APC1  CONSERVATION PHYSIOLOGY: A CHANGING WORLD – PROBLEMS AND SOLUTIONS

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SESSION SUPPORTED BY: CONSERVATION PHYSIOLOGY

APC1.1 PHYSIOLOGY MEETS ECOLOGY: CORAL REEF FISHES, PERFORMANCE, DISTRIBUTION, AND GLOBAL CHANGE

MONDAY 4 JULY, 2016  11:00

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Globally, coral reefs are more at risk to human-induced stressors - such as climate change, including ocean warming, acidification, and hypoxia events, and altered water quality due to coastal development - than at any other time in recorded history. Dramatic effects on fish performance, distribution, and overall ecosystem health are predicted. While the success of the fishes over their long evolutionary history is thought to have hinged on key adaptations for maintaining oxygen transport and physiological performance under challenging conditions, whether they possess the necessary plasticity and/or adaptations to keep pace with the large-scale, rapid changes plaguing their habitats today is not known. Moreover, the coral reef fishes in particular diversified more recently on the geological time scale, with most species radiating within the last 23 million years, a period characterised by relatively stable environmental conditions. Evolving and existing under stable environmental conditions may heighten the vulnerability of coral reef fishes to the rapidly changing conditions coral reefs are facing today. By harnessing geographic gradients, such as the latitudinal thermal profile along the Great Barrier Reef, and local extreme environments, such as the volcanic CO₂ seeps in the reefs of Papua New Guinea, as analogues for future change and integrating physiological, biochemical, and molecular techniques, the mechanisms that fish use to acclimate and adapt to these stressors can be identified. Such responses may become potential targets of natural selection and will determine which species and populations may be most at risk from climate change and other human-induced stressors.

APC1.2 THERMAL PERFORMANCE OF SIX EQUATORIAL INLAND FISHES FROM THREE CONTINENTS IN THE FACE OF CLIMATE CHANGE

MONDAY 4 JULY, 2016  11:40

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Global climate change interacts with and adds to a myriad of stressors exerting pressure on inland aquatic environments, and thus may impede the ability of these systems to support diverse and sustainable fish stocks. Inland fisheries are often critical to food security and poverty alleviation strategies of developing nations of the tropics. However, climate warming is predicted to have a greater impact on equatorial fishes than temperate species because they live in less seasonally fluctuating thermal environments and may live closer to their upper thermal limits. However, this prediction has little empirical support. Therefore, we studied the thermal performance of six species of indigenous fishes, two species from each of three continents. We measured their capacity to supply oxygen to tissues at the prevailing temperatures as well as up to 4°C higher. We used established physiological endpoints to address a time sensitive conservation problem: how environmental change, specifically increases in water temperature, influence culturally and socio-economically important tropical freshwater fish species.
The climate is changing rapidly, and terrestrial ectotherms are expected to be particularly vulnerable to changes in temperature, but also to an increase in extreme weather events. In conservation, physiological responses of terrestrial gastropods to such events are poorly studied. This is surprising, because terrestrial gastropods are the third-most successful group of terrestrial animals, and they are of biodiversity significance among litter-dwelling species, as both invaders and native species, playing important roles in ecosystem function. When assessing threats of climate change, four different categories are used by the IUCN and can be applied to terrestrial gastropods: (i) Extreme temperature, (ii) droughts, (iii) storms and flooding, and (iv) habitat alteration. (i) In winter, terrestrial gastropods use different strategies to survive sub-zero temperatures in buffer refuges, like the litter or the soil. Absence of the insulating snow cover exposes species to high variability in temperature. The extent of cold tolerance might influence the potential of local extinction, but also of invasion. (ii) Physiological responses to droughts involve high-cost processes that protect against heat and dehydration. Some species decrease activity periods thereby reducing foraging and reproduction time. Costs and physiological limits increase mortality. (iii and iv) Although terrestrial gastropods are able to survive hypoxic conditions for several hours, storms and flooding as well as habitat erosion represent threats. Low capacity to migrate towards zones of favourable conditions might be the most limiting factor in the response to such climate change effects; specialist species are more vulnerable to habitat alteration than generalists.

Ectotherms face an ever-increasing risk of losing functional performance as ongoing climate change drives environmental temperatures beyond physiological limits. The threat of overheating may be particularly salient for ectothermic divers (e.g. crocodilians, marine/freshwater turtles and iguanas), with increased temperatures reducing their potential to perform obligate underwater activities. We explored the efficacy of physiological compensation in buffering the negative impacts of elevated temperatures on dive capacity in juvenile estuarine crocodiles (Crocodylus porosus). Crocodiles were exposed to one of three long-term thermal treatments, designed to emulate water temperatures under differing climate change scenarios (i.e. current summer, 28°C; moderate climate warming, 31.5°C; high climate warming, 35°C), and dive capacity was subsequently tested. We show how metabolism, blood-oxygen carrying capacity and thermal acclimation treatments interact to determine the thermal sensitivity and plasticity of dive capacity. These findings are compared to body temperature and dive durations of free-ranging C. porosus.
Habitat fragmentation will preclude many large terrestrial mammals from shifting their range in the face of climate change. Predicting how trapped large mammals will respond to environmental change requires measurement of their sensitivity and exposure to changes in the environment, as well as the extent to which phenotypic plasticity can buffer them against the changes. Methods used to assess the responses of laboratory mammals to changing physical environments do not adequately predict how mammals living in their natural habitats, and subject to a complex array of stressors, will respond. In free-living mammals, behavioural modifications, such as a shift to nocturnal foraging or selection of a cool microclimate, may buffer the mammals against thermal and water stress, but may carry a cost, for example by reducing foraging time or increasing predation risk. Large mammals also use autonomic responses to buffer themselves against changing environments, but those buffers may be compromised by a changing physical environment. Restriction of food energy or water, likely to become more prevalent, especially in arid areas, with climate change, leads to a trade-off in which the precision of thermoregulation is relaxed, resulting in large daily fluctuations in body temperature. We propose use of the amplitude of the 24h body temperature rhythm as an index of the performance status of mammals. Long-term biologging of body core temperature in large free-living mammals provides a tool to investigate which species will cope physiologically, or not cope, when confronting a changing physical world.

We have little understanding as to why some species thrive and others perish in urban habitat. Small mammals that tolerate urbanisation likely take advantage of biological traits that allow a quick response to environmental disturbance. The European hedgehog Erinaceus europaeus is a species that shows higher population densities in cities than in rural areas. The physiological mechanisms responsible for its ecological success in urban environments remain unknown, yet these data are crucial for informing conservation strategies. We aimed to address this knowledge gap by studying several physiological and behavioural variables of free-ranging individuals in a large city in northern Germany. Specifically, we monitored skin temperature, activity patterns, metabolic rates and nest microclimate throughout the year. Additionally, we assessed health risks using a long-term dataset collected at a hedgehog care station. Our results show that hedgehogs were flexible in some thermoregulatory and behavioural traits (e.g. individual differences in torpor patterns, temporal organisation of activity in gardens vs. parks, smaller homeranges and more simply constructed nests than rural conspecifics), while other variables remained more conservative (e.g. hibernation duration, rates of metabolism and rewarming from torpor). The primary health concerns were abscesses developed from physical injuries caused by anthropogenic hazards (e.g. fences, nets, pits) or gardeners disturbing nests. Our study provides important baseline data highlighting the importance of ecophysiological flexibility in the successful persistence of hedgehogs in disturbed environments, which will be useful for advising conservation strategies for small mammals in general.
**APC1.8 OCEAN ACIDIFICATION AFFECTS LOCATOR MOTOR BEHAVIOUR AND LATERALIZATION OF A KEYSTONE MARINE MOLLUSC**

**MONDAY 4 JULY, 2016 14:50**

PAOLO DOMENICI (CNR, ITALY), RODRIGO TORRES (CIEP, CHILE), PATRICIO H MANRIQUEZ (CEAZA, CHILE)

We investigated the effect of elevated levels of pCO₂ and temperature on locomotor behaviour during prey searching in the marine gastropod Concholepas concholepas, a rocky-shore keystone predator from the south-eastern Pacific Coast of South America. Several locomotor and behavioural traits such as movement duration, decision time, obstacle avoidance and lateralization were measured using a T-Maze tank with a prey item positioned behind a barrier at the end of a runway. Two contrasting pCO₂ levels and temperatures representing present-day (control conditions: pCO₂ 500 μatm, temperature 15°C) and near-future scenarios (pCO₂ 1400 μatm, temperature 19°C) were used to rear the experimental individuals for 6 months. Regardless of the experimental conditions, no significant differences were found in the relative and absolute lateralization before and after 6 months of treatment. However, regardless of temperature, relative lateralization was significantly repeatable for animals tested after 6 months at control pCO₂, while elevated pCO₂ appears to affect the individual ability to retain relative lateralization at both experimental temperatures. We suggest that these effects may be related to malfunctioning at the neurotransmitter level caused by elevated pCO₂. Other measures of locomotor behaviour were not repeatable. However, movement duration and decision time were significantly increased and obstacle avoidance was decreased at elevated pCO₂, suggesting that elevated pCO₂ may have a negative effect on the locomotory behaviour and sensory ability of C. concholepas and similar species in the presence of a prey odour and thus decrease their ability to forage efficiently.

**APC1.9 DIGESTION PHYSIOLOGY PREDICTS SENSITIVITY TO OCEAN ACIDIFICATION IN NON-CALCIFYING MARINE LARVAE**

**MONDAY 4 JULY, 2016 15:05**

MARIAN Y HU (INSTITUTE OF PHYSIOLOGY UNIVERSITY OF KIEL, GERMANY), MEIKE STUMPP (HELMHOLTZ CENTRE FOR OCEAN RESEARCH KIEL (GEOMAR) GERMANY, GERMANY)

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Marine larval stages are often the weakest link when a species is confronted with acidified seawater as predicted for near future ocean acidification scenarios. Special attention has been dedicated to marine calcifiers which were predicted to be particularly sensitive to changes in seawater carbonate chemistry. However, recent studies demonstrated that some non-calcifying species also respond sensitively to acidified seawater but the underlying physiological processes remain unexplored.

We used larvae of the hemichordate (Ptychodera flava) and the sea star (Archaster typicus) to assess the effects of near future acidification levels on these non-calcifying marine organisms. Larval stages of the hemichordate respond highly sensitively (100% mortality after 8 days) to simulated near-future acidification levels. Microelectrode measurements demonstrated that this species has highly regulated alkaline (pH 10.13 ± 0.04) digestive systems and metabolic rates increase 4-fold in response to acidified seawater. In contrast, the sea star larvae are less sensitive, showing only a slight developmental delay. Larval stages of A. typicus do not regulate gastric pH, but conform to the surrounding seawater.

Our results demonstrate that non-calcifying marine larvae may respond very differently to simulated near-future ocean acidification. Interspecific comparisons within the Ambulacraria indicate that the alkaline gastric pH and the rigidity to maintain gastric pH, and thus functionality, represents a unifying physiological feature for the sensitivity to ocean acidification. These findings highlight the importance of understanding fundamental physiological processes in marine species to generate hypothesis driven approaches to unravel potential adaptation mechanisms in times of rapid climate change.

**APC1.10 THE EFFECTS OF SIMULATED OCEAN ACIDIFICATION ON GLOBAL TRANSCRIPTOMIC PROFILING IN A MARINE TELEOST**

**MONDAY 4 JULY, 2016 15:20**

COSIMA S PORTEUS (UNIVERSITY OF EXETER, UNITED KINGDOM), TAMSYN UREN-WEBSTER (UNIVERSITY OF SWANSEA, UNITED KINGDOM), EDUARDA SANTOS (UNIVERSITY OF EXETER, UNITED KINGDOM), ROD WILSON (UNIVERSITY OF EXETER, UNITED KINGDOM)

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Marine fishes exposed to end-of-the-century levels of ocean acidification (OA) show altered sensory behaviour that is likely to affect survival of both individuals and populations. Recently we have found that elevated CO₂ seawater can have a direct negative effect on the olfactory sensitivity of European sea bass (Dicentrarchus labrax), an economically important species. The main objective of the current research was to elucidate the molecular mechanisms underlying the negative effects of OA on sea bass sense of smell using high-throughput sequencing. Sea bass were exposed for 2 and 7 days to either control (~400 μatm) or OA (~1000 μatm) seawater and 4-6 tissues replicate samples for each treatment were sampled from the olfactory epithelium (OE) and the olfactory bulb (OB-brain). Samples were sequenced using an Illumina HighSeq 2500 platform and a high quality de novo transcriptome was built using the Trinity pipeline. After 2 days of exposure, differentially expressed genes in the OE predominantly included those involved in sodium bicarbonate transport. After 7 days of exposure many more genes were differentially expressed including those involved in ion transport, peptidase activity, olfactory receptors, and alternative splicing. Overall fewer genes were differentially expressed in the OB. These data highlight the temporal dynamics of the response to OA at the molecular level underpinning the decrease in olfactory sensitivity. This study provides a better understanding of which genes are involved in coping with elevated CO₂, helping us predict which species are more likely to be affected by OA in the future.
APC1.11 STEPPING INTO THE WILD: TUNING OXIDATIVE BALANCE TO CHANGING ENVIRONMENTS

MONDAY 4 JULY, 2016  16:10

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The world is changing dramatically. This current pace of change is such that many organisms face rapid, severe and, often, unpredictable fluctuations in their physical and biotic environments. The emerging field of conservation physiology explores the physiological responses of organisms to human-induced environmental changes and attempts to develop physiological markers that can help to predict how these changes will impact on the viability of natural populations in the short-to, possibly, the long-term. Recent research in evolutionary ecology and conservation physiology has shown that the assessment of oxidative status metrics, such as oxidative damage and antioxidant molecules, may provide conservation practitioners additional physiological tools to predict individual perspectives of reproduction and survival and to assess aposteriori the effect of environmental stressors on fitness-related traits of a given species of conservation concern. To foster awareness of conservation practitioners, recent studies on the link between life-history traits and oxidative stress and on the impact of environmental perturbations on oxidative status metrics will be presented.

APC1.12 ANTI- AND PRO-OXIDANT GENE EXPRESSION AND OXIDATIVE DAMAGE IN THE BLUBBER TISSUE OF GREY SEAL (HALICHOERUS GRYpus) PUPS DURING SUCKLING AND THE POST WEANING FAST

MONDAY 4 JULY, 2016  16:40

HOLLY C ARMSTRONG (PLYMOUTH UNIVERSITY, UNITED KINGDOM), AILSA J HALL (SEA MAMMAL RESEARCH UNIT, UNIVERSITY OF ST. ANDREWS, UNITED KINGDOM), SIMON E. W MOSS (SEA MAMMAL RESEARCH UNIT, UNIVERSITY OF ST. ANDREWS, UNITED KINGDOM), PADDY P POMEROY (SEA MAMMAL RESEARCH UNIT, UNIVERSITY OF ST. ANDREWS, UNITED KINGDOM), KIMBERLEY A BENNETT (ABERTAY UNIVERSITY, UNITED KINGDOM)

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The ability to respond adequately to stress is crucial to fitness and survival. Cellular defences play key roles in protecting against natural and anthropogenic stressors. Grey seal pups experience rapid physiological changes during development. They feed on 40-60% fat milk, triple in body mass during their first 18-21 days of life, and undergo a post weaning fast of up to four weeks. High fat intake, rapid fat deposition and prolonged food deprivation can stimulate reactive oxygen species production in other species. We used qPCR to investigate changes in gene expression of pro- and antioxidant enzymes in blubber tissue during suckling and fasting in pups on the Isle of May, Scotland, during October to December 2013 (n = 15). Glutathione peroxidase (GPx), superoxide dismutase (SOD) and NADPH oxidase4 (NOX4) were significantly upregulated during the post weaning fast, whereas catalase (CAT) and glutathione-S-transferase (GST) were down regulated during this period (LME; p<0.05). There was no difference in malondialdehyde (MDA) concentration, an index of oxidative damage, during suckling or fasting. MDA was not related to gene expression changes. This suggests antioxidant defences are important and effective in avoiding oxidative stress in blubber during fasting. These mechanisms mirror those in muscle tissue of fasting Northern elephant seal pups. Our data highlight that suckling is not associated with higher antioxidant gene expression, despite high fat intake and rapid fat tissue expansion. The ability of pups to avoid ROS production and oxidative damage in blubber under these conditions warrants further attention.

