From the Subprogram Leader

2017 FRDC Aquatic Animal Health & Biosecurity Scientific Conference

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

It was great to catch up with regular Cairns conference participants and to see some new faces, particularly those from various industry sectors. The feedback form has been emailed to all registrants. We are interested in your views to improve the conference so please take the few minutes to complete and return the form to Joanne (joanne.slater@csiro.au) as soon as you can. Feedback during the conference on the quality of the presentations, including the keynote presentations (by Dr Grant D Stentiford (Centre for Environment, Fisheries and Aquaculture Science, Weymouth Laboratory, UK) and Dr Brian Jones (Ministry of Primary Industries, NZ)), was very positive which was good to hear.

Congratulations to the winners of the Student Awards for the top three student presentations:

Chloe English, The University of Queensland and CSIRO Agriculture & Food, Bribie Island Research Centre

David Vaughan, Marine Parasitology Laboratory, James Cook University

Giana Gomes, Marine Parasitology Laboratory, James Cook University

As usual, all the student presentations were of a high quality, making the job of selecting the top three very difficult.

STC/SAC Meetings

The AAHBS met in March and July. Items for discussion included:

- 2017 Scientific Conference
- Review of R&D Expressions of Interest
- DAWR Aquatic Animal Health Training Scheme
- AAHBS committee membership
Further information on these items is provided in the Announcements section of this newsletter.

**Health Subprogram Website**

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


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**

**AAHBS Scientific Advisory Committee**

FRDC has appointed two new members to the Aquatic Animal Health & Biosecurity Scientific Advisory Committee:

Dr Jeremy Carson, Principal Microbiologist, Aquatic Animal Health & Vaccines Centre of Excellence, Department of Primary Industries Parks Water & Environment, Tasmania

Dr Kate Hutson, Marine Parasitology Laboratory, Centre for Sustainable Fisheries and Aquaculture, College of Marine and Environmental Sciences, James Cook University, Townsville, Queensland

**DAWR Aquatic Animal Health Training Scheme**

The Aquatic Animal Health Training Scheme has received some further funding from DAWR. The 2017 round of funding is complete. There will be an announcement about the 2018 round 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**

<table>
<thead>
<tr>
<th>Project No. 2013/002: Identifying the cause of Oyster Oedema Disease (OOD) in pearl oysters (<em>Pinctada maxima</em>), and developing diagnostic tests for OOD (PI: David Raftos)</th>
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**Executive Summary**

The goal of this FRDC project was to investigate the cause of oyster oedema disease (OOD) in Australian pearl oysters so that diagnostic tests and management practices for the disease can be developed. OOD has been associated with mortalities in some pearl oyster farming areas. However, the cause of these mortalities has remained unknown, hampering efforts to study the disease and develop effective control strategies. The project described in this report was conducted by researchers from Macquarie University, Fisheries Western Australia and the New Zealand Ministry for Primary Industries (Manatū Ahu Matua), working in collaboration with the Australian Pearl Producers Association and the Australian pearlling industry.

We used next generation nucleotide sequencing and high throughput qPCR to compare OOD-affected oysters with healthy control oysters to identify any nucleotide (cDNA from total and mRNA) sequences in the OOD-affected oysters that might come from an infectious agent such as a virus, bacteria or parasite. Our logic was, if OOD is caused by an infectious agent, cDNA sequences from the pathogen should be far more abundant in OOD-affected oysters than in healthy controls. That genetic material would act as a fingerprint for the disease and may provide information about its cause. We found clear differences between the cDNA sequences present in oysters affected by OOD when compared to healthy controls. A number of sequences were strongly associated with OOD and the abundance of some of these sequences was correlated with increasing mortality. None of the differential sequences were closely related to any known infectious agents. However, the strong relationship between these sequences, OOD and mortality means that they may...
be very useful predictors of mortality. Their lack of resemblance to known infectious agents leaves open the possibility that OOD is not an infectious disease and may have some other cause.

