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Health Highlights

Aquatic Animal Health Subprogram Newsletter
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In this issue

From the Subprogram Leader

FRDC Aquatic Animal Health Subprogram 2008-2012

The current FRDC Aquatic Animal Health Subprogram (AAHS) is due to finish in May 2012 and so this will be the last edition of *Health Highlights* within the current project. It is appropriate that I, as Subprogram Leader, look back over the past four years and provide a brief summary of the achievements of AAHS.

During this period, AAHS has been involved in providing advice to FRDC on aquatic animal health project proposals (submitted to FRDC) that has resulted in over 20 projects on aquatic animal health being approved by the FRDC Board. Subsequently, AAHS has had oversight of these projects to ensure that they deliver the promised outputs and outcomes to the relevant stakeholders and end-users. A listing of the projects managed during this 4-year period is provided on page 5 of this newsletter.

In addition to project oversight, AAHS has organised two Aquatic Animal Health Scientific Conferences (the "Cairns Conference") in 2009 and 2011. These were the fourth and fifth conferences of the Cairns series (previous conferences were held in 2003 (Geelong), 2005 (Cairns) and 2007 (Cairns)) and, based on participant feedback, these conferences are increasing in their significance not only nationally, but also internationally.

In 2011, in association with the Cairns conference and with additional support from FRDC, AAHS also organized an international workshop on Ostreid Herpesvirus in response to the POMS outbreaks in New Zealand and Australia. The report from this workshop has been made available to researchers, regulators and oyster growers around the world.

The Pullman Reef Hotel, Cairns
Venue for the First Australasian Scientific Conference on Aquatic Animal Health & OsHV-1 International Workshop, July 2011

For me, these have been the highlights of the past four years. It only remains for me to acknowledge and thank the aquatic animal health community in Australia for their support, particularly the research providers that have generated the high quality research, the presenters at the Cairns conferences and OsHV-1 workshop, the AAHS Steering and Scientific Advisory Committee members, the staff at FRDC Head Office, and the FRDC Board.

STC/SAC Meetings

The FRDC AAHS met on 20 March 2012 to provide FRDC with AAHS' recommendations concerning the research full proposals for the 2012-13 funding cycle. It is anticipated that the results of this funding round will be announced by May 2012.

Health Subprogram Website

Our website is located of the FRDC site and can be accessed directly under:

http://www.frdc.com.au/research/Animal-Health

There you can view this issue and all previous issues of *Health Highlights* - in addition to finding other information about the FRDC Aquatic Animal Health Subprogram.

Announcements

All final reports are available through the FRDC. Go to www.frdc.com.au to obtain a copy.

Newsletter submissions

The Aquatic Animal Health Subprogram welcomes contributions to *Health Highlights* on all aquatic animal health R&D news and events – both within and outside the FRDC. We aim to assist the

widespread exchange of information by including any of the following in each bi-annual edition: Project updates, milestone reports, final reports, research papers, project communication and extension outputs, info sheets, and letters to the editor. Announcements of conferences, workshops, meetings, etc. are also welcome.

Please forward contributions for the next edition of *Health Highlights* (December 2012) to Joanne Slater before 15 November 2012.

Mailing list

Health Highlights is distributed biannually to stakeholders via hard copy and email as well as being posted on the FRDC website at: http://www.frdc.com.au. To change contact details or to ensure inclusion on the *Health Highlights* mailing list, contact Joanne at:

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Completed AAHS Project Summaries

Project No. 2008/030: AAHS: Development of a DNA microarray to identify markers of disease in pearl oysters (*Pinctada maxima*) and to assess overall oyster health (PI: Brian Jones)

OBJECTIVES:

1. To construct a cDNA library using healthy and stressed oysters.
2. To design and print DNA microarray slides for the analysis of diseased states in pearl oysters (*P. maxima*).
3. To use the DNA microarray to identify molecular markers that differentiate between pearl oysters that are stressed from those that appear to be healthy.
4. To use the DNA microarray to test for markers of adverse health in pearl oysters that appear to be affected by environmental stressors other than OOD.

NON TECHNICAL SUMMARY

This project used DNA-based technology to study the effects of environmental stress on pearl oysters. During the oyster farming process, oysters are subjected to a range of changes in their environment, such as fluctuating temperature and salinity. These changes are exacerbated by farming practices, during which oysters are exposed to additional stresses, such as routine antifouling and nucleation. All of these factors have the potential to stress oysters, potentially affecting their growth, susceptibility to disease and other important characteristics that decrease productivity. At its most basic level, stress causes changes in the activity of genes in oysters, switching some on and turning others off. It is these changes in gene activity that alter characteristics such as growth rate and susceptibility to disease.