APC1.13 HYPERCAPNIA, BRAIN IONS AND FISH BEHAVIOUR: GABAERGIC NEUROTRANSMISSION IN FISHES APPEARS FINE TUNED TO THE PREVAILING CO2 LEVELS IN THEIR HABITAT

MONDAY 4 JULY, 2016  16:55

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Projected increases in atmospheric CO2 levels appear to trigger acid–base regulatory responses in fishes that lead to altered GABAergic neurotransmission and disrupted behaviour. It is thought that changes in Cl- and HCO3- gradients across neural membranes interfere with the function of GABA-gated anion channels (GABA-A receptors). So far, such alterations have been revealed experimentally by exposing species living in low-pCO2 environments (around 400µatm), like many oceanic habitats, to elevated pCO2 (usually around 1000µatm). We have now explored the opposite situation, hypothesising that fishes living in typically hypercapnic environments also display behavioural alterations if exposed to low CO2 levels. This would indicate that ion regulation in the fish brain is fine-tuned to the prevailing CO2 conditions. We quantified pH regulatory variables and behavioural responses of Pangasianodon hypophthalmus, a fish native to the hypercapnic Mekong River, acclimated to high-pCO2 (30 000 µatm) or low-pCO2 (400µatm) water. The brain and blood pH were found to be actively regulated and the low-pCO2 fish displayed significantly higher activity levels, which were reduced after treatment with gabazine, a GABA-A receptor blocker. This indicates an involvement of the GABA-A receptor and altered Cl- and HCO3- ion gradients.

Goldman calculations suggested that low levels of environmental CO2 can cause significant changes in neural ion gradients in P. hypophthalmus. We conclude that brain ion regulation in fishes is fine-tuned to the prevailing ambient CO2 conditions and is prone to disruption if these conditions change.

APC1.11 STEPPING INTO THE WILD: TUNING OXIDATIVE BALANCE TO CHANGING ENVIRONMENTS

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Projected increases in aquatic CO2 levels appear to trigger acid–base regulatory responses in fishes that lead to altered GABAergic neurotransmission and disrupted behaviour. It is thought that changes in Cl- and HCO3- gradients across neural membranes interfere with the function of GABA-gated anion channels (GABA-A receptors). So far, such alterations have been revealed experimentally by exposing species living in low-pCO2 environments (around 400µatm), like many oceanic habitats, to elevated pCO2 (usually around 1000µatm). We have now explored the opposite situation, hypothesising that fishes living in typically hypercapnic environments also display behavioural alterations if exposed to low CO2 levels. This would indicate that ion regulation in the fish brain is fine-tuned to the prevailing CO2 conditions. We quantified pH regulatory variables and behavioural responses of Pangasianodon hypophthalmus, a fish native to the hypercapnic Mekong River, acclimated to high-pCO2 (30 000 µatm) or low-pCO2 (400µatm) water. The brain and blood pH were found to be actively regulated and the low-pCO2 fish displayed significantly higher activity levels, which were reduced after treatment with gabazine, a GABA-A receptor blocker. This indicates an involvement of the GABA-A receptor and altered Cl- and HCO3- ion gradients. Goldman calculations suggested that low levels of environmental CO2 can cause significant changes in neural ion gradients in P. hypophthalmus. We conclude that brain ion regulation in fishes is fine-tuned to the prevailing ambient CO2 conditions and is prone to disruption if these conditions change.
Changes in the function of the main inhibitory neurotransmitter GABA has been suggested as a general mechanism behind the sensory and behaviour alterations seen in ocean acidification studies on fish. When exposed to elevated pCO₂, fish regulate their acid-base balance by accumulating HCO₃⁻ in the blood and tissues, accompanied by a release of H⁺ and Cl⁻ to the water. These ion-regulatory changes might affect the ion gradient across the neural membranes and interfere with the GABAergic receptor function, possibly making it excitatory rather than inhibitory. We here present the first comprehensive analysis of expression of genes involved in the GABAergic transmission and of genes involved in transmembrane ions transport in fish brain. mRNA transcripts were quantified in brains of three-spined stickleback (Gasterosteus aculeatus) kept under control (333 ± 30 μ atm CO₂) or high CO₂ tension (991 ± 57 μ atm CO₂) for 43 days. In the high-CO₂ group there was an increased mRNA expression of some GABAergic receptor subunit isoforms. Moreover, exposure to elevated CO₂ altered the expression of NKCC1 and NDAE, two transporters involved in regulating intracellular Cl- and in HCO₃- neurons.
UNDERSTANDING THE CAUSES OF GLOBAL AMPHIBIAN DECLINES: HOW IMPORTANT IS ENVIRONMENTAL CONTEXT?

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Around the world, hundreds of amphibian populations are disappearing despite the availability of pristine habitat. These mysterious population declines exemplify the severity of the current biodiversity crisis and present one of the greatest challenges for conservation: they demonstrate that the influence of humans on the environment is so pervasive that even species in protected habitats are not safe from extinction. One possible explanation for these declines is exposure to increased ultraviolet-B radiation (UVBR) caused by human-induced ozone depletion. Studies on the impact of increased UVBR have predominantly considered UVBR in isolation of other environmental factors. Such studies have shown that exposure to UVBR is detrimental to the health of amphibians, causing mortality, malformations and reduced growth. In nature, however, amphibians often contend with numerous abiotic and biotic factors simultaneously. For instance, amphibians must cope with variations in temperature and aquatic oxygen while also competing with others for resources and avoiding predation, all of which can be detrimental to their health and survival. Importantly, such natural challenges are present in the pristine habitats where amphibians are declining. I will present examples of our research showing how these natural challenges alter the impact of UVBR on amphibians. I will also discuss how the effect of UVBR combined with other factors varies across response variables and across levels of UVBR. Our research demonstrates that consideration of additional environmental factors together with anthropogenic factors is vital for understanding the contribution of human-induced environmental change to biodiversity loss.

EVIDENCE FOR CHRONIC STRESS IN MARGINAL POPULATIONS: A CASE STUDY OF THE CAPE MOUNTAIN ZEBRA

JESSICA LEA (UNIVERSITY OF MANCHESTER, UNITED KINGDOM), SUSANNE SHULTZ (UNIVERSITY OF MANCHESTER, UNITED KINGDOM), GRAHAM KERLEY (NELSON MANDELA METROPOLITAN UNIVERSITY, SOUTH AFRICA), SUZIE WALKER (CHESTER ZOO, UNITED KINGDOM), JOHN JACKSON (UNIVERSITY OF SHEFFIELD, UNITED KINGDOM), SHELBY MATEVICH (UNIVERSITY OF MANCHESTER, UNITED KINGDOM)

Many species have undergone long-term range contraction caused by anthropogenic activities, resulting in their conservation in areas of low ecological suitability. A core issue lies in the active management of species in suboptimal habitat due to an inaccurate perception of its historical distribution and ecology. The Cape mountain zebra has been identified as one such species, with several populations classified as ‘ecological refugees’. These populations are characterized by low habitat quality and diet quality and poor performance. Establishing the links between habitat marginality, population performance and individual physiology is crucial for managing vulnerable populations. We use faecal hormone sampling techniques to assess the physiological status of Cape mountain zebra individuals in populations with both high and low quality habitat. Faecal glucocorticoid levels were significantly elevated in populations with poor habitat quality and performance. In addition, we found a significant interaction between habitat quality and rainfall season, where during the dry season faecal glucocorticoids remain high across all populations. Our results indicate that populations of Cape mountain zebra in ecologically unsuitable conditions are chronically stressed, and that this may be negatively impacting reproductive rates. This highlights the potential cost of confining a species to marginal habitat, whether knowingly or not, and is particularly alarming when considering critically endangered species that have only one or a few populations left. We emphasize the importance of taking into account historical distribution and ecology when undertaking conservation planning on any scale.

ENVIRONMENTAL DRIVERS OF IMMUNE FUNCTION IN ECTOTHERMS

REBECCA L CRAMP (UNIVERSITY OF QUEENSLAND, AUSTRALIA), LESLEY A ALTON (MONASH UNIVERSITY, AUSTRALIA), CRAIG E FRANKLIN (UNIVERSITY OF QUEENSLAND, AUSTRALIA)

As a consequence of rapid environmental change, the world is facing its sixth major biological extinction event. A recent surge in the rate of emergence of infectious diseases of wildlife has contributed significantly to this biodiversity crisis. In addition to the cost to biodiversity, there is a significant risk to human health from emerging infectious diseases with a wildlife origin (zoonoses), with more than 60% of recent human emerging infectious diseases being
zoonotic. Consequently, understanding the drivers underpinning the emergence of novel diseases in animals is important as a ‘first line of defence’ for managing the emergence of potential zoonotic diseases of humans. How organisms respond immunologically to pathogens and how their environment shapes this response is one consideration likely to determine the impact of emerging diseases, not only at the level of the organism, but at the community and species levels as well. Recent work in our laboratory has examined two key environmental drivers of physiological function, temperature and solar UV-B radiation, and their influence on various aspects of immune function and disease susceptibility in frogs and fish. In this presentation, we will present several examples of how these two key environmental factors affect or influence aspects of immune function and how the effect of one can be modulated by the presence of the other.

**APC1.19 MALE MONKEYS GET KICKED WHEN THEY’RE DOWN: INCREASED INJURY RATES DURING FEVERS**

*ROBYN HETEM (UNIVERSITY OF THE WITWATERSRAND, SOUTH AFRICA), RICHARD MCPARLAND (UNIVERSITY OF WISCONSIN–MADISON, UNITED STATES), DUNCAN MITCHELL (UNIVERSITY OF THE WITWATERSRAND, SOUTH AFRICA), SHANE K MALONEY (UNIVERSITY OF WESTERN AUSTRALIA, AUSTRALIA), PETER S HENZI (UNIVERSITY OF LETHBRIDGE, CANADA), LOUISE BARRETT (UNIVERSITY OF LETHBRIDGE, CANADA), CHRISTOPHER YOUNG (UNIVERSITY OF SOUTH AFRICA, SOUTH AFRICA), ANDREA FULLER (UNIVERSITY OF THE WITWATERSRAND, SOUTH AFRICA)*

Fever when there is sickness is a common mechanism employed by vertebrates to control the spread of pathogens and to fight infection. Fevers can modulate the metabolism of tissues, alter the circadian rhythms, and increase the level of sickness behaviors. Understanding these mechanisms and how they operate is important for understanding ecological implications of disease.

**APC1.40 LIFE ON A MISMATCHED DIET: LESSONS FROM FARMED ATLANTIC SALMON**

*THURSDAY 7 JULY, 2016  12:25*

*ELZBIETA KROL (UNIVERSITY OF ABERDEEN, UNITED KINGDOM), ALEX DOUGLAS (UNIVERSITY OF ABERDEEN, UNITED KINGDOM), CHRISTOPHER J SECOMBS (UNIVERSITY OF ABERDEEN, UNITED KINGDOM), SAMUEL AM MARTIN (UNIVERSITY OF ABERDEEN, UNITED KINGDOM)*

Increasing numbers of animals are exposed to diets they did not evolve to digest, absorb and utilise. These evolutionarily mismatched diets typically come with novel sets of toxins, antigens and microbial challenges, which have been implicated in the predisposition to gut inflammation (enteritis) and other gastrointestinal diseases in humans, zoo animals and pets, domesticated livestock and poultry. Evidence is also growing that many wild animals are facing dietary shifts and nutritional challenges resulting from global climate change. Understanding the impacts of mismatched diets on animal health and performance has been limited by the lack of rodent models for diet-induced enteritis. Here, we argue that important insights can be gained by studying carnivorous fish that are typically fed plant protein diets in aquaculture settings. We examined the gut transcriptome responses to different plant proteins in Atlantic salmon and demonstrated that these responses were plant-specific, with relatively few transcriptomic alterations common for all plant proteins used. When different plant proteins were simultaneously included in the diet, they induced less extensive alterations of the gut transcriptome than single plant protein diets. The mixed plant protein diets were also associated with improved body composition of fish relative to the single plant protein diets, providing evidence for a link between the magnitude of changes in the gut transcriptome and whole-animal performance. Our results indicate that farmed fish provide an attractive animal model for investigating the complex interactions between the digestive system and evolutionarily mismatched diets in vertebrates, at both whole-animal and molecular levels.
**APC1.20** PATHOGENS OF PLENTY: INTEGRATION OF MOLECULAR, PROTEOMIC, CELLULAR, AND ORGANISMAL-LEVEL ASSESSMENTS OF WILD MIGRATING SALMON TO DISCERN THE PATHOGENIC POTENTIAL OF DOZENS OF MICROBES

**KRISTI M MILLER (PACIFIC BIOLOGICAL STATION FISHERIES AND OCEANS CANADA, CANADA), ANGELA D SCHULZE (PACIFIC BIOLOGICAL STATION, CANADA), AMY TABATA (PACIFIC BIOLOGICAL STATION, CANADA), SHARONG LI (PACIFIC BIOLOGICAL STATION, CANADA), KARIA K KAUKINEN (PACIFIC BIOLOGICAL STATION, CANADA), EMILIANO DI CICCO (PACIFIC BIOLOGICAL STATION, CANADA), SCOTT G HINCH (UNIVERSITY OF BRITISH COLUMBIA, CANADA), NATHAN FUREY (UNIVERSITY OF BRITISH COLUMBIA, CANADA), ARTHUR BASS (UNIVERSITY OF BRITISH COLUMBIA, CANADA), AMY TEFFER (UNIVERSITY OF VICTORIA, CANADA), BRIAN RIDDLE (PACIFIC SALMON FOUNDATION, CANADA)**

Wild salmon populations have been declining across multiple species in many countries around the world. Cumulative and/or synergistic stressors affecting salmon in their vulnerable smolt out-migration stage are suspected to be important determinants of year-class strength, but which factors are most important is still not known. A role for infectious disease in salmon decline is suspected, but insufficient data exists on disease impacts on wild salmon to determine which, if any, diseases may cause substantial losses in the ocean. We devised a multidisciplinary program integrating broad-scale microfluidic monitoring with physiological impact assessments at the molecular, cellular and organismal levels to tackle these questions. Central to the research was the development of a high-throughput pathogen monitoring tool based on microfluidic quantitative PCR to simultaneously detect dozens of salmon pathogens. This platform is being applied to determine which pathogens causing diseases in salmon worldwide are carried by BC salmon, to assess their distributional shifts over time and space in wild, enhancement hatchery, and farmed salmon, and to identify pathogens associated with salmon survival in tracking, predation, and stress-challenge studies. Disease phenotypes are being identified by merging pathogen monitoring with host gene expression profiling and histopathology. Ultimately, this research will identify pathogens of greatest biosecurity risk to wild salmon.

