Health Highlights

Aquatic Animal Health Subprogram Newsletter
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From the Subprogram Leader

2015 FRDC Australasian Scientific Conference on Aquatic Animal Health

Planning for the 2015 FRDC Australasian Scientific Conference on Aquatic Animal Health held in Cairns at The Pullman Reef Hotel, on **6**-10 July 2015 is well underway. Registration and abstract submission information has been distributed. If you have not received your copy please contact Joanne Slater, AAHS coordinator (joanne.slater@csiro.au).

I am pleased to announce that the conference keynote speakers will be Professor Larry Hammell, Director - AVC Centre for Aquatic Health Sciences, University of Prince Edward Island, Canada, and Dr Charles Caraguel, School of Animal & Veterinary Sciences, Roseworthy Campus, University of Adelaide.

As in previous conferences there will be prizes for the top three student presentations by students registered at Australian universities. Please note that previous winners of these student prizes will not be eligible to win any of the 2015 prizes.

STC/SAC Meetings

Following stakeholder consultation, the AAHS met in March 2015 to consider R&D priorities for the 2016 FRDC open funding round. These were submitted to FRDC and a call for EoIs for the 2016 round will be issued no later than May 2015. Please make sure that Joanne has your current contact details to ensure that you receive this announcement.

It's on again – Cairns July 2015

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 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

Changes to the Steering Committee

Dave Ellis resigned from the Subprogram Steering Committee in 2014. Members of the Steering Committee thank Dave for his conscientious commitment and valuable input to the Subprogram while he was a member of the Steering Committee and we wish him all the best for the future.

New Steering Committee Member

It is a pleasure to welcome David Mills, Paspaley Pearling Company, as a new industry member to the Subprogram Steering Committee. David steps in to the vacancy created by the resignation of Dave Ellis.

Aquatic Animal Health Technical Forum

You should all have received, by email, a notice concerning the 5th Aquatic Animal Health Technical Workshop to be held on 17-19 June 2015 and hosted by James Cook University, Townsville.

Program: The workshop program will consist of presentations from participants and will cover a number of disciplines including molecular biology, histology, microbiology and virology.

Participant cost: Project funding will be used to subsidise participant costs for travel and, depending on participant numbers, some accommodation costs.

Please note that participant numbers are limited to a maximum of 30. Please contact Nette Williams (lynette.williams@csiro.au) for further information.

Newsletter submissions

The Aquatic Animal Health Subprogram welcomes contributions to *Health Highlights* on all aquatic animal health R&D news and events – both within and outside the FRDC. We aim to assist the widespread exchange of information by including any of the following in each 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 annually to stakeholders via hard copy and email as well as being posted on the FRDC website at: http://www.frdc.com.au. To change contact details or to ensure inclusion on the *Health Highlights* mailing list, contact Joanne at:

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Health Highlights is funded by the Fisheries Research and Development Corporation.

Completed AAHS Project Summaries

Project No. 2009/032: Aquatic Animal Health Subprogram: Characterisation of abalone herpes-like virus infections in abalone (PI: Serge Corbeil and Mark Crane)

Executive summary

What the report is about

Scientists at CSIRO Australian Animal Health Laboratory, the Department of Environment and Primary Industries Victoria, South Australian Research and Development Institute, the Department of Primary Industries, Parks, Water and Environment Tasmania, and the University of Adelaide, collaborated on a major project investigating various aspects of the biology, detection and identification of abalone herpesvirus, the causative agent of abalone viral ganglioneuritis. The various aspects under investigation included determining the stability of the virus under various physico-chemical conditions, providing new information on the current diagnostic tests for the detection and identification of the virus including sub-clinical infections, and determining the susceptibility of various abalone and other molluscan species to infection and disease.

Much of the work built on research undertaken on previous FRDC-funded projects and involved using diagnostic tests and the *in vivo* infectivity model developed previously. All *in vivo* research was undertaken at the CSIRO Australian Animal Health Laboratory, a high-level bio-security laboratory located in Geelong.

Background

The project was undertaken in response to a scientific forum that was convened in 2006 involving national and international experts to discuss R&D requirements in the face of this newly emerging viral disease of abalone. A second scientific forum was convened the following year to develop a national work plan based on the outputs of the previous forum. The prioritised work plan was developed by the National Abalone Health Management Advisory and Coordinating Body (NAHMACB) for the then Federal Government Department of Agriculture, Fisheries and Forestry's Aquatic Animal Health Committee (AAHC). This project was developed to address many of the priorities identified in the consultative process coordinated by Fisheries Victoria.

