

JCU ePrints

This file is part of the following reference:

Elliott, Elisabeth (2008) *Role of shock protein 70 (hsp70), ubiquitin and gill-associated virus in loss of production on prawn farms.*
PhD thesis,
James Cook University.

Access to this file is available from:

<http://eprints.jcu.edu.au/5379>



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



<http://www.csiro.au/news/ps1pp.html>

© Copyright CSIRO Australia

Thesis submitted by

**ELISABETH ELLIOTT
BAppSc (Hons) JCU**

in March 2008

**for the degree of Doctorate of Philosophy
in the School of Veterinary and Biomedical Sciences,
Discipline of Microbiology and Immunology,
James Cook University
Townsville, Queensland
Australia**

STATEMENT OF ACCESS

I, the undersigned, author of this work, understand that James Cook University will make this thesis available for use within the University Library and, via the Australian Digital Theses network, for use elsewhere.

I understand that, as an unpublished work, a thesis has significant protection under the Copyright Act and I do not wish to place any further restriction on access to this work.

Signature

Date

ELECTRONIC COPY

I, the undersigned, the author of this work, declare that the electronic copy of this thesis provided to the James Cook University Library is an accurate copy of the print thesis submitted, within the limits of the technology available.

Signature

Date

STATEMENT OF SOURCES

DECLARATION

I declare that this thesis is my own work and has not been submitted in any form for another degree or diploma at any university or other institution of tertiary education. Information derived from the published or unpublished work of others has been acknowledged in the text and a list of references is given.

Signature

Date

DECLARATION ON ETHICS

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)

Signature

Date

ACKNOWLEDGEMENTS

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.

Firstly I would like to thank Pacific Reef Fisheries for allowing me onto their farm over several months and kindly donating prawns for the laboratory based trials. Thanks also to Daniel Zamykal for doing the CART analysis for me on very short notice and to James Munro for the use of his monoclonal antibodies for GAV.

I would like to thank Brenda and Ray for their support, help, advice, coffee and continued friendship without which I think I would have quit long ago. I would also like to thank Julie for her help with the setting up of the experimental system very early in my PhD and her assistance, both professionally and personally when I needed it.

Thanks also to my helpers during the farm trial including (not in any particular order) Kathy, Karen, Sarah, Ketheesan, Marshall, Leigh, Ray (the only person I have ever witnessed successfully catching prawns on the outside of the cast net) and my two fantastic kids, Michelle and Jason.

I was also fortunate enough to be surrounded by a group of extraordinary people who stimulated my mind and helped me out when insanity was looming. Thanks to Jen, Vicky, Jo, Kirsty, Jenny and especially Helen.

A special thanks to Jan who not only employed me during my PhD and allowed me time to complete it, but was also a great source of support during some personal perturbations. And to Leigh who thought up this investigation (using apocryphal ideas) and allowed me to begin this long and blustery journey.

And of course last but certainly not least, is my family. Thanks to Greg, Jason and Michelle for being supportive and encouraging even when my floccinaucinihilipilification of research was at its peak.

Thanks a googolplex everyone

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-indicators 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.

TABLE OF CONTENTS

STATEMENT OF ACCESS	I
ELECTRONIC COPY	II
STATEMENT OF SOURCES	III
DECLARATION ON ETHICS	IV
ACKNOWLEDGEMENTS	V
ABSTRACT	VI
TABLE OF CONTENTS	IX
LIST OF TABLES	XVII
LIST OF FIGURES	XX
LIST OF ABBREVIATIONS.....	XXIV
<i>CHAPTER 1</i>	1
GENERAL INTRODUCTION	1
<i>CHAPTER 2</i>	7
REVIEW OF SHOCK PROTEINS AND UBIQUITIN AND THE STRESS RESPONSE IN AQUATIC ANIMALS.....	7
2.1 Introduction	7
2.2 Disease in Penaeid Prawn Aquaculture	8
2.2.1 The immune response of penaeid prawns.....	9
2.3 Shock Proteins	13
2.3.1 Current knowledge on shock proteins	17
2.4 Aquatic Temperature Stress.....	19
2.4.1 Non-thermal stresses	22