**APC1.21** LATE-PROGRESSION AMOEbic GILL DISEASE IMPAIRS TEMPERATURE TOLERANCE IN INFECTED ATLANTIC SALMON (SALMO SALAR)

**TUESDAY 5 JULY, 2016  14:20**

**ALYSSA BOWDEN (IMAS UNIVERSITY OF TASMANIA, AUSTRALIA), T D CLARK (IMAS UNIVERSITY OF TASMANIA, AUSTRALIA), S J ANDREWARTHA (CSIRO AGRICULTURE, AUSTRALIA), N ELLIOTT (CSIRO AGRICULTURE, AUSTRALIA), P FRAPPELL (IMAS UNIVERSITY OF TASMANIA, AUSTRALIA)**

Amoebic gill disease (AGD) is the most prevalent health issue affecting Atlantic salmon industries in southeast Tasmania and is a major cause of mortalities in farmed populations. The amoeba attaches solely to the gills and cause hyperplastic lesions which generally lead to lamellar fusion. AGD-associated mortality is presumed to be related to respiratory failure due to the loss of functional gill area, but this has yet to be conclusively confirmed. Tasmanian outbreaks proliferate in the summer months in parallel with increasing temperature. Infected fish suffer higher mortality rates at high temperatures, so this study took the first step to investigating the host response to AGD under elevated temperatures through a critical thermal maxima (CTmax) test. It was hypothesised that infected individuals would be less tolerant to elevated temperatures than naïve fish. Water temperature was increased at a rate of 2°C/hr and CTmax was recorded at loss of equilibrium. Subsequently, fish were gill scored using the standard farm criteria to determine level of infection and blood samples were taken to measure blood parameters and stress hormones. Preliminary results support the hypothesis of lowered thermal tolerance in diseased individuals, but this was only manifest once the disease had progressed substantially such that gill scores reached high values of 4–5.

**APC1.22** LONG-TERM ENVIRONMENTAL INFLUENCE UPON HYPOXIA TOLERANCE IN FISH: DOES THE CARDIORESPIRATORY SYSTEM PLAY A ROLE?

**TUESDAY 5 JULY, 2016  14:35**

**GUY CLAIREAUX (UNIVERSITÉ DE BRETAGNE OCCIDENTALE, FRANCE), FLORIAN MAUDUIT (UNIVERSITÉ DE BRETAGNE OCCIDENTALE, FRANCE), HÉLÈNE OLLIVIER (UNIVERSITÉ DE BRETAGNE OCCIDENTALE, FRANCE), NICOLAS LE BAYON (IFREMER, FRANCE), OLIVIER MOUCHEL (IFREMER, FRANCE), JOSÉ L ZAMBONINO (IFREMER, FRANCE)**

Using a population of 400 individually tagged European seabass we designed an experiment with the objective of travelling down the levels of biological complexity to highlight some entry points of the environmental influence upon fish tolerance to hypoxia. This experiment spanned over 2 years and targeted the cardiorespiratory system. Young-of-the-year seabass were submitted to a hypoxia challenge test which allowed the determination of individuals’ incipient lethal oxygen saturation. Our experimental population
was then divided into two subgroups. One subgroup was kept in the laboratory (LAB), while the second was transferred to semi natural tidal ponds (POND) for a period of six months. Fish were then recovered from the ponds, brought back to the laboratory and regruped with those that remained in the laboratory. After 4 months under common garden conditions, we observed that fish from the POND group displayed higher tolerance to hypoxia than fish from the LAB group, and that this difference persisted after 1 year. Respirometry showed no difference between LAB and POND with regards to maximal and standard metabolic rates as well as critical oxygen level. ECG of anaesthetised individuals submitted to a standardized hypoxia revealed that fish from POND and LAB groups responded differently. However, maximal heart rates measured following atripine injection were similar. Ventricular strip preparations tested under hypoxic conditions showed no difference between POND and LAB. We conclude that the cardiorespiratory system poorly explains the improved by hypoxia tolerance observed in the POND. Capacity for metabolic depression and anaerobic metabolism will have to be examined.

APC1.23 PHENANTHRENE IS THE CARDIOTOXIC POLYCYCLIC AROMATIC HYDROCARBON

TUESDAY 5 JULY, 2016  14:50

HOLLY SHIELS (UNIVERSITY OF MANCHESTER, UNITED KINGDOM), FABIEN BRETTE (UNIVERSITY OF BORDEAUX, FRANCE), GINA GALLI (UNIVERSITY OF MANCHESTER, UNITED KINGDOM), CAROLINE CROS (UNIVERSITY OF BORDEAUX, FRANCE), JOHN INCARDONA (NOAA, UNITED STATES), NATHANIEL SCHOLZ (NOAA, UNITED STATES), BARBARA BLOCK (STANFORD UNIVERSITY, UNITED STATES)

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The Deepwater Horizon disaster drew global attention to the toxicity of crude oil and the potential for adverse health effects among spill responders and the numerous animals in the northern Gulf of Mexico. Crude oil from the spill released complex mixtures of polycyclic aromatic hydrocarbons (PAHs) into marine areas including pelagic spawning habitats, for example, billfish, and other ecologically important top predators. PAH exposure of whole fishes during development and exposure to heart cells from adults, reveals the heart is vulnerable to oil toxicity. However, the precise PAHs that cause cardiotoxicity, as well as the mechanisms underlying contractile dysfunction, are not known. Here we used electrophysiological and confocal microscopy techniques in tunas (Pacific bluefin tuna, Thunnus orientalis, yellowfin tuna, Thunnus albacares) and Pacific mackerel (Scomber japonicus) to demonstrate that phenanthrene, a PAH with a benzenoid ring structure, is the key compound disrupting cardiac function. Phenanthrene prolongs the action potential due to potassium channel blockade and decreases the amplitude of the cellular Ca2+ transients that drive force generation. Because there are many important environmental sources of phenanthrene in addition to petroleum based oil spills, including urban air pollution, our findings suggest that phenanthrene may be a major worldwide cause of vertebrate cardiac dysfunction.

APC1.30 THE ENERGETIC TRIANGLE: A PHYSIOLOGY-BASED LIFE-HISTORY CLASSIFICATION SCHEME FOR REEF CORALS

TUESDAY 5 JULY, 2016  16:00

MIA HOOGENBOOM (JAMES COOK UNIVERSITY, AUSTRALIA)

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Conserving and restoring ecosystems requires understanding of how environmental changes affect populations and communities. Many of the environmental changes associated with global warming impact on organisms at the physiological level and, therefore, predicting changes in population dynamics and community structure requires the effects of environmental change on the physiology and demographics of individuals to be quantified. Here I describe a novel functional classification of stony corals, the ecosystem engineers of coral reefs, that is based explicitly on physiological energetics and that helps to bridge between effects on individual organisms and communities. All of the myriad functional classification schemes described in the literature are founded on the underlying concept that organisms apportion resources between reproduction, survival and growth. However, few of these schemes explicitly link organismal traits to energy allocation. In this study I present new data, and review several decades of literature data, to identify patterns of energy acquisition and allocation among stony corals, and to ordinate species based on their energy allocation to structural tissue biomass, reproductive tissues and skeleton growth (a metric for space acquisition by these organisms). I then explore how the position of species in this ordination changes as individuals grow over time and, using general relationships between temperature and metabolic rates, I demonstrate how patterns of energy allocation might change to maintain e.g. growth at the expense of reproduction in a changing environment.

APC1.31 METABOLIC AND GROWTH EFFECTS OF DAILY ACUTE HEAT CHALLENGE ON A COLD WATER PISCIVORE

TUESDAY 5 JULY, 2016  16:30

MATTHEW GUZZO (UNIVERSITY OF MANITOBA, CANADA), NEIL MOCHNACZ (FISHERIES AND OCEANS CANADA, CANADA), TRAVIS DURHACK (FISHERIES AND OCEANS CANADA, CANADA), BENJAMIN KISSINGER (UNIVERSITY OF MANITOBA, CANADA), JASON TREBERG (UNIVERSITY OF MANITOBA, CANADA)

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Temperature is an important environmental factor influencing fish physiology. In small north-temperate lakes, nearshore prey makes up the majority of cold-water piscivore diets; however, because these lakes thermally-stratify during summer, nearshore regions often exceed the optimal temperature limits for most cold water piscivores, but not for the prey. To cope, piscivores make short excursions into the nearshore to feed and quickly return to cold water to digest. With air temperatures predicted to increase, many historically-isothermal northern lakes may begin to stratify and lakes that already stratify may achieve longer, warmer stratified periods. To understand the impact of these limnological changes on cold water fish, we performed lab experiments to test: (1) if daily short excursions into...
supra-optimal temperatures impact growth and metabolic rate; and, (2) if increasing supra-optimal temperatures would strengthen impacts. Juvenile lake char (Salvelinus namaycush) were held at \(-10^\circ\text{C}\) (optimum temperature) with treatments exposed to 17 or 22 \(^\circ\text{C}\) for ~5 min daily over 64 days. All groups were offered a ration of ~1.5 % body mass daily. Heat exposures were mild but recurrent, with treatments fish subjected to supra-optimal temperatures for only ~1% of the experimental duration. Control fish consumed more food than the daily heat challenged fish but growth was similar across treatments suggesting the daily acute heat challenge may increase food conversion efficiency. Standard metabolic rate was similar among treatments; however, control fish had lower maximum metabolic rates and lower metabolic scope than fish that were exposed to warm water.

**APC1.32 ARE RESPIRATORY EFFECTS OF GLOBAL WARMING AND OCEAN ACIDIFICATION EXPLAINED BY A UNIFYING OCLTT CONCEPT?**

**TUESDAY 5 JULY, 2016 ☑️ 16:45**

**S.JANNIE LEFEVRE (UNIVERSITY OF OSLO, NORWAY)**

The changing climate prompts a desire to understand and thereby need to study and ultimately predict the outcome for marine ectothermic animals. The concept of “Oxygen and Capacity Limited Thermal Tolerance” (OCLTT), which is inspired by the Fry paradigm of a bell-shaped “increase-optimum-decrease” type response of absolute aerobic scope (AAS) to increasing temperature, while also including proposed negative and synergistic effects of elevated CO₂ levels, has been suggested as a unifying framework. In my talk, I will present a meta-analysis of available data examining the following questions: does AAS in general follow a bell-shaped curve i.e. is there always an optimum temperature? Does CO₂ in general cause an increase in resting oxygen demand (O₂rest), and thereby reduce AAS? And is the combined effect of CO₂ and temperature on AAS generally larger than expected from their sum i.e. is the interaction synergistic? I calculated log response ratios to be able to compare results from a wide range of studies, including both fish and invertebrates. When examining the data as a whole, from the perspective of the above predictions, it becomes evident that there is a heterogeneity that is difficult to reconcile with the idea of a single unifying principle. While it is clear that climate change can have severe physiological effects, and that AAS might be a useful variable for predicting the outcomes in some cases, malfunction of other physiological mechanisms must be considered and generalizations such as the OCLTT concept should be used with caution. This presentation will explore the role of experimental biology in evidence-based conservation.

**APC1.45 WHY IS THE HATCHING SUCCESS OF GREEN TURTLES ON RAINE ISLAND SO LOW?**

**TUESDAY 5 JULY, 2016 ☑️ 17:00**

**DAVID T BOOTH (UNIVERSITY OF QUEENSLAND, AUSTRALIA), ANDY DUNSTAN (QUEENSLAND GOVERNMENT, AUSTRALIA)**

Raine Island is the biggest green turtle rookery in the world. However, in recent years the hatching success of nests has been exceeding low < 30%, with considerable death occurring very early in incubation. Initially increased frequency of nests being inundated with sea water at high tides due to sand loss from the beach was hypothesised to be responsible for this high within nest mortality. However, beach manipulation that increased the level of nests well above the water inundation level also resulted in a high level of within nest mortality. We hypothesize that either low oxygen gas tensions and/or high microbial load may be responsible for high within nest mortality and have begun measurements to investigate if low oxygen tensions are associated with high mortality. Interestingly, hatching success increased remarkably in a low nest density year, suggesting that a nest density dependent process is involved in determining the within nest mortality of developing embryos.

**APC1.34 SENTINEL OYSTERS: MONITORING REAL-TIME PHYSIOLOGY TO INFORM AQUACULTURE PRODUCTION**

**THURSDAY 7 JULY, 2016 ☑️ 09:40**

**SARAH ANDREWARTHA (CSIRO AGRICULTURE, AUSTRALIA), JOHN W MCCULLOCH (CSIRO MARINE LABORATORIES, AUSTRALIA), ANDREW HELLICAR (CSIRO MARINE LABORATORIES, AUSTRALIA), PETER B FRAPPELL (CSIRO AGRICULTURE, AUSTRALIA), NICK G ELLIOTT (UNIVERSITY OF TASMANIA, AUSTRALIA)**

Monitoring stock welfare and productivity remains a key challenge for most aquaculture sectors. Integrating sentinel animals fitted with biosensors that monitor heart rate and other relevant variables with environmental sensing can address this challenge. Timely estimates of daily energy expenditure and indicators of stress/pathology resulting from multi-parameter environmental changes can be provided and enable management actions. A thorough understanding of how physiology and behaviour respond to the variety of environmental and production stressors experienced is required to enable on-farm data to be interpreted. Here we present the effects of temperature, salinity and dissolved oxygen on the relationship between heart rate and metabolic rate in summer acclimated Pacific oysters (Crassostrea gigas). The laboratory calibration data are used to predict daily energy expenditure in sentinel oysters in two locations: on a commercial lease and in a more estuarine environment. Integrating these into production and wellbeing models drive decision support systems that predict animal condition and wellbeing in the context of current and projected environmental conditions.
a methodology to evaluate health in fish populations. We first applied high-throughput, non-lethal challenge tests on a population of 700 juveniles of sea bass to assess hypoxia tolerance, temperature susceptibility and critical swimming speed as proxies of individuals’ functional integrity. Experimental population was then transferred into semi-natural tidal ponds and correlates of Darwinian fitness (growth and survival) were monitored over a period of 4 months. We found that hypoxia tolerance and swimming capacity, but not temperature susceptibility, were predictive of fish ecological performance in the field. However, we have identified several pitfalls that must be taken into consideration. Interpretation of fish responses to health assessment tests must take into consideration such as inter-population variability, the environmental shaping of phenotypic diversity and the modulating effect of behaviour and learning.
**APC1.38 MODELLING THE SPREAD OF PARASITOID WASPS FROM POINT RELEASE**

**THURSDAY 7 JULY, 2016  11:25**

- CHRISTOPHER STRICKLAND (SAMSI AND THE UNIVERSITY OF NORTH CAROLINA CHAPEL HILL, UNITED STATES), NADIAH P KRISTENSEN (NATIONAL UNIVERSITY OF SINGAPORE, SINGAPORE), LAURA MILLER (UNIVERSITY OF NORTH CAROLINA CHAPEL HILL, UNITED STATES)

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Parasitic hymenoptera are a group of insects which are critical for biological pest control and increasingly being used in agriculture to protect crops via direct release. However, due to their small size (often less than 1 mm), movement and long-distance dispersal of these wasps have long been poorly understood and likely underestimated. Recent data collected by Kristensen et al. (2013) on the wind-borne dispersal pattern of Eretmocerus hayati (0.7 mm long) provides a new and significant opportunity to finally develop a detailed, validated, multi-scale model for the initial spread of invasive insects and biological control introductions. In this talk, I will present a new mathematical model for parasitoid wasp dispersal from point release, as in the case of biocontrol. The model is derived from underlying stochastic processes and, as a special case of the Fokker-Planck equation, is fully deterministic. The Python implementation of this model is capable of running month-long simulations on the scale of 15 km² while maintaining a resolution of 10 m², all within two minutes on a common workstation. Speed is an essential component to our model because it allows flexibility in fitting parameters to data. Validation of the model includes comparison with two multi-scale, first-release datasets described in Kristensen et al. (2013).

