FRDC
FISHERIES RESEARCH & DEVELOPMENT CORPORATION

Health Highlights

Aquatic Animal Health & Biosecurity Subprogram Newsletter
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In this issue

From the Subprogram Leader

New name for the Subprogram

The FRDC Aquatic Animal Health Subprogram has a new name; in recognition of the subprogram's portfolio which, in addition to research on aquatic animal diseases, includes projects that support development of systems for improved bio-security, the subprogram is now known as the FRDC Aquatic Animal Health & Biosecurity Subprogram (AAHBS). Biosecurity can be defined as the resources and procedures in place to prevent pests and diseases from entering, or escaping from, a specified area. Thus there are aspects of biosecurity that are relevant to all of the subprogram's key research areas within the R&D Plan:

- Nature of disease and host-pathogen interaction
- Aquatic animal health management
- Disease diagnostics
- Surveillance & monitoring
- Aquatic animal disease therapy & prophylaxis
- Training & capacity building

STC/SAC Meetings

The AAHBS had its first meeting on 1 September 2016. Items for discussion included:

- 2017 Scientific Conference
- AAHBS procedures and processes including new arrangements for submission of research applications
- DAWR Aquatic Animal Health Training Scheme

Further information on these items is provided in the ***Announcements*** section of this newsletter.

2015 AAHTF Workshop, James Cook University, Townsville: Participants

Health Subprogram Website

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

http://www.frdc.com.au/research/aquatic_animal_health/Pages/default.aspx

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. For Final Reports see http://www.frdc.com.au/research/final-reports/Pages/default.aspx.

Please contact FRDC if you have problems with this website.

Announcements

2017 Aquatic Animal Health Scientific Conference

The 2017 FRDC Australasian Aquatic Animal Health & Biosecurity Scientific Conference will be held in Cairns, at The Pullman Reef Hotel, on 10-14 July 2017.

The first announcement has been released. If you missed it please see the announcement at the end of this newsletter. It is important that you respond to the first announcement if you wish to receive further information (second announcement with registration details).

I am pleased to announce that the keynote speakers for the conference will be:

Dr Grant D Stentiford FRC Path, Director, European Union Reference Laboratory for Crustacean

Diseases, Team Leader, Pathology and Molecular Systematics at Centre for Environment, Fisheries and Aquaculture Science (Cefas) Weymouth Laboratory, United Kingdom.

and

Dr Brian Jones, A/Manager, Bacteriology and Aquatic Animal Health, Ministry of Primary Industries, New Zealand, Adjunct Professor, Murdoch University, Western Australia, Australia.

Grant will present:

1. New paradigms to solve the aquaculture disease crisis
2. The Microsporidia – emergent pathogens in the animal-human food chain

Brian will present:

1. New and emerging infectious diseases of molluscs
2. New and emerging diseases of molluscs with a non-infectious aetiology

DAWR Aquatic Animal Health Training Scheme

The Aquatic Animal Health Training Scheme has received some further funding from DAWR. Further information about this scheme will be provided in the near future.

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, 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: 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. 2011/004: Aquatic Animal Health Subprogram: Aquatic Animal Health Subprogram: Development of improved molecular diagnostic tests for *Perkinsus olseni* in Australian molluscs (PI: Nick Gudkovs)

Executive Summary

Background

Perkinsus is the widespread cause of disease and lost production in mollusc fisheries world-wide. Found mostly in temperate waters, two species are listed internationally as notifiable by the OIE and *also appear on Australia's National List of Reportable Diseases of Aquatic Animals. Although Perkinsus marinus is exotic to Australia, Perkinsus olseni* is enzootic and well-known as the cause of serious infections in various wild abalone populations in south-eastern Australia.

The rapid identification and reliable differentiation of species is a major issue in the diagnosis and management of Perkinsosis in Australia. Traditional methods of *Perkinsus* diagnosis, such as histology and Ray's thioglycollate culture, are straightforward and practical, however they lack sensitivity and fail to differentiate specific species. The molecular methods currently recommended by the OIE are based on conventional 1-step PCR which is generally more labour intensive, slower and less sensitive than real-time PCR.

The primary aim of this project was to develop and validate a *species-specific* real-time PCR (qPCR) assay for *Perkinsus olseni*.

Aims/Objectives

The aims and objectives of the project were:

1. Undertake a targeted molecular, histological and cultural examination of known *Perkinsus*-infected wild abalone populations to compare existing methods of detection.
2. Establish representative axenic (single species) cultures of *Perkinsus* spp. from infected abalone.
3. Use established PCRs and DNA sequencing methods to confirm the presence of *P. olseni* and

determine the genetic diversity, including other *Perkinsus* spp. from these populations.

4. Develop and validate qPCR methods for the detection and identification of *P. olseni* in infected abalone.
5. Compare and evaluate the performance of the objective 4 qPCR with existing conventional PCR methods for detection of *P. olseni*.

Methodology

Scientists from the CSIRO Australian Animal Health Laboratory in Geelong, have undertaken a study of *Perkinsus* in blacklip abalone to develop a new qPCR system for the diagnosis of *P. olseni*. The design of primers and probe for the *P. olseni* Taqman® assay was based on DNA sequence from infected abalone collected from South Australia over the course of the project and sequence data publically available through GenBank.

Single species (axenic) cell cultures of *P. olseni* from abalone were established using standard methods of propagation. Cell cultures were confirmed by sequencing and phylogenetic analysis of the ITS, LSU and actin genes. DNA sequence was independently confirmed by experts in Japan.

The accumulated test data were used to compare the new qPCR assay with exiting test methods. Validation of the *P. olseni* qPCR was based on a wide range of samples including mollusc field samples, cloned plasmids and axenic reference cultures of *Perkinsus*. The analytical sensitivity (ASe) of the test system was determined using serially diluted plasmid DNA and genomic DNA from cultured cells. All calculations of diagnostic sensitivity and specificity were based on testing of naturally infected animals. The diagnostic performance characteristics of the assay were also determined by ROC and Bayesian analysis to obtain estimates of diagnostic sensitivity and specificity.

Results

A species-specific Taqman® based real-time PCR for *P. olseni* was developed. The analytical specificity (ASp) of the system was 100% with respect to a range of non-target *Perkinsus* reference samples (n = 42). The system could detect 1 to 2 copies/µl of target DNA in a background of abalone genomic DNA, and plasmid dilutions between 2 and 20 copies per PCR reaction represent the 95% confidence limit of analytical sensitivity as recommended by the OIE. The C_T values from the equivalent amount of *P. olseni* genomic DNA (600 pg to 1 fg) were linear over this range.

ROC analysis provided an estimate of the relative diagnostic sensitivity (DSe) of 94.6% and diagnostic specificity (DSp) of 92.8% at a preliminary cut-off C_T of 41.29 for the new AFDL *Pols* qPCR using the OIE species-specific PCR assay as a reference. When different tissues were used our analysis shows that the DSe obtained with the AFDL *Pols* qPCR with gill and muscle were similar, 0.88 and 0.92, respectively.

While the primary focus of the project was the development of the qPCR, cell cultures were to be used as the basis of DNA sequencing. Although this strategy was modified, two cryopreserved axenic cultures of *P. olseni* were established. These are the first *in vitro* cultures of *P. olseni* propagated from abalone in Australia. The culture from Thistle Island, South Australia 2012 (12:978-11T) was obtained from a site near Memory Cove, South Australia and was accepted as the holotype culture for *P. olseni* by the ATCC.

The sequencing and phylogenetic analysis undertaken in this project confirmed the taxonomy and identification of the field samples used for test development and the identity of the *in vitro* cultures. Analysis of DNA sequence from the ITS region indicated that there had been little change in this region since 1989 and that this region remains a stable and useful target for molecular detection.

Implications for relevant stakeholders

The molecular assay developed in this project provides rapid detection and identification of species and has application for testing individuals from outbreaks or high-throughput surveillance of populations for certification and management of stocks.

The development of a highly sensitive and highly specific diagnostic test has the potential to provide state government authorities, their diagnostic laboratories, and their fisheries managers and regulators with improved diagnostic capability not only for the diagnosis of Perkinsosis (specifically, rapid identification of the causative agent) but also for the detection and identification of sub-clinical infections. The provision of a validated diagnostic test with estimates of diagnostic specificity and sensitivity allows the design of surveillance programs and sampling protocols to be based on reliable scientific data. The test can be implemented in the recently developed abalone farm accreditation program.