2.5 Shock Proteins in Aquaculture	25
2.5.1 Shock proteins and disease	26
2.6 Osmotic Stress and Organic Osmolytes	31
2.7 Ubiquitin.....	33
2.7.1 The ubiquitin-proteasome pathway	34
2.7.2 Proteasomes	39
2.7.3 The ubiquitin-proteasome pathway and disease	40
2.7.4 Ubiquitin research in aquatic invertebrates.....	42
2.7.5 The role of the ubiquitin-proteasome pathway and apoptosis	47
2.8 Conclusion.....	49
CHAPTER 3.....	53
GENERAL MATERIALS AND METHODS	53
3.1 Introduction	53
3.2 Laboratory Trials	53
3.2.1 Experimental animals	53
3.2.2 Experimental system	53
3.2.3 Husbandry	55
3.3 Sample Collection and Preparation	56
3.3.1 Sample collection	56
3.3.1.1 Laboratory trial sample collection	56
3.3.1.2 Field trial sample collection.....	56
3.3.2 Sample preparation	57
3.4 Protein Estimations.....	57
3.4.1 Protein assay for bicinchoninic acid	57
3.5 Protein Electrophoresis	58

3.6 Western blots	59
3.6.1 General technique for protein transfer	59
3.6.2 Immunostaining of Western blots	60
CHAPTER 4	61
DEVELOPMENT OF AN ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA) FOR THE DETERMINATION OF UBIQUITIN (Ub) AND OPTIMISATION OF AN ELISA FOR THE DETECTION OF SHOCK PROTEIN 70 (HSP70) IN <i>PENAEUS MONODON</i>.	61
4.1 Introduction	61
4.2 Materials and Methods	65
4.2.1 Experimental animals	65
4.2.2 Sample collection and preparation	65
4.2.3 Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE)	65
4.2.4 Western blot	66
4.2.5 Antibodies.....	66
4.2.6 ELISA	67
4.2.6.1 Indirect ELISA.....	67
4.2.6.2 Assay validation	68
4.2.6.3 Microtitre plates investigated for shock protein ELISA.....	69
4.2.6.4 ELISA for detection of hsp70	69
4.2.6.5 Microtitre plates investigated for ubiquitin ELISA	72
4.2.6.6 ELISA for detection of ubiquitin.....	72
4.3 Results	73
4.3.1 SDS-PAGE.....	73
4.3.2 Western blotting	74
4.3.3 ELISA	75
4.3.3.1 Assay validation	75
4.3.3.2 Hsp70 checkerboard titration 1	77
4.3.3.3 Hsp70 titration 2.....	79
4.3.3.4 Ubiquitin checkerboard titration 1	80
4.3.3.5 Ubiquitin checkerboard titration 2	81

4.4 Discussion	83
CHAPTER 5.....	87
EFFECT OF TRANSPORTATION, DISEASE AND HYPOOSMOTIC STRESS ON HSP70 AND Ub EXPRESSION IN <i>PENAEUS MONODON</i> .	87
5.1 Introduction	87
5.2 Materials and Methods.....	90
5.2.1 Experimental animals	90
5.2.2 Experimental system	90
5.2.3 Experimental design	90
5.2.4 Disease stress	90
5.2.5 Transport stress	91
5.2.6 Hypoosmotic stress	92
5.2.7 Sample collection and preparation	92
5.2.8 ELISA	93
5.2.9 Determination of sample size	94
5.2.10 Statistical analysis	94
5.3 Results	95
5.3.1 Animal husbandry.....	95
5.3.2 Determination of sample size	96
5.3.3 Statistical analysis for hsp70 and Ub ELISAs.....	96
5.3.3.1 High and Low Yield ponds	96
5.3.3.2 Transport stress	97
5.3.3.3 Osmotic stress	97
5.4 Discussion	99