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)

Executive Summary
This project was developed by The University of Sydney in collaboration with James Cook University and the Centre of Excellence for Biosecurity Risk Analysis. Prior research showed that despite the biosecurity measures in place since 2000, there have been several incidents of exotic pathogens from ornamental fish affecting wild and farmed fish populations in Australia. There is a need to acquire new knowledge to support policy reform as the ornamental fish industry advances and new pathogens emerge. During 2015, a cross sectional survey was completed for the nationally and internationally significant pathogens carried by ornamental fish entering Australia. The report contains the first comprehensive survey of the parasite assemblages affecting imported ornamental fish under quarantine. The research findings will be used to refine biosecurity policy to support safe trade and protect Australia’s natural resources and its fisheries and aquaculture industries, including the developing local ornamental fish breeding sector. The project was funded by the Australian Government Department of Agriculture and Water Resources, through the Fisheries Research and Development Corporation.

Background
Imported ornamental fish are known to be subclinical carriers of exotic pathogens of biosecurity concern. Nearly 18 million ornamental fish are imported annually under a policy based on an Import Risk Analysis published in 1999. The potential for these aquatic animals to be released into the environment presents additional risk to Australia’s ecosystems. Determination of the range of infectious agents carried by imported fish is required so that appropriate regulations can be put in place to manage the risk. In recent years, there has been particular interest in the risk associated with imported ornamental fish infected with iridoviruses, specifically those belonging to the genus Megalocytivirus.

This project was developed from the need to acquire new knowledge to support policy reform as the ornamental fish industry advances and new pathogens emerge. There is little information available on viral and bacterial disease agents carried by imported ornamental fish. Additionally, there is a paucity of information available on parasites of imported fish and their potential risk to Australian industries and ecosystems. There is a need to determine whether pathogens of biosecurity significance associated with ornamental fish are entering Australia despite the import conditions.

Objectives
1. Determine if pathogens of potential biosecurity concern on the national list are entering QAPs in Australia through the trade in ornamental fish
2. Determine if current import conditions for goldfish requiring freedom from specific pathogens are being met
3. Document parasites of potential biosecurity concern that are associated with imported ornamental fish
4. Develop efficient pooled sample strategies for testing imported fish

Methodology
A repeated cross sectional survey was conducted to examine imported ornamental fish under quarantine for the presence of nationally listed aquatic pathogens that are associated with at least one ornamental fish host. All fish collected were considered pre-import and under quarantine at the time of testing. We assessed whether or not the existing import conditions for goldfish requiring sourcing from populations free from specific diseases were being met, and identified parasites of potential biosecurity significance. To support surveillance testing for freedom from infection with megalocytiviruses (MCV), diagnostic sensitivity was examined using clinically-relevant fish tissue homogenates.

Results
Australia’s biosecurity policy framework for the importation of ornamental fish was being met for the OIE-listed pathogens koi herpesvirus (KHV), spring viraemia of carp virus (SVCV) and viral haemorrhagic septicaemia virus (VHS) as there was no evidence of subclinically infected fish being imported to Australia. Similarly, there was no evidence of the bacteria, Edwardsiella ictaluri and Aeromonas salmonicida which are listed on national list of aquatic pathogens.

Australia’s biosecurity policy framework for the importation of ornamental fish was not being met for the nationally listed MCV and Nervous necrosis virus (NNV). MCV was detected in more than half of the populations (13%) of marine ornamental fish which were all infected with the virus. NNV was detected in 3 populations (13%) of marine ornamental fish which were all exported from Indonesia.

No parasites (e.g. Gyrodactylus salaris and Myxobolus cerebralis) were detected that are listed in the list of aquatic pathogens.
on Australia’s National List of Reportable Diseases of Aquatic Animals. Current import conditions for Goldfish, which had been treated with an effective parasiticide to eliminate infestation by the gill flukes Dactylogyrus vastator and D. extensus are not being met. Dactylogyrus vastator was detected in three populations of Goldfish received from the same exporter in Thailand on two separate occasions. Further, six other dactylogyrid monogenean species (but not D. extensus) and one Gyrodactylus were detected in imported Goldfish.