Aims/objectives

1. Validate the developed *in situ* hybridisation diagnostic test including roll-out to other States.
2. Develop a quantitative assay (qPCR) for determining the infectious dose for this virus.
3. Determine the sensitivity of the virus to physico-chemical conditions including its stability in water/on fomites and its sensitivity to

inactivation agents.
4. Determine the role of mucus in viral transmission.
5. Determine whether or not a latent stage exists in AVG.
6. Determine the susceptibility of remnant populations of abalone previously exposed to AVG and known unexposed wild populations in South Australia.
7. Using all three available qPCR tests, determine their relative sensitivities and specificities by re-testing previously collected samples from the abalone populations in Tasmania (additional objective added during the project).

Methodology
In the absence of *in vitro* culture systems such as molluscan cell lines for the replication of infectious virus, the research undertaken in this project made use of molecular technology (*in situ* hybridisation, polymerase chain reaction, dot-blot hybridisation), an archive collection of samples obtained from naturally infected abalone and experimentally infected abalone using the previously developed infectivity model for this virus (see FRDC Project 2007/006: Aquatic Animal Health Subprogram: Development of molecular diagnostic procedures for the detection and identification of herpes-like virus of abalone (*Haliotis* spp.)).

Results/key findings
The project was successful in achieving all its objectives. Firstly, the developed *in situ* hybridisation diagnostic test was validated and made available to diagnostic laboratories in Australia and overseas.

Secondly, it has been shown that a quantitative polymerase chain reaction (qPCR) can be used to determine the infectious dose for this virus. An analysis of the qPCR tests has determined that the ORF49 and ORF66 qPCR tests, when used in parallel, have a diagnostic sensitivity (DSe) of 86% and a diagnostic specificity (DSp) of >98% for the detection of sub-clinical infections.

Moreover, the sensitivity of the virus to various physico-chemical conditions including its stability in water/on fomites and its sensitivity to inactivation agents has been determined. While the virus can be present in mucus secreted by infected abalone, mucus does not appear to play a significant role in prolonging viral infectivity in seawater.

Available data indicate that abalone herpesvirus (AbHV) can exist as a sub-clinical infection, and this is supported by investigations into disease events.

In addition, it was shown that remnant populations of wild abalone (in Victoria) previously exposed to AbHV (studied here and in FRDC Project 2009/075: Determining the susceptibility of remnant populations of abalone previously exposed to AVG) remain susceptible to infection and disease. Furthermore, it has been shown that known unexposed wild populations in South Australia are susceptible to infection and disease.

Implications for relevant stakeholders
New information on technical aspects of the diagnostic tests will be of interest to diagnostic laboratories. In addition, results on the performance of the diagnostic tests provide industry and regulators with important information on the detection of sub-clinical infections that needs to be considered when designing surveillance programs e.g. sampling regime.

Moreover, results on stability of the virus under various conditions provide information of importance when developing control and management strategies.

To date, the known host range includes greenlip abalone (*Haliotis laevigata*), blacklip abalone (*H. rubra*), hybrids of these, and Roe's abalone (*H. roei*) which occur in Australia. Other mollusc species tested, *Turbo undulatus* (marine snails) and *Mimichlamys bifrons* (scallops), do not appear to be susceptible to infection with AbHV.

Recommendations
Information from this project should be used by industry and regulators when considering various aspects of this disease threat, e.g., during development of biosecurity plans, surveillance programs, and management and control strategies.

Keywords
Abalone viral ganglioneuritis (AVG); abalone herpesvirus (AbHV); diagnostic methods; *in situ* hybridisation (ISH); polymerase chain reaction (PCR); diagnostic test validation; virus transmission; virus inactivation; virus stability

Project No. 2010/034: Aquatic Animal Health Subprogram: Investigation of an emerging bacterial disease in wild Queensland grouper, marine fish and stingrays with production of diagnostic tools to reduce the spread of disease to other states of Australia (PI: Rachel Bowater)

Objectives
1. To create a library of different *Streptococcus agalactiae* strains enabling utilisation by scientific researchers.
2. Develop reliable, rapid and accurate diagnostic tools to enable detection of the bacterial disease streptococcosis caused by *Streptococcus agalactiae* in marine fish.
3. To perform phylogenetic comparison of Australian fish, human and animal strains of *Streptococcus agalactiae* with overseas fish and animal *S. agalactiae* strains, to determine their genetic relatedness and origin of the grouper strain (introduced or endemic).
4. Perform a challenge infectivity trial in Queensland grouper to prove experimentally that *S. agalactiae* causes mortalities in Queensland grouper to fulfil Koch's postulates.
5. To determine the potential food source of infection for Queensland grouper in Trinity Inlet, Cairns.

