Role of shock protein 70 (hsp70), ubiquitin and gill-associated virus in loss of production on prawn farms

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The research presented and reported in this thesis was conducted within the guidelines for research ethics outlined in the *National Statement on Ethics Conduct in Research Practice* (1997), the *James Cook University Policy on Experimentation Ethics, Standard Practices and Guidelines* (2001), and the *James Cook University Statement and Guidelines on Research Practice* (2001). The proposed research methodology received clearance from the James Cook University Experimentation Ethics Review Committee (approval number A925)

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After years of saying ‘I will never do a PhD’, here I am.
There are so many people I would like to thank I will have to be epigrammatic or I will end up with a 1000 page thesis, and I only want to write one page as I have already done the Table of Contents, so here I go.
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ABSTRACT

Shock protein 70 (hsp70), ubiquitin (Ub) and gill-associated virus (GAV) were chosen as bio-indicators in an attempt to determine if they could be used to predict production of *Penaeus monodon* on a farm. To investigate the response of these bio-indictors with respect to changes in environmental factors, an ELISA for Ub was developed and previously developed ELISAs for hsp70 and GAV were optimised.

The utility of the ELISAs with respect to farm conditions, changes in the expression of hsp70 and Ub relative to health status, transportation and laboratory-induced hypoosmotic stress in cultured *P. monodon* was investigated. Protein expression as determined by ELISA, showed samples from the high yield pond had significantly lower optical density for hsp70 and Ub than the low yield pond ($p<0.001$, $p<0.001$ respectively). Transport ($p<0.001$, $p<0.05$) and osmotically stressed ($p<0.001$, $p<0.001$) groups showed a significantly higher response for hsp70 and Ub when compared to the control group. These results indicated that further investigations using farm data were justified.

A trial was undertaken in collaboration with a commercial prawn farm who supplied all the environmental and production data for the trial period. Two investigations were undertaken using this data. The first was to investigate changes in the hsp70, Ub and GAV responses in relation to environmental factors. There were significant correlations between all factors, the greatest number were associated with hsp70 (22 significant correlation coefficients) followed by GAV (18 significant correlation coefficients) and then Ub (17 significant correlation coefficients). In general the correlations between bio-indicators were positive and the environmental factors showed mostly negative correlations with the bio-indicators.

To determine the biological significance of these interactions, correlation analysis was conducted for each bio-indicator and environmental factor for all ponds daily from six days prior to sampling up to and including the day of sampling. The major environmental factors identified were pH (am) and salinity (am).

Morning pH was negatively correlated to hsp70 at day of sampling and four days prior to sampling with a dramatic correlation coefficient increase at five and six days prior to sampling. A similar pattern was noted with Ub. Salinity (am) was negatively correlated to hsp70, Ub and GAV at all days.

Principal component analysis was used in an attempt to better understand the underlying factors that explained the correlations and to reduce the data necessary for farmers to monitor. Five components were produced. Component one consists of four factors; days in pond, salinity (am), hsp70 and GAV. Components two to five consists of two factors in each component being temperature (am and pm) in component two, secchi (am and pm) in component three, pH (am and pm) in component and DO (am) and Ub in component five. The total cumulative variance explained by the five components was 74.3%.

The next study investigated changes in the hsp70, Ub and GAV responses in relation to production factors. There were significant correlations between all factors, the greatest number were associated with hsp70 and GAV (28 significant correlation coefficients) and then Ub (10 significant correlation coefficients). In general, correlations between hsp70 and GAV and production factors were positive. Survival was the only exception with negative correlations for hsp70 and GAV.

Production factors and the bio-indicators were subjected to principal component analysis. Two components were produced. Component one consisted of five factors, being days in pond, average body weight, yield, hsp70 and food conversion ratio. Component two consisted of two factors, survival and Ub. The total cumulative variance explained for the two components was 61.7%.

Discriminant analysis was performed to determine 1) if the bio-indicators and environmental factors could be used to distinguish between specified production outcomes and 2) which factors contribute most to these outcomes. Production factors were separated into the mutually exclusive categories of < or > 70% survival and < or > 7 tonnes/hectare (yield).

