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Health Highlights

Aquatic Animal Health Subprogram Newsletter
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In this issue

From the Subprogram Leader

2013 FRDC Australasian Scientific Conference on Aquatic Animal Health

The Proceedings (on CD-Rom) for the 2013 FRDC Australasian Scientific Conference on Aquatic Animal Health held in Cairns at The Pullman Reef Hotel, on 8-12 July 2013 have been distributed to all delegates. If you have not received your copy please contact Joanne Slater, AAHS coordinator (joanne.slater@csiro.au).

Here are a few statistics from the conference. There were 125 registrations with, as expected, the vast majority from Australia:

Country	No. delegates
Australia	105
New Zealand	7
Singapore	3
Hong Kong	2
Brunei	2
Taiwan	1
West Indies	1
Canada	1
India	1
Denmark	1
Malaysia	1
Total	**125**

We received 51 returns of the conference feedback form. Thank you to all those who responded. These will help us with the planning for the 2015 Conference. A summary of the responses are shown in the following table. Most of the feedback was positive. The location/venue/date/frequency received strong support. With respect to the format, there was some support for posters especially if the conference continues to grow. There was also support for longer breaks (30 minutes for coffee/tea breaks and 1 hour for lunch), a significant number of responders suggested that we do not need a conference bag, and there were mixed views concerning the on-line registration.

2013 Australasian Scientific Conference on Aquatic Animal Health, Pullman Reef Hotel, Cairns, 8-12 July 2013: Conference dinner.

ITEM	Excellent	Good	OK	Poor
Location (Cairns)	38	12	1	
Venue (Reef Hotel)	44	7		
Dates (July)	33	13	3	1
Frequency (biennial)	34	10	1	
Format	33	13		
Room layout	24	17	7	
Program session topics	23	13		
Length (4 days)	30	15	2	
Keynote speaker	18	4	1	
Abstract format	35	12	2	
Abstract book	36	11	1	
Conference bag	16	13	13	2
Food (breaks/lunches)	31	14	6	
Happy hours	35	10		1
Conference dinner	35	8	1	
On-line registration	30	9	6	1
Registration fee value	32	12	2	

Finally, congratulations again to the winners of the student awards:

M. Stride, U. Tasmania (supervisor: B. Nowak)
A. Brazenor, James Cook U. (supervisor: K. Hutson)
M. Blumhardt, U. Tasmania (supervisor: B. Nowak)

STC/SAC Meetings

The AAHS met in July 2013 to consider the 2014 Annual Competitive Round Expressions of Interest (EoIs) and Tactical Research Fund (TRF) proposals, and in September 2013 to review the final EoIs and the aquatic animal health training program applications. Recommendations were submitted to FRDC and applicants will have received notification from FRDC concerning their EoIs and applications.

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

Aquatic Animal Health Technical Forum

You should all have received, by email, a notice concerning the 4th Aquatic Animal Health Technical Workshop to be held Wednesday 19 to Friday 21 February 2014 and hosted by Sydney University, Camden Campus.

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.

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 bi-annual edition: project updates, milestone reports, final reports, research papers, project communication and extension outputs, info sheets, and letters to the editor. Announcements of conferences, workshops, meetings, etc are also welcome.

Please forward contributions for the next edition of *Health Highlights* (August 2014) to Joanne Slater before 15 July 2014.

Mailing list

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

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Completed AAHS Project Summaries

Project No. 2008/041: Aquatic Animal Health Subprogram: tools for investigation of the nodavirus carrier state in marine, euryhaline and freshwater fish and control of NNV through integrated management (PI: Richard Whittington)

OBJECTIVES:

1. To develop and validate a real-time PCR method for the detection and identification of betanodaviruses
2. To develop and evaluate the applicability of serological tests for detection and identification of betanodaviruses
3. To transfer developed technology to Australian diagnostic laboratories
4. To provide a basis for development of a national proficiency testing scheme for the detection and identification of betanodaviruses
5. To provide recommendations for improved biosecurity protocols in relation to nodavirus infection and fish translocation

NON TECHNICAL SUMMARY

OUTCOMES ACHIEVED TO DATE

The overall outcome of this project has been the successful development and transfer of technology to accurately identify NNV infection in barramundi and Australian bass, and to accurately identify the main means of infection of hatcheries, leading to reduced impact of disease in hatcheries, and lower risk of spread of disease to natural ecosystems, recreational and commercial fisheries. In addition, awareness of NNV has been enhanced in industry and also in government biosecurity agencies, and management approaches can now be aligned more strongly with scientific evidence. Thus Australia's capacity to prevent and control NNV is greatly improved.

This project was developed following national consultation and a scientific workshop convened by FRDC to ensure that research undertaken addressed the highest priorities of industry, governments and other stakeholders.

As a result of this project a new sensitive and specific test (qPCR) to detect NNV was successfully developed and transferred to five aquatic animal health diagnostic laboratories in Australia. The new test was established and run according to specifications and each laboratory has since participated in the Australian National Quality Assurance Program (ANQAP) for NNV with good results. The program is underpinned by an Australian and New Zealand Standard Diagnostic Test Procedure which was revised by researchers based on the findings of this project. The new qPCR test was shown to detect all of the known types of NNV and so can be used throughout Australia.

A disadvantage of qPCR test is that it requires samples of brain/eye from dead fish. However, another type of test was also developed which uses blood samples from live fish. This ELISA test measures the level of antibody against NNV. It was applied successfully in barramundi and Australian bass and individual fish were found that had both antibody in their blood and NNV present in their brain. Over time (months to years), the proportion of fish with antibodies in blood and virus in nervous tissue decreased, as did the levels of both antibodies and virus.