6. To determine the distribution and prevalence of *S. agalactiae* in fish along the North Queensland coast.

NON TECHNICAL SUMMARY

OUTCOMES ACHIEVED TO DATE

This project has assisted in ensuring the future sustainability and profitability of the aquaculture industry and natural fisheries resources in Queensland, Australia (including the Great Barrier Reef Marine Park), by providing industry, the public, State and Commonwealth governments with improved understanding of the occurrence of *Streptococcus agalactiae* in fish and crustaceans in coastal Queensland. Specifically, the project as shown how *S. agalactiae* may be transmitted experimentally in Queensland grouper, the relatedness between Australian *S. agalactiae* strains from animals and humans and has developed diagnostic tools for Australian State Veterinary Laboratories and Universities, that will assist in State and National aquatic animal disease detection, surveillance, disease monitoring and reporting.

The specific outcomes of this project include; 1. Provision of a bacterial strain collection of *S. agalactiae*, for utilization by scientists for further studies on various immunological, microbiological and genetic aspects of *S. agalactiae* from Australia; 2. Provision of evidence on the genetic relatedness of the Queensland grouper *S. agalactiae* strains to other Australian fish, animal and human strains, enabling inference on the origin of the bacterium in Queensland grouper; 3. Provision of four infection models of *S. agalactiae* in fish, demonstrating the pathogenicity and transmission pathways of *S. agalactiae* in juvenile Queensland grouper; 4. Provision of evidence on the prevalence of *S. agalactiae* in a wide range of fish and crustacean species in the Cairns region, and coastal North Queensland.

Knowledge gained from this project will assist in developing biosecurity, health and disease management plans and programs relating to disease control for *S. agalactiae* in aquaculture facilities, commercial marine aquaria and live reef fish holding facilities. The project has resulted in outcomes that support consumer confidence in the safety of Australian seafood as the *S. agalactiae* isolates from fish are genetically different from those isolated from mammals and have never caused disease in terrestrial animals to date. Outcomes of this project have assisted in protecting recreational fisheries, through improved knowledge on prevalence and distribution of *S. agalactiae* within fish species of the Great Barrier Reef Marine Park in Northern Australia.

Queensland grouper, *Epinephelus lanceolatus*, is a protected species under the *Fisheries Regulation 2008*, under the Queensland Fisheries Act 1994. Between 2007 and 2012, 96 wild, adult, giant Queensland grouper were reported dead in Queensland, most occurring in urban coastal regions of Cairns, Port Douglas and Townsville (Bowater *et al*. 2012). Emerging infectious diseases pose a threat to ecosystem biodiversity, and there is increasing evidence supporting the link between human environmental disturbances and emerging infectious diseases of wildlife populations (Daszak *et al*. 2001).

From 2009 to 2012, Queensland Department of Agriculture, Fisheries and Forestry (QDAFF) veterinarians examined many adult (breeding size) Queensland grouper that washed up dead on public beaches or in creeks, and discovered several fish were infected with *Streptococcus agalactiae.* This bacterium had caused bacterial septicaemia and meningitis (brain infection) in numerous wild adult Queensland grouper (Bowater *et al*. 2012). In the same year, sick javelin grunter *Pomadasys kaaken,* catfish *Arius thalassinus,* and a diamond scale mullet *Liza vaigensis* in Trinity inlet, Cairns, were also found infected with *S. agalactiae*. This caused public concern over seafood safety, since grunter are an edible, recreational fish species. In 2009, a disease epizootic occurred at *Sea World,* with hundreds of different species of stingrays dying in '*Ray Reef*', a public display and touch tank pool. Veterinary investigations found the stingrays were infected with *S. agalactiae. Sea World* had recently introduced several wild stingrays into '*Ray Reef*' from North Queensland. The introduced stingrays were originally wild-caught, from the same geographical region of North Queensland, where many Queensland grouper had washed up dead on coastal beaches, and were infected with *S. agalactiae*.

S. agalactiae causes neonatal infection in humans and bacterial septicaemia in a wide range of terrestrial animals including horses, cows, dogs, rabbits, guinea pigs, lizards, dolphins, crocodiles, fish, stingrays and bullfrogs (Amborski *et al*. 1983; Domingo *et al*. 1997; Keefe 1997; Schuchat 1998; Hetzel *et al*. 2003; Zappulli *et al*. 2005; Evans, et al. 2006a, 2006b; Bishop *et al*. 2007; Filho *et al*. 2009; Harris *et al*. 2011; Bowater *et al*. 2012; Huang *et al*. 2013; Ren *et al*. 2013). This knowledge fuelled public concern regarding seafood safety. Multiple enquiries to QDAFF and Queensland Health arose from the general public, seafood outlets, recreational, commercial and traditional fishers, state and national media, the Great Barrier Reef Marine Park Authority (GBRMPA), and the Department of Environment and Resource Management (DERM), regarding the zoonotic potential of *S. agalactiae* via human contact with sick dying or dead grouper carcasses on beaches, or from handling or ingestion of other fish species or crustaceans (of unknown disease status) occurring in the vicinity of, or feeding on, grouper carcasses. There was a clear need for more research to answer these questions relating to public health and food safety. Information was also needed

