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## **The pattern and mechanism of nutrient assimilation in the Australian plague locust (*Chortoicetes terminifera*)**

William Armour    *Supervisors:* Dr F Clissold, Professor R Overall & Professor S Simpson  
School of Biological Sciences, University of Sydney, NSW 2006.

Recent work has discovered that nutrient assimilation per unit intake in the Australian plague locust (*Chortoicetes terminifera*) differs with insect age and between its two main natural host plants, the C<sub>4</sub> grasses button (*Dactyloctenium radulans*) and Mitchell grass (*Astrebla lappacea*). As both grasses contain similar levels of protein and non-structural carbohydrates differences in nutrient assimilation may result from an interaction between mandible size and grass cellular structure. This system provided an opportunity to understand how herbivorous insects assimilate nutrients, which is fundamental to determining plant susceptibility to insect attack.

This project had three main aims; to determine:

- 1) If leaf particle sizes generated by *C. terminifera* differed by age (third or fifth instar), grass species (button or Mitchell), sex (male or female) or nutritional state prior to feeding (deprived or un-deprived).
- 2) The number of bundle sheath cells (BSCs) that maintained membrane integrity or had plasmodesmata blocked by callose after being chewed by *C. terminifera*.
- 3) If *C. terminifera* gut enzymes could degrade callose in plasmodesmata, a barrier to nutrient exchange from BSCs, in transverse sections of button and Mitchell grass leaves.

Results showed that third instars created significantly smaller particles for both grasses than larger fifth instars. Third and fifth instars created smaller particles of Mitchell, which is less digestible, than button grass. Sex had a significant effect on particle size only at fifth instar where larger females produced larger particles. Nutritional state prior to feeding had no significant effect on particle size. On average less than 6% of BSCs maintained membrane integrity and less than 0.03% of BSCs had callose deposition. Enzymes from *C. terminifera* could not degrade callose in transverse sections of both grass species. Mechanical degradation appears important as differences in particle size between grasses and instars correlate with the differences nutrient assimilation per unit intake.

## **The use of compatibility as a measure for rate-heterogeneity across sites, with an application to butterflies**

Leonore Carr    *Supervisor: A/Professor L Jermin*  
School of Biological Sciences, University of Sydney, NSW, 2006

Since its origin in 1965, molecular phylogenetics has led to a better understanding of the relatedness and evolution of species. A variety of phylogenetic methods have been developed to interpret historical signals stored in alignments of nucleotide or amino acid sequences, all with their own benefits and pitfalls. Often, these methods assume that sites have evolved under independent and identically distributed (IID) conditions. However, different sites may encode components that serve different roles in the gene product, so it is likely that the rate of evolution varies across sites. The core aim of my thesis was to provide means to detect and overcome violation of the assumption of rate-homogeneity across sites.

Using synthetic data and randomisation techniques, I show that compatibility between sites can be used as a tree-independent measure of rate-heterogeneity across sites. Sites were defined as fast evolving if their compatibility score was not significantly greater than that of a random site with the same character composition. By allowing sites to evolve at different rates on known trees, I found that removal of fast-evolving sites improved the probability of recovering the correct tree.

I used the compatibility-based method to reanalyse an alignment of nucleotides (3159 characters; three gene regions: *COI*, *EF-1 $\alpha$*  and *wingless*) from 57 butterfly species, including representatives of the seven accepted families. These data were provided, and previously analysed, by Wahlberg et al. (Proc. R. Soc. B 272:1577-1586). The monophyletic family groupings and relationship were consistent between the original phylogenetic estimate and the estimate based on the refined alignment (i.e., with fast-evolving sites removed). There were, however, discrepancies within family clades, including within the Nymphalidae, Riodinidae, and Papilionidae. Parametric bootstrap support increased significantly on refinement of the alignment. These results illustrate how the compatibility measure can be used to clear up the historical signal and allow for the estimation of greater supported (and plausibly more accurate) trees.