Using these new tests it was found that brood stock did not appear to be a source of infection for batches of larvae, even though some brood stock had been exposed to NNV. In fact there was no apparent relationship between infected batches of larvae and the test results of brood stock. Four batches of NNV-infected larval barramundi were found to have become infected during larval rearing or during grow out.

Based on these findings recommendations were developed to prevent outbreaks of NNV in hatcheries and to prevent spread of NNV through aquaculture. Emphasis should be placed on protecting larval fish from exposure to NNV rather than brood stock testing. The most likely source of infection is the water supply to the hatchery. Larval fish should be provided with UV-treated, filtered water to reduce the likelihood of exposure to NNV for as long as possible before transfer of fish to grow out. Hatcheries should adopt all-in-all-out batch culture and should develop individualised biosecurity protocols to reduce the risk of transmission of NNV associated with introduced brood stock, live feed, personnel movements, visitors and other factors.

Regardless of whether fish are required for grow out or for restocking, protocols to prevent translocation of NNV with movements of live cultured fish should recognise that subclinical infections may occur in young fish and that there is a high risk that the survivors of clinical NNV outbreaks will be sub-clinical carriers. Objective laboratory tests must be used to screen batches of fish for NNV infection. Histopathology should not be used for certification of freedom from infection.

Recommendations were made for further development to determine whether the ELISA can be used as a test for exposure to NNV infection at population level. This is important with respect to restocking waters for recreational fishing, as it is important to conduct surveys of wild fish to confirm that NNV is not already present. The biological significance of qPCR test positive fish that contain very low levels of virus also requires further investigation to avoid unnecessary regulatory action such as destruction of batches of fish or quarantine. The effect of pooling tissues on test sensitivity in small fish also needs to be examined.

Other recommendations include developing better understanding of the distribution and abundance of wild fish with NNV infection, the behaviour of NNV in marine finfish, developing methods for disinfecting seawater to remove viable NNV, and determining the period of susceptibility of young fish to NNV.

In summary, this project achieved the successful development and transfer of new technology to accurately identify NNV infection in barramundi and Australian bass, and to accurately identify the main means of infection of fish in hatcheries. Thus Australia's capacity to prevent and control NNV is greatly improved.

KEYWORDS: nervous necrosis virus, disease, barramundi, sea bass

Project No. 2009/044: Aquatic Animal Health Subprogram: surveys of ornamental fish for pathogens of quarantine significance (PI: Joy Becker)

OBJECTIVES:

1. To determine whether DGIV is entering Australia despite quarantine practices

2. To determine whether CyHV2 is entering Australia despite quarantine practices
3. To determine whether DGIV is already established in farmed gourami in Australia
4. To determine whether CyHV2 is already established in farmed goldfish in Australia
5. To determine whether DGIV is already established in wild gourami in Australia
6. To determine whether CyHV2 is already established in wild goldfish in Australia
7. To determine whether domestic goldfish free of CyHV2 succumb to disease when cohabitated with imported goldfish carrying CyHV2
8. To extend the findings of this study to the ornamental fish sector in Australia and provide information for use by DAFF

NON TECHNICAL SUMMARY

OUTCOMES ACHIEVED TO DATE

This project will assist in ensuring the sustainability and profitability of the aquatic industry and the health of natural resources by providing industry and governments with knowledge of the entry of DGIV and CyHV2 in Australia. The overall outcome of this project was successful through the provision of scientific evidence of the incursions of exotic viruses from ornamental fish in Australia. This will assist in the design of improved quarantine policy for live imported ornamental fish, disease prevention strategies, improved policy regarding domestic aquaculture production and facilitate the certification of farms as being free of these viruses. Also, outcomes of this project will help protect recreational fisheries through improved conservation management of threatened freshwater fish and help promote aquaculture of native fish.

Previous R&D funded by FRDC developed molecular diagnostic tests for dwarf gourami iridovirus (DGIV) and cyprinid herpesvirus 2 (CyHV2). These viruses were considered exotic to Australia, although disease outbreaks were reported from domestic farms and the viruses were readily detected at retail outlets selling ornamental fish. This project was developed to use the validated PCR assays to determine whether DGIV and CyHV2 were in fact entering Australia despite quarantine practices and to further determine if these viruses were established in domestic populations of fish.

This project is of national significance. It was developed in close consultation with Commonwealth Department of Agriculture, Fisheries and Forestry (DAFF), the Murray Darling Basin Authority and in consultation with FRDC.

As a result of this project, DGIV was consistently found in several species of gourami imported from six different countries. The virus was also found in stocks of gourami from wholesale premises, at retail outlets and one domestic fish farm. The findings indicate that the health certification at exporting countries was insufficient to detect and prevent fish with DGIV being exported to Australia. Once fish arrive in Australia, quarantine and visual inspection were insufficient to identify fish with DGIV infections. Finally, DGIV was found in a group of platy at a domestic ornamental fish farm. At the time of collection, there was no reported outbreak and the infection was presumably sub-clinical. The detection of DGIV at a domestic farm is concerning due to the risk of spreading (and potentially amplifying) the virus through the live fish trade to other farms and retail outlets and the risk of releasing the virus into natural waterways through contaminated effluent and other waste. The lack of a plan to deal with such an incursion of an exotic pathogen is concerning. These pathways increase the opportunity for DGIV to become established in wild populations, which would impact on recreational fisheries, biodiversity and aquaculture development.