to determine the prevalence and distribution of *S. agalactiae* in fish and crustacean populations in the Cairns region, and in other coastal regions of Queensland (many of which are part of the Great Barrier Reef Marine Park) as they are highly valued for recreational fishing, tourism, and general recreation.

Research was needed to determine the potential source of infection for Queensland grouper, since most Queensland grouper were dying in highly urbanized coastal areas, such as Trinity Inlet in Cairns. Trinity Inlet was therefore considered a potential site, for infection for Queensland grouper. Furthermore, overseas studies have showed the confirmed transmission of human pathogenic GBS to wild fish via contaminated sewage. Water discharged into Trinity Inlet includes treated sewer, storm water, and seepage from the town dump. Molecular studies were needed to elucidate the genetic relationships between the different isolates of *S. agalactiae* found in grouper, grunter, catfish, mullet, stingrays, and to compare with the human and other animal strains of *S. agalactiae,* to assist in determining the potential origin of the bacterium and therefore any potential hazard to human health. Possible routes of transmission of the bacterium in wild Queensland grouper were unknown at the time of wild grouper deaths, hence there was a need for research to develop infection models in Queensland grouper, to determine how the bacterium is spread in Queensland grouper, and to fulfill Koch's postulates ie. to provide a complete scientific basis for conclusion that *S. agalactiae* was the pathogen responsible for killing wild Queensland grouper.

In summary, this project achieved all of its objectives. The project demonstrated that *S. agalactiae* is highly pathogenic to juvenile Queensland grouper, *E. lanceolatus* and can be spread via infected water, infected food, by injection, or by cohabitation (of *S. agalactiae*-infected fish with non-infected fish). The project provided a collection of 96 different *S. agalactiae* strains at UQ and QDAFF, from a variety of fish, humans and land animals, to support ongoing scientific research.

This project produced rapid, reliable and accurate diagnostic tools, including PCR and an Immuno-histochemistry (IHC) method to specifically detect *S. agalactiae* in fish, thus increasing the States', Territories', and Australia's capability for disease testing, surveillance, monitoring and reporting. Histopathology with use of special stains also proved to be useful for providing additional diagnostic information.

In spite of targeted surveillance and sampling of over 1300 wild fish and crustaceans *S. agalactiae* was not detected in wild fish and crustaceans in Trinity inlet, Cairns, and other coastal regions of Queensland, between 2010 and 2012. This information, combined with molecular results from this project, gives assurance on seafood safety for fish and crustaceans in Trinity Inlet, Cairns, and coastal Queensland, and the results of this project informed Queensland's risk assessment process.

The project found no evidence to indicate that *S. agalactiae* is present in species commonly used as bait. Sampling of over 200 frozen baitfish and mullet failed to detect *S. agalactiae*. Frozen baitfish also poses a negligible-to-very low risk as a potential source of *S. agalactiae* infection to other marine fish and mammalian marine species that rely on frozen baitfish as a source of food, at commercial marine aquaria, wildlife parks or zoos.

Molecular studies showed the *S. agalactiae* isolates from Queensland grouper were genetically distinct from human, cow, dog, cat or crocodile strains. Molecular studies further showed the Queensland grouper *S. agalactiae* isolates were most closely related to the grunter, mullet, catfish and stingray strains isolated from north Queensland. All North Queensland fish isolates belonged to strain type ST-261, a strain type that has been identified from Nile tilapia and hybrid tilapia species from Indonesia, China, Brazil, Israel and other countries that culture tilapia. Importantly, this sequence type has not been associated with human infection anywhere in the world and is substantially different from all terrestrial isolates of GBS. It is possible that this strain type was imported to Australia with tilapia, or hybrid tilapia species, that were introduced into North Queensland by aquarists over 30 years ago (Arthington *et al*. 1984). This would be consistent with the very high degree of genetic similarity across the whole genome between the Australian fish isolates and tilapia isolates from the US and Israel.