## **Detecting dinner: a structural and behavioural examination of the antenna of the shallow-water isopod *Cirolana harfordi***

Hannah Elstub    *Supervisors: Dr A Pile & Dr M Thomson*  
School of Biological Sciences, University of Sydney, NSW 2006

Exploitation of deep sea resources has dramatically increased over the last century. It is essential that we increase our understanding of how new structures such as oil rigs may be affecting habitat utilisation and migration of deep sea organisms. All isopods have two key life history features: they have a brooding reproductive strategy, and are actively mobile scavengers. As the deep sea environment is often difficult to study, in order to understand how movement to locate food occurs in the deep sea and how this affects individual distribution, it is useful to have relevant shallow water models. The shallow water isopod *Cirolana harfordi* provides a good model, as it shares key life history traits with deep sea isopods, and is also an introduced species. This allows the study of movements in a species where a habitat is encountered for the first time, as occurs when new habitats are built in the deep sea. This seminar will focus on food detection by *C.harfordi*. Electron microscopy was used to structurally describe the antenna and antennule of *C.harfordi*. There were differences in type and distribution of setal and non-setal features, the most prominent being the presence of aesthetascs on the antennule – considered to be the primary setae involved in chemoreception in crustaceans. Behavioural assays were used to determine whether the antennae or antennules were necessary for food location. Four groups of isopods ((i) antennae ablated, (ii) antennules ablated, (iii) all ablated, (iv) none ablated) were tested for their ability to locate a food source in a re-circulating flume. Results show that both antennae and antennules are used for locating food, but may have different roles depending on distance to the food source. These results show that chemoreception is the most prominent method used by *C.harfordi* to locate food, and shows that chemoreception may be important in facilitating movements of individuals between suitable habitats.

## **Hunting lateral root architecture genes in plant model organism *Arabidopsis thaliana***

Christopher Gaal    *Supervisor: Dr J Saleeba*  
School of Biological Sciences, University of Sydney, NSW 2006

The development of root systems is poorly understood in terms of the genes involved and their various functions. In part this is because of a lack of attention until recent times, but also the process' complexity is responsible. This is a complexity necessary to perform many functions vital to whole plant survival including water and nutrient uptake, support and anchorage. Within the *A. thaliana* species there is an extreme plasticity of the root structure in response to both external biotic and abiotic factors, and structural diversity due to selective pressures which have shaped the genomes of various ecotypes. Root branching is a commonly affected in both instances. As part of my project I undertook a search to identify genes involved in lateral root development.

Using data gathered from a Quantitative Trait Loci analysis and a novel bioinformatics program amongst other sources, a list of candidate root architecture genes was identified based on a number of selection criteria. Growth conditions for an assay of root branching in seedlings of *A. thaliana* were optimised. Then this assay was used to identify aberrant branching of T-DNA insertion lines each with a candidate root architecture gene mutated. A single gene locus was identified to drastically increase branching in the mutant. Under conditions of environmental stress the mutant was observed to show a different response to wild type plants in some circumstances. Additionally, root branching was found to be indistinguishably different at early stages of lateral root development. Further work to understand to the function of this gene will assist in the elucidation of the mechanisms of root system architecture in *A. thaliana* and potentially agriculturally significant crop species

## Pieces of the phylogeographic and taxonomic puzzle of elderberry panax (*Polyscias sambucifolia*)

Kerry Gibbons    *Supervisors: Dr M Henwood & Dr M Pye & Dr A Perkins\**  
School of Biological Sciences, University of Sydney, NSW 2006

Phylogeography is the study of genetic variation within species over space and time, and provides insights into the historical processes that led to contemporary geographic species distributions and associated genetic structure. This relatively new field is developing rapidly, and its core methodologies and assumptions are under constant revision. With the identification of highly variable markers from the chloroplast genome (cpDNA), phylogeographic studies of plants are now becoming more common.

