A microarray approach to understanding stress in a coral reef fish

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Statement of the contribution of others

All chapters of this thesis include collaborative work with my supervisors Prof. Ross H. Crozier and Dr. M. Julian Caley. The thesis chapters two, three, and four also include collaborative work with Dr. Alister C. Ward, Dr. Glenn Stone and Dr. Ashley R. Connolly. While undertaking these collaborations I was responsible for the project concept and design, the collection of the majority of the samples, the laboratory work, the statistical analyses, synthesis, and the preparation of manuscripts and this thesis.

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This work conforms to ethical requirements at relevant authorities and collection of animals was authorised from the Great Barrier Reef Marine Park Authority, permit number G04/12331.1.

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General Abstract

Coral reef fishes are expected to experience a rise in sea surface temperatures due to climate change. How well tropical reef fishes will respond to these increased temperatures and which genes are important in the response to elevated temperatures is not known. Microarray technology provides a powerful tool for gene discovery studies, but the development of microarrays for individual species can be expensive and time-consuming. There often are, however, microarrays available for related species. I show that inter-species genomic hybridisation experiments can be used to assess which genes are conserved enough for microarray analysis across species, and thus introduce a novel application of microarray technology. I performed a series of tests to determine whether a microarray developed for the zebrafish *Danio rerio* is useful for measuring gene regulation in the coral reef fish *Pomacentrus moluccensis*. I hybridised genomic DNA from both taxa onto the *D. rerio* microarray, and based on significant cross-hybridisation, inferred that most genes share significant sequence similarity between the two taxa. I also sequenced eight nuclear genes. These genes showed an average sequence similarity of 81%. Finally, I used quantitative real-time PCR to validate the microarray data for differential expression. The results of the genomic hybridisation experiments, direct sequence comparisons, and quantitative real-time PCR indicate that the *D. rerio* microarray is useful for measuring gene regulation in *P. moluccensis*. I then used the *D. rerio* microarray to characterise the transcriptional responses of *P. moluccensis* to elevated temperatures over five days. Heat stress elicited differential expression of 324 genes. The functions of heat-responsive genes indicated that prolonged heat exposure leads to oxidative stress and protein damage, challenges the immune system, and causes re-allocation of energy sources. I have shown that a temperature increase of three degrees above normal can lead to significant gene regulation in a coral reef fish suggesting that climate change will have measurable impacts upon coral reef fish physiology. In order to identify upstream regulators of the transcriptional responses observed and test for the presence of a general stress response, I measured the early gene responses of *P. moluccensis* to hypoxic, hyposmotic, cold and heat shock. Early stress responses three hours after exposure were generally associated with a

suppression of transcription, but the responses of individual genes varied depending on the type of stressor applied. Only a few genes showed consistent regulation across stress treatments. However, a series of gene functions showed consistent responses across stress treatments, suggesting that there are common effects of stress on biological function. I present a conceptual model of the interactions between stress responses at different levels of biological organisation. I propose that stress commonly leads to a reduction in cellular oxygen levels and oxidative stress. Reduced cellular oxygen levels initiate endocrine, cardio-respiratory, and cellular responses many of which are aimed at restoring cellular oxygen balance. Oxidative stress in turn activates certain signal transduction pathways, immediate early genes, and transcription factors. The transcriptional stress profiles measured in environmental genomic studies are likely the result of the activation of these redox-sensitive signalling pathways and transcription factors. Further, I tested whether genes with stress-related functions evolve at accelerated rates. To do this, I competitively hybridised genomic DNA from *D. rerio* and *P. moluccensis* to a *D. rerio* microarray. 985 genes showed evidence of accelerated rates of sequence evolution between *D. rerio* and *P. moluccensis*. Rapidly diverging genes were over-represented for receptor, transcription co-activator, and cell signalling functions, but not for stress-related gene functions. I obtained orthologous sequences to *D. rerio* for the teleosts *Takifugu rubripes* and *Gasterosteus aculeatus*. A selection of rapidly diverging candidate genes showed accelerated rates of sequence evolution across multiple teleost lineages. I have shown that genomic hybridisation experiments on microarrays can be successfully used to identify rapidly diverging genes, in particular in species that currently lack genome sequence data and for which bioinformatic approaches are thus not applicable.

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