AIMS@JCU
2011 Student Seminar Day

7th April, 2011

Australian Institute of Marine Science

9:00am	Opening Address	Lyndon Llewellyn
9:15 am	Influence of DMSP on coral associated bacteria	Jean-Baptiste Raina
9:30 am	Predicting the distribution of mesophotic reef communities in the Great Barrier Reef Marine Park across spatial scales	Tom Bridge
9:45 am	Chemically mediated interference of bacterial signals by Australian soft corals	Marnie Freckleton
10:00 am	Extraordinary tissue regression and recovery in the marine sponge *Ianthella basta*	Heidi Luter
10:15 am	Adaptation of *Symbiodinium* populations defines the fitness of coral symbioses	Emily Howells
10:30 am	Sea urchin predation on Ningaloo Reef	Charlotte Johansson
10:45 am	Morning Tea	
11:15 am	Nitrogen fixing bacteria associated with 3 corals of the Great Barrier Reef	A. Kimberley Lema
11:30 am	A novel assay for the detection of the coral pathogen Vibrio coralliilyticus	F. Joseph Pollock
11:45 am	Benthic nitrogen cycling in a prawn farm settlement pond	Sarah Castine
12 midday	The role of live coral in the recruitment and recovery of reef fish communities	Darren Coker
12:15 pm	Rapid genetic assay for the identification of two *Pocillopora* species and its application in a population genetic study	Gergely Torda
12:30 pm	Exploring the species boundary in the Genus *Seriatopora* on the Great Barrier Reef	Patricia Warner
12:45 pm	Multiplex PCR protocol for simultaneous detection of *Vibrio harveyi*-related pathogens	Ana Cano-Gómez
1:00 pm	Lunch and tour of AIMS facility	
2:15 pm	Prize Presentations	

Influence of DMSP availability on coral associated bacteria

Jean-Baptiste Raina[1,2,3], Adrian Lutz[1,2,3] , Dianne Tapiolas[1], Cherie Motti[1], Francois O. Seneca[1,2,3], Bette L. Willis[2] and David G. Bourne[1]

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Dimethylsulfide (DMS) emissions, derived from the breakdown of dimethylsulfoniopropionate (DMSP), are integral for cloud formation and exert potentially major cooling effects on climate at local scales. Reef-building corals are major contributors to the production of DMSP and DMS, and these compounds also potentially have an important role in structuring coral-associated bacterial communities. The dynamics of DMSP production during thermal anomalies and its effect on coral-associated microbes was investigated. Twelve colonies of the reef-building coral Acropora millepora were subjected to two different water temperatures (27ºC and 32ºC) for a period of three weeks. A novel quantitative-NMR approach was developed to measure DMSP concentrations in coral tissue and demonstrated a two fold increase in DMSP in corals exposed to 32°C compared to controls held at 27°C. However, deep sequencing of the bacterial 16S rRNA genes did not reveal a shift in the coral-associated bacterial community. These results suggest that these communities might me more resilient to changes in water temperature than previously thoughts, maybe due to the persistent availability of DMSP throughout this stressful event. This integrated approach increases our current understanding of coral-microbial associations, the effect of temperature stress on coral microbial symbioses and the role organic sulfur compounds play in the resilience of the coral holobiont.

Predicting the distribution of mesophotic reef communities in the Great Barrier Reef Marine Park across spatial scales

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Mesophotic coral reef ecosystem (MCE) is a term that has recently been used to describe warm-water coral reef communities in the deeper regions of the photic zone (30-150 m). In recent years there has been a rapid increase in research effort on MCEs, both because of their unique biodiversity and also for the potential to provide refugia from environmental stress. There is growing evidence that deep reef communities may afford significant protection for corals and associated species from disturbance events such as warm-water bleaching events and tropical cyclones, both of which are expected to increase in frequency and severity in coming decades as a result of global climate change. MCEs may therefore play a vital role in the resilience of coral reef ecosystems. Unfortunately, MCE research in Australia is lagging behind other developed countries such as United States. The submerged reefs occurring along the shelf-edge of the Great Barrier Reef may support some of the most extensive MCEs in the world, however the vast majority of potential habitat is currently not recognised as reef habitat by management authorities. This study uses information collected on a 2007 expedition to the GBR shelf-edge to model the distribution of MCE taxa and communities on the Great Barrier Reef, and indicates that the amount of reef habitat in the GBRMP may be underestimated by up to 30%. Given the potential importance of MCEs to provide both refuge and a source of colonists to shallow-water habitats following disturbance, understanding the spatial extent and ecology of MCEs should be a priority for scientists and managers.