*Polyscias sambucifolia* (Sieber ex DC.) Harms occupies a range of habitats, including coastal and subalpine, in south eastern Australia. Throughout its distribution, *P. sambucifolia* exhibits a number of distinctive leaf forms, some of which appear to covary with habitat. Two cytotypes have also been recorded from *P. sambucifolia* which, together with its geographic distribution and morphological variation, makes this species an ideal taxon with which to explore the phylogeography of south eastern Australia.

Seventy-nine samples, representing the morphological, cytological and geographic extent of *P. sambucifolia* were collected. Two non-coding cpDNA markers (*trnT-L* and *psbA-trnH*) and one non-coding nuclear marker (External Transcribed Spacer) were sequenced for these samples and for three outgroup species. The two cpDNA markers were combined and subjected to maximum parsimony (MP) phylogenetic analyses and to statistical parsimony network analyses. Substitution data recovered several lineages, only one of which broadly corresponds with a recognisable morphotype. Inclusion of characters derived from insertions/deletions enhanced the resolution of phylogeographic inferences in *P. sambucifolia*, revealing a lineage linking Tasmanian populations with areas of Victoria. Statistical parsimony network analyses reveal a putative ancestral haplotype centred on Victoria, although MP analyses indicate an ancestral haplotype centred on the Northern Tablelands (NSW). Regardless of the location of the ancestral haplotype, subalpine populations are unequivocally derived from lowland ancestors. These hypotheses and those concerning the origins of isolated populations in Tasmania and Mt Kaputar will be discussed.

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## **The role of woodland patches as micro-refugia in a desert sand dune environment**

David Gregory    *Supervisors: Dr G Wardle & Professor C Dickman*  
School of Biological Sciences, University of Sydney, NSW 2006

Large scale periodic events such as wildfires, droughts and flooding are key processes driving species dynamics and the distribution of resources across desert landscapes.

Many species seek refuge in small retreats or micro-refugia which act as buffers against the climatic extremes. In the Simpson Desert of South Western Queensland, extensive sampling suggests that small stands of gidgee (*Acacia georginae*) woodland support rich assemblages of plants, invertebrates, mammals and reptiles and may in fact provide critical refugia for desert mammal species whilst resources are limited in the surrounding sand dune habitats. My aims were to compare mammal diversity and species composition in gidgee woodland to the adjacent sand dunes and to relate any patterns to the availability of food and habitat resources to identify key habitat features which may act as micro-refugia. Animal movements and microhabitat use were identified for two species of rodent, the Sandy Inland Mouse; *Pseudomys hermannsburgensis* and the Spinifex Hopping Mouse; *Notomys alexis* to gain knowledge on whether animals move between gidgee and sand dune habitats and what habitat features are important to the survival of these species.

Over two sampling periods (April and June 2008) 408 mammals were caught including 3 species of rodent and 5 species of dasyurid. An overall temporal difference in mammal abundance was found, i.e. mammal abundance in April was greater than in June. However, no difference in abundance was detected between gidgee and sand dunes in both sampling periods. No significant spatial differences in mammal abundance have been detected thus far. Species composition was similar throughout both habitats in June and April, and no significant difference in temporal species composition was detected between the April and June sampling periods.

Observations using light tracking and analysis of spool and line data indicate that the sandy inland mouse targets micro-habitat with high percentage cover and spends very little time in the open. It was observed that seeds are abundant at the base of vegetation and other forms of structure which may serve as a natural trap. Small rodents may not necessarily need to stray far from cover as it seems to provide protection from predators and also serves as an important food source.

Assessment of the structural and biotic attributes of multiple gidgee woodland patches suggests that this habitat does contain habitat features characteristic of micro-refugia such as abundant animal burrows, logs, ample food resources (seeds and invertebrates) and rich assemblages of plants and animals. My research was undertaken during a period where resources were plentiful and mammal abundance was high. It would be interesting to replicate this study at a time when resources become limited to see what changes occur.