Recommendations

It is recommended that details of this test be provided to diagnostic laboratories for immediate implementation in state jurisdictions that have a need for *Perkinsus* diagnostics. Moreover, the method and validation should be published in a peer-reviewed scientific journal to facilitate wider adoption of the test through inclusion in the OIE Manual of Diagnostic Tests for Aquatic Animals.

Keywords

Australia, mollusc, protozoa, *Alveolata, Perkinsozoa, Perkinsea, Perkinsida, Perkinsidae, Perkinsus olseni*, *Perkinsus atlanticus*, Perkinsosis, PCR, real-time PCR, qPCR, cell-culture, *in vitro* culture, *Haliotis rubra*, blacklip abalone, PCR validation, ROC analysis, Bayesian analysis, *in vitro* culture, diagnostic validation.

Project No. 2012/001: Aquatic Animal Health Subprogram: Strategic planning, project management and adoption (PI: Mark Crane)

Executive Summary

What the report is about

The Aquatic Animal Health Subprogram (AAHS) is a FRDC national subprogram that was established in 2001 and since then has been renewed on a continual basis. Since 2004, AAHS has been managed by the CSIRO AAHL Fish Diseases Laboratory with input from a steering committee (STC) and a scientific advisory committee (SAC). During the current term (2012-16), AAHS has managed 35 projects concerned with various aquatic animal health issues including pathogen/host interaction, aquatic animal health management, development of diagnostic techniques and various training projects and targeting wild and farmed molluscs, crustaceans and finfish.

Background

Industry and government have recognised the importance of an integrated and planned approach to aquatic animal health. This recognition led to an industry/government cooperative effort in developing *AQUAPLAN 1998-2003*, Australia's first five-year National Strategic Plan for Aquatic Animal Health, and its successor *AQUAPLAN 2005-2010*, and the current *AQUAPLAN 2014-2019*. A common theme within these strategic plans is recognition of the need for research, and the adaptability of the plan to include emerging aquaculture industries. Compared to the terrestrial animal industries, the state of knowledge of aquatic animal health management is limited. Research has a critical role in expanding this knowledge and enhancing management practices to prevent disease or limit its impact on the expanding fisheries/aquaculture sector, including recreational fisheries and natural resources.

Since its establishment AAHS has managed over 100 projects within 6 key research areas:

- Nature of disease and host-pathogen interaction
- Aquatic animal health management
- Endemic and exotic aquatic animal disease diagnostics
- Surveillance and monitoring
- Aquatic animal disease therapy and prophylaxis
- Training and capacity building

The major stakeholders, the fisheries and aquaculture sectors and state and federal governments, have continued to express strong support for the subprogram and have co-invested in the vast majority of the projects within its portfolio, demonstrating the subprogram's importance to the aquatic animal health community.

Aims/objectives

The mission of AAHS is stated in the R&D Plan:

"To provide leadership to aquatic animal health R&D and its adoption in Australia".

To achieve this the current project had three objectives:

1. To manage a portfolio of R&D projects that are directly concerned with aquatic animal health and are not covered by other FRDC subprograms.
2. In consultation with key stakeholders (industry and aquatic animal health specialists) develop strategic directions for R&D.
3. Facilitate the dissemination of information and results

Methodology

AAHS is led by a Subprogram Leader who is assisted by a Subprogram Coordinator and guided by a STC which is advised by a SAC. The Subprogram Leader, along with these committees, conducted AAHS business through either face-to-face meetings or by teleconference. The schedule of meetings is aligned with the normal FRDC funding cycle to ensure that project preproposals are canvassed and evaluated, full proposals are invited and evaluated, and recommendations are submitted to the FRDC Board, as required.

The Subprogram consults on aquatic animal health R&D priorities and strategies with Animal Health Committee (AHC) - Australia's primary government advisory committee for policy, communication and awareness related to animal health. Consultation is primarily through AHC's Sub-committee on Aquatic Animal Health (SCAAH) and the National Industry Reference Group for Aquatic Animal Health (NAAHIRG). In addition, communication with industry peak bodies, e.g. Australian Abalone Growers' Association, Australian Prawn Farmers' Association, Oysters Australia, is maintained on a regular basis.

The dissemination of information and results was achieved through:

- *Health Highlights*, the Subprogram's newsletter
- Scientific workshops
- Scientific Conferences
- FRDC Website
- Formal and informal communication with industry peak bodies, other Subprograms, RACs, SCAAH, as needed
- Use of developed databases

Results/key findings

In the period 2012-16, AAHS has managed 35 projects concerned with aquatic animal health.

AAHS reviewed, in-house, the R&D Plan on an annual basis which included consultation with major stakeholders (industry and governments). The revisions accounted for the changing aquatic animal health R&D needs at the state and national levels.

Consultation with State Government aquatic animal health specialists included formal face-to-face meetings, e.g. at the annual face-to-face meetings of SCAAH. During these meetings R&D priorities were discussed and taken to AAHS March meeting of the same year for discussion by the STC and SAC. The AAHS R&D was then up-dated in time for the annual call for expressions of interest issued the same year.

During the period 2012-16, there were six issues of the Subprogram newsletter, *Health Highlights*, which has a broad distribution list (>300 subscribers) that includes industry associations, research providers and regulators both domestically and internationally. *Health Highlights* includes information about scientific conferences and workshops, progress reports on active and completed research projects and notices submitted by subscribers.

FRDC AAHS has conducted scientific workshops and conferences on various aquatic animal health issues, as needed. In 2013, FRDC provided funds in support of an international KBBE Workshop on Mollusc Disease Diagnosis (FRDC Project 2009-315-24: People development program: Aquatic Animal Health Training Scheme – KBBE workshop on diagnostics for mollusc diseases) coordinated by the Subprogram Leader. In addition, during the 2012-16 period two FRDC Australasian Scientific Conferences on Aquatic Animal Health were convened (in 2013 and 2015) attracting 125 (112 from Australia and New Zealand and 13 from other countries) 75 (71 registrations from Australia and New Zealand and 4 from other countries), respectively. Workshop and conference proceedings are produced and distributed electronically.

Implications for relevant stakeholders

The overall planned outcome of the FRDC Aquatic Animal Health Subprogram was an increased ability to manage aquatic animal disease in the commercial, recreational and traditional fishing industry sectors and thus assist Australia's aquaculture and fisheries industries become more competitive, profitable and sustainable. In addition, there is a broader responsibility towards the Australian community to ensure the sustainability of Australian aquatic natural resources.

This overall outcome was achieved through improved diagnostic capability and/or disease management for a number of aquatic animal pathogens; development of enhanced awareness of emergency aquatic animal disease response arrangements and other training to improve the disease emergency management capability of industry and government personnel.

The AAHS has been able to enhance aquatic animal health R&D outputs, strengthen the network of aquatic animal health experts and research providers, and provide training opportunities for young scientists interested in aquatic animal health. Furthermore, AAHS has maintained its linkages to Animal Health Committee, through the Sub-committee on Aquatic Animal Health as well as peak industry groups to ensure that the strategic direction for investment in aquatic animal health is maintained.

Keywords

Aquatic animal health; disease; diagnostics; training and capacity building

Project No. 2012/002: Aquatic Animal Health Subprogram: Aquatic Animal Technical Forum (PI: Nette Williams)

Executive Summary

What the report is about

The three-year project for the Aquatic Animal Health Technical Forum from 2013 to 2015 was to provide persons working in the aquatic animal health field the opportunity to meet annually in a workshop environment, gain experience in making oral presentations and participate in training, and to develop a network that can be used for obtaining information and advice on technical matters concerning aquatic animal health. The workshops involved participants making presentations on current work projects and to gain experience from others in the aquatic field in laboratory methods. Three annual workshops were held at three locations in different States over the duration of the project and provided a wide variety of presentations in various disciplines and different opportunities for field trips to local aquaculture facilities. Participants included staff from aquatic animal health diagnostic laboratories, university laboratories as well as a number of aquaculture industry staff. Participant numbers varied between 23 and 32 over the three workshops.

Workshop locations and dates were:

2013 workshop (20-22 March, 2013) was hosted by South Australian Research and Development Institute (SARDI) and the University of Adelaide Veterinary School, Roseworthy Campus.

2014 workshop (19-21 February, 2014) hosted by the University of Sydney, Camden campus (Veterinary School).

2015 workshop (17-19 June, 2015) hosted by James Cook University Veterinary and Biomedical Sciences School.