CHAPTER 6.....	104
FARM INVESTIGATION OF CHANGES IN HSP70, Ub AND GILL-ASSOCIATED VIRUS (GAV) EXPRESSION IN <i>PENAEUS MONODON</i> WITH RESPECT TO ENVIRONMENTAL FACTORS.	104
6.1 Introduction	104
6.2 Materials and Methods.....	107
6.2.1 Experimental animals	107
6.2.2 Experimental design	107
6.2.3 Sample collection	108
6.2.4 Sample preparation	108
6.2.5 Antibodies.....	109
6.2.6 ELISA	109
6.2.6.1 ELISA for detection of GAV	109
6.2.6.2 ELISA for detection of hsp70 and Ub.....	112
6.2.7 Statistical analysis	112
6.3 Results	113
6.3.1 Checkerboard titration for GAV	113
6.3.2 Statistical analysis for hsp70, Ub and GAV ELISAs	114
6.3.3 Correlation analysis.....	115
6.3.3.1 Correlation analysis for hsp70	118
6.3.3.2 Correlation analysis for ubiquitin.....	119
6.3.3.3 Correlation analysis for gill-associated virus	120
6.3.4 Principal component analysis	121
6.4 Discussion	123
CHAPTER 7.....	127
FARM INVESTIGATION OF CHANGES IN HSP70, Ub AND GAV EXPRESSION IN <i>PENAEUS MONODON</i> WITH RESPECT TO PRODUCTIVITY.	127
7.1 Introduction	127

7.2 Materials and Methods	129
7.2.1 ELISA.....	129
7.2.2 Statistical analysis.....	129
7.3 Results	130
7.3.1 Correlation analysis.....	130
7.3.2 Principal component analysis.....	133
7.3.3 Discriminant analysis.....	134
7.3.4 Predictive ability of discriminant analyses	139
7.4 Discussion	141
CHAPTER 8	146
USE OF CLASSIFICATION AND REGRESSION TREE (CART) ANALYSIS TO PREDICT PRODUCTION OUTCOMES IN CULTURED <i>PENAEUS MONODON</i> WITH RESPECT TO ENVIRONMENTAL FACTORS, HSP70, Ub AND GAV	146
8.1 Introduction	146
8.2 Materials and Methods	149
8.2.1 Statistical analysis.....	149
8.3 Results	150
8.3.1 CART analysis for average body weight.....	150
8.3.2 CART analysis for survival	151
8.3.3 CART analysis for FCR	153
8.3.4 CART analysis for yield	155
8.3.5 CART analysis for biomass	157
8.3.6 CART analysis for growth.....	159
8.4 Discussion	159

CHAPTER 9.....	163
GENERAL DISCUSSION AND CONCLUSIONS	163
REFERENCES	171
APPENDIX 1: Major stress protein families of multicellular eukaryotes.....	191
APPENDIX 2: Major stress protein families and their expression in aquatic animals during times of environmental stress.	192
APPENDIX 3: Aerial view of penaeid culture.....	195
APPENDIX 4: ELISA protocol for the detection of hsp70 and ubiquitin in <i>Penaeus monodon</i>.....	196
APPENDIX 5: ELISA protocol for the detection of gill-associated virus in <i>Penaeus monodon</i>.....	197
APPENDIX 6: Line graphs for hsp70, ubiquitin and gill-associated virus. .	198
APPENDIX 7: Significant correlation coefficients.	201
APPENDIX 8: Principal component analysis: Environment.	202
APPENDIX 9: Principal component analysis: Production.	204
APPENDIX 10: Discriminant analysis: Survival.	205
APPENDIX 11 Discriminant analysis: Yield.	206
APPENDIX 12: Discriminant analysis: Average body weight.	207
APPENDIX 13: Discriminant analysis: Survival 3 weeks prior to harvest. .	208