## **Inter-specific parasitism between the dwarf honey bees *Apis andreniformis* and *Apis florea***

Jessica Higgs    *Supervisors: Professor B Oldroyd & A/Professor M Beekman*  
School of Biological Sciences, University of Sydney, NSW 2006

The honey bee queen communicates her presence to the colony via queen pheromones. In the presence of their queen workers remain sterile, forgoing direct reproduction to rear the queen's offspring. In contrast, a socially parasitic worker ignores the pheromonal signal, activates her ovaries and lays unfertilised haploid male eggs. Intra-specific parasitism of this type has been demonstrated in the western honey bee *Apis mellifera*. Furthermore, of the three honey bee species examined thus far, high levels of social parasitism have been observed in queenless colonies.

Sympatric in South East Asia, *Apis florea* and *A. andreniformis* comprise a subgenus of honey bees known as the dwarf bees. I examined undisturbed nests of *A. andreniformis* and *A. florea*, confirming reports that hetero-specific workers are often present at low frequency. The presence of hetero-specific workers in nests raises the possibility of inter-specific social parasitism in which hetero-specific workers fail to respond to the resident queen's pheromonal signal, laying eggs that potentially develop into males.

To examine this question I developed a molecular test of species, which allowed me to assess the species of eggs and larvae. I then created species-blended colonies with, and without queens. I assessed worker ovary activation rates within blended colonies and the species of the male brood they produced.

In queenright species-blended colonies hetero-specific workers responded to the resident queen's pheromones and did not activate their ovaries. When queenless, the majority species in the blended colonies had greater reproductive success than the minority species, presumably because the majority policed hetero-specific eggs by eating them. Examination of hetero-specific workers from wild nests showed that none had activated ovaries. I therefore conclude that although *A. andreniformis* and *A. florea* workers drift between nests they respond to the pheromonal signals within a hetero-specific nest as if it was their own. Thus, despite strong predictions from kin selection theory that heterospecifics would benefit from parasitising other nests, policing is apparently a strong defense against such behaviour, which is apparently extremely rare. The implications of my findings for the ongoing debate as to whether functional worker sterility arises from pheromonal castration or workers responding to an honest signal of their queen's fertility will be discussed.

## **Analysis of a plasmid segregational stability determinant from *Staphylococcus aureus***

Evelyn Lai    *Supervisors:* Dr N Firth & Dr S Jensen  
School of Biological Sciences, University of Sydney, NSW 2006

Strains of *Staphylococcus aureus* resistant to multiple antimicrobial agents are a major cause of hospital acquired infections globally. In many cases, resistance genes are carried on extrachromosomal DNA elements known as plasmids. In the absence of antibiotic selection, plasmids with low copy numbers are effectively maintained in bacterial populations through segregational stability determinants such as active partitioning systems. A novel putative partitioning system that enhances plasmid segregational stability, *par*, is present on the multiresistance plasmid pSK1; homologous genes are found on most *S. aureus* multiresistance plasmids. It is hypothesised that this single protein-encoding gene may be functionally analogous to the two gene partitioning systems found on other characterised plasmids.

Two putative domains within Par have been identified by sequence analysis; an N-terminal helix-turn-helix (HTH) DNA-binding domain and a centrally located coiled-coil (CC) protein-protein interaction domain. The aim of this study was to assess the significance and roles of these domains through site-directed mutagenesis and subsequent functional analysis. A mutation was created in the HTH domain and in vivo segregational stability assays showed that it abolished function; mutations in the CC domain have previously been shown to have a similar effect. In vitro studies were then utilised to determine the specific roles of the two domains. Gel-mobility shift assays with purified proteins revealed that the HTH mutant was unable to bind to its known DNA-binding site. Two different mutations in the CC domain had no significant effect on DNA binding. Protein cross-linking studies suggested that Par oligomerises into high molecular weight species, and that mutations in the CC domain affected their formation. Taken together, these results confirm the importance and predicted roles of the HTH and CC domains of Par. The findings of this study may permit the advancement of strategies that interfere with plasmid maintenance, which could improve the efficacy of existing antibiotics.