Protozoan or metazoaan parasites were detected in all populations of fish (33/33) examined. We found that significant parasitic disease agents including ciliates (e.g. Trichodina), myxozoans (e.g. Ceratomyxa, Kudoa and Myxobolus spp.), helminths (primarily larval trematode metacercariae of the family Heterophyidae) and a crustacean (e.g. Argulus japonicus, a known exotic parasite) infected freshwater and marine imported ornamental fish.

The diagnostic sensitivity of the qPCR assay to detect MCV was substantially reduced when testing fish tissues in pools of five or 10 compared to individual testing. The sensitivity for a single positive sample in a pool of five was 68% (95% CI, 55-80%). The sensitivity was further reduced for one positive in a pool of 10, being equal to 63% (95% CI, 51-74). In addition to prevalence of infection in the source population, pooled sensitivity was strongly influenced by the pathobiology of the infection. For example, the nominal surveillance sensitivity was achieved by testing 40 pools of five (200 fish, 40 tests) or 26 pools of ten (260 fish, 26 tests) instead of 150 tests on individuals for a population of Dwarf gourami (Trichogaster lalius) with subclinical infection. However, this surveillance sensitivity (95% confident of detecting 2% prevalence assuming a perfect test) could not be achieved when using pooled samples for a population of Kissing gourami (H. temminckii) also with subclinical infection.

Implications for relevant stakeholders
All of the freshwater species that were positive for MCV fall under the revised biosecurity policy framework requiring batch testing for freedom from infection. However, none of the marine fish species that were MCV-positive require batch testing under existing policy.

The risk imported ornamental fish present to Australian aquatic animal industries and natural resources is high with respect to parasitic agents. The recovery from Goldfish of numerous Dactylogyrus species indicates the pre-export health requirement for a parasiticide treatment was not effective at eliminating monogeneans. The project results show that freshwater and marine ornamental fish arrive with a diversity of external and internal parasite fauna. Host specificity is a significant component of parasite ecology and determining if the parasite can become established in a new environment and on novel fish hosts. There was a reduction in diagnostic sensitivity for MCV when fish tissue homogenates were pooled for testing. However, pooling strategies were effective for maintaining surveillance sensitivity when the prevalence of MCV infection exceeded 10%. In some cases the lower pooled diagnostic sensitivity was compatible with the desired surveillance sensitivity if the sample size was increased above 150 yet fewer assays were required. This study demonstrated that the pathobiology of MCV infection influenced pooled diagnostic sensitivity, thus the applicability of pooled testing must be considered on a case-by-case basis.

Recommendations
1. Revision of biosecurity policy to prevent incursions of Megalocytivirus virus from marine ornamental fish
2. Revision of biosecurity policy to require the application of an effective parasite treatment of all ornamental fish being exported to Australia.
3. Revision of biosecurity policy to require health certification to be free of the parasitic agents, Argulus japonicus and Lernaea cyprinacea.
4. From the project findings, visual inspection of consignments was not effective at detecting misidentified fish species and did not prevent importation of live parasite fauna. Revision of biosecurity policy to include import conditions that state a maximum number of fish that can be transported in a bag based on fish species and size.
5. Pooled testing cannot be applied empirically for surveillance using PCR assays. Factors additional to prevalence and viral load affect the pooled diagnostic sensitivity and need to be better understood.