APPENDIX 14: Discriminant analysis: Yield 3 weeks prior to harvest.	209
APPENDIX 15: CART analysis: Average body weight.	210
APPENDIX 16: CART analysis: Survival.	211
APPENDIX 17: CART analysis: Food conversion ratio.	212
APPENDIX 18: CART analysis: Yield.	213
APPENDIX 19: CART analysis: Biomass.	214
APPENDIX 20: CART analysis: Growth.	215

LIST OF TABLES

Table 2.1: Methods for the control of environmental stressors known to produce shock proteins on aquaculture farms.....	29
Table 2.2: Identified components for environmentally triggered apoptosis pathway in penaeid prawns 'accommodating' active viral pathogens. Modified from Owens (1999).	30
Table 4.1: Description of the monoclonal antibodies (MAb) used in the development and optimisation of an ELISA for the detection of ubiquitin and hsp70 in <i>P. monodon</i>	67
Table 4.2: Summary of the average OD recorded from the hsp70 checkerboard titration. The positive control sample was in its reduced form, with blocking buffer. MAb conjugate at 1/1000 dilution. Displayed times represent the hour after the addition of ABTS. The highest acceptable OD recorded is highlighted.	77
Table 4.3: Summary of the average OD recorded from the hsp70 checkerboard titration 1. The positive control sample was in its reduced form, with blocking buffer. MAb conjugate at 1/2000 dilution. Displayed times represent the hour after the addition of ABTS.....	77
Table 4.4: Summary of the average optical density recorded from the Ub checkerboard titration 1. The positive control sample was in its reduced form, with blocking buffer. MAb conjugate was at 1/2000 dilution. Displayed times represent the hour after the addition of ABTS. The highest OD recorded is highlighted.	80
Table 4.5: Summary of the average optical density (OD) recorded from the Ub checkerboard titration 2. Displayed times represent the hour after the addition of ABTS. The highest OD using a Ub MAb dilution of 1/2000 and conjugate dilution 1/1000 between the ABTS incubation times of four and eight hours.....	82
Table 6.1: Summary of ponds sampled weekly. Pond numbers were assigned according to sampling regime. DIP = days in pond.....	107
Table 6.2: Significant correlation coefficients for hsp70, Ub and GAV with environmental factors for individual ponds (grouped weekly). Coloured numbers represent individual ponds. Bold numbers represent: 1) number of significant correlations, 2) positive and negative average correlation coefficients (calculated separately). DO = dissolved oxygen	117
Table 6.3: Component matrix generated from average weekly data using principal component analysis for hsp70, Ub, GAV and environmental factors. Output produced five components incorporating all factors except dissolved oxygen am. Only correlations above 0.5 are included in the analysis. Salinity pm was excluded from the analysis.	122
Table 6.4: Total variance explained from average weekly data using principal component analysis for hsp70, Ub, GAV and environmental factors from five components. Salinity pm was excluded from the analysis.	122
Table 7.1: Summary of the production factors and categories used in the discriminant analysis. t/ha = tonnes per hectare	130

