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Health Highlights

Aquatic Animal Health & Biosecurity Subprogram Newsletter
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In this issue

From the Subprogram Leader

This is indeed strange times that we live in. I hope you and your families, friends and colleagues are all well and coping with the circumstances in which we find ourselves.

As for me, this is the last *Health Highlights* issue that I will produce – the current term of FRDC AAHBS will end in June this year and I will be stepping down as Subprogram Leader. It has been a privilege to work with you, as the Subprogram stakeholders, and FRDC in our attempt to deliver high quality and cost-effective research to the aquatic animal health and biosecurity community. While there have been very many positive experiences, the standout highlight has been the FRDC Aquatic Animal Health & Biosecurity Conference – colloquially known as "the Cairns Conference". The conference has been held biennially since 2003 and it has been very gratifying to witness its development from, in 2003, a FRDC-focussed meeting to, now, an international conference that provides a unique forum for mainly (but not exclusively) Australian and New Zealand researchers, regulators and industry to discuss aquatic animal health and biosecurity issues. A special part of the conference is the support provided to the students that are coming through "the system". In addition, the quality of the keynote presenters has been outstanding, and it has been wonderful to be able to invite international experts to headline the conferences. Furthermore, the recent increase in industry attendance at the conferences is an important development. Communication between researchers, governments and industry is key to keeping the Subprogram relevant.

It is impossible to thank individually all of the researchers, regulators, industry representatives, managers and colleagues (particularly the Subprogram committee members) that have supported me during my tenure as Subprogram Leader. The list is long and your support over the last sixteen years is greatly appreciated.

2019 FRDC Australasian Aquatic Animal Health & Biosecurity Scientific Conference student award winners

A proposal to renew the Subprogram is currently being developed and I fully expect the Subprogram to continue in some form – it has been, and I'm sure it will continue to be, an important part of the development of aquatic animal health and biosecurity capability in Australia.

STC/SAC Meetings

The AAHBS met in March. Items for discussion included:

- Review of R&D Expressions of Interest
- Review of R&D full applications
- AQUAPLAN
- Renewal of the FRDC Aquatic Animal Health & Biosecurity Subprogram

Subprogram Website

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

http://www.frdc.com.au/frdc-stakeholders/national-priorities-and-subprograms/aquatic-health-and-biosecurity

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 & Biosecurity Subprogram and related issues.

For Final Reports see:

http://www.frdc.com.au/research/final-reports

Please contact FRDC directly (Email address: frdc@frdc.con.au) if you have any problems with this website.

Announcements

Aquatic Animal Health Technical Forum

Due to the current COVID-19 situation, the 2020 AAHTF workshop has been cancelled. At this stage it is unclear if the workshop can be rescheduled until later in the year, or 2021. You will be kept informed of any updates.

Further Information: contact Nette Williams (email: lynette.williams@csiro.au)

Newsletter submissions

The Aquatic Animal Health & Biosecurity Subprogram welcomes contributions to *Health Highlights* on all aquatic animal health & biosecurity R&D news and events – both within and outside the FRDC. We aim to assist with the widespread exchange of information by including any of the following in each 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, job vacancies etc. are also welcome.

Mailing list

Health Highlights is distributed biannually to stakeholders via email as well as being posted on the FRDC website at:

http://www.frdc.com.au/en/partners/national-priorities-and-subprograms/aquatic-health-and-biosecurity

To change contact details or to ensure inclusion on the *Health Highlights* mailing list, please contact Joanne:

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Completed Project Summaries

Project No. 2015/001: Aquatic Animal Health and Biosecurity Subprogram: Bonamiasis in farmed Native Oysters (*Ostrea angasi*) (PI: Tracey Bradley)

Objectives

1. Obtain nucleic acid sequence and compare with other, described *Bonamia* sp. and determine their taxonomic relationship and ensure that available diagnostic tools are suitable.
2. Improve understanding of Bonamiasis infestations in Native Oysters including the determination, under controlled conditions, of the stressors that induce clinical disease in sub-clinically infected oysters.
3. Develop a biosecurity plan and farm management practices to manage the risk of infestation and the mitigation of clinical infection with *Bonamia* sp.

