that can be directed to other activities, or whether the use of heterothermy indicates that an animal is manifesting some strain, and that physiological systems other than thermoregulation may be similarly impaired.

A study on disease transmission in wild rabbits, which involved implanting 80 wild rabbits with small loggers that collected core temperature every 5 minutes for up to a year, provided us the opportunity to test whether heterothermy is associated with physiological performance of the animals, by assessing the number of reproductive episodes.

Using cosinor anlaysis to characterise the mesor (mean) and daily variability (amplitude) of the circadian rhythm of core body temperature, we found that females that had a smaller amplitude of the circadian core temperature rhythm prior to the breeding season had more litters during the subsequent nine months than those females that had a larger amplitude of the circadian core temperature rhythm prior to the breeding season.

Our results lend support to the contention that the characteristics of the circadian core temperature rhythm provide information on the level of physiological strain being experienced by an individual, and may be correlated with fitness outcomes. In well nourished and hydrated mammals, homeothermy seems to be the default condition while heterothermy can result from many different stressors.

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Maximum metabolic rate, relative lift, wingbeat frequency, and stroke amplitude during tethered-flight in the adult locust *Locusta migratoria*

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Flying insects achieve the highest mass-specific aerobic metabolic rates of all animals. However, few studies attempt to maximise the metabolic cost of flight and so many estimates could be sub-maximal, especially where insects have been tethered. To address this issue, oxygen consumption was measured during tethered-flight in adult locusts *Locusta migratoria*, some of which had a weight attached to each wing (totalling 30-45% of body mass). Mass-specific metabolic rates (μmol O_2 g^{-1} h^{-1}) increase from 28 $\pm$ 2 at rest, to 896 $\pm$ 101 during flight in weighted locusts, and 1032 $\pm$ 69 in unweighted locusts. Maximum metabolic rate of locusts during tethered-flight (MMR; μmol O_2 h^{-1}) increases with body mass (M_b; g) according to the allometric equation, $MMR = 994 M_b^{0.75 \pm 0.19}$, whereas published metabolic rates of moths and orchid bees during hovering free-flight (HMR) is approximately 2.8-fold higher, $HMR = 2767 M_b^{0.72 \pm 0.08}$ [1, 2]. The modest flight metabolic rate of locusts is unlikely to be an artefact of individuals failing to exert themselves, because mean maximum lift was not significantly different from body mass (95 $\pm$ 8%), mean wingbeat frequency was 23.7 $\pm$ 0.6 Hz, and mean stroke amplitude was 105 $\pm$ 5 degrees in the forewing and 96 $\pm$ 5 in the hindwing – all of which are close to free-flight values. Instead, the low cost of flight could reflect the relatively small size and relatively modest anatomical power-density of the locust flight motor, which is a likely evolutionary trade-off between flight muscle maintenance costs and aerial performance.

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Thermoregulatory Physiology of Australian birds

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Birds live an energetically-expensive lifestyle as they are mostly small, diurnal and use flight. However they are long-lived, dominate most Australian environments and survive successfully in harsh and highly-variable conditions. Despite these contradictions little is known about the mechanisms Australian birds use to cope with these constraints, particularly thermoregulatory adaptations. By comparison, strategies of co-occurring mammals are reasonably well-known; they utilise a variety of behavioural and physiological strategies to buffer energy requirements. To better understand how birds meet their energetic and thermoregulatory demands, we examined body temperature (T_b), metabolic rate (MR) and evaporative water loss (EWL) of three species of Australian bird. Standard open flow-through respirometry was used to gather continuous metabolic data at a range of ambient temperatures (T_a; 10 – 32.5°C) for three species; Spotted Dove *Streptopelia chinensis*, Red Wattlebird *Anthocaera carunculata* and Rufous Whistler *Pachycephala rufiventris.* Continuous core T_b was logged using Passive Implantable Transponders. Despite differences in taxonomy, size and diet all three species showed similar T_b responses over the range of T_as tested. They maintained a constant T_b at and below thermoneutrality, achieved via a typical endothermic increase in MR. Similarly, wet thermal conductance (C_{wet}) and EWL remained constant at and below thermoneutrality and increased sharply at high T_a for all three species. The implications of these physiological responses for maintaining homeothermy will be considered in relation to the strategies of similar-sized sympatric mammals.

