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

Thesis submitted by
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ABSTRACT

This study characterises various aspects of barramundi (*Lates calcarifer*) humoral immunity, including ontogeny, temperature modulation and kinetics following challenge with Stre*ptococcus 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 (<23^0C) 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 28^0C 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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PBS	phosphate buffered saline
SDS PAGE	sodium dodecyl sulphate polyacrylamide gel electrophoresis
UV	ultraviolet
ELISA	enzyme linked immunosorbent assay
CAGE	composite agarose-acrylamide gel electrophoresis
PVDF	polyvinylidene flouride
ADH	arginine dihydrolase
VNN	viral nervous necrosis
BDS	bovine donor serum
FBS	fetal bovine serum
OPI	oxaloacetate-pyruvate-insulin
PEG	polyethylene glycol
Ig	Immunoglobulin
FIA	Freund's incomplete adjuvant
FCA	Freund's complete adjuvant
bIgM	barramundi immunoglobulin
IP	Intraperitoneal
IV	Intravenously
CCB	carbonate coating buffer
ABTS	2,2'-azino-di-(3-ethylbenzthiazoline-6-sulphonic acid)
DAB	3, 3'-diaminobenzidine tetrahydrochloride
AEC	amino-ethyl-carbozole
HRPO	horseradish perioxidase
PH	post-hatch
ppt	parts per thousand
ANOVA	Analysis of Variance
RPS	relative percentage survival
HAT	hypoxanthine-aminopterin-thymidine
HT	hypoxanthine-thymidine
DMSO	dimethyl sulphoxide
OD	optical density