Summary

This project was a collaborative study across three research organisations: Agriculture Victoria, CSIRO (Victoria) and the South Australian Research and Development Institute (SARDI). The overall aim of this collaborative project was to better understand many aspects of infection with the parasite *Bonamia exitiosa* in Native Oysters (*Ostrea angasi*). The genesis of this project was the detection of clinical bonamiosis in farmed Native Oysters in Port Phillip Bay, Victoria in 2015. At the time the species of this parasite was unknown, and it had caused extensive mortalities (presumed over 80%) in stock on one farm.

CSIRO demonstrated that current diagnostic PCR assays are sufficient and effective in the detection and identification of *Bonamia* species from farmed *O. angasi* in southern Australia. The species of *Bonamia* parasite present in farmed *O. angasi* between 2013 and 2017 in Victoria, New South Wales and South Australia was confirmed to be *B. exitiosa*. Next generation sequencing (NGS) and bioinformatic analysis of nucleotides extracted from *B. exitiosa* infected and uninfected *O. angasi* facilitated assembly of the first draft genome of a member of the family Haplosporidiidae, *B. exitiosa.* For the first time a draft genome was also assembled for *O. angasi* as a result of the sequencing strategy undertaken to identify the *B. exitiosa* genome. Unexpectedly, a near complete genome was also assembled for an Epsilon proteobacterium, *Poseidonibacter* from *O. angasi* tissues infected with *B. exitiosa*. This organism may have been an adventitious discovery or potentially proliferated in flat oysters weakened by *B. exitiosa* infection.

In Victoria, 4 individual tank and field trials were undertaken between 2016 and 2018. All trials were designed to investigate proposed risk factors for the

development of clinical bonamiosis under normal farming or controlled laboratory conditions. The tank trials utilised presumed sub-clinically infected and healthy oysters and subjected them to stressors such as heat, starvation and turbulence. Further tank trials examined oyster origin and size as risk factors. The field trials utilised existing farm sites and practices to investigate a number of proposed risk factors of interest to the farmers. The field trials were conducted on a known clinically infected farm and examined the risk factors including basket density, basket depth in the water column, oyster size and level of fouling. Concurrently, the project validated the diagnostic performance of the *Bonamia* sp. qPCR and established the optimal epidemiological qPCR cycle threshold (C_T) value to differentiate between a positive and negative result.

SARDI undertook a range of trials in both the field and under laboratory conditions. Oysters were tested from 3 farms to assess diagnostic sensitivity (DSe) and specificity (DSp) of heart smears, histopathology and qPCR individually or in combination, and to assess prevalence. Tank trials were utilised to develop a cohabitation infection model using uninfected hatchery-reared recipient animals and infected donor animals from farms to better understand infection dynamics. The Pacific oyster, *Crassostrea gigas*, was shown to be susceptible to *Bonamia exitiosa* by cohabitation in the laboratory. A decontamination trial was undertaken in the laboratory using heavily infected Native Oysters to assess processes for decontaminating equipment that may have been exposed to *Bonamia exitiosa.* These approaches were combined in a field trial where *O. angasi* were deployed at Cowell, Coffin Bay and Streaky Bay to examine the prevalence of *B. exitiosa* over time related to measured environmental parameters and growth rates. SARDI also assessed 3 different diagnostic tests for detecting *Bonamia* sp: heart smears, histology and qPCR. In this work the effect of combining tests to maximise overall diagnostic performance was also investigated.

Keywords: *Bonamia exitiosa*, Native Oysters (*Ostrea angasi*), *Bonamia* sp. qPCR; epidemiology; decontamination.

Project No. 2015/003: Aquatic Animal Health and Biosecurity Subprogram: Development of standard methods for the production of marine molluscan cell cultures (PI: Andrew Read)

Objectives

1. Describe standard methods for the development and storage of marine molluscan cell cultures.
2. Production and characterisation of molluscan cell cultures.
3. Examination of resultant cell cultures for the ability to sustain growth of endemic molluscan viruses and protozoa.
4. Examination of resultant cell cultures for the ability to sustain growth of exotic molluscan viruses and protozoa.