Beating hypoxia: depressed free radical release from heart mitochondria of hypoxia-tolerant epaulette sharks

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Hypoxia and warm ischemia are primary concerns in ischemic heart disease and transplant and trauma. Hypoxia impacts tissue ATP supply and can induce mitochondrial dysfunction that elevates reactive species release. The epaulette shark, *Hemiscyllum ocellatum*, is remarkably tolerant of severe hypoxia at temperatures up to 34^{o}C, and therefore provides a valuable model to study warm hypoxia tolerance. Heart mitochondrial function was tested using high-resolution respirometry coupled with purpose-built fluorospectrometers. Ventricular mitochondrial function, stability and reactive species production of the epaulette shark was compared with that of the hypoxia-sensitive shovelnose ray, *Aptychotrema rostrata.* Fibres were prepared from each species acclimated to normoxic water conditions, or following a 2 h, acute hypoxic exposure at levels representing 40% of each species' critical oxygen tension. Although mitochondrial respiratory fluxes for normoxia-acclimated animals were similar for both species, reactive species production in the epaulette shark was approximately half that of the shovelnose ray under normoxic conditions, even when normalised to tissue oxidative phosphorylation flux. The hypoxia-sensitive shovelnose ray halved oxidative phosphorylation flux and cytochrome c oxidase flux was depressed by 34% following hypoxic stress. In contrast, oxidative phosphorylation flux of the epaulette shark ventricular fibres isolated from acute hypoxia exposed the animals remained similar to those from normoxia-acclimated animals. However, uncoupling of respiration revealed depressed electron transport systems in both species following hypoxia exposure. Overall, the epaulette shark ventricular mitochondria showed greater oxidative phosphorylation stability and lower reactive species outputs with hypoxic exposure, and this may protect cardiac bioenergetic function in hypoxic tropical waters.

Fish and Chips: Using lab-on-a-chip technology to investigate acid-base regulation during embryonic development in zebrafish

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Organismal pH regulation in fish has experienced a surge of interest in recent years, and with the advance of some technologies, the role of the gill and cellular mechanisms have been greatly clarified in adults. However, little is known about acid-base regulation during early development, despite the likelihood of adverse conditions that promote a general acidosis, such as hypoxia or hypercapnia, occurring in areas typically encountered by fish eggs. This information gap is likely due to the difficulty in assessing physiology of embryonic fish with an appropriate experimental methodology. In this work, we demonstrate a novel application of microfluidic technology (i.e., lab-on-a-chip, LOC) for investigating larval physiology. This flow-through preparation allows visual examination of basal physiological status of many embryos under identical conditions, and assessment of alterations in these parameters as conditions change. To demonstrate the merit of LOCs, we investigated the relationship between acid-base regulation and systemic function, by measuring pH_i (as measured by fluorescence), cardiac function (heart rate, blood flow), and mitochondrial performance (O_2 consumption, fluorescent dyes) during a general acidosis induced through exposure to hypercarbic water in 24-48 h zebrafish embryos. We believe this example will promote the use of LOC technology to address the many hypotheses surrounding larval physiology, both within the field of acid-base regulation and in a broader evolutionary context.