**APC1.39 CONSERVING IMPERILED FISHES: FINDING SOLUTIONS THROUGH PHYSIOLOGICAL AND BEHAVIORAL STUDIES**

**THURSDAY 7 JULY, 2016  13:50**

- NANN A FANGUE (UNIVERSITY OF CALIFORNIA DAVIS, UNITED STATES), DENNIS E COCHERELL (UNIVERSITY OF CALIFORNIA DAVIS, UNITED STATES), JAMILYNN B POLETTO (UNIVERSITY OF CALIFORNIA DAVIS, UNITED STATES)

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Approximately 80% of freshwater fishes are facing extinction over the next 100 years, and in California, native freshwater and anadromous fishes reflect this trend. Anthropogenic threats to species viability can alter habitats beyond native species’ environmental tolerances and may result in extirpation. California has a long history of habitat fragmentation and degradation (i.e., the presence of dams, and water withdrawals associated with agricultural and urban use), and in combination with climate change, suitable habitat for fishes has been greatly reduced. In this talk, I will highlight results from two main areas of research. The first addresses the impact of water diversions on the susceptibility of several California native fishes to entrainment (i.e., becoming sucked into diversion pumps and structures), as well as the evaluation of the efficacy of behavioral deterrents designed to minimize entrainment. In the second example, I will discuss a variety of approaches that we use to define suitable thermal habitat for native fishes, as well as the challenges associated with then translating these measures of thermal performance into regulatory numeric criteria. In both examples, I will emphasize how we transform our physiological and behavioral results into actionable outcomes for fishes in order to promote conservation and achieve biodiversity goals.
**APC1.41** EFFECTS OF TEMPERATURE AND FEEDING RATE ON THE GROWTH OF LARVAL GREEN STURGEON: IMPLICATIONS FOR SURVIVAL OF EARLY LIFE STAGES

**THURSDAY 7 JULY, 2016  0  14:30**

JAMILYN B. POLETTO (UNIVERSITY OF CALIFORNIA DAVIS, UNITED STATES), BENJAMIN MARTIN (NOAA SOUTHWEST FISHERIES SCIENCE CENTER, UNITED STATES), ERIC DANNER (NOAA SOUTHWEST FISHERIES SCIENCE CENTER, UNITED STATES), DENNIS E. COCHERELL (UNIVERSITY OF CALIFORNIA DAVIS, UNITED STATES), JOSEPH J. CECH JR. (UNIVERSITY OF CALIFORNIA DAVIS, UNITED STATES), NANN A. FANGUE (UNIVERSITY OF CALIFORNIA DAVIS, UNITED STATES)

Both temperature and food availability have significant effects on the growth and survival of native fishes, particularly during early developmental stages. Therefore, we reared larval green sturgeon (initial age ca. 27 days post hatch) at four different temperatures (11, 14, 17, and 20°C) and two different food rates (100% and 25% of optimal) to assess the effects of these stressors and their interactions on larval growth. We compared the overall size (fork length, total length, and mass), specific growth rate (cm/day), and condition factor of larval fish after being held in rearing conditions for 3 and 6 weeks. Our results can be used to develop models of the early life history requirements of green sturgeon and to inform management actions seeking to increase larval and juvenile recruitment success.

**APC1.42** IMPROVING FISH PASSAGE THROUGH CULVERTS: INTEGRATION OF HYDRODYNAMICS AND SWIMMING PERFORMANCE

**THURSDAY 7 JULY, 2016  0  14:45**

CRAIG E FRANKLIN (THE UNIVERSITY OF QUEENSLAND, AUSTRALIA), HUBERT CHASON (THE UNIVERSITY OF QUEENSLAND, AUSTRALIA), REBECCA L CRAMP (THE UNIVERSITY OF QUEENSLAND, AUSTRALIA), MATTHEW GORDOS (NSW DPI FISHERIES, AUSTRALIA), PIPPA KERN (THE UNIVERSITY OF QUEENSLAND, AUSTRALIA), ESSIE M RODGERS (THE UNIVERSITY OF QUEENSLAND, AUSTRALIA), CAROLINE THOMPSON (THE UNIVERSITY OF QUEENSLAND, AUSTRALIA)

A key component of river and stream management is to ensure that native migratory fish have unimpeded access to required habitat to complete necessary life cycle stages. Regrettably, over the past two centuries, man-made instream structures (e.g. dams, weirs, and road crossings) have significantly impeded habitat connectivity and have created barriers to fish movement. While the impacts of large dams and weirs on fish populations are well acknowledged, smaller-scale in-stream structures like waterway crossing culverts have until recently been overlooked despite having as great or greater impact on fish populations. To address these concerns, many agencies responsible for healthy waterways have created guidelines for the design and construction of fish-friendly culverts.

However, problematically these guidelines are often based on limited empirical data from a few select large-bodied fish species, or come from anecdotal information from field observations. A critical factor in considering the design and effectiveness of waterways crossings in passing native fish is quantifying the swimming performance of fish, and in particular their ability to swim against the artificial flows created by instream structures. In this study, biologists and engineers have been integrating data on the swimming ability of Australian fish species with culvert hydrodynamic modelling to better understand fish requirements in and around road crossings. Results will also be presented on the effects of culvert roughening on swimming performance. These data are being used to strengthen national design guidelines and provide the tools engineers and planners need to balance fish migration with effective water management.

**APC1.43** FOG, FORAGING AND THE FUTURE OF TWO SYMPATRIC LIZARD SPECIES IN THE NAMIB DESERT

**THURSDAY 7 JULY, 2016  0  15:45**

DUNCAN MITCHELL (UNIVERSITY OF THE WITWATERSRAND, SOUTH AFRICA), IAN W MURRAY (UNIVERSITY OF THE WITWATERSRAND, SOUTH AFRICA), STEPHAN WOODBORNE (ITHEMBA LABORATORIES AND UNIVERSITY OF PRETORIA, SOUTH AFRICA), ANDREA FULLER (UNIVERSITY OF THE WITWATERSRAND, SOUTH AFRICA), HILARY M LEASE (WHITMAN COLLEGE, UNITED STATES), ROBYN S HETEM (UNIVERSITY OF THE WITWATERSRAND, SOUTH AFRICA)

Rhoptropus bradfieldi and Pedioplanis husabensis are endemic lizards of similar size (snout-vent length ~50mm) that live in the same rocky Namib habitat. Both are threatened by climate change and by uranium mining. We studied their activity, body temperature, body water turnover using $\delta^18O$, and diet using $\delta^13C$ and $\delta^15N$ analysis of body tissues and items in their trophic cascade. We noosed stationary lizards (31–62 in a cohort) in summer and autumn. At lower ambient temperatures, R. bradfieldi was significantly cooler than was P. husabensis, the difference resulting mainly from their foraging behaviour and consequently their microclimate. R. bradfieldi is a sit-and-wait forager on rocks, while P. husabensis is an active forager, venturing off rocks onto nearby hot sand. Stable isotope analysis showed that the summer diet of P. husabensis was 63% termites, and that of R. bradfieldi 58% ants and 35% wasps. The high water content of termites allowed metabolic and pre-formed water to exceed the water needs of P. husabensis, but metabolic and pre-formed water contributed only 30% of the daily water influx rate (0.07 mL $^{-1}$) of R. bradfieldi. Consequently R. bradfieldi must have been consuming free water, with advective fog the most likely source. The prey of both species was deriving energy almost entirely from CAM plants, presumably mainly grasses. If, under climate change, fogs become less frequent and regular, the future for R. bradfieldi is bleak. Also, climate change will lead to a loss of CAM plants, potentially compromising the future of both species.
The influence of copper pollution on the natural environment constantly increases. This trace element accumulates in plants and water what may be especially dangerous for herbivores.

In contaminated areas, lower density of small rodents is observed. This decline of rodent number may be caused by decline in reproductive abilities or dysfunctional sexual behavior. My previous research has proved, that copper mainly debilitates males’ reproductive system and prolongs sexual maturation.

The aim of the presented study was to assess the effect of copper exposure on small rodents’ sexual behavior using bank vole (Myodes glareolus, Schreber 1780) as a model species.

In the presented research, copper was administered at concentrations similar to those recorded in industrial districts (Cu I 1-150 mg/kg, Cu II 600 mg/kg, C-control). After 12 weeks of copper exposure, the preference test was performed; female behavior was recorded in the following combinations: Cu 1 & C, Cu 2 & C, Cu I vs C, Cu II vs C. The assessed parameters were: total activity, number of approaches and sniffs and the time of sniffing. Additionally, the behavior and vocalization of male-female pairs were recorded during open-field tests in the following combinations: Cvs C, Cvs Cu I, Cvs Cu II. The assessed parameters were: latency to first approach, attack, number of aggressive and non-aggressive approaches, and sniffs and ultrasonic calls.

Results have shown that copper did not affect intersexual behaviors. However, the preference tests proven that copper, especially in higher concentration, decreased males’ sexual attractiveness depicted by the decline of total activity, number of female approaches and sniffs.

An increase in the partial pressure of oxygen, at the time of oviposition, is the trigger that breaks turtle eggs from pre-ovipositional embryonic arrest. Significant levels of mortality occur when eggs are moved within 12 hours to 14 days after oviposition in some species. This movement-induced mortality is likely to be linked to the recommencement of embryonic development. We aimed to identify the amount of time an embryo takes to recommence development following oviposition and whether embryos can re-enter embryonic arrest. Green sea turtle (Chelonia mydas) eggs were randomly placed into hypoxia at one of ten time intervals between 30 mins to 72 hrs post oviposition. Each treatment group remained in hypoxia for three days. An 11th group of eggs remained in normoxia throughout incubation, serving as a control for the experiment. Development and survival rate of embryos was then compared to determine both the time taken to break arrest and ability to re-enter arrest. Our experiment identified that, after oviposition, between 12 to 16 hours of exposure to normoxic conditions is required to recommence embryonic development in green sea turtles. The information gained from this experiment enables turtle conservation groups to better understand the ideal time to relocate eggs. In addition, the experiment also revealed that green sea turtle embryos are unable to re-enter a state of arrest following recommencement of development. The findings of this research have the potential to improve conservation outcomes, through reducing rates of embryonic death or reproductive failure during translocation.

Appropriate regulation of energy balance is crucial for health, fitness and survival. Evidence in humans and other mammals suggests persistent organic pollutants (POPs) can disrupt energy balance, and alter adiponectin levels, contributing to obesity related disorders. Seals experience high POP burdens as a result of their position as top predators and the bioaccumulation and magnification of POPs up the food chain. We investigated whether a suite of 11 circulating POPs, including the pesticides lindane, DDT and its metabolites, PCBs and PBDEs were associated with plasma adiponectin levels, blubber adiponectin mRNA abundance or mass changes in suckling and fasting grey seal pups (n=9). After accounting for differences between feeding and fasting, adiponectin levels were positively related to circulating CB101 levels (LM: p = 0.0323). Adiponectin mRNA abundance was not related to circulating POP concentrations. Mass increase rate during suckling was positively related to maternal postpartum mass (LM: T = 0.328; F(1,7) = 10.76; R² = 0.5495; p = 0.0135) and none of the POPs improved the model fit. CB52 was negatively related to weaning mass (LM: T = 3.063; F(1,7) = 9.38; R² = 0.54; p = 0.0138). Our data suggest POPs disrupt normal energy balance in grey seal pups. Altered adiponectin levels may influence inflammatory responses and insulin sensitivity, and lower weaning mass may impact on survival. Causal relationships between POPs and energy balance effects and their consequences require investigation in a larger sample, and mechanisms through which POPs alter energy balance in seals need to be identified.
In 2010, the Deepwater Horizon oil spill released 210 million gallons of crude oil into the Gulf of Mexico. Crude oil has been shown to have cardiotoxic effects on fish and prior work on mahi mahi has highlighted a reduced cardiac output in response to short-term oil exposure. We investigated the effects of acute and chronic crude oil exposure on the contractility of isolated cardiac tissue from adult mahi mahi and used chemical inhibitors to identify any effect on calcium cycling targeting the sarcoplasmic reticulum and adrenergic signalling pathways. Direct exposure of cardiac muscle to high energy water accommodated fractions (HEWAF) of crude oil at 10% concentration had no effect on muscle contractility. Further, cardiac tissue collected from fish exposed to 10% HEWAF solution for 24h did not display reduced contractility, compared with controls. Concurrent with this, there was no difference in the contribution of the sarcoplasmic reticulum to contractility between fish exposed to HEWAF for 24h and unexposed fish. Our data is consistent with the effects of oil on cellular calcium cycling in mahi mahi but contrary to data previously obtained for bluefin tuna and suggests that the reduced cardiac output previously observed in juvenile mahi mahi after oil exposure may result from some factor other than contractility.

Macroalgal blooms (‘green tides’) are a growing problem on many coastlines worldwide, including here in the UK. These blooms can suffocate marine life and decimate local populations, but their environmental and anthropogenic drivers remain poorly understood. Accordingly, we are using the bloom-forming green seaweed species, Ulva spp. (a.k.a. ‘sea lettuce’) to understand how the physiology of their growth and reproduction can both lead to blooms and be exploited for conservation. Using a combination of mathematical modelling, high-resolution imaging, and molecular approaches, we ask two main questions: a) how do physiological responses at the cellular scale drive seaweed thallus growth and b) how can patterns of thallus growth inform marine conservation efforts? Currently, the main method of tracking the growth of macroalgal biomass is its physical removal and measurement on weighing scales. This method is limited by its poor temporal resolution and intrusiveness. We have therefore developed an optical imaging system linked to automated image analysis software to allow real-time measurement of circadian growth rhythms in response to the environmental factors that determine Ulva spp. proliferation. These macroscopic growth results are supplemented by Light Sheet Microscopy data of stained samples, which correlates overall growth rates with patterns of cellular size and distributions. This combination of methods provides much greater resolution of the growth responses of Ulva in various conditions; this is now being linked to global bloom formation using hydrodynamic models.
One of the hypotheses explaining species invasiveness states that invasive species demonstrate rapid response to natural selection. Thus, we can expect differences in fitness related traits between populations of invasive species from latitudes (different climatic regions). We measured growth rate and metabolic rate, both traits that influence other traits such as development time and body size, and thus fitness of an individual.

*Arión vulgaris* is regarded as one of the 100 most invasive species in Europe. We collected adult specimens of *A. vulgaris* in 12 sites across Europe (from Northern Norway to Southern France) transferred them to identical conditions of 15°C (optimal temperature for this species), 80% RH and standard food and let them reproduce. Similar aged juveniles were kept individually and weighed regularly to estimate growth rate and respiration rate was measured at rearing conditions.

In our common garden experiment we observed significant population differences in standard metabolic rate (SMR) statistically corrected for body mass. Individuals derived from the most Northern population had the lowest SMR, but growth rate was intermediate and did not differ from other populations. In general, we did not observe clear patterns in SMR and growth rate between populations that origin from different latitude. Although, we don’t observe clear latitudinal patterns in our common garden experiment our result of progeny growth and SMR of the most extreme Northern population suggest physiological adaptations to new environments, which may support invasive potential of *Arión vulgaris*.
Sustained crop yields are dependent on inorganic fertiliser application, but it comes at a high price, both in the cost of the fertiliser and the environmental damage that results from its use.

