Shrimp Pathology Short Course: Disease diagnosis and control, University of Arizona and visit to the Shrimp Biotechnology Business Unit, Thailand

Daniel Pountney

AUSTRALIAN SEAFOOD COOPERATIVE RESEARCH CENTRE

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NON-TECHNICAL SUMMARY

PROJECT NO: RTG 2011/713

PRINCIPAL INVESTIGATOR: Daniel Pountney

ADDRESS:

NCMCRS
University of Tasmania,
Locked Bag 1370 Newnham,
Tasmania, 7250

(PROJECT) OBJECTIVES OF RESEARCH TRAVEL GRANT/ INDUSTRY BURSARY

To attend and participate at the 2011Shrimp Pathology course: Disease diagnosis and control, University of Arizona and visit the Shrimp Biotechnology Business Unit, Thailand.

NON TECHNICAL SUMMARY: I visited Bunjonk shrimp hatchery which is located in the Chachoengsao province of Thailand. I toured the farm and spoke with Mrs Bunjonk who has been involved with growing shrimp for approximately 20 years. In the past the Chachoengsao province has been a white spot syndrome virus (WSSV) 'hotspot'. For this reason the shrimp farmers within the area have changed from culturing *Penaeus monodon* to culturing *Litopenaeus vannamei* which are less susceptible to WSSV and taura syndrome virus (TSV), which occurs every 4 years years (TSV) causing mass mortality of shrimp. There are other viral and bacterial diseases which are now endemic to the province causing reduced growth rather than substantial mortality. Recently the Thailand government introduced 'Good Agricultural Practice' (GAP) for shrimp farmers to abide by, this includes the use of more stringent biosecurity measures such as, fencing around farms to stop poaching, restricting access to visitors, water treatment, bird netting, and certification of 'disease free stock' for hatcheries. The Bunjonk hatchery is a certified disease free shrimp hatchery, and is the only *P. monodon* hatchery in the area. Their success is from stringent biosecurity measures within the hatchery and current research and development associated with Shrimp Biotechnology Business Unit to produce disease resistant *P. monodon* stocks from selectively bred broodstock.

I visited the Shrimp Biotechnology Business Unit (SBBU) located in the Pathumthani province of Thailand. SBBU is a government facility where they specialize in contract research for business, shrimp farmers, feed manufacturers and biotechnology laboratories. SBBU are equipped with 30 x 220 L; 30 x 390 L and 10 x 2000 L tank recirculation systems and they also have functional molecular and microbiology

laboratories for analyzing samples. SBBU also develops shrimp disease diagnostic kits such as, the Ezee Gene Strip Test for yellow-head virus, white spot syndrome virus and *Vibrio harveyi* bacteria; Loop-Mediated Isothermal PCR (LAMP) polymerase chain reaction (PCR), and products used in PCR and RT-PCR. Other contract research includes nutritional research to improve shrimp growth and resistance to TSV and WSSV.

I attended the 2011 Shrimp Pathology Short Course which was run at the University of Arizona, Department of Microbiology and Veterinary Science and Microbiology in Tucson, Arizona. The course has been conducted every year since the late 1990's and has attracted participants from over 100 countries worldwide. The course is run by Professor Donald Lightner and his staff from the Department of Microbiology and Veterinary Science, who are experts in the fields of crustacean disease diagnosis, and techniques used in disease diagnosis. The course involved structured lectures on viral, bacterial, parasitic, and nutritional diseases which affect cultured shrimp and crab species worldwide, including: white spot syndrome virus, taura syndrome virus, gill associated virus, yellow-head virus, bacterial, rickettsial and fungal diseases. Microscopy sessions followed the lectures, which focused on specific disease causative agents presented in each lecture and later assessment using histopathology. After the lectures, there were laboratory sessions or demonstrations which involved practical experience in a number of disease diagnostic methods which are routinely conducted at shrimp farms and diagnostic labs, including: wet mounts of shrimp tissues, blood clotting tests, proper sample preparation for disease diagnosis, microbiology and histology techniques. The last day of the workshop included a practical exam where there were 50 questions and 50 work stations. Participants had 2 minutes to answer each question, changing locations after each question. Questions included theoretical and disease diagnosis questions.