Background

The concept of the Aquatic Animal Health Technical Forum (AAHTF) was informally discussed by some of the participants at the 2007 FRDC National Aquatic Animal Health Scientific Conference held in Cairns. During the discussions it was noted that aquaculture is expanding not only overseas but also in Australia and this has attracted a cadre of young scientists with little experience in aquatic animal health. The establishment of an Aquatic Animal Health Technical Forum (AAHTF) or a network where scientists recent to the field could access information from more experienced scientists was

seen as one way to accelerate their technical and professional development. The AAHTF would include annual training workshops where inexperienced scientists/technologists and industry staff that may feel isolated can network and be encouraged to present aspects of their work in a friendly, non-intimidating environment.

The "aquatic animal heath" discipline involves a relatively small number of specialists that do not get the opportunity to convene at meetings/workshops/conferences as often as those involved in the terrestrial animal health sphere. The forum would be open to all aquatic animal health specialists and industry personnel, e.g. fish farm staff.

At the FRDC Aquatic Animal Health Subprogram (AAHS) business meeting held in July 2008 Mark Crane, FRDC AAHS Leader, presented the proposal to commence an Aquatic Animal Health Technical Forum to the AAHS Steering and Scientific Advisory Committees. The concept was well-supported by the AAHS members. Following this support and an application, the FRDC People Development Program provided funding to subsidise people to attend the inaugural meeting and forum training workshop of the Aquatic Animal Health Technical Forum (AAHTF) which was held at CSIRO, Australian Animal Health laboratory, Geelong in March 2010. The workshop was conducted over 2.5 days and attended by 18 participants. Feedback from participants and their home organizations indicated that the workshop exceeded expectations and it was recommended to be held annually, hosted at various venues, to continue the exchange of technical information. Based on the positive input of participants and support of their organisations, an application was submitted to the DAFF/FRDC Aquatic Animal Health Training Scheme to conduct a second workshop in March 2011. The funding obtained would subsidise participant costs, travel and accommodation and provide some reimbursement to the host laboratory for consumables, reagents and other workshop expenses. On successfully gaining joint funding from DAFF and FRDC, the second workshop was held at the Animal Health Laboratory's Fish Health Unit of the Department of Primary Industry Water, Parks, Water and Environment (DPIPWE) in Launceston, March 2011. Again, the workshop organisers received positive feedback with the participants, host facility and participant home organisations showing strong support and indicating the benefits of the AAHTF including the annual workshops.

After the 2011 workshop, a three-year application was submitted to FRDC to continue the forum and workshops from 2013 to 2015. It was thought that continuing the workshops would build on the previous skills workshops held in 2010 and 2011, which assisted in the development of functional networks for the exchange of information and enhancement of the skills of the aquatic animal health service providers. In addition to developing a valuable national resource - a repository of technical knowledge – the forum provides mentoring to the new generation of laboratory technicians, students and staff at diagnostic laboratories, teaching institutions and aquaculture enterprises. The application was successful and FRDC Project 2012-002 commenced in July 2012.

Aims and Objectives

1. To further develop the email discussion group for the Aquatic Animal Health Technical Forum.
2. To ensure the continuation of technical information transfer between forum members.
3. To organise annual workshops at various institutes that provide specific aquatic animal health services.
4. To open the forum to international participation and thus enhancing the knowledge base of the forum members, for example exotic diseases.
5. To canvass State Departments for potential funding contributions to enable the continuation of the forum beyond 2015.

Methodology

As a network, AAHTF operates very basically via email and telephone communication which is supplemented by training workshops organised annually and hosted by institutes that provide specific high-level aquatic animal health services. Workshops were organised by the Principal Investigator and three staff from DPIPWE, Launceston. Input from a staff member at the host facility proved to be invaluable and provided information with respect to facilities available, potential field trips and accommodation. Suggestions for future workshop locations were made at the completion of each workshop via discussion and the feedback forms. On selecting a suitable location and workshop date, information was distributed electronically to past participants and via the FRDC Aquatic Animal Health Subprogram newsletter *Health Highlights.* On distribution of initial workshop information to people who expressed an interest to attend, they were also asked to provide a potential presentation title. The workshop program was then prepared with participant presentation information and, where available, incorporated practical sessions and field trips. At the three workshops there were additional presentations made by professional members from the host facility. Accommodation for participants was booked by the organising group and partial reimbursements of flight costs processed. For the majority of workshops all participant accommodation was fully covered by project funding in addition to partial flight reimbursement costs.

Results/key findings

The forum has developed from an initial 18 members in 2010 to a current membership of 61 with participants from government departments,

universities and aquaculture industry in all States and Territories. The workshops have continued to provide networking opportunities for aquatic animal health technicians and workers. Networking is more vital in the smaller discipline of aquatic animal health than the mammalian/avian area to encourage these people to learn and share their skills with others who may be new or lack experience in the aquatic field. Through the workshops, technical staff at diagnostic laboratories have developed improved competencies in a broad range of diagnostic procedures.

Implications for relevant stakeholders

The workshops have provided participants with knowledge and tools to respond more effectively in the face of disease outbreaks which appear to be occurring with greater frequency. In this way the negative impact of disease outbreaks on industry productivity and profitability will be reduced.

In addition, the younger generation of less experienced technologists attending the workshops have benefited from the interaction with their more experienced colleagues. In addition, this type of professional training supports the Quality Systems in place at each of the diagnostic laboratories.

Recommendations

Workshop feedback indicated that the AAHTF, including the skills and training workshops, was beneficial to participants and their host institutes. The technical knowledge gained, experience in presenting to peers and the contacts made during and after the workshops are invaluable. It has been recommended that the forum should continue and workshops should be held as stand-alone events on an annual basis preferably at different institutes that have strong aquatic animal health capability. A funding model for the forum needs to be agreed upon by governments and industry and implemented as soon as possible to ensure that the forum is maintained and past achievements can be built on.

Keywords

Aquatic animal health; diagnostics; skills; technology transfer; training

Project No. 2012/032: Aquatic Animal Health Subprogram: Pacific oyster mortality syndrome (POMS) – risk mitigation, epidemiology and OsHV-1 biology (PI: Richard Whittington)

Executive Summary

What the report is about

POMS is a viral disease with the potential to devastate Pacific oyster aquaculture in Australia. This project was an attempt by researchers from the University of Sydney to intervene in the face of a disease outbreak to discover ways to continue oyster farming. It was conducted in partnership with oyster farmers whose businesses had been wiped out, by working in real-time on affected oyster leases in the Georges and Hawkesbury Rivers, and in state of the art laboratories at the University of Sydney. It is the first study in Australia, and one of the few worldwide, to involve very intensive long term field work to find a solution for an oyster disease. The project addressed nationally agreed priorities and complemented research to develop a genetically resistant oyster. The solution to POMS will require both genetic improvement and science-based modifications to husbandry.

Background

There has been a disturbing pattern of emerging disease in oysters in Australia. Examples include QX disease, winter mortality and bonamiosis. These diseases have spread, persisted or recurred. Too little was known about their cause, or their biology (epidemiology) to enable rational control measures, and research was either not undertaken, or commenced too late to inform policy and strategy. Consequently the opportunity was seized to commence research on POMS at the outset of its emergence in Australia, and to communicate research findings in real time to both government and industry, to enable both policy formulation and commercial decisions. The Pacific Oyster Health Management Working Group under the Subcommittee for Aquatic Animal Health, Department of Agriculture Canberra was established to coordinate the flow of information.

Aims/objectives

The aims of the project were to confirm the identity of the virus, determine the mechanism(s) of transmission of disease, determine the major factors that contribute to outbreaks thereby identifying potential risk-mitigation management practices, to identify the natural reservoir(s) for the virus, its stability in the environment and disinfection guidelines, to develop a laboratory infection model to study the disease and to address future shortages of technical expertise through training a PhD student.

Methodology

The project required DNA sequencing to identify the virus. The samples for this were obtained from oyster leases by the university researchers who worked closely with oyster farmers during the disease outbreaks. Long term field study sites were set up on oyster leases in both the Georges River and the Hawkesbury River at the start of the project. The research team conducted an intensive real time investigation of the first outbreak of POMS in the Hawkesbury River in 2013, and set up controlled experiments in the Georges River to measure key aspects of the disease. Oysters were placed at multiple locations in both rivers over 2 years and sampled every few weeks to accurately determine the seasonal window for the disease. Experimental up-wellers were set up at the Hawkesbury River to

test the idea that the virus could be removed from seawater to protect hatcheries. Wild molluscs were sampled in the Georges River to try to find an environmental reservoir for the virus. Laboratory experiments were conducted to develop a reliable way to induce POMS under controlled conditions, and then these methods were used to study how long the virus remained infective in seawater, how it could be inactivated by disinfectants, and how the disease was dependent on water temperature.