Summary

Cell cultures have been used for many decades in isolation, culture and study of a wide range of human and animal pathogens including viruses, bacteria and protozoa. Cell culture systems for marine molluscs have been lacking in Australia. This report describes work performed by scientists at the NSW Department of Primary Industries' Elizabeth Macarthur Agricultural Institute and the Australian Animal Health Laboratory to produce standard methods for the development and storage of marine molluscan cell cultures. This work was undertaken between 2015 and 2019. Cells from Australian native oysters (Ostrea angasi), abalone (Haliotis spp.) and Pacific oysters (Crassostrea gigas) were used to develop these methods. Cells were produced, grown and stored in a frozen state using modified classical cell culture techniques. Pacific oyster heart and gill cells were shown to be permissive for ostreid herpesvirus-1 (OsHV-1), and using these cells may prove to be a useful model for OsHV-1 infection.

Keywords: Cell culture; infection model, Pacific oyster; native oyster; blacklip abalone; greenlip abalone; ostreid herpesvirus-1

Project No. 2017/117: Aquatic Animal Health Subprogram: Identification of differentially expressed innate immune genes in the New Zealand paua (*Haliotis iris*) and the Australian hybrid abalone (*H. laevigata* X *H. rubra*) upon immersion challenge with the abalone herpesvirus-1 (HaHV) (PI: Serge Corbeil)

Objectives

1. Define the time-line of an anti-viral response in the päua and Australian hybrid abalone for the first time, utilising real-time PCR, and a set of known anti-viral effector genes.
2. Through mRNA sequencing and genomic analysis, identify early genes expressed in päua and Australian hybrid abalone upon HaHV-1 immersion challenge.
3. Establish an immune signature in the early response of the host to the virus that differs between the päua and Australian hybrid abalone, to determine key immune players in HaHV-1 resistance.

Summary

This project was carried out by scientists from AAHL CSIRO (Dr Serge Corbeil) and from LaTrobe University (Drs Karla Helbig and Subir Sarker). After discovering the existence of an abalone species (Päua - *Haliotis iris*) resistant to AVG our research team based in Geelong and Melbourne undertook (2018) to expose the AVG resistant päua and the AVG susceptible greenlip x blacklip hybrid abalone to HaHV-1 (the etiological agent of AVG) and look for differential gene expression between species.

Cutting edge sequencing technology and bio-informatic analysis allowed us to investigate the gene expression of the animals at the molecular level. This approach led to pinpoint abalone genes that are likely to play a role in the protection against AVG in päua. Furthermore, the identification of these genes may facilitate (if applicable) the use of a gene silencing technology such as the CRISPR system in vitro and in vivo to improve immune response to AVG. A breeding program strategy could also eventually be implemented to increase resistance to AVG in susceptible abalone species.

Keywords

Haliotid herpesvirus-1 (HaHV-1), abalone viral ganglioneuritis (AVG), päua, *Haliotis iris*, hybrid abalone (*H. laevigata* x *H. rubra*), gene expression, disease resistance, mRNA sequencing, genomic analysis.

Progress Summaries for Active Projects

Project No. 2014/002: Aquatic Animal Health Subprogram: Development of stable positive control material and development of internal controls for molecular tests for detection of important endemic and exotic pathogens (PI: Nick Moody)

Objectives

1. Produce quantified synthetic RNA positive control material for conventional and real-time RT-PCR assays, available on request.
2. Produce quantified plasmid DNA positive control material for conventional and real-time RT-PCR assays, available on request.
3. Optimised universal internal control based on plant viral RNA and DNA and/or species-specific genes for use in molecular assays developed and implemented.
4. Technology transferred and adopted by participating laboratories.

Progress: Draft final report is in preparation.