## **Moving home: Nest-site selection in the Asian Dwarf Honeybee (*Apis florea*)**

James Makinson    *Supervisors:* A/Professor M Beekman & Professor B Oldroyd  
School of Biological Sciences, University of Sydney, NSW Australia 2006

The Asian Dwarf Honeybee (*Apis florea*), is one of the most basal species within the *Apis* family, and differs in nesting biology to the more derived and better studied European Honeybee (*Apis mellifera*). The process of nest site selection and swarming is well understood in *A. mellifera* and involves three main steps: first a large number of workers headed by a queen split from a colony and form a temporary cluster within a few tens of metres. Individuals then search the surrounding environment for suitable nest sites before returning and performing a waggle dance indicating the location visited. Once a (near) unanimous decision on a nest-site is made the swarm takes off and travels to their new home.

Oldroyd et al. (2008) discovered that *A. florea* swarms do not advertise for specific potential nest-sites, but rather a number of large general areas. They also found that *A. florea* swarms often took off prior to having reached a unanimously decision about the direction of travel.

My aim was to understand the individual interactions that take place during reproductive swarming in *A. florea*, and to determine the process behind the observations made by Oldroyd et al. 2008. To achieve this I created 4 artificial swarms, 2 in which every individual was individually marked, and 2 in which bees were marked when were first seen dancing. I found that dance decay, a process critical in the decision-making process of *A. mellifera*, does not occur in this species.

Through observation of natural swarming events in wild colonies, I found evidence suggesting *A. florea* does not form temporary clusters during nest-site selection, and that the Nasonov pheromone is not used to cause clustering in this species. These differences are discussed in the context of the evolution of decision-making behaviour in this species.

## **Transfer of antibiotic resistance genes in clinical strains of *Staphylococcus aureus***

Christopher McKenzie    Supervisors: Dr B Lyon & Dr N Firth  
School of Biological Sciences, University of Sydney, NSW 2006

*Staphylococcus aureus* is a Gram-positive bacterium that is a frequent cause of hospital- and community-acquired infections. Horizontal transfer of mobile genetic elements, including plasmids, has been a major driving force behind the evolution of antibiotic-resistant strains. When two strains of *S. aureus* are cultured together, it is possible to obtain transfer of plasmids at relatively high frequency. However the mechanism by which such transfer occurs is not well characterised. Conventionally plasmid DNA can be exchanged by three mechanisms: transformation (uptake of naked DNA from the surrounding environment); transduction (transfer of DNA by a bacteriophage viral vector) and conjugation (transfer of plasmids encoding their own mobility, by a mechanism involving cell-to-cell contact). Transfer between mixed cultures of *S. aureus* has been previously reported that does not fit neatly with any of these processes. The aim of this study was to survey Australian clinical *S. aureus* isolates for the ability to transfer plasmids in mixed cultures and to investigate the mechanism by which this transfer occurs.

A range of clinical isolates was obtained, from a number of hospitals around Australia, representing a period of decades. These strains were surveyed for their ability to donate antibiotic resistance genes into a suitable recipient strain. Variation was observed between strains in ability to transfer and transfer frequency. Transfer of plasmid DNA was confirmed by plasmid isolation and polymerase chain reaction (PCR). Of the 16 donor strains, 8 were observed to transfer, however variability in donor capacity was seen even within strains suggesting that transfer in mixed culture is a stochastic event.