Keywords
disease freedom, biosecurity, exotic pathogens, infectious spleen, kidney and necrosis virus, Megalocytivirus, nervous necrosis virus, Koi herpesvirus, spring viraemia of carp virus, viral haemorrhagic septicemia virus, Aeromonas salmonicida, Edwardsiella ictaluri, Dactylogyrus vastator, Argulus japonicus, Gyrodactylus, Myxobolus, Centrocestus, ornamental fish, Goldfish, Carassius auratus, Blue gourami, Trichopodus trichopterus, Dwarf gourami, Trichogaster lalius, Rosy barb, Pethia concinonius, Guppy, Banggai cardinalfish, Pterapogon kauderni, Clownfish, Amphiprion spp.

Progress Summaries for Active Projects

Project No. 2014/002: Aquatic Animal Health Subprogram: Development of stable positive control material and development of internal controls for molecular tests for detection of important endemic and exotic pathogens (PI: Nick Moody)
Positive controls for molecular assays have been designed for 32 assays (22 real-time PCR and 10 conventional PCR assays), detecting 18 pathogens of finfish (*Megalocytivirus*, infectious salmon anaemia virus, nervous necrosis virus, viral haemorrhagic septicemia virus, spring viraemia of carp virus, Tasmanian salmon reovirus, pilchuck orthomyxovirus, epizootic haematopoietic necrosis virus and Tasmanian Aquabirnavirus) crustaceans (white spot syndrome virus, Taura syndrome virus, yellowhead virus, AHPND PirA) and molluscs (*Ostreid herpesvirus, Abalone herpesvirus, Bonamia spp., Perkinsus spp.*). Evaluation of T4 and QBeta bacteriophages for use as heterologous internal positive controls has occurred and implementation is currently underway.

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

*Bonamia ostreae* and *Bonamia exitiosa* are significant pathogens of oysters that cause high mortality rates and substantial economic losses to the oyster farming industry globally. Infection by a *Bonamia sp.* was responsible for the devastation of experimental aquaculture of the Native Oyster (*Ostrea angasi*) and adjacent wild beds in Victoria in the early 1990s and recent monitoring of apparently healthy stock on Victorian aquaculture sites has determined that prevalence of this *Bonamia sp.* is high and, once again, is causing clinical disease. A tank and field trial were undertaken in the first year of a two-year project to examine putative risk factors for clinical expression of Bonamia infection, with the aim of elucidating the best farming practices for mitigation of clinical *Bonamia*.

The tank trial utilised a 2^3 (i.e. 2x2x2) factorial design nested in a randomized control block design (RCBD). Live oysters of approximately the same size were sourced from a known infected site, with a negative control group drawn from a presumed negative site with the unit of interest being the individual oyster (*Ostrea angasi*). Ten oysters were assigned to one of each possible combination of three risk factors, specifically temperature (warm versus ambient), tumbling (none versus one minute oscillation every two days) and nutrition (starvation versus adequate nutrition). Tissues were collected, where available, for qPCR and histopathological examination to confirm the presence of *Bonamia*.

At the end of the 8-week study period, all oysters in the negative control tank (n=10) were alive and negative for *Bonamia* by qPCR, indicating that the tank was not directly responsible for oysters deaths during the trial. Overall, 34.4% (n=86/250) of oysters died, with 79.4% of these with detectable levels of *Bonamia*. In contrast, 48.2% of live oysters had detectable levels of *Bonamia*, with a significant difference in the mean C_7_ value between oysters that were dead or alive at trial end (p<0.01). The association between each risk factor and oyster death was complex, but an oyster was at the greatest risk of death when it was held at high temperature, with increased motion and was starved.

The first year of the field trial was undertaken at a known infected and non-infected site. All oysters (n=4,267) were double graded, sorted and placed into treatment cages using a 2^3 factorial design, evaluating all possible combinations of oyster size (small versus large), depth in the water (2 versus 7 metres) and within-cage density (30% versus 60% filled) with each combination replicated between 3 and 7 times. After approximately 5 months, oysters were counted and sorted into dead and alive. Six control cages, assembled to mimic “normal” risk factor levels at the infected site (i.e. mixed size, 50% cage density and a water depth of 5 metres) were also submerged. Only oyster size was significantly associated with oyster death, with small oysters observed with a 9.9% higher mortality rate relative to large oysters (p<0.01). Within-cage density and depth in the water were not associated with oyster death.

