THE LYMPHOID ORGAN IN PENAEIDS AND ITS INTERACTION WITH MOULTING

Thesis submitted by

R U S A I N I

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School of Veterinary and Biomedical Sciences
James Cook University
Townsville, northern Queensland
Australia

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The research presented and reported in this thesis was conducted within the guidelines for research ethics outline in the *National Statement on Ethics Conduct in Research Involving Human* (1999), the *Joint NHMRC/AVCC Statement and Guidelines on 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 A956).

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DEDICATION

In the name of Allah, the compassionate, the merciful
Praise be to Allah, Lord of the universe
Peace and prayers be upon His final prophet and messenger

Glory to Allah, who created in pairs all things that the earth produces, as well as their own (human) kind and (other) things of which they have no knowledge.
And a sign for them is the night: We withdraw there from the day, and behold they are plunged in darkness.
And the sun runs its course for a period determined, for it that is the decree of (Him), the exalted in might, the all knowing.
And the moon,, We have measured for it, mansions (to traverse) till it returns like the old (and withered) lower part of a date stalk.
It is not permitted to the sun to catch up the moon, nor can the night outstrip the day, each (just) swims along in (its own) orbit (according to law). (The Holy Qur'an, 36: 36 – 40).

If there is
any goodness in this work,
I would dedicate it to
my beloved Mum and Dad,
and my lovely brother and sisters.

ABSTRACT

Prawn immunity is still a mysterious puzzle in immunology. However, this knowledge is important in culture management in order to avoid the devastating impact of infectious pathogens and economic losses. Furthermore, since the effectiveness of vaccination and immunostimulants is unclear, the enhancement of immune capability of prawns might provide a bright light to this industry. Therefore, the objective of this thesis was to develop a simple modified method of quantifying the histopathological changes of a component of the lymphoid organ (LO), the spheroid cells, and apply this technique in a study of the influence of moult cycle, lunar rhythm, and viral infections on spheroid cells quantification.

Moult cycles of *P. monodon* were studied by using setal development (setogenesis) and retraction of epidermis from the setal bases (apolysis) in the inner uropod adjacent to the telson tip. Five stages and four substages of the moult i.e. postmoult (stage A and B), intermoult (stage C), and premoult (stage D0, D1, D2 and D3/D4) and ecdysis (stage E) could be determined by applying these two criteria. However, unsynchronised development of these two criteria in abnormal prawns led to the difficulties in differentiating between stage B, C and D.

A modified transect technique seemed to offer a simple, rapid, and accurate method in analysing the abundance of spheroid cells in the lymphoid organ. Furthermore, one half longitudinal section of the cephalothorax represented the abundance of the spheroid cells in the lymphoid organ of penaeid prawns. Based on this technique, the fluctuation of the lymphoid organ spheroid (LOS) cells during the life of *P.monodon* was investigated. It was found that animal size (weight and total length) had no significant effect ($P > 0.05$; ANOVA) on the spheroid to total tissue (STT) ratio, the prevalence of vacuolated spheroids and the number of vacuoles in the spheroid cells.

Unfortunately, the cyclic phenomena of the prawn's, moult cycle also showed no significant effect on any measure of spheroid cells ($P > 0.05$) rejecting the original hypothesis of this work. The effect of the prawn's sex was variably related to the spheroid cells during the experiments. In the first two trials (Chapter 5 and 6), evidently sex had no significant effect on any measure of spheroid cells ($P > 0.05$).

However, in the last experiment (Chapter 7) female bias on the ratio of STT was obvious ($P < 0.05$).

Lunar related patterns on the spheroid to total tissue ratio were evident during the experiments. It was found that the STT ratio was significantly lower at new moon than first quarter and full moons ($P < 0.05$). This indicated that increased activity of the prawns during the dark moon enhanced immunocompetence of the prawns to eliminate viral diseases. Moreover, apparently, the STT ratio of GAV-injected prawns was significantly higher than control prawns ($P < 0.05$). Together with this, the presence of distinct bacterial granulomas in the lymphoid organ implied that the formation of the spheroid cells in the haemal sinuses of the lymphoid organ was only associated with viral diseases not with bacterial infections.

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LIST OF ABBREVIATION

AMP	Anti-microbial peptide
BGBP	Beta-glucan binding protein
CL	Carapace length
CP	Clotting protein
CPUE	Catch per unit of effort
DHC	Differentiated haemocyte count
DNA	Deoxyribonucleic acid
dpi	Day post-injection
EST	Expressed sequence tag
GAV	Gill associated virus
HDL	High density lipoprotein
HH	Hyaline haemocyte
HLF	Haemocyte lysate fraction
HLS	Haemocyte lysate supernatant
hpi	Hour post-injection
HPLC	High performance liquid chromatography
HPT	Haematopoietic tissue
HST	Head soft tissue
ICC	Immunocytochemistry
ISH	*In situ* hybridization
KBr	Potassium bromide
kDa	kiloDalton
LGBP	Lipopolysaccharide and β-1,3-glucan-binding protein
LGH	Large granular haemocyte
LM	Light microscope
LO	Lymphoid organ
LOS	Lymphoid organ spheroid
LOV	Lymphoid organ virus
LOVV	Lymphoid organ vacuolization virus
LPS	Lipopolysaccharides
LPV	Lymphoidal parvovirus

LSD	Least significant difference
LSNV	Laem-Singh virus
MCMS	Midcrop mortality syndrome
ME	Mercaptoethanol
MSGS	Monodon slow growth syndrome
PG	Peptidoglycan
pH	Puissance d'hydrogene
pI	Isoelectric point
PNR	Peripheral neuropathy and retinopathy
PO	Phenoloxidase
ppA	Prophenoloxidase activating enzyme
proPO	Prophenoloxidase
proppA	Pro-form of prophenoloxidase activating enzyme
RBC	Red blood cell
rER	Rough endoplasmic reticulum
RNA	Ribonucleic acid
ROI	Reactive oxygen intermediate
RV-PJ	Rod-shaped nuclear virus of *Penaeus japonicus*
SDS-PAGE	Sodium dodecyl sulphate polyacrylamide gel electrophoresis
SGH	Small granular haemocyte
SPF	Specific pathogen free
$TCID_{50}$	50% tissue culture infective dose
TEM	Transmission electron microscopy
TGase	Transglutaminase
THC	Total haemocyte count
TMMS	Three main moult stages
TSV	Taura syndrome virus
TUNEL	Terminal deoxynucleotidyl transferase (TdT) – mediated dUTP Nick-End Labelling
VHDL	Very high density lipoprotein
VTG	Vitellologenin
WSBV	White spot associated baculovirus
WSSV	White spot syndrome virus
YHV	Yellow head virus