Investigations into the mechanism of transfer were carried out using donor strain SK656, transferring the pSK1-family plasmid pSK14. Transformation as a mechanism of transfer was found to be unlikely, as transfer was not sensitive to deoxyribonuclease (DNase) and staphylococcal cultures themselves were found to produce sufficient extracellular nucleases to interfere with transformation. Calcium ions were found to be an obligatory requirement for mixed culture transfer, as is the case in transduction, and the inclusion of calcium ions was associated with higher concentrations of bacteriophage. However, when transfers were attempted using a cell-free filtrate of the mixed culture, no transducing phage particles were detected. This suggests that phage is involved in mixed culture transfer, but its role remains uncertain. Conjugative transfer was deemed unlikely as pSK14 lacks the *tra* region required for conjugative plasmid transfer. However, the role of cell-to-cell contact in mixed culture transfer has yet to be fully explored.

## **Investigations into pathogenic *Staphylococcus epidermidis***

Sign Cong Nguyen    *Supervisors: Dr B Lyon & Dr N Firth*  
School of Biological Sciences, University of Sydney, NSW 2006

Though normally a harmless commensal, *Staphylococcus epidermidis* is also an opportunistic pathogen, and is fast becoming one of the leading causes of nosocomial infections worldwide. The ability to form biofilms on medical devices is one of the major determinants in the initial phase of infection, ultimately leading to further complications such as septicaemia. In this project, *S. epidermidis* and other coagulase-negative staphylococci (CNS) strains were isolated from individuals in the community and infected patients. It was hypothesised that the two sets of strains would differ in regard to their ability to form biofilms, as well as in their resistance to antibiotics and the presence of candidate pathogenicity genes.

Biofilm formation was tested using two different techniques, though the two did not always agree for the surveyed isolates. Where there was agreement, it was found that the ability to form biofilms was more common among clinical strains than community strains. A similar trend was observed with antibiotic resistance. The PCR was used to detect the presence of eight known or putative pathogenicity genes, both related and unrelated to biofilm formation. Possession of no single gene was able to fully distinguish clinical strains from community strains. Furthermore, aside from the previously reported relationship between the *icaADBC* operon and biofilm formation, none of the genes was found to be highly correlated with biofilm formation, though functional analyses have yet to be performed. Finally, investigations into biofilm production suggest that cell-surface proteins are required for biofilm formation to occur.

The results and methods developed in this study pave the way for future studies into differences between clinical and community strains, as well as mechanisms behind biofilm formation. Ultimately the ability to screen for potentially pathogenic strains and a further understanding of them would allow for preventative action as well as treatment.

## **Targeting and expression of a symbiotic ion transport protein in soybean**

Andrew Ritchie    *Supervisors: Professor R Overall, Dr P Smith & Professor D Day*  
School of Biological Sciences, University of Sydney, NSW 2006

Symbiosis is one of the most fundamental biological interactions and one which is vital to a wide range of biological systems. One of the most accessible and economically important symbiotic relationships is that which is formed between legumes and nitrogen-fixing rhizobial bacteria. The rhizobia are housed within modified root cells in specialised organs known as nodules and are packaged in facultative, membrane-bound organelles known as symbiosomes. The symbiosome membrane contains a number of membrane-bound transport proteins which allow it to serve as a symbiotic nutrient-exchange interface between the host cell and the endosymbionts, the mechanisms for which remain poorly understood despite extensive research interest. In particular, almost nothing is known about the manner in which these proteins are specifically targeted to the symbiosome membrane. Recent studies have discovered that GmZIP1, a membrane-bound zinc transporter in soybean, is a symbiosis-specific protein which is localised to the symbiosome membrane. This protein has been found to contain a sequence that encodes a putative 28 amino acid cleavable signal peptide which may function in the targeting and localisation of the protein to the symbiotic membrane. I attempted to investigate this signal by expressing targeted and untargeted fragments of the GmZIP1 sequence in soybean nodules and visualising their subcellular localisation using both immunocytochemistry and fusion to a fluorescent marker protein. In addition, I carried out a quantitative real-time PCR analysis of the expression of GmZIP1 over the period of nodule development in an attempt to clarify its proposed status as a symbiosis-specific protein. These investigations have shown that GmZIP1 is strongly upregulated in mature nodules, and have yielded insights both into the function of the proposed signal peptide and the suitability of overexpressed fluorescent markers for future studies in soybean nodules.