Similarly, CyHV2 was found at wholesaler premises, farms and notably in several populations of wild goldfish in the ACT and Victoria. The findings of the project demonstrated that CyHV2 was already established in Australia and were used to inform quarantine policy to revoke the requirement for goldfish exported to Australia to be certified free of CyHV2. The findings provided clear evidence that an aquatic pathogen from ornamental fish with quarantine significance can become established in farmed and wild populations. This is of particular significance to Australia as there are many endemic and ecologically sensitive populations of fish that may be severely affected by exotic pathogens. The incursion of CyHV2 in Australia should be considered a case study to inform pathway analysis for pathogen establishment.

The findings of this project supports revision of policy to prevent incursion of exotic pathogens from imported ornamental fish and previous recommendations that laboratory testing should be carried out as an effective way of detecting exotic pathogens in imported ornamental fish. Additionally, policy revision and new policy development should address operational procedures to minimize biosecurity risks if notifiable agents are found, mandatory reporting of mortalities during quarantine, diagnostic testing of rejected consignments and increased tracking and traceability of ornamental fish. Recommendations were made to complete a risk analysis of the aquarium trade as a pathway for release of DGIV in Australia. This is important given the wide host range for DGIV and the high prevalence of the virus in retail outlets. Experimental studies are also needed to determine the range of native fish species that are susceptible to DGIV with species closely related to Murray cod given a high priority.

Recommendations for further research on the epidemiology of DGIV for freshwater fish living in Australia, sociology research to investigate the role humans play in the dispersal of pet fish in the wild and help protect future aquaculture opportunity.

In summary, this project achieved its objectives in provision of scientific data to support the revision of national policy to prevent the incursions of exotic viruses from the ornamental fish trade. This will help protect Australia's recreational fisheries through improved conservation management of freshwater fish species and help promote aquaculture.

KEYWORDS: dwarf gourami iridovirus, cyprinid herpesvirus 2, ornamental fish, biosecurity

Project No. 2011/003: Aquatic Animal Health Subprogram: Investigations into the genetic basis of resistance to infection of abalone by the abalone herpes-like virus (PI Serge Corbeil)

OBJECTIVES:

To determine whether there is genetic variation in susceptibility to abalone viral ganglioneuritis in abalone family lines

NON TECHNICAL SUMMARY

OUTCOMES ACHIEVED TO DATE

While a genetic basis to resistance has been demonstrated, no commercial selection for resistance has commenced due to outstanding questions on the cost-benefit of selecting for this trait and on the long-term nature of the challenge model, i.e. its ability to select for resistance across consecutive generations in a program of genetic improvement. Further refinement (e.g. better estimation of infectious viral dose) and a better understanding of the challenge system may be necessary for adaptation to further studies on genetic resistance.

Previous research on abalone herpes virus (AbHV) was successful in developing an experimental infection model at CSIRO Australian Animal Health Laboratory for studying pathogenesis in the target species. This model was used to determine whether or not there is a genetic basis for resistance to infection by AbHV and/or abalone viral ganglioneuritis (AVG) - the disease it causes. The study was undertaken using a greenlip (*Haliotis laevigata*) population obtained from a commercial farm (Great Southern Waters Pty Ltd (GSW)). The parents of this population were from known sources (both wild and farmed) and the animals challenged had full records of parentage, making this a very good study population.

The results demonstrate a genetic basis for resistance to AVG for both challenge modes - by immersion and by injection. Data are suggestive of polygenic genetic control, meaning susceptibility is likely to be under the control of a large number of different genes of small effect. Resistance appears moderately heritable (h^2= 0.24).

There was no evidence of differing resistance from different wild greenlip populations. A total of 81 founder animals (founders are the base parents in a breeding population) were represented in the study population and they were sourced from both wild populations and from first generation farm populations. Wild populations, that were represented, included those sourced from Port Philip Bay, Western Port Bay, and locations along the west Victorian coast. Farm populations represented were from Tasmania, Kangaroo Island and Victoria.

In theory, resistance to AbHV should be a trait that can be changed through applied selective breeding. Based on data from this study, cumulative gains in the order of 10% per generation are predicted when selecting for resistance to AbHV alone. However, more knowledge is required before an applied selective breeding program for this trait should commence.

The economic importance and practical significance of these estimated genetic gains are unclear. These gains are based on a laboratory challenge model and it is unknown how many generations of selection would be required to provide protection against catastrophic commercial loss.

If AVG-resistance breeding was to move to an operational phase, there are potentially large improvements that can be made to the rate of genetic gain by refining the challenge system and gaining a better understanding of factors that may influence an animal's response to infection, such as husbandry and seasonal effects.

Furthermore, if there should be a wild population resistant to AbHV/AVG then it would be much easier to source abalone from that population than attempting to incrementally change existing captive populations into something that natural selection has already produced.

KEYWORDS: Abalone viral ganglioneuritis, AVG, Abalone Herpesvirus, AbHV, disease resistance, *Haliotis laevigata*.

Project No. 2011/053: Aquatic Animal Health Subprogram: Pacific oyster mortality syndrome (POMS) – understanding biotic and abiotic environmental and husbandry effects to reduce economic losses (PI: Richard Whittington)

OBJECTIVES:

To correlate biotic and abiotic environmental factors with POMS occurrence in selected oyster populations

NON TECHNICAL SUMMARY

OUTCOMES ACHIEVED TO DATE

This project will assist in ensuring the sustainability and profitability of the aquaculture industry and the health of natural resources by providing new data

on the epidemiology of Pacific oyster mortality syndrome (POMS). Practical management measures based on increasing growing height to reduce adult oyster losses due to POMS are possible but require confirmation through a second season of study because the summer of 2011-2012 was unusually mild and wet, and it is possible that POMS disease expression will be different in a typical hot dry summer. There is a broader responsibility towards the Australian community to ensure the sustainability of Australian aquatic natural resources. This was achieved through the promotion of information about oyster health in general and POMS in particular. This project assisted industry to strengthen biosecurity practices: there were proposals from specific sectors of the oyster industry for voluntary restrictions on oyster movements between estuaries, and objective laboratory testing of oysters for specific pathogens prior to movement to decrease the risk of disease spread. This will protect commercial aquaculture. The need for genetic selection to improve oyster health was addressed by flow of data from this project to the POMS genetic selection program, whereby optimal experimental design for field trials was confirmed to enable identification of a genetic component in resistance. Communication of the most recent findings of the project, namely confirmation that growing height could beneficially affect survival of adult oysters in the face of an outbreak, will be ongoing, including through a fully illustrated website that was established during the project and has proven to be very popular with industry (www.oysterhealthsydney.org).