A number of plant species have evolved beneficial interactions with micro-organisms that facilitate the uptake of nutrients. Legumes form symbiotic interactions with mycorrhizal fungi that facilitate phosphate uptake and with rhizobial bacteria that provide the plant with a source of nitrogen. The establishment of these symbioses involves a molecular communication between the plant and the symbiotic micro-organisms in the soil. Mycorrhizal fungi and rhizobial bacteria release signals that are recognised by the host plant and lead to developmental changes associated with the accommodation of the symbionts. Genetic dissection in the legume Medicago truncatula has defined the signalling pathways involved in these symbioses. A number of the genes required for the mycorrhizal interaction are also necessary for the rhizobial interaction, indicating a conserved symbiosis signalling pathway. This implies that the evolution of nodulation involved the recruitment of a signalling pathway already functioning in mycorrhizal interactions. This signalling pathway is present in most plant species, including cereals suggesting that engineering the perception of rhizobial bacteria in cereals is simplified and requires an understanding of the legume specific components that activate and are activated by the common symbiosis signalling pathway. We are in the process of engineering this signalling pathway in cereals to promote the recognition of rhizobial bacteria as the first step in engineering biological nitrogen fixation into cereal crops.
**PC1.4 RE-WIRING SIGNALING PATHWAYS TO ENHANCE STRESS TOLERANCE**

**Tuesday 5 July, 2016  15:05**

Iulia Gherman (University of Warwick, United Kingdom), Christopher Penfold (University of Oxford, United Kingdom), Mathias Foo (University of Warwick, United Kingdom), David Wild (University of Warwick, United Kingdom), Declan Bates (University of Warwick, United Kingdom), Katherine Denby (University of Warwick, United Kingdom)

I.Gherman@warwick.ac.uk

Abiotic and biotic stress conditions result in large-scale transcriptional reprogramming in plants. We are elucidating these complex regulatory networks from experimentally derived time series data of Arabidopsis leaves after infection with bacterial and fungal pathogens, as well as during senescence. Using a combination of network inference algorithms together with biological knockout and yeast one-hybrid data, we have constructed a high-confidence transcription factor network for each stress individually. This allows us to identify key regulators of the Arabidopsis stress response, as well as genes that are co-regulated across multiple stresses. Knockouts and overexpression of individual transcription factors can be simulated to determine the impact these perturbations have on the rest of the network. Algorithms such as Approximate Bayesian Computation allow us to test different combinations of rewiring the network to achieve the desired gene expression in our key regulators. This is done with the goal of establishing the rewirings that enhance the plant’s defence response to pathogens. In order to validate the conclusions of our network and re-wiring simulations, the target transcription factors will be expressed in a protoplast system under different spatial and temporal promoters. After a few rounds of the design-build-test cycle, the experiments will be performed in Arabidopsis plants and phenotypes with enhanced resistance will be selected for.

**PC1.5 SYNTHETIC APPROACHES TO EXPLORING EVOLUTION AND DEVELOPMENT**

**Tuesday 5 July, 2016  16:00**

Jamie A Davies (University of Edinburgh, United Kingdom)

@JAMIE.DAVIES@ED.AC.UK

Most biology is analytical and aims to understand life as it evolved on Earth. Researchers study organisms, make hypotheses, and test them experimentally usually by perturbing a component with mutation or drugs. The results of many such experiments are brought together in textbooks to formulate general principles, much simpler than the complicated mechanisms by which they are realized in specific instances. A robust way to test correctness of these ideas is to build something that uses them in their simple form, and test whether it works. Recently, a small number of synthetic biologists have started to apply this idea to embryology. They have constructed modules, based on developmental principles, to programme naive cells to make patterns in response to gradients or completely de-novo, and to perform simple morphogenetic tasks: there are even primitive working examples of patterning-followed-by-morphogenesis, driven entirely by designed genetic modules. As well as having applications in tissue engineering, this approach provides a powerful test-bed for basic ideas about developmental mechanisms. Furthermore, interesting evo-devo insights may come from exploration of ‘roads not taken’, through constructing invented mechanisms that work but seem not to have arisen in evolved life. This presentation will illustrate these approaches, drawing from our own work and that of others.

**PC1.6 SYNTHETIC CELL-BASED SENSORS WITH PROGRAMMED SENSITIVITY, SELECTIVITY AND DYNAMIC RANGE**

**Tuesday 5 July, 2016  16:40**

Baoyun Wang (University of Edinburgh, United Kingdom)

@BAOJUN.WANG@ED.AC.UK

We use single bacterial cells, programmed with engineered modular genetic sensors and digital logic or analogue amplifying gene circuits, to sense, integrate and amplify multiple customized environment and health related signals. We have shown that these engineered gene circuits can predictably and significantly increase the selectivity, sensitivity and output dynamic range of cellular sensors for toxic heavy metal ions including arsenic and mercury in an aqueous environment. By example, we engineered a selective double-input AND gate sensor for zinc in E. coli in which the AND gate functions as a filter by filtering out the nonspecific signals from the two promiscuous zinc input sensors. We next engineered a set of orthogonal activator-based high-gain transcriptional amplifiers, and cascaded them in multiple layers to tandem amplify transduced sensor signal to increase sensitivity and output dynamic range. With the amplifier-cascade, we increased the sensitivity > 4 0 0 0 fold with detection limit < 0.01 ppb, and the output dynamic range 500 fold for a mercury sensor (WHO safe limit 2 ppb). Our approach is modular and can be readily applied to improving the sensing limit and performance of a range of cellular sensors to meet their real world detection requirement in environment and healthcare.
PC1.8 REFINEMENT OF THE PHYTOBRICKS STANDARDS FOR PLANT SYNTHETIC BIOLOGY

**WEDNESDAY 6 JULY, 2016  09:40**

**DIEGO ORZAEZ** (IBMCP-UPV-CSIC, SPAIN), MARTA VAZQUEZ- VILAR (IBMCP-CSIC, SPAIN), ASUN FERNANDEZ-DEL- CARMEN (IBMCP-CSIC, SPAIN), JOAN BERNABE (IBMCP- CSIC, SPAIN), ALFREDO QUIJANO (IBMCP-CSIC, SPAIN), ALEJANDRO SARRION-PERDIGONES (IBMCP-CSIC, SPAIN), ROCIO OCHOA (IBMCP-CSIC, SPAIN), PEIO ZIARSOLO (UPV, SPAIN), JOSE BLANCA (UPV, SPAIN), ANTONIO GRANELL (IBMCP-CSIC, SPAIN)

Modular DNA cloning has been quickly embraced by the Plant Biotechnology community due to its efficiency in resolving multigene engineering goals. The adoption of common rules for describing synthetic DNA parts (phytobricks) and for the composition of multigene modules has been proposed as an optimal strategy for speeding up advancements in the field. Recent efforts in this direction include the adoption of a Plant Standard Syntax for Modular Cloning and the development of collections of synthetic parts. Here we propose further refinements in the characterization of phytobricks with the definition of standard experimental conditions and the registering of the information obtained under such conditions as metadata for the description of synthetic DNA elements. To incorporate these new features, we developed a new version (v3.0) of the GoldenBraid assembly system and database, now incorporating experimental data. Consequently, Phytobricks in GB3.0 display associated datasets that inform of their assembly genealogy and also about their functionality under predefined experimental conditions. Thus, we used the dual luciferase/renilla reporter system in Nicotiana benthamiana transient agroinfiltration experiments to estimate the relative transcriptional activities (RTA) conferred by regulatory elements, and showed the reproducibility of this method to characterize synthetic phytobricks. Following this scheme, we constructed a number of small genetic circuits, e.g. (i) a reversible switch based on site-specific recombinases, (ii) a glucocorticoid-regulated myb/bHLH transcriptional activation module, and (iii) several CRISPR/Cas9-based programmable transcriptional factors. All in all, these designs confirm that GB3.0 phytobrick documentation can serve to anticipate the qualitative behaviour of new circuit configurations.

PC1.9 SYNTHETIC EXTRACELLULAR MATRICES FROM EXTRUDED NANOFIBROUS SCAFFOLDS

**WEDNESDAY 6 JULY, 2016  10:10**

**DOROTHEA BRÜGGEMANN** (UNIVERSITY OF BREMEN, GERMANY)

Cells are embedded into the extracellular matrix (ECM), which forms a complex network of different protein nanofibres and polysaccharides. This natural composite material plays a major role in many biological functions like the mechanical support of large tissues or cell adhesion and proliferation. Here, we present a novel synthetic extracellular matrix system based on extruded biopolymer nanofibres, which will help us to understand and control cellular functions on the nanoscale. Using a novel extrusion method we were able to prepare scaffolds from various biopolymer nanofibres, which resemble the native architecture of the ECM. Nanoporous aluminium oxide was employed as template material to extrude different ECM proteins in physiological buffers. Thus, we obtained either randomly oriented meshes or highly aligned bundles of biopolymer nanofibres. Our extrusion approach also facilitated the first-time preparation of nanofibrillar composites, which contain different proteins and polysaccharides within single nanofibres. Adjusting the nanopore diameter and protein concentration enabled us to control the diameter of single nanofibres, which we analysed with scanning electron microscopy. Most interestingly, by adjusting the pore diameter and protein concentration we were able to induce varying degrees of structural changes in extruded fibronectin fibres, which we analysed with Förster resonance energy transfer measurements in a confocal laser scanning microscope. Next, we will study the biological functionality of synthetic extracellular matrices from composite nanofibres on a cellular level to promote the design of novel tissue engineering scaffolds.

PC1.10 GENOMIC ENGINEERING BY TRANSPOSABLE ELEMENTS IN VERTEBRATES

**WEDNESDAY 6 JULY, 2016  11:00**

**ZSUZSANNA IZSVÁK** (MAX DELBRÜCK CENTER FOR MOLECULAR MEDICINE, GERMANY)

DNA-based transposons are natural, non-viral DNA delivery vehicles. Similarly to retroviruses, transposons integrate into the chromosomal host cells, but their life-cycles do not involve reverse transcription, and they are not infectious. Transposon-based, integrating vectors open up new possibilities in genome engineering. Molecular reconstruction of the Sleeping Beauty (SB) transposon represents a milestone in applying transposition-mediated gene delivery in various vertebrate species. During the last decade, the SB system has been developed into a technology platform for vertebrate genetics with application areas including gene therapy, transgenesis, somatic and germline mutagenesis. These efforts revolutionized genomic manipulations in vertebrates, including tissue culture, animal biotechnology, gene function annotation to connect genetics and physiology in vertebrate models, cancer research to identify gene networks protecting against cancer, and gene therapeutic applications. The hyperactive Sleeping Beauty
(SB100X, Molecule of the Year in 2009) transposon system represents the first non-viral vector capable of stable gene transfer coupled with long-term therapeutic gene expression at a comparable efficiency to viral strategies. The SB system has advantageous attributes for stable, long-term expression both in vitro and in vivo. Regarding therapeutic application, the SB system has favourable safety profile as it has intranscriptional activities, it does not target transcriptionally active regions for integration and has reduced immune complications. Importantly, the SB vector does not have no strict limitation of the size of expression cassettes, can tolerate larger and more complex therapeutic genes and exert relative resistance to gene silencing. The SB system has been recently translated for clinical applications.

**PC1.11 SEEING GREEN: ENGINEERING GREEN LIGHT SENSITIVITY FOR PLANTS**

**WEDNESDAY 6 JULY, 2016  11:30**

**MARTIN W BATTLE (UNIVERSITY OF ESSEX, UNITED KINGDOM), MATTHEW A JONES (UNIVERSITY OF ESSEX, UNITED KINGDOM)**

Light is a remarkably versatile and precise tool, the prevalent nature of which has caused it to become a common stimulus in biological processes, ranging from the incredibly complex to the beautifully simple. By engineering photoreceptor pathways into cells which are naturally unresponsive to light we are able to manipulate gene expression directly without introducing intrusive stimuli such as chemicals. The application of optogenetics to plant systems has been limited by the wide range of endogenous photoreceptors present, although plants are typically far less responsive to green light than to other parts of the visible spectrum. We have designed a green-light-sensitive optogenetic construct which will allow for light controlled manipulation of gene expression in planta without influencing wild-type photoreceptors. Using Golden Gate cloning, we have designed this construct with modularity in mind, allowing for the replacement of target genes in the system. Currently the system is being characterised using luciferase and YFP reporter genes as outputs but we see broad potential for future research.

**PC1.12 WE DO IT OUR (PATH)WAY: BRINGING INORGANIC CARBON INTO LIFE WITH SYNTHETIC CO₂-FIXATION**

**WEDNESDAY 6 JULY, 2016  13:50**

**TOBIAS J ERB (MAX PLANCK INSTITUTE FOR TERRESTRIAL MICROBIOLOGY, GERMANY)**

Carbon dioxide (CO₂) is a potent greenhouse gas that is a critical factor in global warming. At the same time atmospheric CO₂ is a cheap and readily available carbon source. Yet, synthetic chemistry lacks suitable catalysts to functionalize the CO₂ molecule, emphasizing the need to understand and exploit the CO₂ mechanisms offered by Nature. Here, I will(1) discuss the evolution and limitation of naturally existing CO₂-fixing enzymes and pathways, I will(2) present strategies for the de novo-design of CO₂-fixing reactions and pathways, and(3) outline how such artificial pathways can be realized in vitro and in vivo to create minimal CO₂-fixation modules and novel CO₂-fixing microorganisms.

**PC1.13 UNTANGLING THE GLYCOSYLATION NETWORK WITH A SYNTHETIC GOLGI REACTOR**

**WEDNESDAY 6 JULY, 2016  14:30**

**KATE E ROYLE (IMPERIAL COLLEGE, UNITED KINGDOM), OLEKSII KLYMENKO (IMPERIAL COLLEGE, UNITED KINGDOM), CLEO KONTORAVOI (IMPERIAL COLLEGE, UNITED KINGDOM), KAREN POLIZZI (IMPERIAL COLLEGE, UNITED KINGDOM)**

Glycosylation is the co- and post-translational addition of oligosaccharides to peptide backbones. As it has important roles in structure and function, it can direct protein folding and degradation, effecting wide ranging processes including the immune response and disease. During N-linked glycosylation, enzymes of the endoplasmic reticulum and Golgi sequentially act to build and trim the oligosaccharide. Due to the numerous enzymes, their overlapping distributions, and substrate promiscuity this complex process can generate hundreds of different glycans, each with ramifications on protein function. To gain insight into the kinetic principles governing this process, we are designing and evaluating a synthetic Golgi reactor. By disengaging the glycoenzymes from the cell, we can linearize the network into individual, sequential modules and characterise them as parts, building on our fundamental understanding of the process. From an industrial viewpoint, such a reactor would allow the design and engineering of protein therapeutics. To date, our research has focused on computationally modelling the process to implement various scenarios. Results have provided guidelines for the design including structural decisions (reactor structure, enzyme immobilisation, column length), process conditions (flow rate, pressure drop and enzyme concentration) in addition to the identification and quantification of co-product inhibition. Concurrently, we are expressing and characterising human glycoenzymes and substrate regeneration pathways with a view to generating a functional prototype. Finally, we are developing a streamlined cloning method to connect with upstream protein expression and, crucially, provide a range of therapeutically relevant proteins with which to challenge and evaluate the system.