OUTCOMES ACHIEVED TO DATE

Attended the 2011 shrimp pathology workshop, which included lectures, practical sessions and a final exam. I also visited Shrimp Biotechnology Business Unit and visited Mr Bunjonk's shrimp hatchery in the Chachoengsao province.

(PROJECT) OUTPUTS DEVELOPED AS RESULT OF TRAVEL GRANT/ INDUSTRY BURSARY:

I was able to enhance my knowledge in the field of shrimp disease using the most current methods to detect diseases, which I will use in the later stages of my PhD research. I learnt new skills and methods for use in my research including, molecular biology, bacteriological assays, histopathology and sampling techniques. I also made numerous contacts from the staff at the Department of Microbiology and Veterinary Science and Microbiology and fellow participants who work in the fields of shrimp research and disease diagnosis and potential for future collaborations.

Visiting a commercial shrimp farm in Thailand provided a better understanding of the region's disease issues involving on a first-hand basis and a better understanding of

the preventative measures which have been implemented over time from case histories of disease outbreaks. Visiting Shrimp Biotechnology Business Unit enabled me to tour a commercial research facility which commonly applies similar techniques and feed ingredient improvement strategies which I will be dealing with in my research, with professional relationships made for potential future collaborations.

ABOUT THE PROJECT/ACTIVITY

BACKGROUND AND NEED

The research aims of my PhD research will assess a range of feed ingredients in *Penaeus monodon* feeds, which provide better growth and /or survival during periods of sub-optimal culture conditions. In Australia sub-optimal conditions like decreased water temperature and low salinity events have great potential to increase expression of gill-associated virus (GAV) and secondary bacterial infections (Vibriosis), causing decreased growth and mortality. The need to gain experience in the theoretical and practical aspects of disease diagnosis methods was essential for my research, which was not possible in Australia. This ensures the research I conduct is conducted and analysed in a manner comparable to that used in commercial shrimp culture, making for better comparisons and explanations between experimental conditions and commercial shrimp farming.

RESULTS

I gained extensive knowledge of the current diseases which are of significant economic importance to shrimp farming worldwide. I learnt about past case studies directly from the researchers that were involved in those situations, involving the spread of pathogens associated with exporting broodstock and seed stock to countries new to commercial shrimp culture,

I also gained new skills which in molecular biology and bacteriology, and I enhanced my histopathology skills involving shrimp as compared to my experience in teleost fish. I learnt techniques that assess the blood (haemolymph) and gut health at a pond-side level, which does not use specialized laboratory experiment and have quick response times.

INDUSTRY IMPACT

PROJECT OUTCOMES (THAT INITIATED CHANGE IN INDUSTRY)

SUMMARY OF CHANGE IN INDUSTRY

(What immediate changes might be expected for business/industry?)

Practical techniques which I learnt will be use in my experiments as part of my research including analysing samples and disease diagnosis. The potential for changes within the Australian prawn industry may present at the completion of my PhD. Start teaching these techniques to graduates in second semester, who will enter the industry at the end of November.

WHAT FUTURE AND ONGOING CHANGES ARE EXPECTED?

(What will be the impact?)

Techniques I have learnt from the course will provide new tools for assessing Australian diseases bringing changes to the industry including a better understanding of how particular feed ingredients may improve prawn survival and growth during periods of challenging culture conditions. The positive outcome for industry may include increased production and an increased survival of prawns. Also there is potential for validating immune response techniques for assessing the feed ingredients which may prove beneficial for prawn growth and survival from pathogens passed on to members of AFPA at completion of my PhD.

WHAT BARRIERS ARE THERE FOR CHANGES TO OCCUR?

This is the first year of research for my project. Therefore, a considerable amount of research is still required and there are many experiments for assessing potential ingredients for further experiments to be done. Only after completion of my research and submission of my thesis will my results provide for a better awareness of potential feed ingredients in prawn feeds. Other barriers to be considered may be how cost-effective are the ingredients which show the highest potential for increasing growth and survival under sub-optimal conditions.

IF NOT ALREADY HAPPENING, WHEN WILL THE CHANGES OCCUR?

(e.g. 2 businesses will adopt project findings and two more are expected to adopt findings within 12 months)

The final conclusions will be available after submission of my thesis in 2013. As experiments are completed I hope to present these to the industry at APFA meetings etc.