Given the diverse aquatic host range of ST261 GBS, recommendations for further research include the development of biosecurity, health and disease management plans for aquaculture facilities to mitigate any potential transfer from infected wild fish to the aquaculture industry. This is particularly relevant to grouper culture in North Queensland which is on-going and well-established in Cairns, and where farm grow-out trials with several different grouper species are already underway. Other recommendations include the use of infection models for barramundi and other cultured fish species in Australia, to determine their susceptibility to *S. agalactiae.*

This project supports the need for improvements in current State Policy regarding translocation and health testing of wild-caught Australian and imported marine fish, since *S. agalactiae* causes large-scale disease epizootics in aquaculture farms in numerous overseas countries. *S. agalactiae* affects a broad range of marine and freshwater fish, stingrays, saltwater crocodiles and dolphins and therefore poses a threat to Australian native fish species through the inter-state and intra-state movement of subclinical carrier fish and elasmobranch species to the aquaculture industry, the marine aquarium trade, zoos and marine aquaria in Australia. Moreover, *S. agalactiae* may be present in subclinical carrier fish,

as demonstrated through the infection models developed in this project. This highlights the need for careful testing of fish prior to transfer.

KEYWORDS: Grouper, *Epinephelus lanceolatus*, *Streptococcus agalactiae*, diagnostic test, PCR, FIHC, histology, pathology, aquaculture, meningitis

Project No. 2011/048: Tactical Research Fund Aquatic Animal Health Subprogram: Determining the susceptibility of Australian species of prawns to infectious myonecrosis (PI: Nicholas Gudkovs and Mark Crane)

Executive Summary

What the report is about

Scientists at the CSIRO Australian Animal Health Laboratory (AAHL) in Geelong Victoria, with assistance from Indonesian scientists at the Centre for Brackishwater Aquaculture Development (CBAD), Jepara, Indonesia have demonstrated that two prawn species of commercial importance to Australia are susceptible to the exotic virus, infectious myonecrosis virus (IMNV). IMNV causes infectious myonecrosis, a disease of penaeid prawns which has been reported to occur in north-eastern Brazil, in the East Java Island, west Java, Sumatra, Bangka, west Borneo, south Sulawesi, Bali, Lombok and Sumbawa in South-East Asia and possibly in other South-East Asian countries (OIE, 2014).

IMNV is known to cause significant disease outbreaks, associated with mortalities, in farmed Pacific white shrimp (*Litopenaeus vannamei*) i.e. by natural infection. In addition, the Pacific blue shrimp (*Penaeus stylirostris*) and the black tiger shrimp (*P. monodon*) are susceptible to experimental infection with IMNV (OIE, 2014). Apart from these data there is no information on susceptibility of other prawn species.

In 2011-12, Australian commercial prawn production was valued at $265 million (ABARES, 2013) and, together, commercial and non-commercial prawns are a significant resource of which the farmed banana prawn (*Fenneropenaeus merguiensis*) and the wild brown tiger prawn (*Penaeus esculentus*) are important species. It is important to know whether prawn species such as these are susceptible to infection by IMNV to assist in determining the risk this exotic virus may pose should there be an incursion.

Thus, in collaboration with MCBAD Indonesia, infectivity trials were undertaken (1) at AAHL to determine the susceptibility of IMNV to the banana prawn and the brown tiger prawn, and (2) at MCBAD, using the natural host the Pacific white shrimp as positive control.

Background

The prawn fishery, including prawn aquaculture, is an important natural resource for Australia that is also the basis for a valuable export industry. Fortunately, the Australian prawn industry is free from many of the diseases that have devastated prawn aquaculture overseas at one time or another, e.g., the estimated impact of white spot disease (WSD), caused by white spot syndrome virus (WSV) in Asia alone after its emergence in 1992 until 2001, was US$4-6 billion (Lightner, 2003). In the Americas, the emergence of WSD in 1999 resulted in immediate losses estimated at US$1 billion to 2001.

Infectious myonecrosis (IMN) is a viral disease that has caused significant disease outbreaks and mortalities in farmed *Litopenaeus vannamei* (Pacific white shrimp) overseas (OIE, 2014). The economic loss in Brazil alone was estimated to be US$20 million in 2003 (Tang et al., 2005). While *L. vannamei* is considered the principal (natural) host, experimental infection of *Penaeus stylirostris* (Pacific blue shrimp) and *P. monodon* (black tiger shrimp) has been reported (Tang et al., 2005). The susceptibility of other shrimp/prawn species is unknown. Information on the susceptibility of prawn species important to Australia is lacking. Using the bio-secure containment facility provided by the CSIRO Australian Animal Health Laboratory, this study provides significant new information on the susceptibility of two commercially important species of Australian prawns, *F. merguiensis* (banana prawn) and *P. esculentus* (brown tiger prawn), following exposure to exotic IMNV. Such information is important to policy-makers, regulators and primary producers with respect to relevant biosecurity issues at all levels of government.