The goal of our project was to identify the genes in oysters that are affected by environmental stress. This had two significant benefits. It allowed the activities of stress-response genes to be monitored in the farming process in an effort to identify and modify particularly stressful components of the farming practice. The project also identified a set of genes that can be used as sensitive monitors of stress in the future to identify the onset of stressful events before they affect productivity.

The project used DNA microarrays to identify stress response genes. In microarrays, the DNA from thousands of oyster genes is printed as microdots onto the surface of glass slides. Our pearl oyster microarrays carried 3,000 spots of DNA. Those spotted microarrays were analysed to see which particular genes were turned on or off under particular environmental conditions. The end result was a list of stress-response genes that are affected by particular types of environmental stress that can be used to gauge the effects of environmental stress during routine farming practices. In terms of immediate advice to industry, our analysis suggests that exposing oysters to air is the most stressful factor in the current farming practice. The microarrays also provide a valuable resource for future research that will be freely available to the broader research community. Despite this, one significant conclusion from this study is that, without a complete genome sequence for pearl oysters, the utility of the microarray that we have developed is limited because it does not allow us to identify some of the genes associated with stress responses in oysters. Hence, one of our key recommendations at the end of this project is that industry and the relevant government authorities should support a nationwide effort to sequence the pearl oyster genome.

OUTCOMES ACHIEVED TO DATE

In terms of practical outcomes for industry, this project has:

- Developed a cDNA microarray that can be used by industry to test the effects of a range of environmental variables in the field
- Identified a suite of gene biomarkers that can be used to assess the relative impacts of different processes within the farming practice, with the intention of ameliorating or mitigating particularly harmful processes
- Provided early evidence that some environmental stressors, particularly exposure to air, may be relatively more harmful than other factors

KEYWORDS: pearl oyster, microarray, gene expression, environmental stress, oyster health

Project No. 2008/031: Aquatic Animal Health Subprogram: Investigation of Chlamydiales-like organisms in pearl oysters, *Pinctada maxima* (PI: Mel Crockford/Brian Jones)

OBJECTIVES:

- To further develop the current conventional PCRs being used to investigate two CLOs in pearl oysters and use these PCRs in an attempt to gain further sequence data than that available. An expansion of the current known sequence data will be used to develop a real-time PCR that is specific and sensitive enough to detect and differentiate between the two CLOs in pearl oysters.
- To test healthy versus OOD-affected pearl oysters to determine if the presence of either or both CLOs play a role in the onset of OOD. The Department of Fisheries WA currently has numerous samples of OOD-infected and some non-infected *P. maxima* oysters that are available to test for the presence of these CLOs using the developed and validated real-time PCR. More samples will be sought as the need arises, in particular *P. maxima* samples from NSW and QLD. Animals will be tested as individuals to determine the prevalence of the two CLOs in *P. maxima* oysters.
- To survey non-maxima shellfish associated with pearl farms to determine the prevalence of these organisms in molluscs in Australian waters, and whether there is a fellow molluscan reservoir host. Sampling will include wild non-maxima shellfish, shellfish stocked in supermarkets and any other shellfish that is available. Any positive samples obtained will be confirmed by sequencing the PCR product.

NON-TECHNICAL SUMMARY

During the initial disease outbreak investigation into Oyster Oedema Disease (OOD), staff from the Department of Fisheries WA visualized Chlamydiales-like organisms (CLOs) in OOD-affected animals by transmission electron microscopy (TEM), suggesting a link between the presence of these CLOs and OOD-affected pearl oysters. Initial molecular research confirmed that two CLOs were present in OOD-affected pearl oysters, one of which appeared to be *Simkania negevensis*, the other to be new and uncharacterised.

The conventional PCR for the detections of the two CLOs was developed as much as possible.

A multiplex qPCR was attempted, which targeted the two CLOs in the one PCR. However, this lacked sensitivity and was ineffective. Separate qPCRs were developed. One qPCR targeted *S. negevensis*., the other qPCR was designed for the detection of the uncultured CLO.

There appeared to be a high prevalence of *S. negevensis* in pearl oysters. However, results showed that *S. negevensis* was prevalent in many samples including both heavily infected animals and healthy oysters and was absent from shellfish sampled during the initial mortality investigation at the index site (Whalebone Island), suggesting that this organism is a commensal and not associated only with OOD-affected oysters.

The uncultured CLO PCR produced different results. Even though this organism appeared to be less prevalent, there appeared to be an association between the presence of this organism and sick oysters, although it is not clear that this organism alone is sufficient to cause OOD. The AusVet report (AusVet 2007) suggested that the cause of OOD is most likely multi-factorial, so it remains a possibility that other factors are also required for the onset of clinical OOD.

