The humoral immune response of *Lates calcarifer* to *Streptococcus iniae*

Thesis submitted by
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in September 2004

for the degree of Doctorate of Philosophy
in the School of Biomedical Sciences,
James Cook University
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ACKNOWLEDGEMENTS

When a PhD is undertaken, it just one person who achieves the final result, however many people lend a hand, or weight, to make this goal achievable. Without the support from people around me over the years this would have never been finished.

To my friend and mentor Leigh Owens. I am glad that many years ago I decided to try your 3rd year aquatic pathology course. This is despite the fact the 2nd year marine microbiology you taught was a struggle. You helped lead me into a field of research that I now truly love, and hope that I can continue in for many years. Oh and by the way, you are right most of the time! Especially finish your PhD before you get a job!

The many people in the department that have lent a hand to get things done, most notably James, Lisa, Brad, Andrew, many thanks for all your assistance. Many thanks must go to Helen Clifton who aided in the rearing of barramundi fingerlings and Laurie Reilly for his invaluable histology assistance. To Jan and Graham, your advice and support throughout my time at JCU was imperative to the success of this thesis.

Finally, I must thank the people who harassed me continuously to get this finished. Mum, Dad, Leigh, and Chantal; OK it’s done now! But most thanks must go to Chantal, who without her brilliant secretarial support, and constant nagging (joking…not), the thesis would have never been finished.
ABSTRACT

This study characterises various aspects of barramundi (*Lates calcarifer*) humoral immunity, including ontogeny, temperature modulation and kinetics following challenge with *Streptococcus iniae*. It was discovered that Staphylococcal protein A (SpA) was able to efficiently isolate antibody from serum, and that all barramundi Ig found in serum is tetrameric with a weight of approximately 800 kDa. This tetramer is composed of 8 heavy chains (72 kDa) and 8 light chains (28 kDa). Denaturing, non-reducing electrophoresis demonstrated differential disulfide polymerization (redox forms) of the tetrameric Ig which was consistent with those observed with other species. Polyclonal and monoclonal antibodies were produced against the protein A purified barramundi Ig, and various ELISA formats were developed. These serological tools were used to investigate aspects of barramundi humoral immunity.

Examination of ontogeny of humoral immunity, revealed that barramundi possess minimal maternal antibody (<10 µg/ml wet weight) post-hatch, which is depleted rapidly (within 3 days). By day 8 systemic Ig is able to be detected, which continues to increase over the following months. However, it is not until seven week post-hatch that barramundi fingerlings are able to mount a prolonged immune response following vaccination with *S. iniae*.

Environmental temperature was also found to significantly impact the ability of barramundi to respond to vaccination with *S. iniae*. Barramundi maintained at low temperatures (<23°C) displayed a diminished, delayed and highly variable humoral immune response following vaccination, with many of the experimental animals failing to respond to primary vaccination. These responses could be mediated by either administering a booster vaccine or by elevating the environmental temperature.

This study also demonstrated that there was a relationship with specific serum antibody and protection against *S. iniae*, with fish possessing high
levels of specific Ig being protected from lethal challenge, while those with low titres being more susceptible to disease. Specific antibody in barramundi could be generated through natural exposure to the bacterium from the environment or through vaccination. Thus bath vaccination of fish (50,000) held at two facilities resulted in elevated systemic antibody levels and lower observed mortality, when compared to the unvaccinated control fish.

Infections due to *S. iniae* were determined to be associated with elevated water temperatures. Laboratory trials and field data indicated that water temperatures between 24 and 28°C resulted in the highest barramundi mortality. A weak association was also determined with low pH and mortality, with fish exposed to low pH’s (<6.0) being more susceptible to infection. No association was observed with mortality and salinity.

Four monoclonal antibodies (Mab’s) were also generated against a 21 kDa protein from cell wall of *S. iniae*. The Mab’s displayed a high level of specificity for *S. iniae*, including those from Australia, Israel and America, and minimal cross-reactivity with other bacterial species tested. The Mab’s were used in an immunohistochemical study that confirmed the neurotropic nature of *S. iniae* infections, as well as demonstrating the presence of the bacterium in the intestine of infected fish.
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<tbody>
<tr>
<td>PBS</td>
<td>phosphate buffered saline</td>
</tr>
<tr>
<td>SDS PAGE</td>
<td>sodium dodecyl sulphate polyacrylamide gel electrophoresis</td>
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<tr>
<td>UV</td>
<td>ultraviolet</td>
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<td>ELISA</td>
<td>enzyme linked immunosorbent assay</td>
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<td>composite agarose-acrylamide gel electrophoresis</td>
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