**PC1.14 TRITERPENE METABOLISM ENGINEERING IN PLANTS**

**WEDNESDAY 6 JULY, 2016  14:50**

**AYMERIC LEVEAU (JOHN INNES CENTRE, UNITED KINGDOM), ANNE OSBOURN (JOHN INNES CENTRE, UNITED KINGDOM)**

The research within our group focuses heavily on plant metabolism and pathways, and(3) outline how such artificial pathways can be realized in vitro and in vivo to create minimal CO₂-fixation modules and novel CO₂-fixing microorganisms.
study and the production of triterpenoids, one of the most numerous and diverse group of plant natural products with a wide range of applications in food, cosmetics, pharmaceutical, and industrial biotechnology sectors. We are currently applying a synthetic biology approach to assemble triterpene biosynthetic pathways in various plant hosts in order to achieve crop bio-protection or production of pharmaceutical compounds. To accelerate gene discovery, functional characterization and facilitate the building of large multi-gene constructs, we have developed a triterpene engineering toolkit relying on the use of the Modular Golden Gate cloning method and the Hypertrans system. Together, these enable rapid assembly and high-level expression of candidate genes and pathways in the model plant Nicotiana benthamiana. In parallel, these tools are being applied for stable transformation of major economically important crop species. Through the incorporation of gene regulatory elements from our library, the capacity to produce simple and complex triterpenes in a coordinated, tissue-specific manner is being investigated.

**PC1.15 IMPROVEMENT OF RESOURCE USE EFFICIENCY AND PRODUCTIVITY IN CROP PLANTS**

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<td>Greta Nölke (Fraunhofer IME, Germany), Marcel Houdelet (Fraunhofer IME, Germany), Christoph Peterhansel (Leibniz-University, Germany), Stefan Schillberg (Fraunhofer IME, Germany)</td>
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The combination of global population growth, an emerging bioenergy economy and the loss of agricultural land to urbanization means that greater agricultural productivity is required per hectare of land to meet demands for food. Although boosting productivity is a significant challenge in agricultural research, one straightforward approach is to enhance the efficiency of photosynthesis and thus the amount of fixed carbon. In C3-plants, photosynthesis reduces the efficiency of photosynthesis by removing carbon from the Calvin-Benson cycle, nitrogen and reducing power. Reducing the metabolic flux through the photosynthesis pathway could increase resource-use efficiency, promote growth and increase yield. Here we discuss our efforts to increase the yield of potato by developing a method to enhance photosynthetic carbon fixation based on expression of a polyprotein (DEFp) comprising all three subunits (D, E and F) of Escherichia coli glycolate dehydrogenase (GlcDH). The recombinant DEFp was active in planta, leading to reduction of photosynthesis and the improvement of tuber yield under greenhouse and controlled field experiments. This approach has the potential to increase the biomass and yield of diverse crops.

**PC1.16 FUNCTIONAL METAGENOMIC MINING AND COMPREHENSIVE PATHWAY OPTIMIZATION USING SYNTHETIC SELECTIONS**

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<td>Hans Genee (BiosynTia APS, Denmark), Anne P Bali (Technical University of Denmark, Denmark), Søren Petersen (Technical University of Denmark, Denmark), Luisa Gronenberg (BiosynTia APS, Denmark), Brian Olson (Joint Genome Institute, Denmark), Sangeeta Nuth (Joint Genome Institute, United States), Leanne Chan (Joint Bio-Energy Institute, United States), Mette Kristensen (Technical University of Denmark, Denmark), Scott Harrison (Technical University of Denmark, Denmark), Nathan Hillson (Joint Bio-Energy Institute, United States), Bo Salomonsen (BiosynTia APS, Denmark), Mads Bonde (Technical University of Denmark, Denmark), Solvej Siedler (Technical University of Denmark, Denmark), Edward Badoo (Joint Bio-Energy Institute, United States), Jay Keasling (Joint Bio-Energy Institute, United States), Christopher Petzhold (Joint Bio-Energy Institute, United States), Samuel Deutsch (Joint Genome Institute, United States), Morten Sommer (Technical University of Denmark, Denmark)</td>
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Efficient optimization of biosynthetic pathways and discovery of novel genes are key to a future of sustainable production of valuable natural products. However, the complex genotype-phenotype relations that govern pathway productivity render the engineering of optimized strains a slow and ad hoc process. In this talk, I present a method that deploys biosensor-based synthetic selection systems to enable high-throughput mining and functional validation of biological functions. We developed a synthetic selection system for thiamine pyrophosphate, a key co-factor of crucial interest for industrial biotechnology and human health. Using this system we mined soil and gut metagenomes for thiamine transporters and identified several members of a novel transporter class (PnuT). Using the synthetic selection approach, we further probed the substrate specificity of PnuT and identified key residues that modulate substrate specificity. Additionally, to probe the sequence-function landscape of the complex and tightly regulated thiamine biosynthesis pathway of Escherichia coli, and to speed up the engineering of optimized strains, we applied the synthetic selection to interrogate 16,384 re-factorized pathway variants that sample the synthetic design space. This approach enabled rapid identification of new and non-intuitive pathway configurations leading to high thiamine production levels. Combined, our results demonstrate how synthetic biology approaches can effectively be deployed to functionally mine metagenomes and elucidate sequence-function relationships of complex transport and biosynthesis systems in bacteria.
**PC1.17 CELL-FREE METABOLIC ENGINEERING OF FINE CHEMICAL ENZYME PATHWAYS**

**WEDNESDAY 6 JULY, 2016  16:35**

**WILFRIED WEBER (UNIVERSITY OF FRIEBURG, GERMANY), KONRAD MÜLLER (UNIVERSITY OF FRIEBURG, GERMANY), MAXIMILIAN HÖRNER (UNIVERSITY OF FRIEBURG, GERMANY)**

Pathway engineering studies often neglect a key requirement to balance the supply and demand of enzyme loading at the biochemical level. Furthermore, toxic intermediates can also hinder growth and promote genetic instability, so fine tuning of gene expression is a key requirement to hit the ‘metabolic sweet spot’ of pathway design. Our main aim is to draw a link in how cell-free prototyping can aid pathway design *in vivo*. Using raspberry ketone as a model pathway, we have designed a synthetic cell-free enzyme competition system to quantify steady state *in vitro* kinetics of ketone synthesis and cofactor regeneration. This has been designed and tested to study the effects of enzyme loading and cofactor availability on metabolic flux, using LC-MS to monitor time-course reactions. We have also discovered a substrate-binding protein that fluoresces and serves as a real-time indicator of pathway activity *in vitro*. We are attempting to engineer this fluorescence protein as a potential small molecule biosensor to aid *in vivo* gene expression optimisation. In summary, the benzoacetone synthase represents a rate-limiting step in the pathway, whilst substrate inhibition occurs by increasing the enzyme levels of the early stage enzymes. Using this cell-free insight, we have implemented an *in vivo* pathway using combinatorial pathway refactoring and natural riboswitch insulators to improve genetic stability. To fine tune enzyme levels in synchrony with pathway flux, we are currently quantifying enzyme-GFP fusions and *N*peptide LC-MS to correlate enzyme levels to pathway performance in parallel with our cell-free design.

**PC1.18 LIGHT-DRIVEN PRODUCTION OF STRUCTURALLY COMPLEX DITEREPENOIDS**

**THURSDAY 7 JULY, 2016  10:55**

**BIRGER LINDBERG MØLLER (UNIVERSITY OF COPENHAGEN, DENMARK), IRINI PATERAKI (UNIVERSITY OF COPENHAGEN, DENMARK), ALLISON MAREE HESKES (UNIVERSITY OF COPENHAGEN, DENMARK), JOHAN ANDERSEN-RANBERG (UNIVERSITY OF COPENHAGEN, DENMARK), AGNIESZKA ZYGADLO NIELSEN (UNIVERSITY OF COPENHAGEN, DENMARK), POUL ERIK JENSEN (UNIVERSITY OF COPENHAGEN, DENMARK)**

With 12,000+ known structures, diterpenoids are a prime example of bio-active natural products produced by plants. Many are used as highly valuable pharmaceuticals, fragrances, natural plant growth promoters, food ingredients such as flavor or as colorants and spices. Unfortunately, they are typically produced in minute amounts in plants and their structural complexity render them difficult to prepare from fossil resources using organic chemical synthesis. Terpenoid synthases, cytochrome P450s and acyl transferases are key multienzyme families involved in diterpenoid synthesis. Using mass spec based imaging of the target plant tissue, tracer studies, single-cell type-based metabolomics and transcriptomics, functional characterization of enzyme candidates using transient expression in tobacco and LC-MS-NMR based structural identification, elucidation of even highly complex biosynthetic pathways is now possible within a reasonably short time frame. Terpenoid metabolism is modular right from assembly of the C5 building blocks to the final structurally complex diterpenoid. Using the approaches of synthetic biology for combinatorial biosynthesis, the functional modules may be assembled in new combinations to expand the landscape of diterpenoid structural diversity in new-to-nature structures. The entire pathway for forskolin was elucidated. Forskolin is a cyclic AMP booster approved for treatment of glaucoma but also used as a weight loss aid. The forskolin pathway is being used as a test model system for large scale light driven production of high value diterpenoids following targeting of the pathway to the thylaloid membrane and using cyanobacteria or moss as photosynthetic production hosts grown in contained photobioreactors.

**PC1.7 OPTICALLY CONTROLLED SIGNALLING PROCESSES IN MAMMALIAN CELLS**

**THURSDAY 7 JULY, 2016  09:00**

**WILFRIED WEBER (UNIVERSITY OF FRIEBURG, GERMANY), KONRAD MÜLLER (UNIVERSITY OF FRIEBURG, GERMANY), MAXIMILIAN HÖRNER (UNIVERSITY OF FRIEBURG, GERMANY)**

Optical control of biological signalling processes in mammalian cells allows dissecting physiological and pathological processes with unmatched spatiotemporal resolution. By functionally rewiring plant and bacterial photoreceptors to growth factors, synthetic polymers, kinases or transcription factors, we achieve optical control along the whole signal transduction cascade. We demonstrate optically controlled reversible administration of extracellular biological and mechanical cues, the reversible activation of kinase cascades as well as the multichromatic induction of different promoters. We apply these optogenetic tools to control cell migration, mechano-signalling as well as cell differentiation.
PC1.21 IN VIVO BIOSENSORS FOR MAMMALIAN BIOPROCESSING

THURSDAY 7 JULY, 2016  14:50

KAREN POLIZZI (IMPERIAL COLLEGE LONDON, UNITED KINGDOM), LISA GOERS (IMPERIAL COLLEGE LONDON, UNITED KINGDOM), SIMON J MOORE (IMPERIAL COLLEGE LONDON, UNITED KINGDOM), LINDA DEKKER (IMPERIAL COLLEGE LONDON, UNITED KINGDOM), PAUL S FREEMONT (IMPERIAL COLLEGE LONDON, UNITED KINGDOM)

The ability to tailor organisms for the synthesis of useful products also allows us to input genetic circuits that can be used to report the internal state of the cell. Genetically encoded biosensors are potentially a very powerful strategy for bioprocess development where sample sizes can be very limited and non-invasive monitoring techniques can vastly improve high throughput screening strategies. We are interested in using biosensors of a variety of types to gain information on cells during bioprocessing.

This talk will focus on the example of therapeutic glycoprotein production using mammalian cell cultures. The process of glycosylation is not templated, meaning the cells produce a heterogeneous mixture of proteins bearing different glycoforms, many of which have different activities in patients. Beyond genetic modification of cells, we can manipulate the environment of cells to try and target specific glycoforms using bioprocess engineering. In my lab, we use a combination of different types of biosensors to get information the metabolism of cells during glycoprotein production to try and develop strategies for manipulating glycoform. By monitoring key metabolite concentrations and applying predictive mathematical modelling of the we can ‘tune’ the glycoform to have the desired effect. In this context, my talk will focus on different types of biosensors for monitoring key metabolites in mammalian bioprocessing.

PC1.22 ENGINEERING AN EXPANDED CHEMICAL PALETTE IN CELLS

THURSDAY 7 JULY, 2016  15:45

HAL S ALPER (THE UNIVERSITY OF TEXAS AT AUSTIN, UNITED STATES)

An industrial biotechnology revolution is approaching. Recent technical advances are leading to a rapid transformation of the chemical palette available in cells making it conceivable to produce nearly any organic molecule of interest—from biofuels to biopolymers to pharmaceuticals. However, these feats require the ability to ‘hijack’ native cellular machinery and metabolism and navigate the complexity inherent in cellular regulation. In this talk, we will describe recent advances in engineering various yeasts for the production of important products, such as organic acids and oleochemicals, with a focus on the synthetic biology tools and paradigms required along the way. Collectively, these case studies demonstrate the power and utility of using yeasts as a production host for chemicals.

PC1.19 SYNTHETIC BIOLOGY FOR THE DESIGN OF MOLECULAR BIOORIGAMI: NEW MODULAR PROTEIN STRUCTURES AND FOLDING PATHWAYS

THURSDAY 7 JULY, 2016  13:50

ROMAN JERALA (NATIONAL INSTITUTE OF CHEMISTRY, SLOVENIA)

Proteins are the most advanced nanostructures, defined by the sequence of amino acids. Nature provides a limited number of protein folds, which have been optimized during evolution. New protein folds are very challenging to design due to the delicate balance of numerous weak long range noncovalent interactions stabilizing proteins structure. Design of poly-peptide-based modular polyhedra was inspired by the DNA-based nanostructures. The folding problem of proteins was by passed by designing topological protein folds by relying on the well-understood specificity of coiled-coil dimers and used them as modules to guide the poly peptide chain between vertices of the selected polyhedral cage. The principle was demonstrated by the construction of a nanoscale protein tetrahedral cage from a single poly peptide chain composed of 12 coiled-coil forming segments. In this assembly 6 edges of the polyhedron are defined by orthogonal coiled-coil dimers. This principle represents a new platform of structural scaffold formation that could be extended to other polyhedra and for different applications. Modularity of designed structures could also allow the design of the folding pathway, which is required particularly for the design of knotted structures as the chain needs to be steered through the previously formed loops in a predefined order. Folding of knots is encoded by the arrangement of modules of different stability based on derived topological and kinetic rules. We anticipate that this strategy could be used to design the folding of other knotted programmable molecules.

PC1.20 INTERFACING GREEN AND RED SYNTHETIC BIOLOGY FOR THE UNDERSTANDING OF SIGNALING PROCESSES

THURSDAY 7 JULY, 2016  14:30

MATIAS ZURBRIGGEN (HEINRICH HEINE UNIVERSITY, GERMANY)

We integrate plant and mammalian cell systems to develop synthetic chemically and light-regulated switches and biosensors for the targeted control and monitoring of cellular processes at high spatiotemporal and quantitative resolution. The synthetic tools and methods are also implemented in orthogonal cellular platforms for studying biological signaling networks, including the reconstruction of light and plant hormone responses. Central to this strategy is the development of mathematical model-descriptions of the systems. Finally we apply the knowledge obtained into biotechnological applications.
Plant development is plastic and is determined by both genotype and the environment. Stomata, the microscopic pores on the leaf surface, are an excellent model for examining how environmental signals modulate plant development. Factors such as light quantity and quality as well as atmospheric carbon dioxide have a major impact on stomatal development. Our work has demonstrated that plant photoreceptors play a critical role in regulating stomatal development in response to environmental signals and consequently, significantly impact on plant performance. We will present data that examines the mechanism by which light regulates stomatal development, as well as how signals such as light and CO$_2$ interact to regulate this process.