OUTCOMES ACHIEVED

1. Optimised conventional PCRs for the detection of the each of the two CLOs in *P. maxima* oysters.
2. Sequence data of two novel CLOs discovered in *P. maxima* oysters, allowing a suggestion of how these two CLOs fit into taxonomic classification.
3. Optimised and validated real-time PCRs for the detection of the two CLOs in *P. maxima* oysters, allowing rapid and specific detection of each CLO.
4. A report on the prevalence of these organisms in *P. maxima* oysters and confirmation/elimination of a link between the presence of one or both of these CLOs with OOD.
5. A report on the prevalence of these CLOs in other molluscs in Australian waters to assess the risk imposed on other shellfish due to the presence or absence of these CLOs.

KEYWORDS: Oyster Oedema Disease, Chlamydiales, *Simkania negevensis*, real-time PCR

Summary of AAHS Projects (2008-2012)

Project No.	Project Title	Principal Investigator
2004/084 **Completed**	Aquatic Animal Health Subprogram: Investigating and managing the *Perkinsus*-related mortality of blacklip abalone in NSW Phase 1 *Associated species: Haliotis* spp.	Dr Geoff Liggins Department of Primary Industries, NSW Phone: 02 9527 8533 geoff.liggins@dpi.nsw.gov.au
2004/086 **Completed**	Aquatic Animal Health Subprogram: Identification and distribution of an intracellular ciliate in pearl oysters. *Associated species:* Pearl oyster	Dr Shane Raidal Murdoch University, WA Phone: 08 9360 6000 Email: Raidal@murdoch.edu.au
2006/064 **Completed**	Aquatic Animal Health Subprogram: Development of diagnostic tests to assess the impact of *Haplosporidium* infections in pearl oysters *Associated species:* Pearl oyster	Dr Philip Nicholls Murdoch University, WA Phone: 08 9360 1394 Email: P.Nicholls@murdoch.edu.au
2007/006 **Completed**	Aquatic Animal Health Subprogram: Development of molecular diagnostic procedures for the detection and identification of herpes-like virus of abalone (*Haliotis* spp.). *Associated species: Haliotis* spp.	Dr Mark Crane AAHL Fish Diseases Laboratory Phone: 03 5227 5118 Email: mark.crane@csiro.au
2007/007 **Completed**	Aquatic Animal Health Subprogram: Optimisation of PCR tests for diagnosis of megalocytivirus (gourami iridovirus) and cyprinid herpesvirus 2 (goldfish herpesvirus). *Associated species:* multi-species	Prof Richard Whittington University of Sydney Phone: 02 9351 1619 Email: richardw@camden.usyd.edu.au
2007/225 **Completed**	Metazoan parasite survey of selected macro- inshore fish of south-eastern Australia, including species of commercial importance. *Associated species:* multi-species	Dr Kate S Hutson University of Adelaide Phone: 08 8303 5282 Email: kate.hutson@adelaide.edu.au
2007/226 **Completed**	Aquatic Animal Health Subprogram: Rapid strain identification of the bacterial fish pathogen *Streptococcus iniae* and development of an effective polyvalent vaccine for Australian barramundi. *Associated species:* barramundi	Dr Andy Barnes University of Queensland Phone: 07 3346 9416 Email: a.barnes@uq.edu.au
2008/030 **Completed**	AAHS: Development of a DNA microarray to identify markers of disease in pearl oysters (*Pinctada maxima*) and to assess overall oyster health. *Associated species: Pinctada maxima*	Dr Brian Jones Department of Fisheries WA Phone: 08 9368 3649 Email: bjones@agric.wa.gov.au
2008/30.20 **Completed**	AAHS: Development of a DNA microarray to identify markers of disease in pearl oysters (*Pinctada maxima*) and to assess overall oyster health. *Associated species: Pinctada maxima*	Dr David Raftos Macquarie University NSW Phone: 02 9850 8402 Email: draftos@rna.bio.mq.edu.au
2008/031 **Completed**	AAHS: Investigation of Chlamydiales-like organisms in pearl oysters, *Pinctada maxima*. *Associated species: Pinctada maxima*	Dr Brian Jones Department of Fisheries WA Phone: 08 9368 3649 Email: bjones@agric.wa.gov.au
2008/039 **Active**	AAHS: Strategic planning, project management and adoption. *Associated species:* multi-species)	Dr Mark Crane CSIRO AAHL Fish Diseases Laboratory Phone: 03 5227 5118 Email: mark.crane@csiro.au
2008/041 **Active**	AAHS: Tools for investigation of the nodavirus carrier state in marine, euryhaline and freshwater fish and control of NNV through integrated management. *Associated species:* multi-species	Prof Richard Whittington University of Sydney, Camden, NSW Phone: 02 9351 1619 Email: richardw@camden.usyd.edu.au