Aims/objectives

1. Import infectious myonecrosis virus (IMNV) of known pathogenicity
2. Determine the susceptibility of banana prawns to IMNV
3. Determine the susceptibility of brown tiger prawns to IMNV

Methodology

An infectious inoculum of IMNV was prepared at MCBAD, Jepara, Indonesia and transferred to CSIRO AAHL, Geelong. At Geelong, the inoculum was inoculated (i.m.) into banana prawns and brown tiger prawns which were subsequently monitored for signs of infection and disease. The prawns were sampled on a daily basis post-inoculation and tissues were processed for determining the presence of IMNV infection and disease using OIE methods. Following this first trial a second series of experiments were conducted to simulate natural modes of viral transmission and confirm susceptibility according to criteria developed by the OIE (OIE, 2014a).

Results/key findings

This investigation has demonstrated that the two commercial species of prawns of Australian origin, *Fenneropenaeus merguiensis* and *Penaeus*

esculentus, are susceptible to infection with the exotic virus IMNV. Such information is important to policy-makers, regulators and primary producers with respect to relevant biosecurity issues at all levels of government.

Implications for relevant stakeholders

While this project was limited to investigating the susceptibility of two important prawn species, the results suggest that the host range for IMNV is broader than previous data had indicated.

Recommendations

It is recommended that industry, regulators at all levels of government and the prawn health community in general note the results of this project and their implications with respect to biosecurity.

Keywords

Infectious myonecrosis (IMN); infectious myonecrosis virus (IMNV); banana prawn (*Fenneropenaeus merguiensis*); brown tiger prawn (*Penaeus esculentus*); *in vivo* infectivity trials; susceptibility; prawn virus

Project No. 2010/036: Aquatic Animal Health Subprogram: Improved fish health management for integrated aquaculture through Better Management Practices (BMP's) (PI: Tracey Bradley)

Objectives

1. Determine risk factors and prevalence of diseases resulting in reduced production on inland integrated aquaculture farms.
2. Develop fish health and biosecurity better management practices (BMPS) for inland integrated aquaculture industries
3. Examine the effect of different standard farmer treatments and frequencies on fish mortality, weight and health under controlled RAS conditions.

OUTCOMES ACHIEVED TO DATE

The project outputs have contributed to or will lead to the following outcomes:

1. Determine risk factors and prevalence of diseases resulting in reduced production on inland integrated aquaculture farms. The project has determined the major causes of mortality events from information provided by farmers and the prevalence and cause of the most important production - limiting factors.
2. Develop fish health and biosecurity better management practices (BMPs) for inland integrated aquaculture industries. The project developed BMPs for inland integrated aquaculture and other associated resources.
3. Examine the effect of different standard farmer treatments and frequencies on fish mortality, weight and health under controlled RAS conditions. The project compared three commonly used farm chemicals and determined the effects on fish in a controlled trial.

The health and production of Murray cod grown on integrated aquaculture farms in the eastern states of Australia was examined during this 2 year project. Eighty–five farm submissions were received with over 400 fish being dissected and examined for diseases. Most of these fish were presumed to be healthy, however there were some submissions from farms where fish were affected by disease or mortality events. The most common health problem seen under the microscope was problems with the gills (in 81% of submissions) and this was most commonly associated with the parasite *Chilodonella.* Farm data was collected, inputted into a computer and analysed from 6 of the project farms. The completeness of this data varied amongst the farms but included mortality rates, water quality parameters and treatments used. It was clear that many of the farms do not collect data of a sufficient quality to generate stock mortality rates and basic financial information for the farms. Most of the major mortality events reported during the project were believed to be caused by management rather than disease events. For example overdosing fish with chemicals during treatments and equipment failure when power was lost. Some disease events were investigated and the cause determined through the project.

A Better Management Practices (BMPs) manual was developed as part of the project. This large and detailed document covers a range of topics pertaining to Murray Cod health and production. There are a series of standard operating procedures and appendices that address topics such as how to submit fish to a laboratory and use of chemicals. Through the project other materials were developed including a video presentation on fish dissection and a poster on the appearance of common parasites.

A treatment trial was conducted on healthy fish with the most commonly used chemicals (formalin, hydrogen peroxide and salt). This trial aimed to determine if any of the chemicals at varying frequency caused problems with the growth, skin and gills of the fish. The trial found that formalin used every 3 days caused the highest mortality rate. Generally treating fish every 10 days did not affect mortality when compared with control fish.

It is apparent from the results of this project that in integrated Murray Cod systems infestations with *Chilodonella* is the greatest cause of mortality and reduced production. Further work into effective treatment regimes for this parasite should assist in addressing this problem. The data quality collected by farmers varied widely. Simple systems should be developed on farms where they don't exist already to ensure a better understanding of fish health and production. Although this industry is small, openness

and cooperation amongst farms could assist the growth of the industry as a whole.