Chemically mediated interference of bacterial signals by Australian soft corals

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It has been well established that many bacteria can communicate through a chemical signalling process known as Quorum Sensing (QS). This process is central to interactions with other bacteria, the environment and higher organisms, and is particularly prevalent in surface associated bacteria and biofilms. It is therefore not surprising that reports are emerging of chemical signals produced by higher organisms that can interfere with this communication system. Soft corals are important members of many GBR communities, averaging approximately 10% of benthic reef cover, but may reach levels of up to 70% cover. In addition, soft corals are known to produce a range of bioactive molecules including cembrenes, many of which share structural similarities to bacterial signalling molecules. We hypothesise that these cembrene molecules may be important to the interaction of the soft coral with its surface associated bacteria. Two genetically modified bacterial biosensors, coupled with chemical separation techniques were used to successfully screen several soft coral species collected from Orpheus Island and their associated bioactive molecules for QS interference. Our results are consistent with the proposal that mediation of the interaction between higher organisms and their surface associated bacteria occurs through interference in QS systems.

Extraordinary tissue regression and recovery in the marine sponge *Ianthella basta*

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Given the totipotent nature of their cells, sponges have the ability to recover from damage due to physical disturbances, with a capacity to regenerate damaged cells within 24 hrs of injury. Here we demonstrate the remarkable ability of sponges to recover from substantial tissue loss in a short period of time. Six specimens of the marine sponge *Ianthella basta* that appeared health-compromised (tissue necrosis and lesions) were collected at Orpheus Is., northeastern Australia and transported to the Australian Institute of Marine Science where they were kept in a 1,000 l flow-through outdoor aquarium. After 12 hrs, sponges displayed substantial tissue loss with visible gaps evident between sponge fibres. However, within 72 hrs the sponges displayed rapid recovery and regeneration of tissues. In addition to visual comparisons of the sponges, images were compared using the integrated density measurement of Image Tool for Windows (UTHSCA). The integrated density of the sponge tissue effectively doubled within 72 hrs (increasing by 92%), confirming extraordinary tissue recovery in *I. basta*. Histological analysis revealed that sponges exhibiting regressed tissues had significantly fewer choanocyte chambers and more densely packed, granulated cells than sponges with non-regressed tissues. In addition, the microbial symbiont profiles between *I. basta* with regressed tissues were consistent with sponges having non-regressed tissues. The mechanisms behind this tissue loss and subsequent regeneration warrant further investigation as they have significant implications for our understanding of health and recovery in sponge communities.

Adaptation of *Symbiodinium* populations defines the fitness of coral symbioses

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The capacity of corals to respond to climate change is partly dependent on the ability of their obligate dinoflagellate symbionts (*Symbiodinium* spp.) to undergo adaptation to changing thermal environments. To determine the extent to which *Symbiodinium* are adapted to their local thermal environment and how such local adaptation governs thermal tolerance of the coral host, we infected coral juveniles with the same *Symbiodinium* strain from populations that differ in thermal history. *Symbiodinium* populations from a warm and a cool reef differed from one another in their photo-physiology, growth and mortality across a range of temperatures (27 to 32°C), where optimal performance was observed under conditions that reflected their native thermal environment. Physiological differences between *Symbiodinium* populations were maintained over multiple asexual generations in culture and strongly influenced the thermal tolerance of the coral host. At high temperature (32°C), corals inoculated with *Symbiodinium* C1 from a warm location resisted bleaching and maintained rapid growth, whereas corals inoculated with *Symbiodinium* C1 from a cooler location suffered severe bleaching and ceased growing. Acclimatory responses of both the *Symbiodinium* and coral host were controlled for during experiments, therefore we conclude that variation in the response of *Symbiodinium* C1 populations has a genetic basis. This study highlights that characteristics of thermal tolerance and sensitivity exist not only among different *Symbiodinium* strains but between populations belonging to the same strain. Furthermore, this population level adaptation influences the fitness of the coral host.