2009/032 **Active**	AAHS: Characterisation of abalone herpes-like virus infections in abalone. *Associated species: Haliotis* spp.	Dr Mark Crane CSIRO AAHL Fish Diseases Laboratory Phone: 03 5227 5118 Email: mark.crane@csiro.au
2009/044 **Active**	AAHS: Surveys of ornamental fish for pathogens of quarantine significance. *Associated species:* multi-species	Prof Richard Whittington University of Sydney, Camden, NSW Phone: 02 9351 1619 Email: richardw@camden.usyd.edu.au
2009/072 **Completed**	AAHS: Risk Analysis – Aquatic Animal Diseases Associated With Bait Translocation (*Associated species:* multi-species)	Dr Ben Diggles DigsFish Services Pty Ltd Phone/fax 07 3408 8443 Mob. 0403 773 592 Email: ben@digsfish.com
2009/075 **Completed**	TRF AAHS: Determining the susceptibility of remnant populations of abalone previously exposed to AVG (*Associated species:* Abalone)	Vin Gannon Victorian Abalone Divers Association Phone: 03 5529 2001 Mob. 0418 292 004 Email: vin@vada.com.au
2009/315 **Active**	PD Program: scholarship program for enhancing the skills of aquatic animal health professionals in Australia. *Associated species:* multi-species	Jo-Anne Ruscoe FRDC Phone: 02 6285 0423 Email: jo-anne.ruscoe@frdc.com.au
2010/034 **Active**	AAHS: Investigation of an emerging bacterial disease in wild Queensland gropers, marine fish and stingrays with production of diagnostic tools to reduce the spread of disease to other states of Australia. *Associated species*: multi-species	Dr Rachel Bowater DEEDI, Biosecurity Queensland Phone: 07 4760 1592 Email: rachel.bowater@deedi.qld.gov.au
2010/036 **Active**	AAHS: Improved fish health management for integrated inland aquaculture through Better Management Practices (BMPs). Associated species: *Maccullochella* spp.	Dr Tracey Bradley DPI Victoria Phone: 03 9217 4171 Email: tracey.bradley@dpi.vic.gov.au
2011/003 **Active**	AAHS: Investigations into the genetic basis of resistance to infection of abalone by the abalone herpes-like virus. Associated species: *Haliotis* spp.	Dr Serge Corbeil CSIRO AAHL Fish Diseases Laboratory Phone: 03 5227 5254 Email: serge.corbeil@csiro.au
2011/004 **Active**	AAHS: Development of Improved Molecular Diagnostic Tests for *Perkinsus olseni* in Australian molluscs. *Associated species*: multi-species	Mr Nick Gudkovs CSIRO AAHL Fish Diseases Laboratory Phone: 03 5227 5456 Email: nicholas.gudkovs@csiro.au
2011/005 **Active**	AAHS: Investigation of inclusions in Australian prawns. *Associated species*: multi-species	Dr Melanie Crockford Dept Fisheries WA Phone: 08 9368 3205 Email: mcrockford@agric.wa.gov.au
2011/043 **Active**	AAHS: understanding and planning for the potential impacts of OsHV1 on the Australian Pacific oyster industry. *Associated species*: Pacific oyster	Dr Tom Lewis RDS Partners Pty Ltd Phone: 03 6231 9033 Email: tom.lewis@ruraldevelopmentservices.com
2011/046 **Active**	Tactical Research Fund - AAHS: Disease risk assessment for abalone stock enhancement program. *Associated species*: *Haliotis* spp.	Mr Richard Stevens Western Australian Fishing Industry Council Phone: 08 9432 7777 Email: richards@wafic.org.au
2011/048 **Active**	Tactical Research Fund - AAHS: Determining the susceptibility of Australian species of prawns to infectious myonecrosis. *Associated species*: multi-species	Dr Mark Crane CSIRO AAHL Fish Diseases Laboratory Phone: 03 5227 5118 Email: mark.crane@csiro.au

2011/053 **Active**	AAHS: Pacific oyster mortality syndrome (POMS) - understanding biotic and abiotic environmental and husbandry effects to reduce economic losses. *Associated species*: Pacific oyster	Prof Richard Whittington University of Sydney, Camden, NSW Phone: 02 9351 1619 Email: richardw@camden.usyd.edu.au
2011/245 **Active**	Tactical Research Fund: Aquatic Animal Health Subprogram Research methods to manage pathogenic microbiological and biological organisms within a redclaw (*Cherax quadricarinatus*) egg incubator hatchery to improve survival and reliability	Mr Colin Valverde AquaVerde Redclaw Hatchery and Farm PO Box 830 Atherton QLD 4883

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