Short term lab placement in Aberdeen, UK

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PROJECT NO: 2009/751, Short term lab placement in Aberdeen, UK

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OBJECTIVES OF RESEARCH TRAVEL GRANT

To conduct a short term lab placement at the Marine Laboratories in Aberdeen to gain skills in new techniques including producing and maintaining primary cell cultures from gill explants and tissues.

NON TECHNICAL SUMMARY:

Following the short term lab placement in Aberdeen, I have developed techniques to isolate and culture gill cells from Atlantic salmon. To date, cultures have been maintained for up to 15 days. Ongoing work will involve further development of these techniques to enable isolation of high cell densities of gill cells. In addition adapting cell culture conditions to allow changes in salinity will facilitate the development of assays involving the agent of amoebic gill disease (AGD) in Atlantic salmon, the marine amoeba, *Neoparamoeba perurans*. This will in the longer term enable attachment assays to be developed, potentially leading to identifying attachment receptors that may be important in disease development.

OUTCOMES ACHIEVED TO DATE

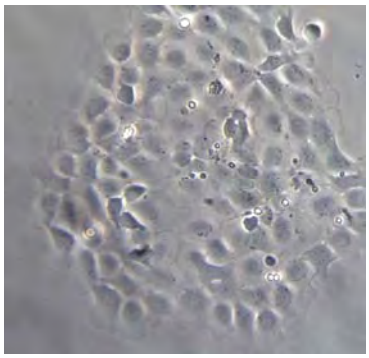
On return to CMAR Hobart in late October, I have refined a technique to obtain primary gill cell lines from Atlantic salmon gill tissue using trypsin digestion. To date, I have maintained several primary cultures of gill cells morphologically similar to epithelial cells for up to 15 days.

BACKGROUND AND NEED

There have been many years of research towards better understanding of AGD, including investigations in the development of prophylaxis and treatments for AGD of marine cultured Atlantic salmon in Tasmania. However better understanding of host-parasite interactions are required and in particular, knowledge of the mechanisms of attachment of the parasite to the gill tissue. Closing this knowledge gap may increase our ability to identify new candidate antigens that could be incorporated into a vaccine for AGD.

By undertaking a lab placement at the Marine Laboratories in Aberdeen, I aim to conduct research that will begin to fill this knowledge gap. During the placement I gained skills in the development of cell lines from gill explants for *in vitro* studies.

RESULTS



To date, cells from Atlantic salmon gill tissues have been cultured and maintained for up to 15 days. These cells appear morphologically similar to epithelial cells from fish gills (pictured). The current technique has been repeated, and improved on, with greater cell yields and healthier cells each time.

Further development of these techniques is needed to obtain preparations of higher cell density. This will potentially allow for assays to be developed and conducted within 14 days of cell isolation. Reducing the time gill cells are in culture before application in cell assays avoids changes that can occur to cells over time in culture affecting results.

EXTENSION ACTIVITIES

These techniques will be further developed to obtain highly reproducible results. It will be necessary in the future to characterise cell lines used for assays. In addition, the development of an in vitro attachment assay that may identify possible mechanisms of attachment of wild-type *Neoparamoeba* spp. to gill tissues is planned. Initially studies will involve testing cell viability with varying media salinity, and seeding cells to filter inserts where media salinity can be varied on either side of the filter insert. Attachment assays will incorporate wild-type isolates and cultured strains of *Neoparamoeba* spp., and pure cultures of *N. Perurans* that are currently being developed at the University of Tasmania may be utilised in attachment assays when available.

PROJECT OUTCOMES

The nature of this work does not pose an immediate change for the Atlantic salmon industry. However, ongoing development of this project may further our knowledge of host-pathogen interactions associated with AGD of Atlantic salmon and lead to identifying potential targets for disease mitigation.

ACKNOWLEDGEMENTS

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