There is a disturbing pattern of emerging diseases in commercial molluscs nationally that has required a succession of government/industry responses. Pacific oyster mortality syndrome (POMS), which appeared in NSW in 2010, is an internationally significant disease that has severely impacted Pacific oyster production in Europe and New Zealand. This project was based on the premise that the oyster industry will need to learn to live with POMS by managing husbandry.

We investigated the epidemiology of POMS during its second summer in Australia to identify factors which may be exploited to reduce the impact of this viral infection. We describe the outbreak of POMS in Woolooware Bay near Sydney NSW, which started in November 2011 and in which virus associated mortalities were observed until late April 2012. The distribution of disease was non uniform, clustered, highly variable in time and space, and clearly dependent on the age of oysters and their growing height or position in the water column. Implementation of different farming practices, in this case modification of the growing height, could play a role in disease management and help reduce mortality of adult oysters during an OsHV-1 outbreak. The pattern of infection and disease was different on different leases suggesting that underlying environmental factors influence disease expression. Differences in mortality among sites, ages and growing heights were evaluated in relation to the intensity and prevalence of viral infection and the environmental data recorded during the outbreak. The epidemiological observations are of considerable importance and inform future strategies to control OsHV-1, including the methodologies to be applied in the genetic selection program.

C. gigas were stocked into 3 different oyster leases in Woolooware Bay and allocated into groups to study growing height and age. Two heights were used: a standard growing height, and one 300mm higher than this. Oysters comprised adults (12 month old; 67-93 mm length) and spat (2-3 month old; 21-38 mm) and were placed in plastic trays with lids. Other oysters were kept in floating baskets at each site. The sites were managed by oyster growers with the assistance of researchers and all sampling was conducted by the researchers. Oysters were placed during the late spring in October 2011, before POMS had recrudesced. The level of OsHV-1 virus, *Vibrio* sp., mortality rates and environmental parameters such as temperature and salinity were studied in detail before, during and after the outbreak.

Spat were highly susceptible to the virus and all those kept in trays died regardless of growing height. In contrast, the high growing height reduced the deaths of adult oysters by 40%. The pattern of results was consistent at all three sites. In a second smaller experiment, spat survived in floating baskets but not in trays.

Environmental factors may affect POMS disease events. We found a slight decrease in salinity and variable changes in water temperature just before three mortality events in Woolooware Bay in the Georges River. Importantly, salinity and temperature readings in the Hawkesbury River system were similar to those in the Georges River and therefore may be suitable for POMS to establish.

Overall the observations suggest that OsHV-1 is a necessary but insufficient factor in the mortality event or that OsHV-1 is a sufficient cause but with a very strong dose effect. The virus was detected in oyster tissues up to 2 months before mortalities commenced therefore other factors may be required. These factors could include environmental conditions/triggers or other as yet undiscovered pathogens. *Vibrio spp.* bacteria, which have been suggested to be involved with OsHV-1 virus in POMS disease in France, did not appear to be involved in this outbreak in Woolooware Bay because their intensity did not increase until after the POMS outbreak had started, the species of *Vibrio* present did not change over time and a similar number of *Vibrio* spp. bacteria were present in tissues of healthy oysters in the Hawkesbury River.

Importantly, the virus did not appear to be transmitted free in water. There was considerable epidemiological evidence that its distribution was clustered in Woolooware Bay, and that it behaved as if it was moving together with still to be defined planktonic particles. These important results have already been used by other researchers to confirm the optimal design of field experiments to study the resistance of different *C. gigas* family lines, to support a genetic selection program.

During the outbreak up to 92% of the oysters tested positive for OsHV-1 but afterwards the infection prevalence decreased over time suggesting that surviving adults can clear the virus. Some appeared to be resistant as they survived three separate mortality events during the summer 2011-2012. This suggests possible immunological mechanisms and underlying potential for genetic resistance to the infection.

It can be concluded from this study that husbandry factors may strongly influence the survival of adult *C. gigas* during an outbreak of POMS. It is possible that measures to reduce the level of exposure of oysters to OsHV-1 as distinct from preventing exposure may be sufficient to prevent mortalities.

Recommendations were made for further development. Further studies are indicated to confirm the effect of growing height on mortality rates, as the present research trial was conducted during an unusually wet and cool summer. If the same results are obtained when the trial is repeated in a more typical summer, oyster growers can confidently take steps to reduce the risk of losses of valuable adult oysters should POMS spread in Australia. Further studies are also required to investigate why some oysters appear to be resistant and how some clear the virus from their organs, to precisely identify the seasonal window of infection, to confirm the mode of transmission of the virus in the environment, to evaluate the risk of transmission with equipment and by handling oysters, to understand how environmental factors combine with the virus to cause mortality, whether a certain level of viral load in the environment is needed to initiate mortalities, and to identify potential wild mollusc hosts for the virus.