## **The relative importance of fixed and plastic responses to climate variability in a fish (*Gambusia holbrooki*)**

Nicholas Roosen    *Supervisors:* A/Professor F Seebacher & Dr A Ward  
School of Biological Sciences, University of Sydney, NSW 2006

Populations can compensate for environmental change through responses such as genetic adaptation or phenotypic plasticity. Individuals capable of reversible phenotypic plasticity in morphology, physiology and behaviour might have a selective advantage to environmental change within a lifetime. The aim of this experiment was to assess whether mosquitofish (*Gambusia holbrooki*) from climatically separated populations have specialised to their thermal environment and thus display a fixed response, or whether any differences that may exist are plastic. Mosquitofish were introduced into coastal Sydney in the 1920's and have recently radiated into the inner hinterland and mountain climates. How populations of mosquitofish survive the abrupt thermal gradient that exists in the relatively small area that they have dispersed into is not known. I assessed five populations of mosquitofish along an altitudinal gradient from coastal Sydney to the Blue Mountains. Male mosquitofish from each of five populations were acclimated to 10 and 25°C for four weeks. We found in endurance swimming performance assays that populations originating from higher elevations acclimated to 10°C conceded performance at 25°C. Similarly we found in assays of aerobic metabolism higher altitude fish experienced a trade-off in enzyme performance at 25°C indicating an acclamatory response. However this trade-off was not found in assays of anaerobic metabolism suggesting that endurance rather than burst metabolic activities are more important during cold temperature stress periods. For conflict behaviour acclimation temperature was important in determining the outcome of a fight. However 10°C acclimated fish from cooler climates won more conflict bouts in 25°C than 10°C acclimated fish from warmer climates. A molecular phylogeny indicated that the populations studied were genetically indistinguishable. Our results show that the populations did respond to acclimation on a number of different levels but any changes that did occur are because of plasticity. We anticipate our research to provide a starting point for future studies that focus on what specific environmental factors are responsible for the observed differences in plasticity between populations.

## **'Drilling mud' – is it really all that bad? An ecotoxicological study of drilling fluids used in the oil and gas industry on shallow and deep water crustaceans**

Helen Smith    *Supervisors: Dr M Thomson & Dr A Pile*  
School of Biological Sciences, University of Sydney, NSW 2006

Drilling activity has increased significantly over the last century and there is growing concern that the extraction of oil and gas may alter the marine ecosystem. Compared to other OECD countries, Australia lags behind in studying the impact of drilling activity on native benthic fauna. Marine environments beyond SCUBA depth are difficult to access, so it is important to trial experimental protocols in the laboratory before embarking on *in situ* studies. This study used the brine shrimp *Artemia franciscana* as a convenient study organism to develop a protocol to test the effects of drilling fluid extract on (a) survivorship, (b) locomotion and (c) stress protein expression. Analogous protocols were used on the shallow water isopod *Cirolana harfordi*. Finally, *in situ* observations of the locomotion of the shrimp *Plesionika sp* in drilling and non-drilling regions were taken at 222m on the Australian Northwest shelf. Regardless of drilling fluid extract concentration, *A. franciscana* (a) lived longer, (b) swam faster and (c) expressed less heat shock protein 70 than the controls. Unlike *A. franciscana*, the survivorship and swim speeds of *C. harfordi* was not different from sea water controls when exposed to drilling lubricant, and similarly the *in situ* studies of *Plesionika sp*. showed no change in locomotion in drilling and non-drilling regions. This study contradicts the common misconception that modern synthetic based drilling lubricants are toxic, and supports the notion that drilling companies are moving towards more environmentally friendly drilling practices. Future long term studies on other deep sea organisms will determine the effect of drilling fluids on the basic biology of deep water animals.