Using am data only, the number of factors required to correctly classify greater than 70% of the ponds for both survival and yield was reduced to seven and five respectively.

Of these, the factors most important in distinguishing between the categories were days in pond, hsp70, salinity and to a lesser extent, dissolved oxygen. Using classification and regression tree analysis, decision trees were developed for the production factors. A maximum average body weight of 23.9 g was predicted if the minimum morning salinity can be kept below 38 ppt and the morning pH can be kept below 7.8. A minimum average body weight of 13.4 g was predicted if the minimum morning salinity was >= 38 ppt and the prawns had been in the pond for < 129.5 days. A maximum survival of 85% was predicted if the morning dissolved oxygen level can be kept at >= 4.4 ppm and the optical density of GAV is >= 0.191. A minimum survival of 50.8% was predicted if morning dissolved oxygen levels are < 4.4 ppm and the minimum evening salinity is <32.8 ppt. Food conversion ratio appears to be largely dependent on days in pond. The best food conversion ratio (1.43) was predicted if the prawns were in the pond < 113.5 days and the worst (1.8) was if the prawns had been in the pond between 113.5 and 136.5 days and the morning pH was >= 7.95. A maximum yield of 8.01 tonnes/hectare was predicted if the prawns had been in the pond for >= 166.5 days and the minimum was 3.11 tonnes/hectare at < 119 days in pond. Secchi and GAV also played a role in yield outcomes. A maximum biomass of 6650 kg/pond was predicted if morning salinity was kept below 40 ppt and the prawns were in the pond for >= 168 days and a minimum biomass of 2740 kg/pond was predicted is morning salinity was above 41.5 ppt.

It is concluded that hsp70 may be a useful indicator relating to transport stress, survival and yield of *P. monodon* in a commercial setting. The results presented here show the successful development of statistical models based on environmental factors for the prediction of production outcomes that are both practical and interpretable at farm level. Continued investigation and development of predictive methods for production outcomes and profitability associated with prawn farms is recommended.

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ABTS	2,2’-azino-di-(3-ethylbenzthiazoline-6-sulphonic acid)
ABW	Average body weight
APC	Antigen presenting cell
ATP	Adenosine 5'-triphosphate
B	Blank
BCA	Bicinchoninic acid
C	Control
CART	Classification and regression tree
CC	Correlation coefficient
CMV	Cytomegalovirus
CWE	Control with water exchange
DAB	3,3’,-diaminobenzidine tetrahydrochloride
DIP	Days in pond
DNA	Deoxyribonucleic acid
DO	Dissolved oxygen
DOS	Day of sampling
DUB	Deubiquitinating enzyme
EBNA-1	Epstein-Barr nuclear antigen 1
ELISA	Enzyme linked immunosorbent assay
FBS	Foetal bovine serum
FCR	Food conversion ratio
GAV	Gill-associated virus
HRPO	Horseradish peroxidase
HSE	Heat shock element
HSF	Heat shock protein transcription factor
hsp	Heat shock protein
HYP	High yield pond
JCU	James Cook University
LYP	Low yield pond
MAb	Monoclonal antibody
MARFU	Marine and Aquaculture Research Facility Unit

MHC	Major histocompatibility complex
MoV	Mourilyan virus
MSLP	Mean sea level pressure
MW	Molecular weight
NC	Negative control
OD	Optical density
OS	Osmotic stress
PAb	Polyclonal antibody
PBS	Phosphate buffered saline
PC	Positive control
PCR	Polymerase chain reaction
PG	Positive gill
ppm	Parts per million
ppt	Parts per thousand
PVDF	Polyvinylidene fluoride
RNA	Ribonucleic acid
RT	Room temperature
RT-nPCR	Reverse transcription polymerase chain reaction
SDS	Sodium dodecyl sulphate
SDS-PAGE	Sodium dodecyl sulphate-polyacrylamide gel electrophoresis
SP	Shock protein
SUMO1	Small ubiquitin-related modifier 1
t/ha	Tonnes per hectare
TP	Total protein
TS	Transport stress
Ub	Ubiquitin
UCRP	Ubiquitin cross-reactive protein
UV	Ultraviolet
WSSV	White spot syndrome virus
YHV	Yellow head virus