Note added in press: A new outbreak of POMS was detected in the Hawkesbury River on 21st January 2013

KEYWORDS: POMS, ostreid herpes virus, Pacific oyster, *Crassostrea gigas*, aquaculture, disease control

Project No. 2011/245: Tactical Research Fund: Research methods to manage pathogenic microbiological and biological organism within a redclaw (*Cherax quadricarinatus*) egg incubator hatchery to improve survival and reliability (PI: Colin Valverde)

OBJECTIVES:

1. Bacterial & fungal identification & management
2. Test commercial probiotics/develop in-house probiotics and best practice
3. Determine critical time when hatched larvae need to start feeding
4. Identify causes for unexplained mortalities at all life stages (egg, larvae, crayling)
5. Develop methods to identify viable/unviable eggs

NON TECHNICAL SUMMARY

OUTCOMES ACHIEVED TO DATE

Production in the hatchery has already significantly improved due to a greater understanding of the microbiological implications researched in this project. This is demonstrated in better management of the micro flora / fauna within the egg incubators, which leads to higher consistency in survival of the craylings and reliability of the system.

The extra hatchery production has been immediately taken up by redclaw growers with increasing demand for next season. The outcomes so far:

Farm husbandry

- Increased yields and extended season for crayling production
- Increased profitability for growers by freeing them up from using brood stock / juvenile ponds
- Improved efficiency by simplifying production, reducing duplication and work load on the farmer
- For the first time farmers are able to stock exact quantities of same age craylings and so will be better able to predict growth rates and bio-mass, to better manage feeding rates / water exchange and predict production
- Facilitate the move from an extensive to a more intensive aquaculture industry
- Earlier stocking of craylings in spring to increase summer grow-out period

Re-vitalising a stalled industry

- Through our growing knowledge and expertise we can provide more guidance and consultation to growers
- New entrants to the industry can save the time it would take to grow their own brood stock and can have an immediate start up with hatchery produced craylings.
- Increasing production (more profitable for producers)
- Farmers report reduced work load

Better control of the lifecycle of redclaw crayfish opens new areas of research

- This technology has already allowed the North QLD Crayfish Farmers Association (NQCFA) to implement a selective breeding project that would have been far less efficient (with less control over family lineage) if traditional crayling

production methods were used. As we have obtained a high level of confidence AquaVerde has now taken over the selective breeding project with the incubation technique being the pivotal part of the process.

It has been identified by the Queensland Crayfish Farmers Assoc. (QCFA) and acknowledged by DAFF (Fisheries Queensland) that for our redclaw industry to grow and develop beyond "extensive farming practices" we require a reliable hatchery for producing disease free seed stock all year round, which will lead to radical changes in farm husbandry which in turn increases production and supports the re-vitalisation of a stalled industry.

AquaVerde secured a crayfish egg incubation system that was originally developed in Scandinavia for disease mitigation (producing crayfish populations free of *Aphanomyces astaci*). It became clear that we could use such an incubator with redclaw to provide the basis for a hatchery. The system basically works by harvesting the fertilised eggs from females and incubating them in baskets in a controlled aquatic environment. Within the baskets, the eggs hatch and progress through two larval stages before they finally become morphologically adults, termed Stage 3 Juveniles (S3J's) and what we also refer to as "craylings". These techniques afford an unprecedented level of control over the lifecycle of freshwater crayfish and have many advantages over the old traditional methods of "just throwing brood stock into ponds and seeing what you get after 12 months".

Good progress was made with the hatchery but microbiological infections occasionally caused high mortalities in predominantly Stage 2 Larvae (S2L) that we were unable to solve. It became clear that to build a reliable hatchery for our industry we needed expert help. Support from leading figures in aquaculture encouraged us to apply for this FRDC Tactical Research Fund to engage microbiologists for research. We found just the people we needed at the School of Veterinary and Biomedical Sciences, James Cook University Townville. The main priority was to identify pathogenic bacteria and find ways to manage them. The use of probiotics has worked well in various aquaculture facilities around the world and most notably in prawn hatcheries. Therefore our main focus was to find and culture suitable non pathogenic bacteria that could be used as a probiotic(s) to competitively exclude pathogens to sublethal dose. The best place to look for suitable probiotic bacteria is within the system itself. A regime of sampling from the hatchery began to identify the pathogenic bacteria and at the same time to identify suitable candidate bacteria for use as a probiotic.

During the studies the main culprit was identified as *Aeromonas hydrophila.* An exception was the first isolation, when *Chromobacterium* species was found to be the cause of disease. Many other bacteria were isolated and some selected to test their suitability as a probiotic. All the candidate probiotic bacteria selected so far have failed to completely contain *A. hydrophila* when tested under laboratory conditions. However we will still trial them in the hatchery to evaluate if they are able to inhibit *A hydrophila* below lethal levels for long enough to allow Stage 2 Larvae to reach Stage 3 Juveniles, at which point they become better able to resist infections.

The use of a commercially available probiotic containing *Bacillus* species was shown not to inhibit the development of the *A. hydrophila*-associated bacterial septicaemic disease in the S2L's. Nor did they appear to inhibit the proliferation and biofilm formation of *Aeromonas hydophila* in the water. A laboratory study showed that the *Bacillus* species were not able to inhibit the growth of both *Chromobacterium*, which was responsible for a single outbreak of disease, and the more common *Aeromonas hydrophila*. Although the probiotic bacteria were able to result in the formation of early biofilms in the hatching tank after cleaning, *Aeromonas hydrophila*, once introduced, was able to out-compete the *Bacillus* species. This was in spite of the fact that the dose and rate of administration of the probiotic *Bacillus* species had been higher than the standard recommended dosages.