Sea urchin predation on Ningaloo Reef

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Predation by fishes has been identified as a major determinant of sea urchin densities. Lack of predators has resulted in unsustainable increases in sea urchin populations around the world, with sea urchins becoming detrimental to the ecosystem. Their herbivorous feeding mode turns from a beneficial feature to becoming a destructive force. Our study quantified the distribution of sea urchin predators on Ningaloo Reef, Western Australia, to see if the availability of predators may account for the significant among-habitat variation in the density of Echinometra mathaei urchins. Studies were conducted in three habitats; slope, back reef and macroalgal lagoons, in 2010 and 2011, with belt transects being used to estimate density and biomass of sea urchins and predators. A video experiment was also conducted to identify sea urchin predators on Ningaloo Reef. We identified 7 species of predators feeding on tethered sea urchins. These species belonged to the Balistidae, Diodontidae, Labridae, Lethrinidae and Tetradontidae. However only 3 of these species were encountered during belt transects, highlighting the shy and cryptic natures of some predators. Sea urchins were 10 times more abundant on the slope compared to the back reef and lagoon habitats. However, the density of predators was also highest where sea urchin abundance was highest. This suggests that higher urchin abundances cannot be explained by the lack of fish predators. Habitat suitability, substratum availability and reef exposure may be more important factors in shaping urchin densities.

Nitrogen fixing bacteria associated with 3 coral species of the Great Barrier Reef

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Nitrogen fixation is thought to be one of the key functional roles of coral-associated bacteria. Given the nitrogen limited status of coral reefs, nitrogen fixing (diazotrophic) bacteria are hypothesised to provide an important source of nitrogen to endosymbiotic dinoflagellates in the genus *Symbiodinium* (zooxanthellae). To better understand the nature and specificity of coral–diazotroph symbioses, diazotrophic bacterial communities were compared among three coral species (*Acropora millepora*, *Acropora muricata* and *Pocillopora damicormis*) at three mid-shelf reefs on the Great Barrier Reef (Australia). Profiling of the nif H fragment of the nitrogenase gene revealed diverse diazotrophic bacterial communities, with each of the three coral species harbouring a characteristic suite of diazotrophic species. Moreover, diazotrophic communities characteristic of each species were similar at all three locations, suggesting that diazotrophic bacteria associated with corals are species specific. Phylogenetic analysis revealed that the dominant diazotrophic bacteria (67% of total retrieved sequences) are closely related to bacterial species symbiotic with plants, further suggesting a close relationship between diazotrophs and zooxanthellae. Coral-associated diazotrophs have also been isolated, and the functional roles and benefits these organisms provide to the coral holobiont are the subject of ongoing studies.

A novel assay for the detection of the coral pathogen *Vibrio coralliilyticus*

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Coral diseases represent an emerging threat to Indo-Pacific reefs. To enhance understanding of the impact, spread, and underlying causes of coral disease, rapid and highly sensitive diagnostic tools are required to specifically detect and quantify coral pathogens. *Vibrio coralliilyticus* has been implicated as the aetiological agent responsible for bleaching and tissue lysis of *Pocillopora damicornis* in the Indian Ocean and for a number of white syndrome disease epizootics throughout the Indo-Pacific, and therefore represents a good model system for the development of coral pathogen diagnostics. A quantitative PCR (qPCR)-based *V. coralliilyticus* detection assay has been successfully developed, which targets the dnaJ gene, encoding for heat shock protein 40. Six out of seven *V. coralliilyticus* strains isolated from white syndrome infected corals showed positive amplification and no qPCR amplification was observed in 12 related outgroup strains. The assay was highly sensitive, able to detect as little as 1 picogram of *V. coralliilyticus* DNA and 10^2 cfu per 20 μL reaction and as little as 105 cfu per mL of seawater. Inhibition of the assay with DNA and cells derived from bacteria other than *V. coralliilyticus* was minimal, validating the applicability of this assay when targeting the pathogen within the complex coral holobiont. This assay represents a novel approach to coral disease diagnosis and provides a useful tool for coral pathogen detection and accurate diagnosis. The assay will enable monitoring of pathogen loads in individuals and ecosystems and will ultimately be used to identify pathogen sources, vectors, and reservoirs. Accurate detection and diagnosis capabilities will play a vital role in advancing the field of coral disease research and will provide important knowledge to help effectively manage the world's coral reefs.