Results/key findings

The virus that causes POMS in Australia is *ostreid herpes virus 1* µVar (OsHV-1 µVar). It is similar to the virus that has devastated Pacific oyster aquaculture in France, other European countries and New Zealand. The disease outbreaks in the Georges and Hawkesbury Rivers were caused by the same virus.

Detailed investigation of the Hawkesbury River outbreak revealed novel features of the disease: the virus was first detected months before the disease began, but it is likely that this was due to several separate infection events, the last one being massive and leading to widespread mortalities; the source of virus was not the farming operation, and was most likely to have been from a distant environmental source; once introduced *en masse* from an external source, local spread of the disease from oyster to oyster and lease to lease was minor; large adult oysters were relatively resistant.

A consistent seasonal pattern of disease was observed in both rivers. POMS was seen between October and May each year and was not present in the other, cooler months. Water temperatures in NSW when POMS occurred were about 4^{o}C warmer (i.e. 20^{o}C) than those observed in France when the disease occurs there (i.e. 16^{o}C). This is a very significant difference in disease behaviour between Europe and Australia. It was confirmed in an experimental infection trial at the University of Sydney where mortality was minimal at temperatures less than 18^{o}C.

Analysis of long term weather and environmental records revealed that the outbreaks in the Georges and Hawkesbury Rivers were not associated with anomalies in air temperature, water temperature, salinity, or chlorophyll-a levels in water. Harmful algae were variably present and did not explain disease occurrence.

The major factors determining the extent of mortalities during an outbreak were found to be the age of oysters (spat are highly susceptible, adults relatively resistant); growing height/immersion time (raising growing height by 300 mm in the intertidal zone reduced mortalities of adults by 50%); and location (some sites within an infected river were not affected at all). The type of cultivation system and the presence of non-susceptible bivalve species on adjacent leases were not important factors. Host energy status (feeding) and cultivation density were not able to be investigated and could be important. POMS disease expression can be variable and the replication of trials over sites and over time was critically important to reveal risk factors.

Wild oysters, both Pacific and Sydney rock, tested positive for the virus, as did other mollusc species and other marine organisms. However, the levels of virus in their tissues were low, and their potential role in storing virus and amplifying and releasing it to infect farmed oysters is debatable. Further study is required.

The virus appears to remain stable in seawater for less than 48 hours. This was confirmed in laboratory experiments and in field trails with spat in up-wellers in the Hawkesbury River. Water treatments based on ageing water for 48 hours and filtration to 5 µm were successful and can be used to protect hatcheries. Several disinfectants were effective and will be useful for decontamination of equipment. Chlorine is commonly available but was not effective in the presence of organic matter.

Implications for relevant stakeholders

Industry can adopt some findings from this study immediately. Hatcheries can treat incoming seawater using simple procedures to prevent mortality of spat due to the virus. It is possible to safely farm oysters in affected estuaries except between late October and mid-May. Farmers who already have suitable and flexible infrastructure, or who will make investments in infrastructure, can provide elevated intertidal growing height to substantially reduce mortalities in adult oysters. Partnerships between farmers in different regions and strategic movement of stock would allow for two stage farming to take advantage of feed availability and to avoid the danger period in POMS affected estuaries.

Policy makers should consider the findings related to disinfection guidelines for equipment. In addition, the potential for further spread of the disease due to unknown sources, probably oceanic, should be considered.

Recommendations

Further research is required to identify strategies to enable spat to survive in infected estuaries during the danger period. Identifying the reason for their profound susceptibility will fill a major knowledge gap.

The role of wild molluscs in outbreaks in farmed oysters, and identification of the main environmental sources of OsHV-1 requires further research.

Exposure of oysters to OsHV-1 at any given place or time is highly variable. This has major implications for surveillance, for prediction of risk and for assessment of the benefits of husbandry modifications and genetically selected oysters. We recommend that all field assessments be conducted using adequate experimental design involving independent professional supervision, multiple sites,

replication, frequent observations and laboratory confirmation.

Active surveillance is required in regional oyster farming locations in Australia as an early warning system, for example to enable an orderly emergency harvest and rational business decisions (such as whether or not to purchase spat). A reliable active surveillance system needs to be devised based on understanding the spatial dynamics of OsHV-1 discovered in this project.

All outbreaks of POMS in Australia have occurred in relatively warm water. Further study of the consistency of seasonal patterns of POMS, the periodicity of infection within season, inter-estuary temperature variation in Tasmania and South Australia, and integration of this information to predict POMS behaviour is warranted.

It is important to determine whether water treatments prevent OsHV-1 infection of spat or merely prevent mortality, and whether they can be applied for biosecurity of hatchery effluent. In order to produce OsHV-1–free spat in an endemic region, and have batches certified for movement to disease free regions, greater confidence than that provided in the current project is needed to assess whether the virus can be excluded from hatcheries.

There is a question about the potential for persistence of infective OsHV-1 in fresh-frozen commercial oysters, and whether this could be a means of translocation of virus. Oysters may be diverted from the human food chain to be used as bait, or shells may be discarded in coastal waters from pleasure boats. Studies are required in Australia to assess the infectivity of OsHV-1 in imported fresh-frozen oysters, including those from New Zealand.

Sequencing of additional samples of OsHV-1 and additional parts of the genome of samples from Australia is recommended to further understand infection and disease spread patterns.

Keywords

Crassostrea gigas, Ostreid herpes virus-1, Pacific oyster mortality syndrome, POMS, disease

Project No. 2012/050: Aquatic Animal Health Subprogram: A survey of *Edwardsiella ictaluri* in wild catfish populations in Australia (PI: Alan Lymbery)

Executive Summary

This report contains the findings of the first survey of the exotic bacterium *Edwardsiella ictaluri* in wild freshwater fish populations in Australia*. Edwardsiella ictaluri* causes enteric septicaemia of catfish (ESC), which is a serious disease of farmed channel catfish in the USA. The bacterium has previously been detected in imported ornamental fish and in native catfish held in Australian aquarium facilities, but wild fish populations in Australia are considered free of the disease. The Australian Government Department of Agriculture, through the Fisheries Research and Development Corporation, funded an active surveillance program to provide further evidence for this claim of disease freedom.

Background

It is never possible to *prove* that a population is free from a disease-causing pathogen. It is possible, however, to estimate the probability that the population is free from the pathogen at a given level of infection. This requires an appropriate sampling strategy to survey high-risk individuals and a diagnostic test of sufficient sensitivity to detect the pathogen if it is present. This study targeted wild catfish species (because catfish are known to be susceptible to *E. ictaluri*) in northern Australia (because acute ESC disease occurs at higher temperatures) and concentrated our sampling around population centres (because the most likely source of *E. ictaluri* is from the release of infected ornamental fishes).

Aims

1) Design a targeted survey for *E. ictaluri* in wild catfish in rivers in northern Australia to establish disease freedom with 95% confidence at a prevalence of less than 5%.
2) Conduct an active survey of wild catfish populations in river systems in northern Australia for the presence of *E. ictaluri* by appropriate laboratory tests.

Methodology

We developed a risk-based sampling model and used this model to test different survey designs. Our final design, based on the model, involved a mean sample size of 18 fish from each of 15 sites, providing a probability of 95% that wild populations of catfish in northern Australia are free of *E. ictaluri* at an overall prevalence 1%, given negative survey results.

Catfish were sampled from these sites, with the assistance of a large network of collaborating freshwater fish scientists. Tissue samples were cultured for evidence of *E. ictaluri* and, if potentially positive cultures were found, DNA sequencing was used to confirm identity.

Results

Edwardsiella ictaluri was detected in eight *Tandanus tropicanus* catfish sampled at one site in the Tully River in northern Queensland. Catfish examined from all other sites in Queensland, the Northern Territory and Western Australia were free from infection.

Implications

Wild fish populations in Australia cannot be considered to be free from *E. ictaluri*. This may affect the export of native ornamental fishes from Australia to countries where the bacterium has not been

found, and has potential implications for the imposition of quarantine restrictions on live fishes imported into Australia. From an environmental perspective, *E. ictaluri* has been found to cause acute disease in a number of fish species and may therefore present an additional stressor on endangered wild fish populations in Australia. Because the bacterium can survive in the bottom sediments of rivers, eradication is not a viable option. Management actions should therefore aim to minimise the potential for spread from infected to uninfected rivers.