Dietary Docosahexaenoic Acid (22:6) Incorporates into Cardiolipin at the Expense of Linoleic Acid (18:2): Analysis and Potential Implications

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Cardiolipin is the signature phospholipid of mitochondria. It is involved in facilitating oxidative phosphorylation and in the triggering mitochondrial-induced apoptosis. The fatty acid composition of cardiolipin is commonly viewed as highly regulated due to the presence of a dominant tetra linoleic acid (4x18:2n-6) molecular species. However, analysis of data from a comprehensive compilation of studies (using rats and mice) reporting changes in fatty acid composition of cardiolipin in heart and liver mitochondria in response to dietary fat shows that in heart the accrual of 18:2 into cardiac CL conforms strongly to its dietary availability between 2-20% of total dietary fatty acid and thereafter is regulated. In liver, no dietary conformer trend is apparent for 18:2 with regulated but lower levels across the dietary range for 18:2. When 18:2 and docosahexaenoic acid (22:6n-3) are present in the same diet, 22:6 incorporates into cardiolipin at the expense of 18:2 when 22:6 is up to ~20% of total dietary fatty acid for heart and 10% for liver. Fatty acid composition of the two other main mitochondrial phospholipids, phosphatidylcholine and phosphatidylethanolamine in response to dietary fat are also compared, and the potential consequences of replacement of 18:2 with 22:6 in cardiolipin is discussed including its potential use in the treatment of cancer.

Climate Induced Restrictions on Ectotherm Activity and Reproduction: The Case of a Rapidly Declining Endangered Grassland Lizard

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Recent modeling predicts heliothermic reptiles are likely to experience a high rate of climate induced extinction due to constraints of their thermal physiology. Increases in ambient temperatures (Ta) are likely to contract the diurnal window suitable for lizard activity and drive individuals to seek thermal refugia for longer periods, compromising opportunities to secure resources and mates. Oviparous species will potentially experience added pressure as higher temperatures reduce egg and hatchling viability. The Grassland Earless Dragon (Tympanocryptis pinguicolla) is an endangered species that has experienced both gradual long-term, and dramatic short-term declines in both its range and population size. Given the recent long-term drought experienced by most of south eastern Australia, it is likely that climatic factors are implicated in this decline. The critical maximum temperature for this species is unknown, however preliminary data indicates individuals seek thermal refugia in burrows or tussocks at Ta >40 °C. We propose to undertake field research to quantify the thermal microhabitats used by this species, characterize thermal profiles for nests and determine operative field skin temperatures and voluntary thermal maxima of free-ranging individuals. A captive breeding colony has been established to enable robust experimental approaches, including evaluating egg viability and hatchling success at varied incubation Ta, quantifying thermal limits of captive adults, and determining metabolic rates over a range of Ta to enable calculation of daily energy requirements. These data will inform projections of the available daily activity window required for this species to meet energetic demands and maintain reproductive viability under increased Ta.

Targeting autophagy to chemosensitise prostate cancer cells

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For men, prostate cancer (PCa) is the second most common cancer and the sixth leading cause of cancer death worldwide. Drug treatments of PCa can be difficult due to the low proliferation rate of prostate cancer cells, which make conventional chemotherapy less effective, but still exert significant toxicity. There is therefore an urgent need for new effective treatments that target androgen dependent and independent cells, or treatments that sensitise the tumour to current treatments and enhance their clinical efficacy. Given the key role that autophagy plays in modulating either tumour cell survival or cell death targeting this pathway may represent a new therapeutic approach. Therefore, the aim of this study was to identify the role of autophagy in modulating chemo-sensitivity of androgen-dependent and –independent prostate cancer cells.

To achieve this we treated PC-3 (androgen-independent) and LnCAP (androgen-dependent) prostate cancer cell lines for 24 hours with docetaxel (0-10µM) gemcitabine and doxorubicin (0-100µM) in combination with the autophagy inhibitor 3-MA (5mM). Cell viability was measured using resazurin reduction, while autophagy was determined fluorometrically using acridine orange. Doxorubicin, gemcitabine, and docetaxel decreased PC3 and LnCAP cell viability in a dose-dependent manner with doxorubicin (100µM) producing the greatest reduction in cell viability (37.7% and 63.4% reduction in viability in PC-3 and LnCAP cells, respectively) compared to untreated control. Treatment with 3-MA significantly protected PC-3 cells against doxorubicin (0.001-100µM) and gemcitabine (0.1-100µM) induced cytotoxicity and acidic vesicular organelle formation (AVO). In contrast 3-MA had no appreciable effect on cell viability in the presence of docetaxel. Similarly, 3-MA afforded LnCAP prostate cancer cells protection against doxorubicin (100µM). In conclusion, the initial findings from this study suggest that modulating autophagy may provide a useful strategy in improving the clinical efficacy of conventional chemotherapeutic regimens in treating androgen-dependent and –independent prostate cancers.