Morphogenesis in plants occurs through a combination of cell growth and cell division. These processes are highly regulated in both time and space throughout development. In leaves and other organs with determinate growth, cell division mainly occurs in the so-called ‘proliferation zone’ located towards the base of the leaf. As cells proliferate and the organ grows, it has been proposed that cells are propelled out of this putative ‘proliferation zone’ and consequently stop mitotic divisions and initiate endoreduplication, differentiation and expansion. However, very little evidence, either cell biological or mechanistic, exist to support this growth model.

We have carried out a quantitative characterisation of leaf growth in Arabidopsis using a set of mutants that appear to prolong the duration of cell proliferation. Leaf growth was analysed using a combination of techniques. Whole organ confocal imaging and advanced image analysis, including custom image segmentation methods, were employed to extract quantitative data. A recently published mathematical tensor approach was used to quantify the different processes contributing to morphogenesis and our data allows us to infer cell divisions and directly measure cell shape and cell size during growth.

These measurements quantify development at the cellular level, and have generated exciting new insights into leaf growth and the mechanisms influencing cell proliferation during organ formation.

Lightsheet microscopes avoid several problems of confocal microscopy such as photobleaching but they come with their own challenges especially regarding sample preparation. Several labs have built their own lightsheet microscopes but a few commercial options are available now. We have been working to adapt one of the commercial set-ups to cater to our needs using readily available Fluorinated Ethylene Propylene (FEP) tubes which are customised in our lab without the need of specialist equipment. A simple lighting system is used for different day length settings and a perfusion system allows different treatments and supplements. This enables us to grow the plant inside the microscope chamber over several days and we can follow its development throughout. The possibility to rotate the sample allows imaging from different perspectives. Using various fluorescent markers, we can follow in vivo the development and specification of the vasculature in the root tip and emerging lateral roots.
Plant cell walls fulfill essential functions during plant development and stress responses. Cell wall integrity (CWI) has to be maintained during these different processes. Although a number of possible CWI maintenance sensors and mediators have been identified, neither their specific functions nor their relationships with each other have been well resolved.

We impaired CWI by inhibiting cellulose biosynthesis using isoxaben (ISX) and analyzed the responses in mutant alleles for 22 Arabidopsis candidate genes. Quantitative data for phytohormone (jasmonic acid and salicylic acid) accumulation, ectopic lignification and root growth was integrated through phenotypic clustering to determine candidate gene functions in CWI maintenance. The results show that receptor-like kinases (RLKs) mediating plant immunity apparently only modulate the response to CWI impairment. In parallel, treatment with cell wall-degrading enzymes only partially mimicked ISX-treatment, suggesting that responses to CWI impairment are not dependent on intact immune signaling. The putative stretch-activated calcium channel MCA1 and the plastid-localized mechanosensitive channels MSLS2/3 are required only for phytohormone accumulation, whereas the RLKs THE1 and FE12 are required for both phytohormone and ectopic lignin accumulation. The nitrate reductases NIA1 and NIA2 are required for all ISX-induced responses, suggesting an essential function of either nitrate reductase in nitrite or nitrate reduction to nitric oxide during CWI maintenance. Our results provide a global overview of CWI maintenance signalling processes, insights into the mode of action of the mechanism and assign functions to particular candidate genes.
Lysine acetylation of non-histone proteins is an emergent post-translational modification. The number of identified Lys-acetylated proteins has been increasing steadily over the past years, and this reversible modification is now considered crucial in the regulation of important metabolic pathways. Despite advances in the characterization of mammalian and yeast Lys-acetyltransferases, the identity of their plant counterparts remains elusive. The Arabidopsis thaliana protein HOOKLESS1 (HLS1) contains an acetyltransferase domain and is localized in the nucleus and the cytoplasm of cells. This protein has previously been described as an integrator of multiple hormonal signals during plant development. When expressed in yeast, HLS1 can complement the phenotype of mutants impaired in acetylation. We show that HLS1 interacts with the A. thaliana inositol-hexakisphosphate (IP6) transporter ABCC5 (ABC subfamily C transporter 5) using a Yeast Two-Hybrid system and Co-Immunoprecipitation. Moreover, A. thaliana plants defective in HLS1 and ABCC5 proteins share similar defects in guard cell function, which suggests that both proteins act together in guard cell signal transduction. We are currently testing the hypothesis that HLS1 can acetylate ABCC5 and thus modulate its transport activity. To our knowledge, this would be the first description of a Lys-acetyltransferase that can regulate the function of a non-histone protein in plants. It would also provide an example of how a transporter can be regulated by Lys-acetylation, and contribute to clarify the role of Lys-acetylation in the hormonal control of cellular functions.
Metabolic changes associated with quiescent meristem reactivation are being studied using a metabolomics analysis (using an ESI-TOF-MS system) of a staged developmental series of dissected axillary buds. Initial metabolite markers for early dormancy response have been identified using MS/MS and are being quantified using Ultra Performance Liquid Chromatography (UPLC). Key metabolites will be imaged using MALDI to provide a high resolution spatial analysis of the patterns of metabolism that occur in and around the meristem during dormancy release, generating leads for new approaches to sprouting control.
Deficiency of folate and its derivatives (vitamin B9) is a worldwide problem that can impact human health. Fruits constitute important components of the human diet, fueling the interest for the enhancement of folate content in crops. According to the USDA, tomato is the second most consumed vegetable in the U.S., but provides roughly 4% of the recommended dietary allowance of folate. The focus of this project was to identify loci affecting folate production using mapping data of a recombinant inbred lines (RILs) population by means of quantitative trait loci (QTLs) analysis. Total folate content was determined in 103 lines of a Solanum lycopersicum (NCEBR1) × Solanum pimpinellifolium (LA2093) RIL population using a Lactobacillus rhamnosus microbiological assay whose growth is folate-dependent. The parents of the population showed differences in their folate content: NCEBR1 contains 9.2 μg folates/100 g FW, while the wild LA2093 contains 26.4 μg folates/100 g FW. Furthermore, significant QTLs were detected after multiple QTL mapping (MQM) analysis on chromosomes 6 and 7. Interaction plots reveal that high folate content may result from the combination of LA2093 alleles on chromosome 7 and 6 as well as from a combination of an NCEBR1 allele on chromosome 4 and an LA2093 allele on chromosome 6. These findings parallel previous S. pennellii introgression lines data where a QTL for high folate was observed on chromosome 6. These QTLs, in conjunction with RNA-seq data, will be used to facilitate the identification of candidate genes governing folate content in tomatoes.

Plant species vary enormously in their ability to survive freezing conditions. Many species from the cooler parts of the world can increase their tolerance of sub-zero temperatures through the process known as cold acclimation. During cold acclimation, exposure to low, non-freezing temperatures elicits a program of transcriptional, metabolic and morphological changes necessary for survival of subsequent freezing. Although studies have shown that cold acclimation results in compositional changes to the cell wall, there has been little evidence to date that these changes have any functional significance with respect to freezing tolerance. We demonstrate that a mutation known to alter cell wall structure and composition also decreases plant freezing tolerance. Arabidopsis mur1 mutants lack the enzyme that catalyses the synthesis of L-fucose and consequently exhibit reduced fucosylation of the cell wall polysaccharide rhamnogalacturonan-II (RG-II). This results in the failure of RGII monomers to dimerise via borate ester cross-links between adjacent fucosylated side chains and leads to reduced growth and tensile strength of plants. We have demonstrated that a chemical inhibitor of fucose synthesis renders wild type plants freezing-sensitive, mimicking the mur1 mutation. Restoring RGII cross-linking by the application of supplemental boric acid reverses the freezing-sensitive phenotype of mur1 mutants, suggesting that freezing tolerance is dependent upon RGII cross-linking specifically, rather than due to other consequences of L-fucose depletion. We discuss the results of further experiments to determine whether pectin cross-linking is necessary for basal freezing tolerance or plays a role in the gain of freezing tolerance through cold acclimation.
PC2.28 PLANTS CHANGE COLD PERCEPTION SYSTEM DEPENDING ON THE ENVIRONMENT

HAYATO HIRAKI (THE UNITED GRADUATE SCHOOL OF AGRICULTURAL SCIENCE, IWATE UNIVERSITY, JAPAN), MATSUO UEMURA (CRYOBIOFRONTIER RESEARCH CENTER, IWATE UNIVERSITY, JAPAN), YUKIO KAWAMURA (CRYOBIOFRONTIER RESEARCH CENTER IWATE, UNIVERSITY JAPAN, JAPAN)

Cold perception is important function for environmental adaptation of plants. Ca$^{2+}$ signal acts as a second messenger in cold perception. Some studies, including our own, showed that several cooling conditions caused quite different shape of Ca$^{2+}$ signals, indicating that Ca$^{2+}$ signals may be a clue how plants sense cold. However, the detailed mechanism of cold perception is still unknown. Here, we focused on the Ca$^{2+}$ signal to estimate the plant cold perception system through the Ca$^{2+}$ signal. We developed the experimental system to observe Ca$^{2+}$ signals by combining confocal cryomicroscope and transgenic Arabidopsis plants expressing FRET-based Ca$^{2+}$ sensor Yellow Cameleon 3.60. To know the temperature range which plants can increase Ca$^{2+}$ concentration, both non-acclimated and 2°C 7-day-acclimated plants were cooled from 2°C. In these results, non-acclimated plant showed the delay of beginning of increase in Ca$^{2+}$ concentration, but cold-acclimated plant showed a rapid Ca$^{2+}$ elevation when cooled from 2°C. By contrast, cold-acclimated plants could not increase Ca$^{2+}$ concentration when they were cooled from 20°C. These results suggest that plants cannot sense wide temperature range, but can change the range. In addition, to know the difference between leaf and root, both were cooled from 20°C to 0°C. In this result, leaf cells have much longer lag time for beginning of increase in Ca$^{2+}$ concentration compared with root cells, indicating that each cell senses low-temperature independently. In conclusion, each plant cell may sense low-temperature with regulating its sensitivity to low-temperature and temperature range for inducing Ca$^{2+}$ signals depending on the environment.

PC2.29 A PRELIMINARY STUDY OF THE POTENTIAL OF HIGH RESOLUTION THERMAL IMAGING FOR DROUGHT TOLERANCE ASSESSMENT OF MAIZE (Z. MAIZE)

ADRIEN DOCKX (UCL – EARTH AND LIFE INSTITUTE, BELGIUM), LOUISE BERTRAND (UCL – EARTH AND LIFE INSTITUTE, BELGIUM), PHILIPPE-FRANÇOIS FAUX (UCL – EARTH AND LIFE INSTITUTE, BELGIUM), NATHALIE WUYTS (UGENTVIB – PLANT SYSTEMS BIOLOGY, BELGIUM), XAVIER DRAYE (UCL – EARTH AND LIFE INSTITUTE, BELGIUM)

Stomatal conductance and transpiration are critical variables for the assessment of crop behavior under drought, but are rarely used by breeders for practical and throughput-related reasons. Our project evaluates the potential of high resolution thermal imaging systems which provide accurate measurements of leaf temperature, which correlates theoretically, though not necessarily linearly, with stomatal conductance, transpiration and some root characteristics. In this study, we have specifically investigated two main directions. Firstly, we have studied the potential of these systems to evaluate crop response to soil water deficit in the field. Some indices gave good performances like those that are based on the temperature of leaf below cob which was correlated to the yield. Secondly, we have tried to develop methods using thermal imaging systems to evaluate characteristics involved in drought tolerance such as root characteristics. In the latter case, we used an inclined field where groundwater was kept artificially at constant level. The results of this experiment were partly consistent with expected root-derived responses to water deficit.
Stomatal closure is a mechanism by which pathogen infection is prevented. The guard cells forming the stomatal pore are highly specialized cells that translate intracellular signalling cues into a dynamic, reversible biomechanical output. This places constraints on the plasma membrane, where the pathogen-sensing receptors must be present and regulated, resulting in adaptive changes in the trafficking machinery that transport the receptors. To investigate the role of membrane trafficking in guard cells, we performed a large-scale, image-based screen and identified a Rab7 GTPase that is specific to stomatal closure induced by bacterial flagellin (flg22), uncoupled from closure triggered by abiotic stress. Time course experiments revealed that rab7 mutants fail to sustain stomatal closure in response to flg22. A possible role for this Rab7 GTPase in transport from multivesicular bodies (MVBs) to the tonoplast has previously been reported. However, rab7 mutants are not generally defective in FM4-64 uptake to the vacuole as well as vacuole morphology, suggesting a potential receptor-specific role in trafficking. The plasma membrane-localized FLAGELLIN SENSING2 (FLS2) receptor is known to internalize upon activation by its ligand flg22. Preliminary experiments suggest that flg22-induced FLS2 degradation could be altered in rab7 mutants. To corroborate these initial findings, work is underway to characterise FLS2-GFP subcellular dynamics and various endomembrane compartments and selected cargoes in rab7 mutant backgrounds, with stable Arabidopsis transgenic lines expressing fluorescent markers. Overall, our results reveal a specific role of membrane trafficking in the regulation of stomatal aperture in response to pathogen infection.
Sigma factors are bacteria-like RNA polymerase subunits that are responsible for promoter recognition and transcription initiation of chloroplast genes by plastid-encoded plastid RNA polymerase (PEP). In higher plants, sigma factors are encoded by the nuclear genome, which is thought to allow nuclear control of chloroplast gene transcription. The nuclear genome of Arabidopsis thaliana encodes six sigma factors (SIGMA FACTOR1 (SIG1)-SIG6), which are regulated by environmental cues and developmental stage. For example, SIG1 is regulated by the circadian clock and participates in the communication of circadian timing from the nucleus to the chloroplast. The relationship between all sigma factors and plant development and growth is regulated by environmental cues and developmental stage. For example, SIG1 is regulated by the circadian clock and participates in the communication of circadian timing from the nucleus to the chloroplast. The relationship between all sigma factors and plant development and growth is regulated by environmental cues and developmental stage.

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**PC2.9 INTERACTION OF MICRORNA160 AND AUXIN RESPONSE FACTOR10, 16, AND 17 AFFECTS HEAT RESPONSES IN ARABIDOPSIS**

**TUESDAY 5 JULY, 2016  POSTER SESSION**

SHIH-TONG JENG (NATIONAL TAIWAN UNIVERSITY, TAIWAN), JENG-SHAH LIN (NATIONAL CHUNG HSING UNIVERSITY, TAIWAN), CHIA-CHIA KUO (NATIONAL TAIWAN UNIVERSITY, TAIWAN), I-CHU YANG (NATIONAL TAIWAN UNIVERSITY, TAIWAN), WEI-AN TSAI (NATIONAL TAIWAN UNIVERSITY, TAIWAN), YU-HSING SHEN (NATIONAL TAIWAN UNIVERSITY, TAIWAN), CHIH-CHING LIN (NATIONAL TAIWAN UNIVERSITY, TAIWAN), YU-LI CHI (NATIONAL TAIWAN UNIVERSITY, TAIWAN), YU-CHI KING (NATIONAL TAIWAN UNIVERSITY, TAIWAN), YUN-WEI KUO (NATIONAL TAIWAN UNIVERSITY, TAIWAN)

High temperature negatively affects plant growth and development, reduces crop yield, and even causes cell death. MicroRNAs (miRNAs) are one of the most important factors regulating gene expression and involved in plant growth, development, and stress defense. The roles of miRNAs in Arabidopsis under high temperature were analyzed in this study. Results indicated that miR160 and its precursors were induced by heat; conversely, its targets, ARF10, ARF16, and ARF17, were significantly repressed. Transgenic plants overexpressing miR160 precursor (a 1600E) and miR160 target mimic inhibitor (MIM160) were generated to investigate the functions of miR160 in heat stress. The expression of ARF10, ARF16, and ARF17 under heat stress was decreased and increased in 1600E and MIM160 plants, respectively. Under heat stress, the seed germination rates, survival rates, hypocotyl elongation lengths, and rachis lengths of 1600E were better or longer than those of WT. However, these phenotypes of MIM160 plants were reduced after heat treatment. Therefore, miR160 regulates its target genes to regulate the thermotolerance of plants. In addition, arf10, arf16, and arf17 mutants also presented better adaptation to heat than WT. These results indicated that miR160 targets, which are ARF10, ARF16, and ARF17, might function as negative factors in plant under heat stress. Furthermore, the expression of HSP70B, HSP21, HSP17.6A, and HSP17.6II was affected in 1600E and MIM160 plants under heat stress. Conclusively, miR160 and its targets altered plant development and HSPs expression to regulate plant responses in heat stress.