## **Responses to macronutrient selection and thermal acclimation in juvenile Mozambique tilapia (*Oreochromis mossambicus*)**

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An animal's ability to thrive in a naturally variable environment is dependent on an array of biotic and abiotic factors, of which nutrition and temperature are critical components. Macronutrients such as proteins (P), carbohydrates (C) and fats (F) are processed by different metabolic pathways. The thermal sensitivity of these pathways can limit their assimilation and thereby constrain animal performance. However, studies on the interaction of nutrition and temperature in fish have progressed slowly. Using the multi-dimensional 'geometric framework' (GM), I show that juvenile Mozambique tilapia (*Oreochromis mossambicus*) have the capacity to selectively regulate macronutrient intake and that thermal acclimation has some effects on macronutrient choice and physiology. Macronutrient regulation occurred within two days of being presented with foods that varied in composition. Thermal acclimation did not have an effect on food intake; however, C macronutrient intake was affected by acclimation temperature but was dependent on the test temperature. Growth was fastest when fish were acclimated to 28°, irrespective of diet. Acclimation temperature and swim temperature affected locomotion, while diet did not; the critical sustained swimming speed ( $U_{crit}$ ) was greater in fish acclimated and swum at the same temperature. Temperature and diet did not have an effect on aerobic metabolic activity, indicated by citrate synthase (CS); however, temperature did have an effect on anaerobic metabolic activity, indicated by lactate dehydrogenase (LDH). This research further provides supporting evidence towards macronutrient regulation in fish and explores the effects of temperature on macronutrient selection and physiology. Research on the interaction between nutrition and temperature on the basic biology and physiology of tilapia can help mitigate their impacts where they are seen invasive but also improve husbandry techniques where they are cultured for food.

## **War at the water's edge: Evaluating the potential of meat-ants as a biological control of cane toads in tropical Australia**

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Invasive species can have hugely detrimental impacts on native ecosystems, and we urgently need novel approaches to reduce invader densities. One potential vulnerability for any invasive organism is the possibility that some of its traits are poorly suited to the new environment it occupies. My project explores one such suite of potentially maladaptive traits in the invasive cane toad (*Bufo marinus*), and examines whether it might be feasible to exploit that situation to reduce numbers of toads in tropical Australia.

First, my laboratory studies assessed factors affecting the vulnerability to meat ants (the native species *Iridomyrmex reburrus*) of metamorph cane toads, compared to seven species of native frogs. This work confirmed that the young toads tend to select "dangerous" habitats, and to be active at "dangerous" times of day, in ways that render them vulnerable to predation by meat ants. Cane toads were significantly more vulnerable than any of seven species of native anurans that I tested in the same way. For example, metamorph cane toads were more likely to select open microhabitats, and less likely to move away from approaching ants; and hence, were more likely to be injured by ant attack.

Second, my field studies quantified the proximate determinants of toad vulnerability. Larger metamorph toads were more active and suffered higher attack rates, but were better able to escape (primarily reflecting an ontogenetic shift in behavioural response to attack). Ant densities also influenced toad antipredator responses. Overall mortality rates of toads were high following ant attack, even for toads that appeared superficially uninjured after escaping from their encounter with ants. Metamorph body size consistently predicted survival rates both under natural conditions, and in my manipulative experiments.

Third, I applied food baits to waterbody edges in an attempt to increase ant densities and thus, potentially, increase mortality rates of metamorph toads. As intended, baiting substantially increased ant densities and metamorph mortality rates, with no detectable impact on other native taxa. Meat ants thus may provide an environmentally friendly approach to biological control of the invasive cane toad; nonetheless, more work is required to understand potential impacts on other non-target species.