Keywords

Murray Cod, *Maccullochella peelii*, aquaculture, farm surveillance, histopathology, *Chilodonella* spp.

Project No. 2013/004: Aquatic Animal Health Subprogram: The Neptune project – A comprehensive database of Australian aquatic animal pathogens and diseases (PI: Marissa McNamara)

Executive Summary

What the report is about

Aquatic animal health experts from the Queensland Museum (QM) have been completing work on a parasite and disease database called Neptune. Work on Neptune has taken place at QM in Brisbane since May 2013, resulting in the completion of major improvements to the database. These will allow Neptune to become Australia's most comprehensive online resource on aquatic animal health. Improvements were carried out in conjunction with IT staff from three different organisations: the Australian Biosecurity Intelligence Network (ABIN), which was based in Canberra until September 2013; Edith Cowan University (ECU), which is based in Perth; and Pixcelldata, which is based in Ireland and runs digital pathology software. The database was hosted by ABIN until September 2013, when ownership passed to ECU. This project was funded by the Fisheries Research and Development Corporation (FRDC) and the Australian Department of Agriculture (DA), with contributions from QM.

Background

Biosecurity is an increasingly important concern for Australia's aquatic animal resources. The global nature of trade and growing use of aquaculture make the introduction and spread of aquatic diseases a major issue in Australia and worldwide. The Neptune project was created in response to these concerns, and consists of a centralised web-based knowledge store with information on all aquatic animal diseases and parasites reported in Australia. Neptune was first launched in 2011, and three versions of the database were released throughout 2012. However, several key improvements were still required to achieve optimal functionality of the database. Neptune users have also had access to an online slide library that contains 180 key pathology slides of both exotic and endemic diseases. These slides were collected from pathologists around Australia, and were digitised in 2012 using Ultra-Resolution Digital Scanning. Like the Neptune database, the slide library required some upgrades to allow easier and more effective access.

Aims/objectives

The aim of the Neptune project is to enhance our understanding of aquatic diseases and parasites. Neptune does this by giving users access to detailed and easily searchable information on all Australian aquatic diseases and parasites, and by facilitating interactions between biosecurity officials from federal and state governments, as well as researchers and pathologists. Neptune was also created to reduce duplication of effort by eliminating the need for multiple, separate data sets. The objective of this project was to improve the existing version of Neptune so that it could better achieve its intended aim. In particular, eight aspects of the original database required work. These included: improving the user interface to make it more intuitive, completing data entry so that Neptune contained a comprehensive data set, integrating and updating the digital microscopy platform, and running a webinar series that included regular user training.

Methodology

The majority of work on this project was completed by the project manager, with assistance from supervisors at QM and DA. Technical work on the database and the slide library was carried out by IT staff at either ABIN, ECU or Pixcelldata. For example, the Neptune user interface was upgraded by incorporating user feedback to create a list of the most important requests, which were carried out by IT staff at ABIN. Data entry was completed by the project manager by a manual transfer of information from a source database. The digital microscopy platform was integrated more fully into Neptune by IT staff at ECU and staff from Pixcelldata. Experts from the latter company also improved the search page of the slide library. The project manager hosted free webinars and training sessions, first using Adobe Connect, which was provided by ABIN, and then using AnyMeeting. Each webinar contained a presentation from a member of the aquatic animal health community.

Results/key findings

The Neptune database and slide library have been significantly improved over the course of this project. Eight changes were made to the user interface, making it easier to search for and access information. Hundreds of host and parasite species have been added to the Neptune database, along with accompanying disease and publication information, resulting in a data set that now contains 88% of the AAAPD. The digital microscopy platform is now available within the Neptune database, and contains a more detailed search page, with additional columns showing key information on every slide. Fourteen webinars have been held, as well as six training sessions, often with excellent attendance from the aquatic animal health community. During this project Neptune was changed from an Oracle platform to open source software, which reduced the cost of hosting. Long term hosting arrangements have been explored, and discussions have been held with four potential hosting bodies.

Implications for relevant stakeholders

Neptune is likely to become an indispensable national resource for Australia's aquatic animal health professional community. The user community includes researchers, government officers, aquatic animal industries, private veterinarians and consultants. The facility will also be of immense value for student training. Neptune's impact will be sustained and its benefits will far exceed its relatively small establishment costs.

