FINAL REPORT

AWARD RECIPIENT: Chloe English

ADDRESS: BIRC, 144 North Street, Woorim, QLD, 4507

HOST ORGANISATION: CSIRO and Masaryk University, Brno

DATE: 25/01/2019

ACTIVITY UNDERTAKEN

I undertook a one-month internship at Masaryk University, Czech Republic, under the guidance of Professor Iva Dyková, a world leader in amoebic disease research. Three key activities occurred during this trip. I learnt how to identify amoebae based on fine scale cell structures viewed in transmission electron microscopy (TEM) images. I conducted phylogenetic analysis to confirm the identity of amoeba sequences previously acquired during my PhD project. Finally, I learnt new amoeba culturing techniques.

OUTCOMES ACHIEVED TO DATE

This internship was highly beneficial to my professional development and research. The key outcomes achieved include;

- Technical skills were gained from a world leader. The internship has improved my abilities in systematics, taxonomy, cell culture, phylogenetics and disease aetiology, knowledge that is fundamental for improving aquatic animal health.
- Generate new data for the amoebic gill disease (AGD) mix aetiology project. All of these newly acquired skills were directly applicable to my current research project investigating the possible multi-amoeba aetiology of AGD.
- A paper has been published as a direct result of this internship, see English et al. 2019. This publication progresses the understanding of AGD infecting Atlantic salmon in Tasmania.
- These results were further communicated through an oral presentation at the 8th International Symposium on Aquatic Animal Health, PEI, Canada in September 2018.
- A new international collaboration has been established between researchers addressing the same issue of fish health in aquaculture.

Acknowledgments
I would also like to thank Professor Iva Dyková, Doctor Tomáˇs Tyml and Masaryk University for hosting my visit to Czech Republic. I also acknowledge CSIRO for the additional financial support to undertake the internship then communicate the results through publication and conferences.

Background
Amoebic gill disease is a parasite-mediated gill condition affecting many farmed fish species in Australia and overseas. To date, *Neoparamoeba perurans* is considered the only aetiologic agent of AGD (Crosbie et al. 2012). However, gill samples from infected fish

frequently present a mixed assemblage of amoeba species and there possible role in disease development in unknown (Bermingham and Mulcahy 2007; Howard 2001). The Health Team at CSIRO Aquaculture is working with our industry partner, Tassal and European collaborators to investigate whether *N. perurans* is the only causative agent of AGD in Atlantic salmon, and whether the virulence of AGD infection is influenced by other amoebae species. These findings will further our understanding of AGD aetiology and could promote improvements to treatment regimes. With this project in mind CSIRO Aquaculture approached a world leader in amoeba biology (Professor Iva Dyková) and proposed a one-month internship to enable their PhD candidate to develop skills in amoeba systematics, taxonomy and cell culture. The proposed activities and expert collaborator were strategically targeted to upskill the candidate and greatly progress this AGD project. Recent progress within CSIRO has cultured and sequenced the 18s rRNA and COI (cytochrome c oxidase subunit 1) gene of over eight amoeba variants (putative species) from farmed Atlantic salmon infected with AGD. These sequences serve as a genetic barcode specific to each amoeba species and has enabled the development of four qRT-PCR probes used to quantify the load of different amoeba species on the gills of Atlantic salmon. However, these sequences, although they can differentiate between different species, do not provide the species name. Amoeba are extremely difficult to identify based on their morphology because a single species can assume many forms and different species, even genera, often look extremely similar. Thus, a low number of fine structures, such as cell surface structure, nuclear structure and nuclear division pattern are used to differentiate species (Page, 1983). Identification at the cellular structure level requires electron microscopy visualisation and, even when they are visualised, those structures are challenging to describe and differentiate. Hence, differences in cellular morphology can only be interpreted correctly by a trained eye. At the time of this proposal there was a lack of expertise within CSIRO (and the broader AGD research community in Australia) to identify different amoeba species associated with AGD based on cellular morphology. The proposed internship at Masaryk University in Czech Republic was designed to provide the opportunity to learn these amoeba identification skills, as well as improve our knowledge in cell culture techniques and phylogenetic analysis.

Professor Iva Dyková is a world leader in amoebic disease research, with over 35 years of experience working on multiple aquatic amoebic diseases, including AGD, nodular gill disease in freshwater fish and paramoebiasis in sea urchins. Professor Dyková and her laboratory staff were ideal teachers and collaborators to assist our research on amoebic gill disease in Tasmanian Atlantic salmon.

Need

Despite the broad economic impact amoeba inflict through disease in aquatic hosts there is a deficit in information about aetiology and taxonomy. The majority of published AGD research focuses on AGD treatment strategies and *N. perurans* detection, with very few publications available on the biology of the causal agent(s). It is counter intuitive to focus primarily on how to treat a parasitic disease, i.e. how to combat the parasite, without understanding the biology of the pathogen and without being certain it is the sole causal agent. Correct species identification and an understanding of disease causation is imperative to disease management. This deficit of fundamental knowledge is hampering further research efforts on improving AGD treatment and prevention, evidenced by the

industries continued reliance on the same unstainable treatment regime despite 30 years of research.