The ongoing cultures and histological examination of both healthy and affected eggs and larvae enabled us to associate the bacteria cultured with those observed histologically and infer the causative agent of the mortalities, especially in the S2L. Histological examination of the larvae also allowed us to detect whether any viral inclusions were present which may have contributed to disease or increasing the susceptibility of the larvae to opportunistic bacterial infections. In this study, none were observed. Furthermore, histological examination of healthy larvae was able to show that the S2L's had a higher number of bacteria within their intestinal tract and hepatopancreas than the S3J's.

The evidence of an infection by bacteria common in freshwater that enter after hatching and affected predominantly the hepatopancreas of the larvae indicated the necessity of at least attempting to control the most obvious source of the bacteria. This directly led to changes in hatchery management regarding better control of the microbiological quality of the incoming water and the control of aerosolised water droplet formation by reducing relative humidity of air within the hatchery from over 90% to just under 50%.

We also performed feeding trials as part of this research project to try to determine the critical stages when S3J's (craylings) need to be fed to survive their first post juvenile moult. Previous studies on the feeding of S3J's have always begun at the point the craylings were released from the mother's tail, which is not entirely relevant in our situation. Because of this we felt the need to begin

our own experiments. We set up nine small tanks with three feeding regimes (no feed, prawn larvae dust, and blood worms, replicated 3 times) and ran the trials three times each with S3J's craylings of different ages (2, 7, 14 day old). The S3J's were taken from the incubator and stocked into the 9 tanks and feeding began to determine if the time held without feeding in the incubator affected survival till first post juvenile moult. The survivability of redclaw craylings was much higher when fed than when they were not fed. Also, the results indicated that S3J's should be fed, but it may not be necessary to feed them before they are 14 days of age as demonstrated in the feeding trials.

KEYWORDS: redclaw crayling, hatchery, incubation, microbiology, bacteria

Project No. 2012/044: Exercise Sea Fox: Testing aquatic animal disease emergency response capabilities within aquaculture (PI: Shane Roberts)

OBJECTIVES:

1. Raise awareness within government and industry of national and state emergency management obligations

2. Provide government and industry personnel with an opportunity to develop and practice skills and procedures when responding to an emergency aquatic animal disease

3. Develop knowledge within government of the aquatic industry

4. Identify gaps in the government and industry's aquatic disease response capability

NON TECHNICAL SUMMARY

The South Australian State government (Primary Industries and Regions South Australia), together with the South Australian Oyster Growers Association (SAOGA), lead a national aquatic disease response exercise: "Exercise Sea Fox". The exercise scenario was based on a fictitious outbreak of Pacific Oyster Mortality Syndrome (POMS) and was conducted in 3 parts; a field trip, a workshop and a discussion exercise during October and November 2012 in South Australia. A key outcome was the development of an emergency disease response plan specific to POMS. Being an emergent disease of national priority in Australia, POMS represents a significant threat to the seafood industry. Exercise Sea Fox was successful in enhancing prevention, preparedness and response capabilities for the oyster growing sector, providing greater food security and protection for regional communities.

POMS is a disease caused by a microvariant of the Ostreid Herpesvirus (OsHV-1 microvariant), and was responsible for significant oyster mortalities (80 – 100 %) and economic impact in Europe, including France, during 2008 and in New Zealand and Australia (NSW) since 2010. Given the significant threat POMS poses to the oyster growing sector of Australia's seafood industry, building emergency response capacity in the aquaculture sector is a high priority at a State and National level.

The aim of Exercise Sea Fox was to enhance government and industry's preparedness for responding to an emergency aquatic animal disease and identify gaps in current emergency response capabilities.

Exercise Sea Fox followed on from FRDC 2011-043 (Understanding and planning for the potential impacts of OsHV-1 microvariant) and was conducted in 3 parts. Firstly, a field trip provided State government emergency response staff experience and knowledge of the aquaculture industry. Secondly, a workshop provided government and industry participants with technical aspects for responding to aquatic diseases. Thirdly, a discussion exercise provided the opportunity to practice current emergency response arrangements (State and National), procedures and systems for responding to a fictitious disease outbreak. The exercise scenario was based on a simulated outbreak of POMS in South Australia. The disease outbreak was chosen to draw out some of the challenges when responding to this disease, identify gaps in current preparedness arrangements and key risks. Participants included personnel from government, industry and universities, representing DAFF (Canberra), South Australia, New South Wales, Tasmania, Western Australia and New Zealand.

A summary of key issues in current response capabilities comprised the key output from the exercise. Importantly, it was highlighted that no POMS-specific disease response plans (State or National) existed in Australia (at the time), which was highlighted as a gap for effective emergency response. However, generic AQUAVETPLAN manuals (e.g. enterprise manual) can be used to respond to unknown diseases, although they lack detail for responding to specific disease risks. Thus, key components of a disease response plan for POMS were discussed, and include; a case definition, reporting requirements, response strategies, tracing, emergency harvest, destruction-disposal-decontamination, movement controls, legislative powers, surveillance and monitoring.

Exercise Sea Fox improved working relationships within government (State and National) and between government and industry. Outcomes from the exercise broadly cover prevention, preparedness and response capabilities for the oyster growing sector, providing greater food security and protection for regional communities. The economic benefit of prevention and preparedness for an exotic disease threat can be estimated at 1:100 (e.g. for every dollar spent, a return of approximately one hundred dollars can be expected). This compares to 1:25 for eradication and 1:5-10 for containment.

Recommendations from Exercise Sea Fox include:

1. Consider outcomes of this project for the development of disease response plans for POMS

2. Improve surveillance systems
3. Improve stock records
4. Cost sharing arrangement to be considered
5. Commitment to ongoing response training
6. Commitment to awareness campaigns

The outcomes and extension of this project have exceeded the initial objectives (see Chapter 6). All recommendations are currently being addressed. Importantly a State response plan specific to POMS has been developed (Appendix 6), while post Exercise Sea Fox workshops have included risk assessments and presentations to industry. National and industry response plans are now being developed.