Benthic nitrogen cycling in a prawn farm settlement pond

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Effluent from aquaculture ponds is an increasing source of anthropogenic nitrogen (N) to coastal areas worldwide. "Zero net nutrient discharge" regulations proposed for land-based aquaculture across tropical Australia will require sound mitigation measures to treat effluent prior to release. Currently, settlement ponds are used to clarify aquaculture effluent. However, there are opposing biogeochemical processes in settlement ponds. The mineralisation of settled organic matter returns dissolved N back into the effluent. In addition, the processes of dissimilatory nitrate reduction to ammonia (DNRA) and inorganic N immobilisation conserve fixed N within the system. In contrast, denitrification and anammox are the only microbial processes capable of complete removal of fixed N from these ponds. The net result of these opposing processes determines the remediation capacity of the settlement pond, but few studies have ever quantified their rates or potential. In this study we employed isotope tracer techniques to quantify the rates and relative importance of the major biogeochemical N pathways in shrimp farm settlement ponds. Ammonium mineralisation was high near the inlet (817 µmol N $m^{-2}h^{-1}$) and middle (770 µmol N $m^{-2}h^{-1}$) of the settlement pond but lower at the end of the pond (180 µmol N $m^{-2}h^{-1}$). DNRA was not important as an uptake pathway for NO_3^-. However NO_3^- immobilisation did compete with denitrification for available NO_3^-. Of the two processes removing nitrogen, the rate of denitrification was low near the pond inlet (88.4 µmol N $m^{-2}h^{-1}$), was highest at the centre of the pond (203.3 µmol N $m^{-2}h^{-1}$) and showed reasonable activity at the pond outlet (124.5 µmol N $m^{-2}h^{-1}$). Denitrification appeared to be directly related to the availability of organic carbon (TOC) (r^2=0.97). Anammox was not detected in the settlement pond. Overall the uptake of NO_3^- was divided evenly between immobilisation and denitrification and the net cycling of N meant that N was conserved within the settlement pond system.

The role of live coral in the recruitment and recovery of reef fish communities

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Replenishment of fish populations following coral degradation is critical for the recovery of ecosystems through the return of key functional groups that help maintain reef health, promote recovery and provide resilience by preventing phase shifts. Coral reef fishes vary in their reliance on critical features of the habitat (live coral, rubble, algae, complexity) at different stages of their life. An understanding of how impacts through the change in substrate health and complexity will influence the recruitment of many fishes is essential. This is extremely important given that impacts to coral reefs are predicted to increase over the next few decades. By using manipulated manmade patch reefs consisting of six different habitat treatments; three levels of live coral cover crossed with 2 levels of habitat complexity, we were able to elucidate difference in abundance, diversity and functional groups of fishes settling to the different habitats using multivariate analysis and general linear models. Reefs with low levels of live coral cover supported less fish and revealed a difference in species composition to that of reefs with high coral cover. This difference was driven by a decline in small planktivores on reefs consisting of low live coral cover. Understanding these changes in recruitment is vital as recruitment is important for the recovery of fish communities as the spatial scale of disturbance to coral reefs increase, population connectivity (which enables replenishment by recruitment from other parts of the population) will no longer be sufficient to buffer local populations against persistent declines in abundance.