Recommendations

Additional sampling should be undertaken to more precisely determine the geographic range of *E. ictaluri* in Australia. This should concentrate on the Tully River and adjacent river systems in northern Queensland.

Once the geographic range of *E. ictaluri* has been established, a risk assessment should be undertaken to guide future management activities. There are some key information gaps that need to be filled to inform this risk assessment process. Of particular importance is the susceptibility and tolerance of Australian native fish species to infection by *E. ictaluri*.

Keywords

Disease freedom; *Edwardsiela ictaluri*; ESC; catfish; *Tandanus tropicanus*

Project No. 2013/001: Aquatic Animal Health Subprogram: Determination of susceptibility of various abalone species and populations to the various known AbHV genotypes (PI: Serge Corbeil)

Executive Summary

Background

Abalone viral ganglioneuritis (AVG) caused by infection with abalone herpesvirus (AbHV) was first detected in Victorian abalone in late 2005/early 2006 and contributed to a reduced wild-catch fishery from 1614 tonnes in 2004/05 to a low of 827 tonnes in 2010/11 in Victoria. Subsequently, there has been a recovery in volume of production to 1196 tonnes in 2012/13. Interestingly, abalone herpesvirus (AbHV) was also detected in Tasmania from 2008 but there was no disease in wild stocks and volume of production in Tasmania has remained relatively steady at 2500-3000 tonnes since 1998/99. The need to understand the epidemiology of the various virus subtypes and their effects on abalone stocks and species and requirement for biosecurity incited us to undertake this study. Information acquired will allow authorities to improve management plans, including avoiding possible translocation of the disease where susceptible abalone species are present. Previous research on AbHV was successful in developing an experimental infection model at CSIRO Australian Animal Health Laboratory high-biosecure aquarium facility for studying pathogenesis in target species. This model was used to determine whether or not *Haliotis rubra* (blacklip), *H. laevigata* (greenlip) and *H. conicopora* (brownlip), originating from various States in Australia, are susceptible to infection by the five known AbHV subtypes and/or abalone viral ganglioneuritis (AVG). This two-year study was undertaken using a well-studied positive control hybrid Jade Tiger abalone (*H. laevigata X H. rubra*) obtained from a commercial farm (Great Southern Waters Pty Ltd (GSW; now known as Craig Mostyn Group Jade Tiger abalone Pty Ltd)). In addition, the genomes of AbHV subtypes Tas3, Tas4 and Tas5 were fully sequenced. Analysis and alignment with previously sequenced AbHV subtypes (Vic1, Tas1 and Tas2) were performed.

Aims/Objectives

1 Determine the susceptibility of greenlip, blacklip, hybrid and other readily available abalone species to abalone herpesvirus (AbHV) genotypes.

2 Determine the complete genome sequences for AbHV Tas3 and Tas4 to gain insights into how and over what timeframe they have arisen, whether genetic recombination is contributing to this variation and which genome regions might affect virulence, as well as instructing on how diagnostic methods for their detection and differentiation can be refined.

Methodology

Objective 1: The susceptibility of greenlip, blacklip and brownlip abalone species to known AbHV genotypes was determined in bioassays using the biosecure aquarium facilities at CSIRO AAHL. Abalone (1-2 years old), particularly species of high commercial value, were obtained from various States in Australia with the assistance of private aquaculture companies and Government agencies (e.g. Tasmania, Victoria, South Australia and Western Australia). These abalone were sourced from regions with no history of disease and transported to AAHL. Experimental groups of 8 abalone were exposed by immersion to a low, medium or high dose of AbHV inoculum using standardised bioassay procedures developed in FRDC Project 2009/032. Uninfected controls were housed in a separate room of the biosecure aquarium facility. Known susceptible abalone (1-2 year old hybrids sourced from Craig Mostyn Group Jade Tiger abalone Pty Ltd, previously known as Great Southern Waters Pty Ltd (GSW)) were used as positive controls. Abalone were monitored daily for gross disease signs for 9 days post-exposure. Tissue samples were collected from abalone exhibiting disease signs or that had died since they were last observed, as well as any that survived the 9-day bioassay period, and processed subsequently to assess AbHV infection severity by histology and/or PCR. The use of 3 dilutions of each inoculum prepared to the AbHV Vic1, Tas1, Tas2, Tas3 and Tas4 subtypes allowed evaluation of their pathogenicity in different abalone species from

different geographical regions using the susceptible GSW hybrid abalone as a benchmark positive control. Quantitative PCR (qPCR) was used to evaluate the viral loads in each sample tissue.

Objective 2: The complete genome sequences of the most recently identified AbHV Tas3 and Tas4 subtypes were determined from DNA amplified from preparations of AbHV semi-purified from infected abalone using procedures developed in FRDC Project 2009/032 (the whole genome sequences for Tas1 and Tas2 have been obtained previously). Because the Tas-5 sub-type became available during the course of the project we also performed its full sequencing and alignment. Briefly, ganglia in AbHV-infected and diseased abalone were harvested in such a way as to minimise the amount of muscle tissue collected. The harvested ganglia were homogenised and the homogenate subjected to various rounds of gradient ultra-centrifugation. Virus-containing fractions (monitored by qPCR) were pooled to obtain a semi-purified viral preparation. DNA from this semi-purified viral fraction was extracted and amplified using a multiple-displacement method prior to preparation for whole genome sequencing using next gen (454 and/or Illumina) technology. Sequences obtained were used to construct the whole viral genome for Tas3, Tas4 and Tas5 which were compared to each other and to AbHV Vic1, to Tas1 and to Tas2 genotype sequences obtained in FRDC Project 2009/032. Such comparisons helped identify genome regions that are most conserved or most consistently divergent in an attempt to associate specific sequences with differences in infectivity and/or pathogenicity of these subtypes as determined in the bioassays, as well as provide information on any need to refine PCR tests used for diagnosis and epidemiology.

Results

Results demonstrated that all abalone tested are susceptible to infection by the five AbHV subtypes used (Vic1, Tas1, Tas2, Tas3, Tas4). Due to limited availability of healthy greenlip abalone from WA, challenge trials using AbHV Tas2, Tas3 and Tas4 could not be performed. Histopathological analysis performed on moribund abalone did not show differences in AVG lesions between virus subtypes or abalone species.

Sequence analysis of AbHV Tas3, Tas4 and Tas5 revealed high identity/homology with the previously sequenced AbHV subtypes (Vic1, Tas1 and Tas2).

Implications for relevant stakeholders

AVG remains a serious concern to all State jurisdictions with significant commercial investments in abalone fisheries and aquaculture. This study emphasised the potential risk of all known subtypes of AbHV to infect the main Australian abalone species of commercial interest.

Recommendations

All abalone species in Australia, including those that have not been tested in this project, should *a priori* be considered likely to be susceptible to infection with AbHV and the disease AVG since so far no species in Australia has demonstrated significant resistance to infection to AbHV subtypes by experimental infection.

Keywords

Abalone viral ganglioneuritis (AVG); abalone herpesvirus (AbHV); experimental challenge; susceptibility to AVG; abalone; *Haliotis laevigata*; *Haliotis rubra; Haliotis conicopora*.

Project No. 2013/036: Tactical Research Fund: Aquatic Animal Health Subprogram: Viral presence, prevalence and disease management in wild populations of the Australian Black Tiger prawn (*Penaeus monodon*) (PI: Jeff Cowley)

Executive Summary

What the report is about

Reported here are the outcomes of a project to (i) test for enzootic and exotic viruses in wild stocks of Black tiger prawns (*Penaeus monodon*) inhabiting disparate regions in northern Australia and (ii) develop new PCR tests and appropriate test controls to improve upon the specificity of existing PCR tests for the exotic highly-pathogenic yellow head virus genotype 1 (YHV1). *P. monodon* were examined from locations in North Queensland (NQ), the Gulf of Carpentaria (GoC) and Joseph Bonaparte Gulf (JBG). Of the enzootic viruses targeted, there was a particular focus on Gill-associated virus (GAV) and a unique YHV genotype (YHV7) discovered recently in *P. monodon* originating from JBG. Testing for exotic viruses was conducted to provide additional evidence to support Australia's claims of pathogen freedom. Aspects of the project were undertaken within the Aquaculture Program, CSIRO Agriculture, Queensland Bioscience Precinct (QBP) and within the CSIRO Australian Animal Health Laboratory (AAHL) Fish Diseases Laboratory (AFDL).