Objectives

The main objective was to gain technical skills relevant to aquatic animal health and to progress the AGD aetiology project. The original proposal detailed four key activities which would be undertaken during the internship. These activities included learning species identification based of fine scale morphology, species identification through phylogenetic analysis and cell culture techniques. The last goal was to validate the specificity of our newly designed qPCR assays using Professor Dyková's archived amoeba DNA samples. I achieved three of the four key activities. The validation task was not achieved because I could not access a qPCR machine during my visit. Despite this minor setback, the other three goals were met (as detailed below) and allowed us to publish results that were of a much higher standard than what we would have achieved if the internship had not taken place. Hence, we believe the main objective of our proposal was achieved.

Methods

1. **Morphological identification:** I learnt how to identify amoebae based on fine scale cell structures viewed in transmission electron microscopy (TEM) images. TEM and image interpretation of protozoan parasites is one of Professor Dyková's main areas of expertise. She first stepped me through sample fixation, sectioning and TEM imaging. We then studied her text, Dyková and Kostka 2013, to learn the main gross and fine scale morphological characteristic used to distinguish amoeba species. After the visit, Professor Dyková continued to help me interpret TEM images of our AGD-associated amoeba, which were later published.
2. **Molecular identification:** I learnt how to identify amoebae through phylogenetic analysis under the guidance of Doctor Tomáˇs Tyml, a postdoc in the Dyková Lab. We used sequences that I previously acquired before the trip to construct trees of publication standard. As I had no prior experience in phylogenetic analysis it was invaluable to learn from an experienced scientist who knows the intricacies of amoebae phylogeny.
3. **Cell culture:** I learnt a variety of new amoeba culturing techniques, including cell media protocols, aseptic technique, bacterial feeding and cell isolation methods. With these new techniques I was able to isolate an additional amoeba monoculture from the gills of AGD-affected Atlantic salmon farmed in Tasmania.

All of these activities took place in the Dyková Lab which is part of the Institute of Parasitology – Biology Centre at Masarky University, Brno. The Biology Centre was a great place to lean about other aquatic parasite projects and to be immersed in the Czech culture.

Results/Discussion

I gained skills in morphological and molecular identification of amoebae and a variety of cell culture techniques. While acquiring these skills I also directly contributed to what is now published results (English et al 2019). Specifically, the phylogenetic trees, interpretation of the TEM images and the isolation of a new amoeba monoculture were direct outcomes. I also developed broader skills, such as working with international collaborators and

presenting at conferences. I am sure I will continue to draw upon these learnings throughout my career in aquatic animal health research.

Benefits and Adoption

AGD is an increasing issue for farmed fish (Nowak and Archibald 2018) so improved understanding of the disease will have broad, positive impact to fin-fish aquaculture. The wider aim of my project is to investigate whether *N. perurans* is the only causative agent of AGD in Atlantic salmon, and whether the virulence of AGD infection is influenced by other marine or freshwater amoebae species. By understanding the biology of all causal agents we could promote improvements to treatment regimes. Such findings will be of benefit to salmon aquaculture as well as participating research institutes.

Further Development

To further disseminate the findings, I plan to present at the next FRDC supported Aquatic Animal Health Conference in Cairns. I will also endeavour to maintain the connection with Iva Dyková and Tomáˇs Tyml.

The Aquatic Animal Health Training Scheme was great. Thank you for the opportunity, it will have lasting benefit. I will be sure to spread the word about your fantastic scheme.

References

Bermingham ML, Mulcahy MF. 2007. *Neoparamoeba* sp. and other protozoans on the gills of Atlantic salmon *Salmo salar* smolts in seawater. Dis. Aquat. Org. 76, 234–240. https://doi.org/10.3354/dao076231

Crosbie P, Bridle A, Cadoret K, Nowak B. 2012. In vitro cultured *Neoparamoeba perurans* causes amoebic gill disease in Atlantic salmon and fulfils Koch's postulates. Int. J. Parasitol. 42, 511–515. https://doi.org/10.1016/j.ijpara.2012.04.002

Dyková I, Kostka M. 2013. Illustrated guide to culture collection of free-living amoebae. Academia, Prague.

English CJ, Tyml T, Botwright NA, Barnes AC, Wynne JW, Lima PC, Cook MT. 2019. A diversity of amoebae colonise the gills of farmed Atlantic salmon (*Salmo salar*) with amoebic gill disease (AGD). Eur. J. Protistol. 67. https://doi.org/10.1016/j.ejop.2018.10.003

Howard T. 2001. Paramoebiasis of sea-farmed salmonids in Tasmania - a study of its aetiology, pathogenicity, and control. University of Tasmania.

Nowak BF, Archibald JM. 2018. Opportunistic but lethal: the mystery of paramoebae. Trends Parasitol. 34, 404–419. https://doi.org/10.1016/j.pt.2018.01.004

Page F. 1983. Marine gymnamoeba. The Lavenham Press, Great Britain.

Appendices

A few happy snaps