Table 7.2: Significant correlation coefficients for hsp70, Ub and GAV with production factors for individual ponds (grouped weekly). Coloured numbers represent individual ponds. Bold numbers represent: 1) number of significant correlations, 2) positive and negative average correlation coefficients (calculated separately). Shaded cells indicate factors showing seven ponds or more with significant correlation coefficients.	131
Table 7.3: Summary of production factors with significant correlations for hsp70, Ub, GAV within individual ponds. ABW = average body weight, FCR = food conversion ratio	132
Table 7.4: Total variance explained from weekly data using principal component analysis for hsp70, Ub, GAV and production factors from two components (n=88 for each factor).	133
Table 7.5: Summary of the component matrix generated using principal component analysis for hsp70, Ub, GAV and production factors. Output produced two components: Component one consisting of days in pond (DIP), average body weight (ABW), yield, hsp70, food conversion ratio (FCR) and Component two consisting of survival and Ub. Only correlations above 0.5 are shown.....	134
Table 7.6: Summary of the results of discriminant analysis of production factors verses bio-indicators and environmental factors (am and pm grouped) (n=88 for each factor) for all ponds over all weeks. In cross-validation, each pond is classified by the functions derived from all ponds other than that pond. Temp = water temperature, ABW = average body weight, DIP = days in pond, DO = dissolved oxygen.	135
Table 7.7: Standardized canonical discriminant function coefficients generated by discriminant analysis of survival and yield verses bio-indicators and environmental factors. Factors with coefficients greater than 0.5 in bold. Temp = water temperature, DIP = days in pond, DO = dissolved oxygen.....	137
Table 7.8: Summary of the results of discriminant analysis of production factors verses bio-indicators and environmental factors (am and pm separate) (n=88 for each factor) for all ponds over all weeks. In cross-validation, each pond is classified by the functions derived from all ponds other than that pond. Temp = water temperature, ABW = average body weight, DIP = days in pond, DO = dissolved oxygen.	138
Table 7.9: Standardized canonical discriminant function coefficients generated by discriminant analysis of survival and yield verses bio-indicators and environmental factors (am and pm). Factors with coefficients greater than 0.5 in bold. Temp = water temperature, DIP = days in pond, DO = dissolved oxygen.	139
Table 7.10: Summary of the results of discriminant analysis for survival and yield verses bio-indicators and environmental factors (am data only) (n=56 for each factor) using data including all weeks up to three weeks before harvest. In cross-validation, each pond is classified by the functions derived from all ponds other than that pond. Temp = water temperature, DIP = days in pond, DO = dissolved oxygen. ...	140

Table 7.11: Standardized canonical discriminant function coefficients generated by discriminant analysis of survival and yield verses bio-indicators and environmental factors (am data only) (n=56 for each factor) using data including all weeks up to three weeks before harvest. Factors with coefficients greater than 0.5 in bold. Temp = water temperature, DIP = days in pond, DO = dissolved oxygen. ... 141

LIST OF FIGURES

- Figure 2.1: Model of the regulation of HSF1 in intertidal mussels and marine snails (modified from Buckley et al., 2001; Tomanek and Somero, 2002) resulting in the synthesis of shock proteins. Under non-stressful conditions the inactive HSF1 monomer is found in the cytosol and is associated with a complex that consists of at least hsp70, hsp90 (in mussels and snails) and hsp40 (in snails). When thermally stressed the shock proteins disassociate from the complex and bind to proteins that have lost their conformational structure. The free HSF1 monomers move into the nucleus and bind to the HSE where they become hyper-phosphorylated to enable transcription. With the increase of shock protein production their binding to the HSF1 triggers its dissociation from the HSE thus leading to an inhibition in shock protein transcription..... 16
- Figure 2.2: The ubiquitin-proteasome pathway. (1) Initially Ub is activated by E1 (using ATP). (2) The activated Ub is transferred to a member of the E2 family. (3) Ub is then transferred to a substrate-specific member of the E3 family. (4) A substrate-E3 complex is formed and through successive reactions, a polyubiquitin chain is synthesised. (5) The polyubiquitinated substrate binds to the Ub receptor subunit in the 19S complex of the 26S proteasome. The substrate is then degraded by the 20S complex resulting in short peptides. (6) Ub is made available to the system again by the action of isopeptidases. 36
- Figure 2.3: The proposed hierarchical organisation of the Ub conjugating system. A single E1 enzyme activates a Ub molecule and transfers it to one of several E2 enzymes. In some cases the E2 enzyme is E3 specific however, in most instances an E2 enzyme transfers Ub to one of several E3 enzymes. E3 enzymes can be either substrate specific or can recognise several substrates..... 36
- Figure 3.1: Holding and experimental system showing one complete module consisting of nine, 50L tanks, eight tanks for holding animals and one tank used as a biological filter (bottom right tank). 54
- Figure 4.1: Schematic representation of an indirect ELISA. Sample is bound to the well surface. Monoclonal mouse anti-human antibody binds to antigen. Goat anti-mouse conjugate binds to the mouse MAb and with the addition of the chromogenic substrate, ABTS, a green colour will form with an intensity related to the amount of bound conjugate and as such a measure of antigen can be determined. 68
- Figure 4.2: Experimental design for the development of the indirect ELISA for the detection of a SP in *P. monodon*. All procedures were replicated on the IWAKI and MaxiSorp plates. 'B' = blank control wells, FBS = foetal bovine serum, TP = total protein concentration. 70
- Figure 4.3: Checkerboard titration 1 for the development of the indirect ELISA for the detection of ubiquitin in *P. monodon*. Goat α mouse conjugate was used at 1/2000. All procedures were replicated on both the IWAKI and PolySorp microtitre plates. TP = total protein concentration..... 73