WHAT IS THE LIKELIHOOD THAT THESE CHANGES WILL OCCUR?

(e.g. 50% chance that four businesses will adopt project findings)?

This will depend upon the cost involved by including the feed ingredients in prawn feeds, and also by how readily available the ingredients are at the time when they are required. If the cost is feasible, the potential for industry to use the ingredients may be higher than 50%. Direct industry engagement in the project, both with industry supervisors, feeds, industry leaders and farmers. The project aims to validate findings under commercial conditions to demonstrate and promote uptake where results show improvements.

WHAT BARRIERS ARE THERE TO DOPTION OF THESE CHANGES AND WHAT ACTION COULD BE TAKEN TO OVERCOME THESE?

(e.g. to adopt project findings will require group training/sharing equipment/invest additional capital etc.)

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This study will provide recommendations based on laboratory based and experimental scale results. Uptake to industry will depend on the cost effectiveness of designing new feeds with the results they produce on farm. Close consultation with industry through the project aims will keep the results as applicable as possible.
Other potential barriers will be similar to the research and development which is done when designing new commercial feeds, and how feed manufacturers market these new feeds to prawn farmers. Potential barriers at a farm level may include feeding strategy and when is the best time to feed prawns for the highest benefit. Otherwise no additional equipment will be required to incorporate the new feed into the feed management strategy.

COMMUNICATION OF PROJECT/EXTENSION ACTIVITIES

WHAT IS THE OUTPUT THAT NEEDS TO BE COMMUNICATED?

Skills gained from attending the course, including disease diagnostic methods such as: histopathology, pond-side health assessment and molecular techniques. Results of my studies will use these techniques and will be communicated at completion of my PhD.

WHO IS/ARE THE TARGET AUDIENCE/S?

Short-term target audiences are the researchers, staff and students at the University of Tasmania who are involved with my project or attend my annual seminar which is presented at the University as part of PhD. As experimental results are available, the target audience would be the prawn farmers, prawn researchers and feed

manufacturers who attend industry presentations at conferences, or read my thesis after completion of my research.

WHAT ARE THE KEY MESSAGES?

If the skills you require to do your research are not currently available in an area where you live and work, there is always potential to gain them by attending workshops and courses abroad from the experts in that field.

WHAT IS THE CALL TO ACTION?

(What is it you want people to do once you communicate the key message to them – i.e. what change of behaviour or action do you want them to take?)

To be able to replicate the techniques and experiments used in my research for future investigations within the discipline.

COMMUNICATION CHANNELS

(How can these messages be communicated and by who?):

Channel	*Who by*	*When*
Communication with researchers in the field of crustacean health and nutrition from knowledge learnt.	Daniel Pountney	Ongoing
Demonstrating to my colleagues and undergraduate students the new skills gained by attending the course.	Daniel Pountney	Ongoing
Supplying information from the course to my supervisors and other researchers for use in future research and teaching undergraduate students,	Daniel Pountney, Prof. Barbara Nowak, Dr Louise Ward	Ongoing
Incorporating the new techniques and assays learnt in my research.	Daniel Pountney	Submission of thesis

LESSONS LEARNED AND RECOMMENDED IMPROVEMENTS

WHAT IS YOUR FEEDBACK?

(e.g. *What difficulties were experienced in undertaking this research and how did this affect the project*, *what improvements and/or considerations can be recommended for future projects in this area* and *what barriers are there to undertaking further research in this area and how could these be overcome?)*

There is great value in add-ons to visits by having the opportunity to add-on the Thailand side trip which was a positive for my project. To make the most of opportunities abroad, it would be good allow extra time to keep travel flexible particularly when visiting commercial facilities – one planned visit wasn't possible due to scheduling changes.

FURTHER ACTION REQUIRED IN REGARDS TO COMMERCIALISATION?

(e.g. IP protection, licensing, sales, revenues etc)

Intellectual property is governed by the contract between the Seafood CRC and the University of Tasmania for this project.