Project No. 2016/009: Aquatic Animal Health and Biosecurity Subprogram: *Perkinsus olseni* in abalone – development of fit-for-purpose tools to support its management (PI: Cecile Dang)

Objectives

1. To culture *Perkinsus olseni in vitro*
2. To sequence the genome of *P. olseni*
3. To develop an antibody binding test for *P. olseni*

The draft final report for this project has been submitted for review.

Project No. 2016/404: Aquatic Animal Health and Biosecurity Subprogram: Strategic planning, project management and adoption (PI: Mark Crane)

Objectives

1. Manage a portfolio of R&D projects that are directly concerned with aquatic animal health & biosecurity and are not managed by other FRDC subprograms, FRABs or IPAs
2. In consultation with key stakeholders (industry, government and aquatic animal health providers) develop strategic directions for R&D
3. Facilitate the dissemination of outputs (information and results) from R&D projects to key stakeholders

This project commenced 1 July 2016 and will continue until 31 August 2020.

Project No. 2018/144: Aquatic Animal Health and Biosecurity Subprogram: Aquatic Animal Technical Forum and Training Workshops (PI: Nette Williams)

Objectives

1. Source workshop venues, in various States and Territories, that have aquatic animal health capability or services and can accommodate the group size.
2. Organise all aspects of conducting the workshops including, advertising through *Health Highlights* subscription and peer referrals, guest presenter, presentation program, field trips and practical sessions, accommodation and catering.
3. Encourage new and emerging science and production staff to attend the AAHTF and to gain experience in making presentations.
4. Continue to up-date the contact list and email distribution list/group for continued information exchange.
5. Reports and financial acquittals prepared according to milestone schedule.

Progress:

The first training workshop scheduled for this project took place at the Department of Primary Industries, Parks, Water and Environment's Animal Health Laboratory and Centre of Excellence in Aquatic Animal Health & Vaccines, Launceston, Tasmania, 6-8 March 2019. There were 22 participants from Commonwealth and State laboratories, universities, regulatory agencies and industry (from ACT, NSW, QLD, SA, TAS, VIC, WA and New Zealand).

Milestone Progress Report has been approved.

As announced previously, due to the current COVID-19 situation, the 2020 AAHTF workshop has been cancelled. At this stage it is unclear if the workshop can be rescheduled until later in the year, or 2021. You will be kept informed of any updates.

New Projects

Project No. 2018/147: Aquatic Animal Health and Biosecurity Subprogram: Diagnostic detection of aquatic pathogens using real-time next generation sequencing (PI: David Cummins)

Objectives

1. Evaluate if MinION data meets or exceeds the data obtained using established laboratory-based NGS platforms.
2. Evaluate the performance of the MinION using existing diagnostic extraction techniques and produce robust methods and protocols for sample preparation, sequencing and data analysis.
3. Compare the applicability of MinION to standard molecular assays for identification of pathogens in diagnostic samples.

The first objective of this project is to demonstrate if the MinION can obtain quality genome assemblies of known pathogens, such as WSSV, AHPND, OsHV-1 and HaHV that have been created using existing NGS technology. Moreover, determine if the MinION is capable of producing a diagnostic result more rapidly and with greater confidence than traditional techniques. If MinION data does not produce reliable genome assemblies, no improvement in genome quality, or is significantly more laborious to set-up/run or analyse than existing NGS technologies, do not proceed with objective 2.

Objective 2 will optimise MinION protocols for sample pre-processing, optimal sequencing conditions, and data post-processing. We will then evaluate the MinION data produced from a range of aquatic organisms against data produced using traditional techniques from the same samples. If after these optimisations, the MinION cannot detect pathogens as reliably as traditional techniques, do not proceed with objective 3.

For objective 3, diagnostic samples will be tested using existing diagnostics tools (qPCR, cPCR) and MinION sequencing. Analysis between the methods will be detailed, including time-to-result, pathogen identity and genomic information. This objective will not only provide an insight into real-time sequencing for diagnostics, but in addition the feasibility of MinION technology for field application in the future.

Progress: First milestone progress report submitted for review.