Figure 4.4: Protein profile of <i>P. monodon</i> positive control (PC), control (C) and hypoosmotically stressed (OS) pleopod samples as shown by a pre-cast tris-tricine Gradipure stained gradient gel (10-20%). Arrows indicate down-regulation of bands at 170 kDa and 100 kDa in the OS sample when compared to the C sample and additional bands at 130 kDa and 14 kDa in the PC sample. Molecular weight (MW) markers are in kDa.....	74
Figure 4.5: Western blot of hypoosmotically stressed <i>P. monodon</i> pleopod (OS) and positive control (PC) samples. Blot was incubated with the Ub MAb (2µg/ml), hsp70 MAb (1/5000) and conjugate (1/500). Arrows indicated immuno-reactive bands at 72 kDa (hsp70) and 10 kDa (Ub) in both samples. An additional band is visible at 170 kDa in the PC sample only. Molecular weight (MW) markers are in kDa. This combined blot was produced for photographic purposes only.....	75
Figure 4.6: Standard concentration curve for hsp70 using <i>P. monodon</i> positive control (PC) sample (pereopod origin) and two random samples termed sample 1 and sample 2 (pleopod origin). All data based on average OD.	76
Figure 4.7: Standard concentration curve for ubiquitin using <i>P. monodon</i> positive control (PC) sample (pereopod origin) and two random samples termed sample 1 and sample 2 (pleopod origin). All data based on average OD.	76
Figure 4.8: Checkerboard titration 2 for the optimisation of the ABTS incubation time using the ELISA for the detection of hsp70 in <i>P. monodon</i> . All data based on average optical density of 414, 492nm.	79
Figure 4.9: Checkerboard titration 2 for the optimisation of the ABTS incubation time using the ELISA for the detection of Ub in <i>P. monodon</i> . All data based on average optical density of 414, 492nm.	83
Figure 5.1: Schematic representation demonstrating the sampling scheme associated with the different stress treatments. Prawns from two ponds were sampled in situ (high yield and low yield ponds). Additional prawns from the high yield pond were transported to JCU where 14 were sampled immediately. The remaining prawns were placed into the experimental system for an acclimation period. During this time samples were taken and used for the development and optimisation of the hsp70 and Ub ELISAs. Remaining prawns were used later in the osmotic stress trial.	91
Figure 5.2: Schematic representation showing the experimental design associated with the osmotic stress trial. Prawns were separated into three treatment groups being osmotic stress, control and control with water exchange.....	93
Figure 5.3: Plot of <i>P. monodon</i> mortalities and moults per day during the 22 days prior to the osmotic stress trial. Points represent the combined mortalities and moults found from all tanks in the system.	95
Figure 5.4: Plot of average hsp70 and Ub OD in pleopod samples as determined by ELISA in high yield pond <i>P. monodon</i> (n=44). Absorbance measured at 414 and 492nm.	96