Background

Two of the project objectives were undertaken to support the Australian Prawn Farmers Association, the Queensland Department of Agriculture, Fisheries and Forestry and commercial broodstock suppliers; to obtain data on the presence and prevalence of YHV genotypes (Objective 1) and other enzootic viruses in wild *P. monodon* (Objective 4). Regions targeted for sampling had been used or were being considered for use as a source of wild broodstock No systematic studies of what viruses could be inadvertently introduced via broodstock sourced from either NQ or more remote locations such as Joseph Bonaparte Gulf (JBG) or the Gulf of Carpentaria (GoC) have been undertaken. The need

to determine the disease status of wild-caught broodstock was further highlighted after the discovery of YHV7 in *P. monodon* during a disease investigation in a hatchery in NQ, associated with broodstock translocated from the JBG.

The other two project objectives were undertaken to revise GAV/YHV molecular assays (Objective 2) and develop synthetic positive control material (Objective 3). In 2012, PCR testing of moribund *P. monodon* at a farm in Queensland (QLD) produced unexpected results with the OIE YHV protocol 2 test. When sent to AFDL for confirmatory testing the OIE YHV/GAV Protocol 2 reverse transcription (RT)-nested PCR (RT-nPCR) test produced anomalous false-positive results for YHV1 in the nested PCR (nPCR). Sequencing identified amplicons derived from GAV. Due to the identified lack of specificity of the test and concerns that this could lead to erroneous reporting of YHV1, it was identified as a high priority to refine or develop yellow head complex PCR tests.

Aims/objectives

The project had 4 objectives:

1. Determine what GAV/YHV genotypes exist and their relative prevalence in *P. monodon* populations in wild NT and QLD populations from which broodstock are captured for aquaculture purposes.
2. Revise PCR test designs as necessary to ensure their specificity, particularly in discriminating the highly virulent YHV-1 strain that emerged in Thailand in the early 1990s from GAV and the other known YHV genotypic variants that appear to be far less pathogenic, and make these tests available for publication in the OIE Diagnostic Manual for yellow head disease.
3. Acquire and/or prepare appropriate control nucleic acids for the various YHV genotypes suitable for use with the YHV- or other genotype-specific tests so that their diagnostic performance can be validated at key testing laboratories such as CSIRO-AAHL, and for distribution to state and international laboratories with needs for equivalent diagnostic capabilities.
4. Determine the existence and prevalence of other endemic viruses [e.g. Mourilyan virus (MoV), Monodon baculovirus (MBV), Hepatopancreatic parvovirus (HPV), Spawner-isolated mortality virus (SMV) and Infectious hypodermal and hematopoietic necrosis virus (IHHNV)] in wild *P. monodon* populations in the NT and QLD. In addition, test samples for the exotic viruses WSSV and IMNV given some broodstock are sourced from waters with a higher than usual likelihood of incursion from these pathogens.

Methodology

Samples of *P. monodon* from populations inhabiting various locations in NQ, the GoC and JBG were sourced from either hatcheries, directly from a commercial broodstock collector or from fisheries survey trawlers. The goal was to collect appropriate tissues from at least 150 prawns from each region to allow viruses to be detected at 2% prevalence level, with 95% confidence assuming a perfect test. To accommodate the fact that unique genetic variants are known to exist for specific viruses (YHV/GAV, MBV, HPV, and IHHNV), available PCR tests were used where appropriate or PCR tests were designed at the CSIRO-QBP. Due to the recent discovery of YHV7, TaqMan and conventional PCR tests specific for this genotype were also designed and evaluated. Due to the deficiencies identified with the OIE YHV PCR tests, new YHV1-specific TaqMan and conventional PCR tests were also developed and evaluated.

Results/key findings

The exotic viruses (WSSV, IMNV, or YHV1) were not detected in any of the Australian-sourced *P. monodon* originating from any of the locations examined.

GAV was detected among *P. monodon* originating from all NQ study locations. For some prawns originating from Etty Bay (the most southerly NQ location examined) the PCR product sequence obtained was consistent with YHV6. This genotype is most closely related to GAV (= YHV2). Archived sequences derived from PCR products amplified from NQ *P. monodon* were re-examined and results indicated that YHV6 has existed in this region since at least the early 2000s (JA Cowley, unpublished). The GAV TaqMan real-time RT-quantitative PCR (RT-qPCR) detected GAV among *P. monodon* originating from JBG, confirmed by sequence analysis of PCR products. While GAV detections were made in some *P. monodon* from GoC using the GAV TaqMan real-time qPCR test, these detections were not confirmed by conventional PCR.

With regard to YHV7, detections were made using the YHV7 TaqMan real-time qPCR test in three prawns originating from NQ locations and in one prawn originating from a GoC location. These could not be confirmed by conventional PCR. The YHV7 TaqMan real-time qPCR test detected YHV7 in *P. monodon* originating from JBG with confirmation by conventional PCR and sequence analysis. YHV7 was also detected with confirmation by sequence analysis from broodstock and progeny from a hatchery in NQ.

Molecular assays to specifically detect YHV1 were designed and evaluated. Two assays, one in a format for high-throughput screening (YHV-1 TaqMan real-time RT-qPCR test) and one for confirmatory testing (YHV RT-nPCR test) showed appropriate analytical specificity and sensitivity. Limited availability of YHV1 known-positive material has delayed determination of diagnostic sensitivity and technology transfer of the assays to state laboratories. However, requests have been made to the international community for additional YHV1-positive samples. Samples generated through FRDC 2015-005 “Aquatic Animal Health Subprogram:

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" will be used for PCR test validation including tests for YHV1 which will be used as a positive control for infectivity trials. A number of published assays for detection of YHV1 and other YHV-complex genotypes (e.g. GAV) were also evaluated during the project. A lack of specificity was identified indicating the need for improvement of a number of these assays. With regard to YHV7, TaqMan real-time qPCR and conventional nPCR tests were designed and found to be specific for this genotype. Material for validation of the YHV7 assays will also be developed through FRDC 2015-005.

As validation of the new YHV1 and YHV7 TaqMan real-time qPCR and RT-nPCR tests has not been completed, positive control material was not developed and distributed as planned. As more extensive validation is undertaken, assays may need minor modifications which will negate the use of any controls developed. Assays can be transferred to state laboratories with tissue-derived positive controls now, if requested. Development of this material will be completed and distributed to state laboratories through FRDC 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".

The MoV TaqMan real-time qPCR test detected MoV at low to high prevalence among prawns originating from various NQ locations. For SMV, only two of 151 NQ prawns and three of 143 JBG prawns tested positive. However, it is possible the SMV prevalence was an underestimated due to the unavailability of hepatopancreas (HP) tissue for most of the prawns tested. For IHHNV (excluding virus-like homologues in the host genome), PCR detections were made among prawns from each of the three regions studied. For MBV, only two of 151 NQ prawns and one of 37 GoC prawns tested PCR-positive. However, it cannot be discounted that the use of gill DNA resulted in the prevalence of MBV being underestimated. For HPV, PCR detections were made within the 3 study regions. However, the unavailability of HP tissue from prawns captured at some locations might have led to HPV prevalence being underestimated at these locations.

Implications for relevant stakeholders

The APFA supported the testing for exotic viruses and will be reassured as WSSV, IMNV and YHV1 were not detected in any of the *P. monodon* tested from any of the study locations. The 2012 discovery of YHV7 in broodstock originating from JBG was confirmed in 2013 samples from QLD hatcheries. However, as YHV7 was also detected and confirmed in QLD grow-out prawns, targeted surveillance of farms in QLD is required to determine the true prevalence and distribution of this genotype. Whether the detection of YHV7 in prawn farms in QLD is due to recent introductions with broodstock sourced from the Northern Territory or is due to YHV7 already being present in QLD prawns is unknown.

When validated, the revised YHV1 and YHV7 PCR tests and non-infectious synthetic artificial controls will be valuable for State Government diagnostic laboratories as well as commercial diagnostic laboratories undertaking certification testing for imported unprocessed prawns.

Recommendations

To assess what risks are posed by the YHV7 genotype, its pathogenicity to farmed prawns needs to be determined. This will be undertaken as part of FRDC 2015-005 "Aquatic Animal Health Subprogram: 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". This project will also develop additional material required for validation of YHV1 and YHV7 assays and preparation of synthetic positive control material.

In order to determine the current prevalence of YHV7 in QLD farmed prawns and to clarify the translocation risk associated with broodstock from the Northern Territory, additional targeted surveillance should be undertaken of prawns in QLD.