Project No. 2018/180: Aquatic Animal Health Subprogram: Benchmarking for health and productivity in aquaculture (PI: Tracey Bradley)

Objectives

1. Develop a farm data collection and review system for abalone farmers to record health and production parameters.
2. Provide a secure data storage system with the capacity to allow industry benchmarking against industry-approved standards – for example the industry median.

First progress report due in August 2020.

Project No. 2019/005: Aquatic Animal Health Subprogram: Risk analysis to identify and minimise biosecurity risks arising from recycling bivalve mollusc shell waste during shellfish reef restoration projects in Australia (PI: Ben Diggles)

Objectives

1. In conjunction with State and Federal biosecurity authorities and stakeholders, identify hazards (pests and diseases) likely to be associated with recycled bivalve shells in Australia and determine the various risk mitigation methods currently being used in each state jurisdiction.
2. Use risk analysis to identify pest and disease threats and improve knowledge about best practice methods for preventing spread of significant pests and disease agents via shellfish reef restoration projects, leading to enhanced biosecurity management and reduced risk of disease spread into wild and cultured populations of shellfish.
3. Enhance preparedness and capability to prevent spread of aquatic animal pests and diseases of significance to Australia

First progress report due in March 2020.

Summary of Active Projects

Project No.	Project Title	Principal Investigator
2014/002	AAHS: Development of stable positive control material and development of internal controls for molecular tests for detection of important endemic and exotic pathogens (*Associated species*: multi-species)	Dr Nick Moody CSIRO AAHL Fish Diseases Laboratory Phone: 03 5227 5749 Email: nick.moody@csiro.au
2015/005	AAHS: Determining the susceptibility of Australian *Penaeus monodon* and *P. merguiensis* to newly identified enzootic (YHV7) and exotic (YHV8 and YHV10) Yellow head virus (YHV) genotypes (*Associated species*: *Penaeus monodon, P. merguiensis*)	Dr Nick Moody CSIRO AAHL Fish Diseases Laboratory Phone: 03 5227 5749 Email: nick.moody@csiro.au
2016/404	AAHBS: 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
2016/009	AAHBS: *Perkinsus olseni* in abalone - development of fit-for-purpose tools to support its management (*Associated species*: *Haliotis* spp.)	Dr Cecile Dang Department of Fisheries – WA Phone: 08 9363 4825 Email: Cecile.Dang@agric.wa.gov.au
2016/013	AAHBS: Comparative pathogenicity of exotic AHPND and the presumptive bacterial hepatopancreatitis detected in farmed *Penaeus monodon* in Queensland (*Associated species*: *Penaeus monodon* and *P. merguiensis*)	Dr Nick Moody CSIRO AAHL Fish Diseases Laboratory Phone: 03 5227 5749 Email: nick.moody@csiro.au
2017/190	AAHBS: Assessment of gamma irradiation as a feasible method for treating prawns to inactivate White Spot Syndrome Virus (*Associated species*: *Penaeus* spp.)	Dr Stephen Wesche Biosecurity Queensland Phone: 07 3087 8086 Email: stephen.wesche@daf.qld.gov.au
2018/144	AAHBS: Aquatic Animal Technical Forum and Training Workshops (*Associated species*: multi-species)	Nette Williams CSIRO AAHL Fish Diseases Laboratory Phone: 03 5227 5442 Email: lynette.williams@csiro.au
2018/147	AAHBS: Diagnostic detection of aquatic pathogens using real-time next generation sequencing (*Associated species*: multi-species)	Dr David Cummins CSIRO AAHL Fish Diseases Laboratory Phone: 03 5227 5777 Email: david.cummins@csiro.au
2018/180	AAHBS: Benchmarking for health and productivity in aquaculture (*Associated species*: abalone)	Dr Tracey Bradley Agriculture Victoria Phone: 03 9217 4171 Email: tracey.bradley@ecodev.vic.gov.au
2019/005	AAHBS: Risk analysis to identify and minimise biosecurity risks arising from recycling bivalve mollusc shell waste during shellfish reef restoration projects in Australia	Dr Ben Diggles DigsFish Services Pty Ltd Phone: 07 3408 8443 Email: ben@digsfish.com

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