Recommendations

The completion of this project has led to five important recommendations for the future. Specifically, we recommend that the FRDC considers the following conditions: that funding recipients be required to include the associated costs of adding material to Neptune in their application; that Neptune be considered the storage space for supplementary data accompanying aquatic animal health publications; that principal investigators of projects anticipated to generate important histopathology slides be required to submit specimens to the online slide library; and that input from the user community should be collected on an ongoing basis to ensure that the database continues to meet user needs. We also recommend that a copyright agreement addressing the use of whole slide images of donated slides be finalised.

Keywords

Biosecurity, database, digital microscopy, aquatic diseases, aquatic parasites, user community, online resource

Progress Summaries for Active AAHS Projects

Project No. 2012/052: Aquatic Animal Health Subprogram: Development of a laboratory model for infectious challenge of Pacific Oysters (Crassostrea gigas) with ostreid herpesvirus type 1 (PI: Peter Kirkland)

Final Report in preparation.

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

For project progress and results see blog: www.oysterhealthsydney.org

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)

Abalone species (greenlip and blacklip) from Victoria have been challenged with five isolates of abalone herpesvirus (Vic-1, Tas-1, Tas-2, Tas-3 and Tas-4) and susceptibility to the viruses has been evaluated.

Similarly, greenlip and blacklip abalone originating from Tasmania (north) have been challenged with three viral isolates (AbHV Vic-1, Tas-1, and Tas-2) and susceptibility to the viruses has been evaluated.

In addition, greenlip abalone species from South Australia have been challenged with five AbHV isolates (Vic-1, Tas-1, Tas-2, Tas-3 and Tas-4), and susceptibility to these viral isolates has been evaluated.

Preliminary analysis indicates that the abalone species tested to date from Victoria, South Australia and Tasmania are susceptible to all viral isolates.

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)

Final Report in preparation.

Summary of Active Projects

Project No.	Project Title	Principal Investigator
2009/315	PD Program: Scholarship program for enhancing the skills of aquatic animal health professionals in Australia (*Associated species*: multi-species)	Jo-Anne Ruscoe FRDC Phone: 02 6285 0423 Email: jo-anne.ruscoe@frdc.com.au
2011/004	AAHS: Development of Improved Molecular Diagnostic Tests for *Perkinsus olseni* in Australian molluscs (*Associated species*: multi-species)	Mr Nick Gudkovs CSIRO AAHL Fish Diseases Laboratory Phone: 03 5227 5456 Email: nicholas.gudkovs@csiro.au
2012/001	AAHS: Strategic planning, project management and adoption (*Associated species*: multi-species)	Dr Mark Crane CSIRO AAHL Fish Diseases Laboratory Phone: 03 5227 5118 Email: mark.crane@csiro.au
2012/002	AAHS: Aquatic Animal Health Technical Forum (*Associated species*: multi-species)	Nette Williams CSIRO AAHL Fish Diseases Laboratory Phone: 03 5227 5442 Email: lynette.williams@csiro.au
2012/032	AAHS: Pacific oyster mortality syndrome (POMS) - risk mitigation, epidemiology and OsHV-1 biology (*Associated species*: Pacific oyster)	Prof Richard Whittington University of Sydney, Camden, NSW Phone: 02 9351 1619 Email: richardw@camden.usyd.edu.au
2012/050	AAHS: *Edwardsiella ictaluri* survey in wild catfish populations (*Associated species*: catfish spp.)	Prof. Alan Lymbery Murdoch University Phone: 08 9360 2729 Email: a.lymbery@murdoch.edu.au
2012/052	AAHS: Development of a laboratory model for infectious challenge of Pacific oysters (*Crassostrea gigas*) with ostreid herpesvirus type-1 (*Associated species*: Pacific oyster)	Dr Peter Kirkland EMAI NSW DPI Phone: 02 4640 6333 Email: peter.kirkland@dpi.nsw.gov.au
2013/001	AAHS: Determination of susceptibility of various abalone species and populations to the various known AbHV genotypes (*Associated species*: *Haliotis* spp.)	Dr Serge Corbeil CSIRO AAHL Fish Diseases Laboratory Phone: 03 5227 5254 Email: serge.corbeil@csiro.au
2013/002	AAHS: Identifying the cause of Oyster Oedema Disease (OOD) in pearl oysters (*Pinctada maxima*), and developing diagnostic tests for OOD (*Associated species*: *Pinctada maxima*)	Prof David Raftos Macquarie University Phone: 02 9850 8402 Email: draftos@rna.bio.mq.edu.au
2013/036	Tactical Research Fund: AAHS: Viral presence, prevalence and disease management in wild populations of the Australian Black Tiger prawn (*Penaeus monodon*) (*Associated species*: *Penaeus monodon*)	Dr Melony Sellars CSIRO Marine & Atmospheric Research Phone: 07 3833 5962 Email: melony.sellars@csiro.au
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

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