Keywords

Black tiger prawns, *Penaeus monodon*, yellow head virus, gill-associated virus, enzootic viruses, exotic viruses, PCR tests and controls, northern Australia, aquaculture

Project No. 2013/036: Aquatic Animal Health Subprogram: Development of a national aquatic animal health curriculum (NAAHC) for delivery by tertiary institutions - Report from consultative workshop, Adelaide, 13-14 February 2014 (PI: Stephen Pyecroft)

Executive Summary

This report summarises the process and outcomes from an interactive workshop which was convened over 1.5 days in Adelaide in February 2014 to progress the possible development of a national aquatic animal health curriculum (NAAHC) for the education of undergraduate, postgraduate and vocational students, by representatives of the Australian aquaculture industry, state and federal governments, universities and vocational trainers.

It has been indicated by government and the aquaculture industry that trained professionals in aquatic animal health (AAH) are needed to support the continued development and sustainability of the aquaculture sector within Australia.

The Fisheries Research and Development Corporation identified the need for such training capacity within the Aquatic Animal Health Sub Program priorities and the review and potential

subsequent curriculum development was identified as clear objective within AQUAPLAN 2014-2019, Australia's National Strategic Plan for Aquatic Animal Health.

Currently a diverse range of training opportunities and educators with appropriate corporate knowledge exist relatively un-connected across the country resulting in poor coverage of the core attributes required of health managers for industry and government.

A concise review of vocational education and training programs in AAH within Australia was undertaken by Mark Oliver of Australian Aquaculture Support Services Pty Ltd for the FRDC Aquatic Animal Health Sub-Program identifying the key deliveries for AAH training to this sector. The documents for this training are organised in the Seafood Industry Training Package (SFITP) and whilst the training is organised within a training package underpinning the Australian Qualifications Framework and managed by Agrifood Skills Australia, the content development and presentation is independent of a nationally relevant and agreed upon message.

Education of veterinary and non-veterinary scientists in the skills of AAH are currently ad hoc, sporadic and lack a central theme, reflecting more the expertise in the various institutions providing the education and training rather than a nationally recognised cohesive need for the relevant stakeholders.

Previous workshops and studies have indicated the need for a concise, coherent, well-resourced, stakeholder-directed curriculum to deliver a common aquatic animal health message. This project was the first step in the development of such a framework for teaching aquatic animal health.

This workshop aimed to:

- Review the need, scope and end user market for a national curriculum in aquatic animal health
- Develop a general understanding of the current content and provision of aquatic animal health education within the tertiary sector in Australia and how this is utilised by end users within the aquaculture sector and other end users of graduates.

In order to engage all recognised stakeholder groups the workshop was structured around a number of sessions including scene-setting and summary presentations as well as break-out groups and interactive discussion sessions (see Appendix 1 workshop agenda). The workshop was facilitated by the Dr Stephen Pyecroft of the University of Adelaide and attendees were organised in a number of discussion groups from which ideas and conclusions were derived.

The key objective subjects were discussed specifically across the stakeholder sectors.

Having a consensus of understanding of what is meant by a national curriculum in the context of AAH education was a key to carrying forward any development of a curriculum. Delegates identified a number of key attributes of a curriculum.

It must be:

• Harmonised and Standardised

• Transportable

• Recognised

• Flexible being 'fit-for-purpose'

The curriculum would deliver a common message that would have to be defined and be applicable across multiple sectors taking advantage of a multi-disciplinary approach. The road map linked all components in the system allowing specific content development at vocational and the various university levels.

It must also allow the development of skills, attributes, competencies of its graduates and have clearly defined learning outcomes and it should not be seen as is a silver bullet for solving all educational circumstances within AAH training. It was recognised that there is an existing diversity of approaches to AAH education and that developing a prescriptive curriculum fails to recognise not only the investment already made by many in the educational sector but also the scale of investment required to develop a 'one–fits-all curriculum' is in itself prohibitive of its development at all, in the current economic climate.

It was also recognised that there are a diversity of skills required by end users and so the development of different levels of expertise and specialisation of graduates made a prescriptive approach non-viable.

Specifics of the content of the curriculum should remain variable within the context of the learning outcomes and the general core learnings should form the skeleton on which all content is supported.

It was seen that a curriculum could be a resource deposit, library or more, catering for resource sharing for a tiered system of training recognising that complete harmonisation across a range of diverse educational targets with variable legal frameworks and dealing with many aquatic animal species will be difficult. There would also be an increased dissemination of knowledge by breaking down the silos perceived as being present now in the existing system. Students would have an understanding of the different disciplines within the AAH sector allowing them to better direct their interest and receive the appropriate training. Breaking the silos down may also allow greater facilities interaction and so a national approach would allow greater access to existing knowledge at all levels.

The key to the development of a national curriculum would be to produce educated personnel who are capable of fulfilling various roles in aquatic animal health required by the Australian aquaculture industry and other end-users such as governments.

Another key and most important aspect would that it would allow AAH professionals to map pathways to careers. The development of such a system would identify common outcomes and improve visibility of

the sector by identifying career paths, linking career options and allowing recognition of what's available.

The brief gap analysis of existing national AAH education provision undertaken at the workshop can be summarised simply as a lack of a proper facility for the production of appropriately educated AAH professions for industry and government and a lack of a coordinated approach, at a national level, to address this issue. Once this is addressed then a detailed curriculum content GAP analysis can be undertaken during the development of the specific core elements of the curriculum for, and by, each sector.

Delegates by sector, in the short time available at the workshop, identified key attributes of AAH graduates from each educational sector and for each employer sector. These attributes and requirements will form the core educational components of any national curriculum.

Stakeholders at the workshop agreed upon a number of specific recommendations (focusing and confirming previous consultation on this subject):

1. That the development of a national curriculum for aquatic animal health proceeds to delivery.
2. That a mechanism for curriculum review be developed so that timely regular reviews of the content and delivery of the NAAHC are undertaken to ensure relevance and optimal delivery of the curriculum for all sectors of industry and government.
3. Establish a model of funding that will allow initial development and ongoing review of the NAAHC.

The workshop proposal aimed to initiate the development of an NAAHC, which led to this workshop and identified the need for a series of workshops or working group sessions to allow the development of a national curriculum. This proposed process was confirmed at the workshop by stakeholders who expressed enthusiasm in carrying the process forward.

It was agreed that the development and delivery of an NAAHC will only be achieved by undertaking a number of vital tasks in a process toward delivery and are as follows:

A. **EITHER** appoint a project manager to coordinate the curriculum development project **OR** establish a working group made up of representatives of key stakeholders (i.e. universities, vocational educators, government and aquaculture sectors) to oversee the project.
B. Formation of working groups within the university sectors (i.e. veterinary and non-veterinary) to align existing teaching amongst institutes, identify gaps in teaching resource and develop needed modules for undergraduate and post graduate education that specifically fulfil the needs of the curriculum.
C. Use the key competencies identified at this workshop as a foundation for a further survey of end users of AAH graduates, to develop a national key competency list around which the curriculum will be developed.

These elements could be initiated and progressed by an additional workshop aimed at developing a work plan for curriculum development and require a funding model to allow progression.

Keywords

Curriculum, Aquatic Animal Health, training, competencies, education, vocational, university, national, bench-marked

Progress Summaries for Active Projects

Project No. 2014/001: Aquatic Animal Health Subprogram: Strategic approaches to identifying pathogens of quarantine concern associated with the importation of ornamental fish (PI: Joy Becker)

The project is continuing to meet the milestones set out in the application.

Mr. Alejandro Trujillo González has completed his PhD confirmation at JCU. The title of his thesis is *Strategic approaches to manage parasite threats from the ornamental fish trade.*

Sample collection for the project is now complete. In total, 63 laboratory accessions were received consisting of 20 species of fish from 12 consignments representing 9 exporters from 5 different countries. An additional 8 laboratory accessions from Sri Lanka 2 have been processed and tested for viral pathogens according to fish species.

All molecular testing for viruses is completed with a small number of consignments undergoing select re-testing for confirmation. Parasite identification, data entry and data analysis are ongoing.