Key words: Aquatic disease, Pacific Oyster Mortality Syndrome, POMS, OsHV-1 microvariant, Emergency Response

Progress Summaries for Active AAHS Projects

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

Final Report in preparation

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

The project objectives are:

1 To determine/confirm the identity of the one or more variant(s) of Ostreid herpesvirus associated with the recent outbreaks of POMS

2 To determine the mechanism(s) of transmission of disease

3 To determine the major risk factors that contribute to precipitation of disease outbreaks thereby identifying potential risk-mitigation management practices

4 To identify the natural reservoir(s) for the virus

5 To determine the stability of the virus in the environment

6 To identify physical and chemical means for viral inactivation

7 To develop an infectivity model for POMS suitable for selection of resistant oysters and pathogenesis/environmental research

8 To address future shortages of technical expertise through the training and supervision of at least 1 PhD student

To address objective 1, Purified DNA samples collected from OsHV-1 infected oysters from different locations and times have been shipped to France where whole genome sequencing will be undertaken with a view to developing greater epidemiological understanding of outbreaks.

To address objective 2, the pattern of spread and transmission of POMS disease across a range of different scales has been analysed in a large field experiment conducted in the Georges River which involved detailed study of 18,000 oysters. Three distinct mortality events were detected, in November 2012, February 2013 and April 2013. Mortality overall was less severe than in the previous summer. As in the previous summer, there was a reduction in mortality for oysters placed at high height in comparison with low height. Use of a high cultivation height can limit POMS mortality in adult oysters to less than 50%. This is a very significant observation. Detailed observations of mortality patterns were consistent with water-borne infection rather than oyster to oyster spread.

To address objective 3 a parallel experiment was conducted in the Hawkesbury River which included height as a risk factor for non-specific mortality. It revealed substantial tolerance of oysters to placement at high height and confirmed that it is a practical way to avoid or delay exposure to POMS disease, albeit with a growth penalty.

Objectives 2 and 3 were also addressed by conducting an outbreak investigation when POMS appeared in the Hawkesbury River in January 2013. The investigation confirmed point source infection, and revealed that after introduction to a bay with infected oysters, oyster to oyster transmission was relatively inefficient. Of great significance was the observation of low levels of OsHV-1 DNA in oysters weeks-to-months before the outbreak began.

Objective 4 was addressed by sampling wild oysters and other organisms in Woolooware Bay before during and after the outbreak of POMS to check for the presence of OsHV-1 and identify a potential reservoir host. The PCR analyses are ongoing and will provide information on the infectious status of wild species present in the Georges River and their potential role as reservoir hosts; preliminary results showed that OsHV-1 can be detected in wild Pacific oysters and to a lesser extent Sydney rock oysters.

Objectives 2 and 5 were addressed by studying the window of infection for POMS by placing spat in estuaries every 2 weeks after the onset of the POMS season. The window in 2013-2014 was November to late April. Furthermore, experiments have commenced to determine how estuary water can be treated to render it safe for rearing spat. Mortality data and preliminary PCR results suggest that filtration to 5um with UV treatment, or sedimentation of water for 48 hours are effective.

Transmission experiments on OsHV-1 have been completed, and immunology experiments have commenced.

The next milestone report will include final analyses and PCR data for the above studies.

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

The combined results from the oral challenge trial including PCR, bacteriology, histopathology analysis, observed clinical signs and post mortem findings indicate that juvenile grouper *Epinephelus lanceolatus*, fed with both high and low doses of the bacteria *Streptococcus agalactiae*, can become infected via the trophic route. Similarly, the combined results from the cohabitation challenge trial including PCR, bacteriology, histopathology analysis, gross clinical signs and post mortem findings indicate that naive, non-infected juvenile grouper *E. lanceolatus*, cohabited with juvenile grouper infected with *S. agalactiae*, can become infected by cohabitation in the same tank.

In the oral challenge trial, there were no mortalities for the 5-week's duration of the experiment, but two fish (from the high dose treatment group) became moribund, showing darkening of the skin, loss of appetite and separation from the cohort. Clinical signs of infection included spiral swimming, unilateral exophthalmia, ascites, or hyperaemia of the caudal or pectoral fins. In contrast to the clinical signs observed from injected fish in the injection challenge trial, no visible signs of petechial haemorrhaging on the gills, or rectal congestion were observed. Surviving fish showed very few or no external clinical signs of infection. A few fish displayed hyperaemia of the caudal or pectoral fins, and nearly all fish had full stomachs on dissection.

The results from the oral challenge trials, showed a very low level of infection of experimental fish with *S. agalactiae*. Percentages of infected fish varied with different test methods. For replicate 1 (R1) and replicate 2 (R2); 16% and 11% of fish tested positive by bacterial culture, compared to 78% (R1) and 67% (R2) of fish tested positive by PCR, and 33% (R1) and 22% (R2) tested positive by histology for *S. agalactiae*. These results, combined with the fact that all except two fish survived for the entire 5-week duration period of the experiment, suggests that most surviving fish were infected with *S. agalactiae*, and were subclinical carriers of *S. agalactiae*, showing little or no overt signs of infection.

Interestingly, four fish from the oral challenge trials (2 from each replicate system) had an infection with a different bacterium, (a Gram-negative, rod-shaped bacterium), based on histopathology examination and bacterial culture. The infection was confined to the kidneys in one fish, but was systemic, infecting multiple internal organs and tissues, in the other fish. Several Gram-negative bacteria were isolated and cultured on SBA from these fish, and identified to species level by PCR and sequencing.