Low temperature delays demographic aging more than it slows the rate of aging: experimental study in the blowfy *Calliphora stygia*.

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Low temperature extends the lifespan of ectotherms and has been thought to be due to a 'rate of living' effect. We have studied the influence of temperature on ageing in the golden-haired blowfly (*Calliphora stygia)* by measuring egg laying, food consumption and age-specific mortality in replicate populations at 12, 15, 20, 25, 29 and 34°C. This covered the species physiological temperature range (egg laying occurred at all but the two extreme temperatures). Daily food consumption significantly increased from 12 to 20°C but not from 20 to 34°C, suggesting that temperature effects are not 'rate of living' effects. Demographic analysis showed that the mortality rates of flies were best described by a two-phase Gompertz, dividing ageing into an 'early' and 'late' phase of ageing that had significantly different rates of senescence. Demographic senescence at 29°C and 35°C was slowed during old age. In contrast, the early phase of ageing at low temperature (12-25°C) was characterized by an extended period time of minimal change in age-specific mortality (i.e. negligible demographic senescence). This period of negligible senescence varied from 72 days at 12°C to 39 days at 25°C and showed a greater sensitivity to temperature than the slope (i.e. the rate of ageing) of the late phase. A series of temperature cross-over experiments (between 15°C and 29°C) confirmed the conclusion that lowered temperature delays 'senescence' more than it slows the demographic rate of ageing. Cellular damage was measured as fluorescent age-related glycation end-products (AGE pigments). At all temperatures AGE pigment increased with age and the rate of AGE pigment accumulation was slower at low temperatures.

Does habitat matter in a changing climate? A mitochondrial insight to temperature induced heart failure in closely related fish from different habitats.

Fathima I. Iftikar and Anthony J. R. Hickey

Predictions of climate change mediated rises in ocean temperatures suggest that ectothermic hearts may place tight constraints on many marine species.. For many aquatic species, the upper temperature limit (Tmax) and the heart failure (HF) temperature (THF) is only a few degrees away from their current environmental temperatures (1). Especially for tropical fishes the window between Tmax and THF is narrower than for temperate fishes, and only slight temperature increases induce heart failure (HF). While cold-blooded hearts may provide sensitive 'ecological thermometers', an explanation for why hot hearts fail remains unresolved. In fish, HF due to elevated temperatures may result from energy and/or oxygen supply disruptions to and from mitochondria in cardiac cells(2). This study targets mitochondria, as damaged mitochondria may increase their reactive species production and trigger apoptosis, or they may fail to produce enough ATP to sustain a heartbeat. Currently we are testing the impacts of temperature on heart mitochondria relative to HF in three closely related fish wrasse species that occupy cold temperate (Notolabrus cinctus), temperate (Notolabrus celidotus) and tropical (Thalassoma lunare) habitats. In N. celidotus that is endemic to New Zealand, a drop in phosphorylation efficiency (inferred from RCR) was apparent at temperatures prior to Tmax suggesting mitochondrial ATP supply may compromise heart function at elevated temperatures. We also assessed substrates and found that the apparent KM for pyruvate rises from ca. 100 uM to over 1.2 mM at 32.5oC. These data suggest that mitochondrial function and integrities could play a significant role in thermal stress tolerance and perhaps limit species distributions. Clarifying the specific mechanisms that lead to HF may provide a powerful biomarker for predicting the impacts of temperature change on biodiversity.

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