Future work will include evaluation of the validity of the qPCR as a test for *Bonamia* against the current gold standard of histopathology. The association between the risk factors and the time to death of individual oysters from trial start date will be assessed using survival analysis. In the second year of the trial, the unexpected association of oyster size with death at the individual oyster level will be analysed in more detail in an additional tank trial, as well as evaluating oysters sourced from both subclinical and clinical sites.

Similarly, a field trial is underway in South Australia to assess survival and seasonal progression of *Bonamia sp.* infection of one *O. angasi* cohort in oyster farms. Spat were deployed at Franklin Harbor (Cowell), Coffin bay (2 sites) and Streaky Bay in January-February 2017. Oysters will be sampled seasonally beginning in May 2017.

A cohabitation tank trial has also been conducted in South Australia to assess exposure time needed to infect oysters, changes in prevalence of *Bonamia sp.* infection, and mortality. For the four experimentally *Bonamia*-exposed groups, continuously aerated 150L tanks contained 10 oysters from a Coffin Bay farm with a prevalence of 75% and 300 SARDI spat shown to be uninfected by testing using three methods. Exposure began on 26 March 2017. Spat were monitored every third day, provided a 100% water exchange and fed 1.25 L of a mixed culture of *Chaetoceros muelleri, Skeletonema costatum* and *Pavlova lutheri* per tank. Water quality was monitored for temperature, DO and nitrogen compounds. Each tank was also assessed for mortalities. Thirty-eight spat per tank including any mortalities containing tissue were sampled at 10 days, 21 days and 40 days and samples for *Bonamia* testing by tissue smear, histology and qPCR. Sampled spat were replaced with spare animals. The experiment ended on 5 May 2017.

The uninfected control groups consisted of continuously aerated 150L tanks containing 300...
SARDI spat shown to be uninfected by testing using three methods. At the end of the trial survival was 99.7% on controls and 23.6% in exposed animals.

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

To date the project has led to the development of methods for the production and storage of primary and secondary molluscan cell cultures. The exclusion of organisms such as bacteria, fungi, protists and algae from the culture has proved challenging. The methods for eliminating these organisms will be discussed. Cell cultures derived from Pacific oysters, native oysters and abalone have been maintained for up to 10 passages. Media conditions for cell survival and growth (where possible) have been empirically determined.

Ostreid herpesvirus-1 has been successfully amplified in Pacific oyster heart cells *in vitro*. This result gives encouragement that the development of molluscan cell culture systems will enhance the ability to isolate and grow mollusc pathogens (particularly viruses, protozoa and intracellular bacteria), lead to further diagnostic tools, and a better understanding of mollusc pathogens. This should lead to improved knowledge about significant disease agents leading to enhanced preparedness for disease diagnosis, management and control. It is anticipated that cell culture systems would become part of a robust methodology for investigation of mollusc disease outbreaks.

**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)**

No further progress has been made with this project due to the involvement of the project team in the response to the white spot disease outbreak in SE Queensland.

**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: Perkinus olseni in abalone - development of fit-for-purpose tools to support its management (PI: Cecil Dang)**

This is a new two-year project that commenced on 1 January 2017.

**Objectives**

Develop and evaluate optimised diagnostic capabilities for Australian *Perkinus* 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 that commenced on 1 December 2016.

**Objectives**

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


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.

Little progress has been made due to the involvement of the project team in the response to the white spot disease outbreak in SE Queensland.
### Summary of Active Projects

<table>
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<tr>
<th>Project No.</th>
<th>Project Title</th>
<th>Principal Investigator</th>
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| 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 Read  
Elizabeth Macarthur Agriculture Institute  
Phone: 02 4640 6332  
Email: andrew.j.read@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  
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Phone: 03 5227 5118  
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| 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  
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