Figure 5.5: Comparison of average hsp70 and ubiquitin (Ub) OD in pleopods of *P. monodon* as determined by ELISA in a high yield pond, and a low yield pond. Same letters above each column indicate no significant difference between treatments (+/- SE). All treatments n=44. Optical density measured at 414 and 492nm. 98

Figure 5.6: Comparison of average hsp70 and ubiquitin (Ub) OD in pleopod of *P. monodon* as determined by ELISAs in high yield pond, and transport stressed samples. Same letters above each column indicate no significant difference between treatments (+/- SE). High yield pond n=44, Transport stress n=14. Optical density measured at 414 and 492nm. 98

Figure 5.7: Comparison of average hsp70 and ubiquitin (Ub) OD in pleopod of *P. monodon* as determined by ELISAs in high yield pond, control, control water exchange and osmotic stressed samples. Same letters above each column indicate no significant difference between treatments (+/- SE). High yield pond n=44; control, control water exchange and hypoosmotic stressed n=28. Optical density measured at 414 and 492nm. 99

Figure 6.1: Checkerboard titration design for the optimisation of the ELISA for the detection of gill-associated virus (GAV) in *P. monodon*. All procedures were replicated for each of the three monoclonal antibodies tested. S1 = sample 1, S2 = sample 2, PC = positive control (pooled pereopods), PG= positive control obtained from RT-nPCR positive *P. monodon* gills at 1/128, NC = negative control obtained from RT-nPCR negative *P. merguensis* gills at 1/128, B = blank control, TP = total protein concentration per well..... 110

Figure 6.2: Optical densities recorded from the GAV MAb (3A-4) checkerboard titration. GAV PAb used at 1/80, MAb conjugate at 1/1000. S1 = sample 1, S2 = sample 2, PC = positive control (pooled pereopods), PG= positive control obtained from RT-nPCR positive *P. monodon* gills at 1/128, NC = negative control obtained from RT-nPCR negative *P. merguensis* gills at 1/128, NS = no sample, B = blank control, TP = total protein concentration per well..... 113

Figure 6.3: Histogram generated from the GAV ELISA results for Pond 3 illustrating the change in the frequency of OD across weeks. A similar trend was noted across all ponds..... 116

Figure 6.4: Plot of significant correlation coefficients showing correlations between hsp70 and the environmental factors water temperature (temperature am), salinity (am) and pH (am) daily from six days prior to sampling to the day of sampling (DOS)..... 118

Figure 6.5: Plot of significant correlation coefficients showing correlations between ubiquitin and the environmental factors salinity (am) and pH (am and pm) daily from six days prior to sampling to the day of sampling (DOS)..... 119

Figure 6.6: Plot of significant correlation coefficients showing correlations between gill-associated virus and salinity (am) daily from six days prior to sampling to the day of sampling (DOS)..... 120

Figure 8.1: Plot of apparent and cross-validated R-squared values verses number of splits (tree size) for average body weight. 150

Figure 8.2: Tree yielding the minimum cross-validated error rate for predicting average body weight.....	151
Figure 8.3: Plot of apparent and cross-validated R-squared values verses number of splits (tree size) for survival.	152
Figure 8.4: Tree yielding the minimum cross-validated error rate for predicting survival.	153
Figure 8.5: Plot of apparent and cross-validated R-squared values verses number of splits (tree size) for food conversion ratio.	154
Figure 8.6: Tree yielding the minimum cross-validated error rate for predicting food conversion ratio.	155
Figure 8.7: Plot of apparent and cross-validated R-squared values verses number of splits (tree size) for yield.	156
Figure 8.8: Tree yielding the minimum cross-validated error rate for predicting yield.	156
Figure 8.9: Plot of apparent and cross-validated R-squared values verses number of splits (tree size) for biomass.	158
Figure 8.10: Tree yielding the minimum cross-validated error rate for predicting biomass.	158

LIST OF ABBREVIATIONS

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