ACKNOWLEDGEMENTS

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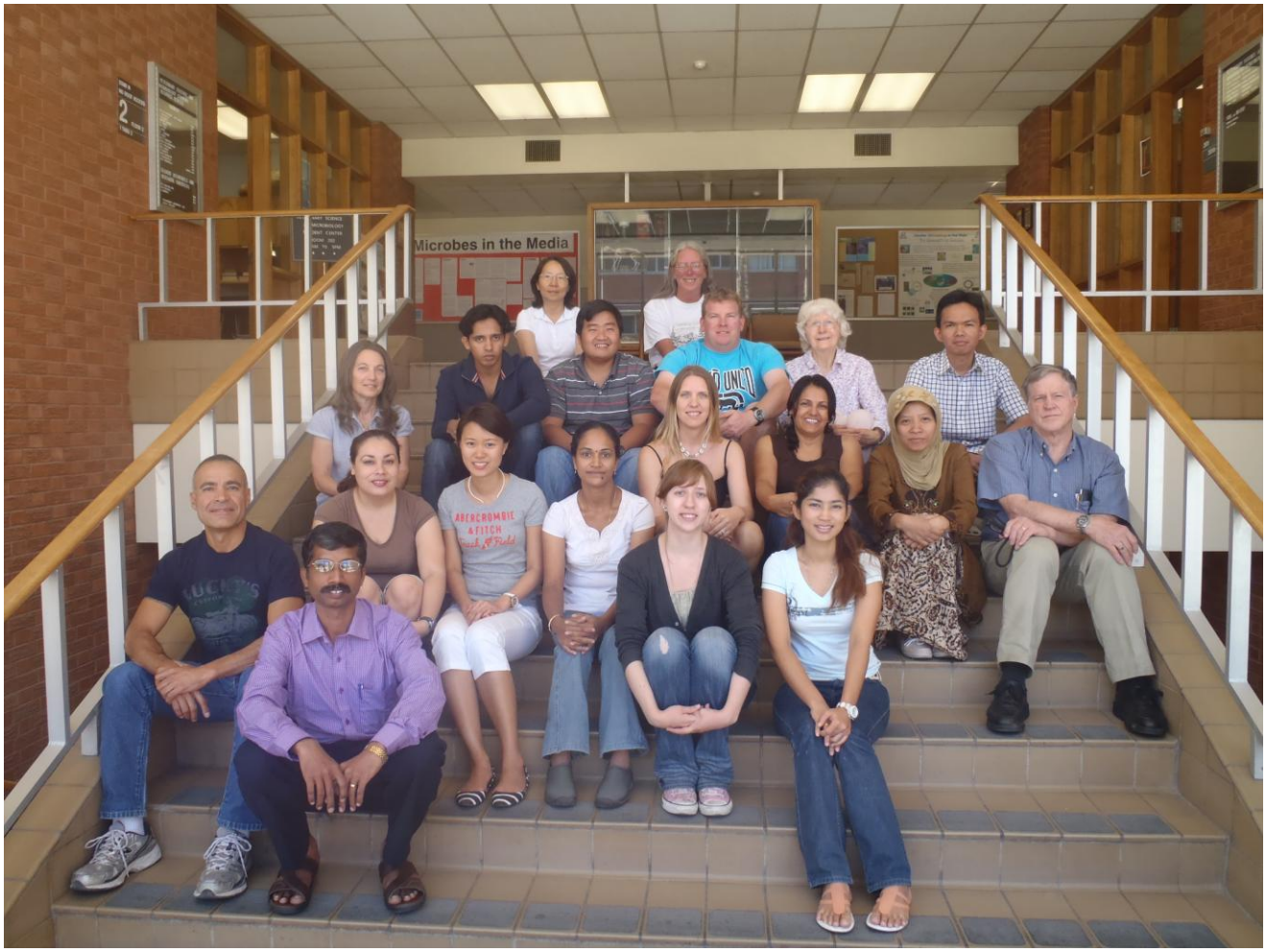
APPENDIX (IF APPLICABLE)

Photos from the 2011 Shrimp Pathology Short Course - participants



Loading a lane for agarose gel electrophoresis of viral PCR and RT-PCR products.

Haemolymph sampling of infected *Penaeus vannamei* with unknown bacterial infection.

Sections prawn tissues using a microtome for histopathology

Examining histology slides of diseased shrimp.

Microbiology – using API 20NE system for bacterial identification from a bacterial colony isolate.