In the cohabitation challenge trials, four fish died during the five-week duration of the experiment. Dead fish were from the injected treatment groups (one fish each from ratio 1:1 & 1:5, both replicates). A further seven fish were moribund at the end of the experiment; six were from the injected treatment groups, one fish from a non-injected fish. Six of the seven moribund fish tested positive by both PCR and histopathology; 2 of these six fish were positive by bacteriology; the seventh moribund fish was negative by all three test methods. Clinical signs of infection observed in the seven moribund fish included anorexia, spiral swimming, unilateral exophthalmia, unilateral corneal opacity, or hyperaemia of the caudal or pectoral fins. Post mortem revealed an empty stomach, with occasional splenomegally, and a reduced size liver or spleen. In contrast to clinical signs displayed by fish from the injection challenge trial, no visible petechial haemorrhaging was observed on the gills, and no reddening of the vent was observed. The remaining fish survived the entire five-week duration of the experiment, and showed little or no signs of infection, with occasional hyperaemia of the caudal or pectoral fins, and nearly all with full stomachs on post mortem. Two fish had mouth deformities, possibly of genetic aetiology.

Statistical comparison and analysis of all three laboratory test methods to detect *S. agalactiae* in fish tissues, from both the oral and cohabitation challenge trials showed differing test results. PCR was found to be the most sensitive test method for detecting *S. agalactiae* in fish.

The laboratory results, observed clinical signs and the fact that most fish survived for the 5-week duration period for both experiments, suggests that most surviving fish were either subclinical carriers of *S. agalactiae*, (showing little or no overt signs of bacterial infection), or were becoming infected with the bacterium, and only just beginning to show signs of infection. Further experimental challenge trials, extending the duration of experiments, would be necessary to determine these factors.

In summary, results from both experiments indicate the transmission routes for the bacterium *S. agalactiae* in juvenile Queensland grouper *E. lanceolatus,* may occur via the trophic route or by cohabitation (i.e. water-borne), but onset of infection is much slower (day 26 and day 33) compared with intra-peritoneal route of infection, which was much quicker and occurred on days 2-3.

In order to understand pathogenicity and to aid diagnosis, specific detection systems that can be used in histological sections are very informative. Two methods were evaluated. Fluorescent *in situ* hybridization (FISH) based on detection of specific

sequences of the 16S rRNA, and immune-labelling using antibodies targeting specific antigens at the sub-cellular level on sections of biological tissue.

The FISH approach proved to be unsuitable for the detection of *S. agalactiae* on formalin-fixed and paraffin-embedded (FFPE) organs. Gram-positive bacteria such as *S. agalactiae* need an additional enzymatic treatment to open the thick peptidoglycan surface layer, in order to allow access of the probes to its target 16S rRNA within the bacterial cells. Multiple attempts using various enzyme treatments and varying formamide stringency failed to give satisfactory results.

Alternatively, immunohistochemistry (IHC) was optimised using a readily available commercial polyclonal antibody that reacts with type-specific carbohydrate on the surface of Group-B *Streptococcus* (GBS). Due to a high level of auto-fluorescence of fish tissues, when excited at wavelengths in the green, yellow and orange portion of the visible spectrum, detection of the primary antibody with minimal background was made possible using a commercial secondary antibody coupled with a photo-stable far-red fluorescent dye.

The IHC approach for the detection and localization of GBS in fish tissues was specific and highly reproducible. Thus both new and archival histological sections from diseased and healthy Queensland groupers, *E. lanceolatus*, were analysed using this method.

Summary of Active Projects

Project No.	Project Title	Principal Investigator
2009/032	AAHS: Characterisation of abalone herpes-like virus infections in abalone (*Associated species*: *Haliotis* spp.)	Dr Mark Crane CSIRO AAHL Fish Diseases Laboratory Phone: 03 5227 5118 Email: mark.crane@csiro.au
2009/315	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
2010/034	AAHS: Investigation of an emerging bacterial disease in wild Queensland gropers, marine fish and stingrays with production of diagnostic tools to reduce the spread of disease to other states of Australia (*Associated species*: multi-species)	Dr Rachel Bowater DAFF Queensland Phone: 07 4760 1592 Email: rachel.bowater@daff.qld.gov.au
2010/036	AAHS: Improved fish health management for integrated inland aquaculture through Better Management Practices (BMPs) (*Associated species*: *Maccullochella* spp.)	Dr Tracey Bradley DEPI Victoria Phone: 03 9217 4171 Email: tracey.bradley@depi.vic.gov.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
2011/005	AAHS: Investigation of inclusions in Australian prawns (*Associated species*: multi-species)	Dr Melanie Crockford Dept Fisheries WA Phone: 08 9368 3205 Email: mcrockford@agric.wa.gov.au
2011/048	Tactical Research Fund - AAHS: Determining the susceptibility of Australian species of prawns to infectious myonecrosis (*Associated species*: multi-species)	Dr Mark Crane CSIRO AAHL Fish Diseases Laboratory Phone: 03 5227 5118 Email: mark.crane@csiro.au
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	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
2012/044	Exercise Sea Fox: Testing aquatic animal disease emergency response capabilities within aquaculture (*Associated species*: multi-species)	Dr Shane Roberts PIRSA Phone: 08 8226 3975 Email: shane.roberts@sa.gov.au
2012/050	*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	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	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	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
2013/004	The Neptune Project: A comprehensive database of Australian aquatic animal pathogens and diseases (*Associated species*: multi-species)	Dr Marissa McNamara Queensland Museum Phone: 07 3842 9173 Email: marissa.mcnamara@qm.qld.gov.au
2013/036	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

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