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**PC2.10 THE ROLE OF NODULE SPECIFIC CYSTEINE RICH (NCR) PEPTIDES IN CONTROLLING NODULATION**

**TUESDAY 5 JULY, 2016  POSTER SESSION**

MINGKKE ACHOM (UNIVERSITY OF WARWICK, UNITED KINGDOM), MIRIAM L GIFFORD (UNIVERSITY OF WARWICK, UNITED KINGDOM), SASCHA OTT (UNIVERSITY OF WARWICK, UNITED KINGDOM)

Legume plants house nitrogen-fixing endosymbiotic rhizobia in specialised polyclad cells forming nodules. The plant genes involved in nodulation process and their regulatory functions have not yet been fully elucidated. Medicago truncatula, a legume with an indeterminate nodule-type has been found to express a large group of nodule-cysteine rich peptides (NCRs) (>500, Nallu et al., 2013) during the different stages of nodulation pathway. Due to their large numbers and sequence diversity, it is possible that these genes are putatively involved as signalling molecules with multiple functions in the control of nodulation and development. Recent work has begun to shed some light on the function of NCRs. However, these initial insights likely represent only a portion of the functional activity of this large family with diverse expression profiles. Analysis of NCR genes that are differentially expressed during nodulation and nitrogen influx discovered six motifs: motif 1 (AAGGGACACA), motif2 (AGAGCAT), motif3 (TCATGAAA), motif4 (TATAA), motif5 (CAACACA) and motif6 (TTTTAC) in the promoters of subsets of differentially expressed NCR genes. Since these putative promoters were strongly rhizobia and nitrogen regulated, we suggest that NCRs may be acting as regulators of nodule numbers depending on the plant N status.
PC2.12 SUPPLEMENTAL MANGANESE REGULATES THE COORDINATED ACTIONS OF ANTIOXIDANT DEFENCE AND GLYOXALASE SYSTEM IN RICE SEEDLINGS TO MITIGATE CADMIUM TOXICITY

TUESDAY 5 JULY, 2016 \ POSTER SESSION

ANISUR RAHMAN (KAGAWA UNIVERSITY, JAPAN), KAMRUN NAHAR (KAGAWA UNIVERSITY, JAPAN), MIRZA HASANUZZAMAN (SHER-E-BANGLA AGRICULTURAL UNIVERSITY, BANGLADESH), MASAYUKI FUJITA (KAGAWA UNIVERSITY, JAPAN)

We investigated the regulatory role of exogenous manganese (Mn) in conferring cadmium (Cd) stress tolerance in rice seedlings. Hydroponically grown 14-d-old rice (Oryza sativa L. cv. BRRI dhan29) seedlings were exposed to 0.3 mM CdCl₂ (Cd) alone and combined with 0.3 mM MnSO₄ (Mn) for three days. Exposure of rice seedlings to Cd caused growth inhibition, chlorosis, nutrient imbalance and higher Cd accumulation. Higher amount of Cd uptake caused oxidative stress through the overproduction of reactive oxygen species (ROS) and methylglyoxal (MG) and resulted in lipid peroxidation and loss of plasma membrane integrity. Cadmium-induced higher ROS and MG production disrupted antioxidant defence and glyoxalase system, respectively. Application of Mn in non-stressed rice seedlings did not show any significant effect, whereas, exogenous application of Mn to Cd-treated rice seedlings partly recovered Cd-induced water loss, chlorosis, growth inhibition and nutrient imbalance by reducing Cd uptake and their further translocation. Supplemental Mn increased the ascorbate (AsA) content, activities of superoxide dismutase (SOD), catalase (CAT), monodehydroascorbate reductase (MDHAR) and dehydroascorbate reductase (DHAR) in the antioxidant system, and increased the activities of glyoxalase I (Gly I) and glyoxalase II (Gly II) of glyoxalase system under Cd stress condition. The Mn-induced improved antioxidant defence and glyoxalase system reduced Cd-induced oxidative damages by reducing ROS production, MG formation, lipid peroxidation and disintegration of plasma membrane.

PC2.13 DEVELOPING MICROBIAL EFFECTORS AS TOOLS FOR ENGINEERING PLANT PATHWAYS

TUESDAY 5 JULY, 2016 \ POSTER SESSION

WEIJIE HUANG (UNIVERSITY OF WARWICK, UNITED KINGDOM), SILVIA LEHMANN (UNIVERSITY OF WARWICK, UNITED KINGDOM), ANA DOMINGUEZ FERRERAS (UNIVERSITY OF WARWICK, UNITED KINGDOM), KATHERINE DENBY (UNIVERSITY OF WARWICK, UNITED KINGDOM), PATRICK SCHAFER (UNIVERSITY OF WARWICK, UNITED KINGDOM), VARDIS NTOUNKAKIS (UNIVERSITY OF WARWICK, UNITED KINGDOM)

Most plant-interacting microbes, either pathogenic or mutualistic, are capable of delivering multiple proteins into host cells to facilitate colonization. These proteins, collectively known as effectors, have an established role in modulating the plant innate immune system. However, it has become clear that the effectors also target multiple physiological pathways and this intrinsic versatility is central for microbes to achieve a successful interaction with their hosts. Here we study the impact of microbial effectors on various plant pathways, with the aim to use them as tools for engineer bespoke pathways in planta. As a starting point, an array of effectors from the pathogenic bacterial 

PC2.14 IDENTIFYING INFECTION-SITE-SPECIFIC TRANSCRIPTIONAL EVENTS IN THE ARABIDOPSIS RESPONSE TO DOWNY MILDEW

TUESDAY 5 JULY, 2016 \ POSTER SESSION

TIMOTHY LR COKER (UNIVERSITY OF WARWICK, UNITED KINGDOM), VOLKAN CEVIK (UNIVERSITY OF WARWICK, UNITED KINGDOM), JIM L BEYNON (UNIVERSITY OF WARWICK, UNITED KINGDOM), MIRIAM L GIFFORD (UNIVERSITY OF WARWICK, UNITED KINGDOM), ANA DOMINGUEZ FERRERAS (UNIVERSITY OF WARWICK, UNITED KINGDOM), PATRICK SCHAFER (UNIVERSITY OF WARWICK, UNITED KINGDOM), VARDIS NTOUNKAKIS (UNIVERSITY OF WARWICK, UNITED KINGDOM)

Changes in gene expression form a crucial part of the plant response to infection, and whole-leaf expression profiling has played a valuable role in identifying genes and processes that contribute to the interactions between the model plant Arabidopsis thaliana and a diverse range of pathogens. However, with some pathogens such as downy mildew caused by the biotrophic oomycete Hyaloperonospora arabidopsidis (Hpa), whole-leaf profiling may fail to capture the complete Arabidopsis response encompassing responses of non-infected as well as infected cells within the leaf. Highly localised expression changes that occur in infected cells may be diluted by the comparative abundance of non-infected cells, or local and systemic responses of a differing nature may become conflated. To address this we applied the technique of Fluorescence Activated Cell Sorting (FACS) to the study of plant-pathogen interactions. We isolated...
haustoriated (Hpa-proximal) and non-haustoriated (Hpa-distal) cells from infected seedling samples using FACS, and measured global gene expression. When compared with a uninfected control, 278 transcripts were identified as differentially expressed, the vast majority of which were differentially expressed specifically in Hpa-proximal cells. By comparing our data to previous, whole organ studies, we discovered many locally responding genes that can be implicated as novel in the Hpa response, and that were uncovered for the first time using our sensitive FACS technique. We are now using transcriptional reporters to further understand expression of a subset of these genes on a spatial scale, and are exploring the effect of gene knockouts on plant susceptibility to Hpa.

**PC2.15 PLANT BIOTECHNOLOGY: EXPLOITING ANTIMICROBIAL POTENTIAL OF SPONDIAS PURPUREA**

**TUESDAY 5 JULY, 2016 POSTER SESSION**

**JOÃO P. A. TEIXEIRA (CENTRO DE EDUCAÇÃO SUPERIOR DE GUANAMBI - FACULDADE GUANAMBI, BRAZIL), BRUNA G. S. SANTANA (CENTRO DE EDUCAÇÃO SUPERIOR DE GUANAMBI - FACULDADE GUANAMBI, BRAZIL), RAYMUNDO FARIA (CENTRO DE EDUCAÇÃO SUPERIOR DE GUANAMBI - FACULDADE GUANAMBI, BRAZIL), ALANNA C. F. PEREIRA (CENTRO DE EDUCAÇÃO SUPERIOR DE GUANAMBI - FACULDADE GUANAMBI, BRAZIL)**

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Plant reproductive growth is an important process, providing food and natural resources for industry. Plant extracts have served as an important source of medicine and cosmetics industries. People have been interested in obtaining active compounds from natural sources, plant extracts, could be a rich source of bioactive compounds. Secondary plant metabolites can exhibit sophisticated activities including antimicrobial activity. In this study, antimicrobial activity of hydroalcoholic extracts obtained from leaves and wood of *Spondias purpurea* were observed. These extracts were tested against bacteria and fungi by disk diffusion, MIC and MBC methods. Both extracts showed different inhibition against microorganisms in the different methods tested as also showed bactericidal or bacteriostatic capacity.

**PC2.16 RESTORED PHENOTYPES BY ATG8 COMPLEMENTATION**

**TUESDAY 5 JULY, 2016 POSTER SESSION**

**ALANNA C. F. PEREIRA (UNIVERSIDADE ESTADUAL DE SANTA CRUZ, BRAZIL), FABIANA A. C. SILVA (UNIVERSIDADE ESTADUAL DE SANTA CRUZ, BRAZIL), CRISTINA PUNGARTNIK (UNIVERSIDADE ESTADUAL DE SANTA CRUZ, BRAZIL), MARTIM BRENDEL (UNIVERSIDADE ESTADUAL DE SANTA CRUZ, BRAZIL)**

**BELLEFP@GMAIL.COM**

Autophagy (ATG) is a cellular process that causes degradation of long-lived proteins and recycling of cellular components to assure survival during periods of nutritional lack or other environmental stresses. In this process, the role of the secretory pathway in autophagy is largely by studies in yeast, the importance of the autophagy process can be verified in mutants *atg8Δ* of *S. cerevisiae* for saw present characterized phenotype already. *M. perniciosa* autophagy gene *MpATG8* was tested by introducing it into yeast mutant *atg8Δ* and testing for heterologous expression via phenotypic sporulation complementation and TcPR-10p sensitivity, the pathogenesis-related protein PR-10 of *Theobroma cacao* has antifungal action and ribonuclease activity *in vitro*. Formation of oxygen radicals (ROS) after exposure to TcPR-10p was observed using fluorescence microscopy with dihydroethidium-stained cells. WT and mutant *atg8Δ* transformed with a single-copy vector containing *MpATG8* gene showed practically the same resistance to TcPR-10p and similar formation of ROS, while mutant *atg8Δ* was sensitive and exhibited increased ROS accumulation. This suggests that the protein codified by *MpATG8* is functionally expressed in *S. cerevisiae* and protects against TcPR-10p whereas mutant *atg8Δ* accumulates ROS under the same conditions, also our results show the sporulation could be restored in *atg8Δ/atg8Δ* diploids when transformed with one copy of *MpATG8*. 
**PC2.17 IDENTIFYING AND CHARACTERISING PUTATIVE ALDOSE 6-PHOSPHATE REDUCTASES IN ARABIDOPSIS THALIANA**

**TUESDAY 5 JULY, 2016 POSTER SESSION**

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In Rosaceae and Plantaginaceae species, sugar alcohols like sorbitol are phloem-translocated and allow more efficient use of carbon, as compatible solutes in abiotic stress and facilitate boron mobilisation. The key enzyme required for sorbitol synthesis is aldose-6-P reductase (A6PR), which reduces glucose-6-P to sorbitol-6-P, which is then converted to sorbitol. Once in sink organs, sorbitol is oxidised by sorbitol dehydrogenase (SDH) to fructose. Curiously, A6PR- and SDH-like enzyme activity is found in families that synthesise and transport sucrose, and in Arabidopsis (a sucrose-translocating Brassicaceae), we have identified two proteins with the structural features and >65% amino acid identity with known plant A6PRs; we call these AtA6PR1 and AtA6PR2. We demonstrate that AtA6PR1 and AtA6PR2 are differentially-expressed in different Arabidopsis organs. By transient transformation of tobacco, we show that GFP-fusion proteins of both reductases are localised in the cytosol. Potential mutant lines have been genotyped, and along with studies of the relative expression of both genes in wild-type plants grown under different abiotic stresses, we are determining their physiological role in this non-sorbitol translocating species. Additionally, when AtA6PR1 is over-expressed in wild-type and sdh- mutant Arabidopsis lines, the starch content increases. Currently, similar experiments are underway with AtA6PR2 with the overall aim of analysing the effects that a potential mis-balancing of sorbitol metabolism has on the plant. Progress in the biochemical characterisation of these proteins will also be presented. Funding: Fondecyt 1140527 and Conicyt Masters scholarship (22160896to KO).

**PC2.18 IDENTIFICATION AND CHARACTERISATION OF TWO PUTATIVE LIPOYL SYNTHASES (LIP1) IN SOLANUM LYPopersicum (TOMATO)**

**TUESDAY 5 JULY, 2016 POSTER SESSION**

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Lipoic acid (LA) is a functional and structural metabolite with powerful antioxidant capacities present in eukaryotic and prokaryotic organisms. LA is both lipid- and water-soluble, and is the prosthetic group of a number of key multi-subunit enzyme complexes, including pyruvate decarboxylase and α-ketoglutarate dehydrogenase. LA synthesis and incorporation into these proteins (lipoylation) proceeds de novo or via a salvage pathway. During de novo synthesis, octanoyl transferase (LIP2) uses recently-synthesised octanoyl groups linked to the acyl carrier protein to transoctanylate target proteins. Subsequently, lipoyl synthase (LIP1) catalyses the final step by inserting two sulphur atoms into the prosthetic group. Whilst a number of the enzymes have been functionally-characterised in Arabidopsis thaliana, the aim of the current work is to identify and evaluate the role of this pathway in a fruit-bearing species. Towards this aim, we identified two proteins in tomato (Solanum lycopersicum) with the molecular characteristics of LIP1. We call these proteins SilLIP1c and SilLIP1m, which possess 78% and 84% amino acid identity with AtLIP1, respectively. Confirming bioinformatic predictions, SilLIP1c has a plastidial localisation whereas SilLIP1m is mitochondrial, as shown by confocal microscopy. Experiments to determine the molecular function of both proteins are underway, by functional complementation of a bacterial mutant, and their stable over-expression in Arabidopsis and tomato. Funding: Conicyt Anillo ACT-1110 (to MH), and Conicyt Doctoral (21160916to JA) and Master (22151178to SM) scholarships.