Rapid genetic assay for the identification of two *Pocillopora* species and its application in a population genetic study

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Pocillopora damicornis (Linnaeus, 1758; Order: Scleractinia, Family: Pocilloporidae) has recently been identified to be a species complex, with as many as 6 potential criptic species on the Great Barrier Reef. Although not only the mitochondrial and nuclear DNA sequences, and microsatellite genotyping, but also the morphological characteristics separate these putative species, their identification based solely on gross morphology is not reliable in most instances, especially not under field conditions, or on live corals. Here we present a quick, cost effective genetic assay to separate putative criptic species in the *P. damicornis* complex. This assay is based on two mitochondrial markers from the putative control region, which show consistent and easily identifiable product size differences among species after Alu I restriction enzyme digestion of the PCR products. We demonstrate the application of this assay in a population genetic study which looks into connectivity patterns of two *Pocillopora* species.

Exploring the species boundary in the Genus *Seriatopora* on the Great Barrier Reef

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The taxonomic delimitation of coral species traditionally relies on morphological characteristics that are often difficult and impractical for field identification. Two species of Scleractinian coral in the Genus *Seriatopora* (Pocilloporidae) have been reported to occur on the Great Barrier Reef (GBR). *S. hystrix* is a widespread and abundant species found in a diverse range of reef habitats, and has recently been the focus of a growing body of scientific research. Comparatively the congeneric *S. caliendrum* is uncommon and has generated less interest. The unclear taxonomic description and separation of these species may often be ignored as a consequence of the apparent rarity of *S. caliendrum*, however for the purposes of population genetic studies in particular, it is imperative that the two can be satisfactorily distinguished. In extensive surveys of the Palm Islands and Lizard Island, we have sampled 10 different sites in both locations for determining the population genetic structure and connectivity of *S. hystrix* populations at various spatial levels. Throughout these surveys the "typical" *S. hystrix* morphology has dominated as expected, however two sites, one in each region, display variability in morphologies inconclusively suggesting that both species may occur. In this study we evaluate gross morphological and genetic evidence for the presence of these different *Seriatopora* species at two sites on the GBR and address how such identification issues should be addressed for future studies.

Multiplex PCR protocol for simultaneous detection of *Vibrio harveyi*-related pathogens

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Bacterial species belonging to the *Vibrio harveyi* clade are considered some of the most pathogenic bacteria to marine reared animals, causing important economic losses in the aquaculture industry worldwide. In the diagnosis of vibriosis, misidentification of *V. harveyi*-like isolates is common due to highly similar phenotypes and genotypes shared by these closely related species. Recently, the Multilocus Sequence Analysis (MLSA) approach has offered an overview of the taxonomy of this group and has also been suggested as a highly discriminative identification tool. The analysis of several housekeeping genes for species of the Harveyi clade in the latter studies allowed the selection of suitable loci to design specific PCR primers targeting *V. harveyi*-related species. This study describes the design of a multiplex PCR assays capable of specifically detecting and discriminating the highly similar *V. harveyi*-related bacterial species, as relevant pathogens of marine aquacultured animals. The four sets of specific primers designed target 3 different protein-coding genes (topA ,mreB and ftsZ) conserved in *Vibrio* species for DNA amplification of *V. harveyi*, *V. owensii*, *V. campbellii* and *V. rotiferianus*. For the multiplex PCR reaction, a single tube contains a mixture consisting in the 4 sets of specific and compatible primers, DNA from 1, 2, 3 or all the 4 target species, and common PCR reagents. Similarly, any combination of DNA templates from 2, 3 or the 4 *Vibrio* species included in the PCR reaction results in a 2, 3 or 4 band PCR pattern observed in the gels. In cases of bacterial isolation from prawns, lobsters and crabs as clinical samples, a qualitative assessment is included in the protocol to evaluate DNA extraction methods and to monitor host DNA contamination in the bacterial genome preparations. The quality assessment is based on the addition of previously designed primers specific for decapods crustaceans targeting the 18S rRNA gene. These primers will amplify the targeted region in cases of complete absence of *Vibrio* DNA or excess of decapod DNA. These monoplex and multiplex PCR-based diagnostic protocols offer fast and reliable single step detection and a discriminative identification of highly related and pathogenic *V. harveyi*-related species.