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)

Positive controls have been designed for 28 assays (19 real-time and 9 conventional molecular assays), detecting 15 pathogens of finfish (nervous necrosis virus, infectious salmon anaemia virus, viral haemorrhagic septicaemia virus, spring viraemia of carp virus, *Megalocytivirus*, Tasmanian salmon *Aquareovirus*, pilchard *Orthomyxovirus*, epizootic haematopoietic necrosis virus, Tasmanian *Aquabirnavirus*), crustaceans (white spot virus, Taura syndrome virus, yellow head virus genotype 1, AHPND PirA) and molluscs (Ostreid herpesvirus type 1, abalone herpesvirus). Preparation of a positive control for the ANZSDP NNV RT-nPCR is still to be completed due to changing priorities requiring implementation of other positive controls (i.e. POMV, YHV1 and AHPND). Evaluation of T4 and MS2 phages for use as heterologous internal

positive controls has occurred and optimisation and implementation is currently underway.

Project No. 2015/001: Aquatic Animal Health Subprogram: Bonamiasis in farmed native oysters (*Ostrea angasi*) (PI: Tracey Bradley)

Both field and laboratory trials have been progressed and data from these are being analysed. Presence of *Bonamia exitiosa* has been confirmed in oyster stocks in both Victoria and South Australia.

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

For each of the 3 species of mollusc (*Crassostrea gigas, Ostrea angasi and Haliotis laevigata x rubra)* and relevant cell types, measurement of contamination levels and suitable techniques to remove contamination and methods to dissociate the various cell types have been assessed.

Project No. 2015/005: Aquatic Animal Health Subprogram: 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 (PI: Nick Moody)

P. monodon and *P. merguiensis* were sourced from North Queensland in November, 2015. Screening of animals prior to experimental infection indicated that both species were negative for YHV7 while *P. monodon* were positive for GAV (YHV2).

Results of inoculation can be summarised as follows:

1. Intra-muscular (i.m.) inoculation with YHV1 caused acute mortalities (100% in 6 days) in both *P. monodon* and *P. merguiensis* with high levels of YHV1 RNA detected by RT-qPCR and pathological changes detected by histology. Both species were highly susceptible to YHV1 via i.m. inoculation.
2. Intra-muscular inoculation with YHV7 caused more chronic mortalities in *P. monodon* (63% after 28 days). While no appreciable increase in mortality, compared to mortality observed in negative controls, were observed in *P. merguiensis*, high levels of YHV7 RNA were detected in both *P. monodon* and *P. merguiensis*. Histological changes were also observed in both species. *P. monodon* are highly susceptible to YHV7, although the appearance of disease was more chronic where compared to inoculation with YHV1.
3. Intra-muscular inoculation with YHV8 caused no detectable increase in mortality compared to negative control mortality for both *P. monodon* and *P. merguiensis*. However, YHV8 RNA was detected in prawns surviving at the end of the trial, indicating that the prawns had been infected with the virus. Histological changes were also observed in both species. Both species may be susceptible to infection with YHV8.
4. Intra-muscular inoculation with the YHV10 inoculum appeared to have no effect on the prawns. However, this inoculum also contained YHV8, and molecular and histology results were similar to those observed in prawns inoculated with YHV8. Additional material testing positive for YHV10 and negative for other YHV genotypes has been obtained by AFDL after the submission of this MPR and this will be used in future infectivity trials to assess the pathogenicity of YHV10.
5. Inoculation of *P. monodon,* already infected with GAV (YHV2), with the various inocula did not lead to a change the level of GAV (YHV2) RNA in animals at the end of the trial period or lead to the appearance of increased disease. No changes in GAV (YHV2) RNA levels or disease were seen in negative control prawns. Inoculation of the GAV (YHV2)-infected *P. monodon* with the YHV7 inoculum, which also contained GAV (YHV2), did not lead to any change in the levels of GAV (YHV2) RNA at the end of the trial. This may be temperature dependent as the temperature of the water in the YHV-genotype inoculated tanks was 24±1°C.

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

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

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

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: Cecil Dang)

This is a new two-year project to commence 1 January 2017.

Objectives

Develop and evaluate optimised diagnostic capabilities for Australian *Perkinsus* spp. isolates for sampling and testing based on estimates of sensitivity and specificity to meet accepted

standards for detecting infection and for testing for freedom

Project No. 2016/011: Aquatic Animal Health and Biosecurity Subprogram: Disinfection measures to support biosecurity for ISKNV at aquaculture facilities (PI: Joy Becker)

This is a new two-year project to commence 1 December 2016.

Objectives

Identify effective disinfection measures to support biosecurity for ISKNV at aquaculture facilities

Project No. 2016/013: Aquatic Animal Health and Biosecurity Subprogram: Comparative pathogenicity of exotic AHPND and the presumptive bacterial hepatopancreatitis detected in farmed *Penaeus monodon* in Queensland (PI: Nick Moody)

This is a new 18-month project that commenced in July 2016.

Objectives

1. Compare the pathogenicity of exotic AHPND and the presumptive bacterial hepatopancreatitis in *Penaeus monodon* and *P. merguiensis*.
2. Compare the pathology caused by exotic AHPND and the presumptive bacterial hepatopancreatitis in *Penaeus monodon* and *P. merguiensis*.
3. Determine the whole genome sequence of the *Vibrio harveyi* strain from farmed *Penaeus monodon* and *P. merguiensis* presumptive bacterial hepatopancreatitis.

Summary of Active Projects

Project No.	Project Title	Principal Investigator
2014/001	AAHS: Strategic approaches to identifying pathogens of quarantine concern associated with the importation of ornamental fish (*Associated species*: multi-species)	Dr Joy Becker University of Sydney Phone: 02 9036 7731 Email: joy.becker@sydney.edu.au
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
2015/001	AAHS: Bonamiasis in farmed native oysters (*Ostrea angasi*) (*Associated species: Ostrea angasi*)	Dr Tracey Bradley Dept Economic Development, Jobs, Transport and Resources - Victoria Phone: 03 9217 4171 Email: tracey.bradley@ecodev.vic.gov.au
2015/003	AAHS: Development of standard methods for the production of marine molluscan cell cultures (*Associated species*: multi-species)	Dr Andrew Reid Elizabeth Macarthur Agriculture Institute Phone: 02 4640 6332 Email: andrew.j.reid@dpi.nsw.gov.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
2016/011	AAHBS: Disinfection measures to support biosecurity for ISKNV at aquaculture facilities (*Associated species*: multi-species)	Dr Joy Becker University of Sydney Phone: 02 9036 7731 Email: joy.becker@sydney.edu.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

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Pheroze Jungalwalla, *National Aquaculture Council, Hobart, Tasmania*	0419 898 852		pherozej@gmail.com
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FOURTH AUSTRALASIAN SCIENTIFIC CONFERENCE ON AQUATIC ANIMAL HEALTH & BIOSECURITY

THE PULLMAN REEF HOTEL, CAIRNS, QUEENSLAND, AUSTRALIA
10-14 JULY 2017

FIRST ANNOUNCEMENT

The Fourth FRDC Australasian Scientific Conference on Aquatic Animal Health & Biosecurity will be held in Cairns (http://www.pullmanhotels.com/gb/hotel-2901-pullman-reef-hotel-casino/index.shtml), Queensland, Australia – gateway to the Great Barrier Reef and Daintree rainforest. The conference provides a forum for presentation of research, diagnostic, epidemiology, management and policy issues encompassing all areas of aquatic animal health and bio-security.

To receive the second conference announcement which will include information on the draft program, registration (registration fee will be AU$500.00; for students registered at Australian universities, there will be a discount registration fee of AU$150.00), abstract submission, and further accommodation details please provide Joanne Slater, FRDC Aquatic Animal Health & Biosecurity Subprogram Coordinator (email: joanne.slater@csiro.au) with the following details as soon as possible:

Your name: ……
Institution: ……
Postal address: ……
……
Email: ……
Fax: ……**Telephone:** ……

Your area(s) of interest (check boxes as appropriate):

☐ Research	☐ Finfish	☐ Viruses
☐ Management	☐ Crustaceans	☐ Bacteria
☐ Policy and regulation	☐ Molluscs	☐ Parasites
☐ Diagnostics	☐ Reptiles	☐ Fungi
☐ Epidemiology	☐ Amphibians	☐ Biosecurity

Draft title of your presentation (if applicable): ……
……
……
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The proposed conference program will in part depend on the range of topics received as abstracts but we anticipate that it will include (but not necessarily limited to) sessions on:

- Bacterial and Fungal Infections of Finfish
- Finfish Viruses
- Finfish Parasites
- Finfish Immunology & Vaccines
- Diseases of Molluscs
- Diseases of Crustaceans
- Invertebrate Immunology
- Emergency Disease Response
- Biosecurity