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**THE LYMPHOID ORGAN IN PENAEIDS AND  
ITS INTERACTION WITH MOULTING**

Thesis submitted by

**R U S A I N I**

in July 2006

**for the degree of Master of Science in  
Microbiology and Immunology  
School of Veterinary and Biomedical Sciences  
James Cook University  
Townsville, northern Queensland  
Australia**

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## DECLARATION OF ETHICS

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).

Signature: Rusaini

Date: July 2006

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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).*

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*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.

## TABLE OF CONTENTS

	Page
STATEMENT OF ACCESS	ii
DECLARATION	iii
ELECTRONIC COPY	iv
DECLARATION OF ETHICS	v
ACKNOWLEDGMENTS	vi
DEDICATION	viii
ABSTRACT	ix
TABLE OF CONTENTS	xi
LIST OF TABLES	xv
LIST OF FIGURES	xviii
LIST OF ABBREVIATION	xxv
CHAPTER 1: GENERAL INTRODUCTION	1
1.1. Overview	1
1.2. Hypothesis	4
1.3. Research Aims	5
CHAPTER 2: REVIEW OF LITERATURE	6
2.1. Introduction	6
2.2. Immunity	6
2.2.1. Haemocytes	6
2.2.2. Haematopoiesis	11
2.2.3. Clotting and wound healing	13
2.2.4. Prophenoloxidase (proPO) activating system	15
2.2.5. Beta-glucan binding protein (BGBP)	18
2.2.6. Penaeidins	19
2.2.7. Lectins/agglutinins	22
2.2.8. Phagocytosis	24

2.2.9.	Encapsulation	25
2.2.10.	Nodule formation	26
2.2.11.	Cytotoxicity	27
2.2.12.	Apoptosis	27
2.2.13.	Conclusion	30
2.3.	Lymphoid Organ as Part of the Immune System	31
2.3.1.	Structure of the lymphoid organ	31
2.3.2.	The structure of the lymphoid organ spheroid cells	34
2.3.3.	The role of the lymphoid organ	37
2.3.4.	Diseases associated with the lymphoid organ	38
2.3.5.	Conclusion	39
2.4.	Moulting as It Interacts with the Immune System	40
2.4.1.	Moult staging	40
2.4.2.	Moult cycle duration	42
2.4.3.	Moulting and prawn immunity	44
2.4.4.	Conclusion	45
2.5.	General Conclusion	46
CHAPTER 3. GENERALS MATERIAL AND METHODS		47
3.1.	Experimental Animals	47
3.2.	Weight and Total Length of Prawn	48
3.3.	Moult Staging	48
3.4.	Histology	48
3.4.1.	Tissue fixation	48
3.4.2.	Tissue embedding and cutting	49
3.4.3.	Haematoxylin and eosin staining	49
3.5.	Analysis of the Lymphoid Organ	49
3.6.	Data Analysis	50
CHAPTER 4: VALIDATION OF QUANTITATIVE ANALYSIS OF THE LOS CELLS IN PRAWNS		51
4.1.	Introduction	51
4.2.	Materials and Methods	52
4.2.1.	Experimental animals	52
4.2.2.	Analysis of the lymphoid organ	53

4.2.3. Data Analysis	54
4.3. Results	54
4.4. Discussion	56
CHAPTER 5: THE RELATIONSHIP BETWEEN THE LOS CELLS AND MOULTING STAGES IN PRAWNS IN AQUARIA	59
5.1. Introduction	59
5.2. Materials and Methods	61
5.2.1. Experimental animals	61
5.2.2. Moulting staging	61
5.2.3. Histology	62
5.2.4. Analysis of the lymphoid organ	62
5.2.5. Data analysis	62
5.3. Results	63
5.3.1. Moulting staging	63
5.3.2. Moulting stages and lunar phases	68
5.3.3. The abundance of the LOS cells	70
5.4. Discussion	83
5.4.1. Moulting staging	83
5.4.2. The lymphoid organ spheroid cells	88
CHAPTER 6: THE RELATIONSHIP BETWEEN THE LOS CELLS AND MOULTING STAGES IN PRAWNS FROM COMMERCIAL FARMS	94
6.1. Introduction	94
6.2. Material and Methods	96
6.2.1. Experimental animals	96
6.2.2. Sampling procedures	96
6.2.3. Moulting staging	96
6.2.4. Histology	97
6.2.5. Data analysis	97
6.3. Results	97
6.3.1. Moulting stages and lunar phases	97
6.3.2. Moulting stages and the LOS cells	90
6.3.3. Lunar phases and the LOS cells	105
6.4. Discussion	110

CHAPTER 7: EFFECT OF VIRAL INFECTION ON THE RELATIONSHIP BETWEEN THE LOS CELLS AND MOULTING STAGES	117
7.1. Introduction	117
7.2. Materials and Methods	118
7.2.1. Experimental animals	118
7.2.2. Viral extraction	119
7.2.3. Viral injection	119
7.2.4. Sampling procedure and moult staging	119
7.2.5. Data analysis	120
7.3. Results	120
7.3.1. Clinical signs and histopathology	120
7.3.2. Moult stages and lunar phases	125
7.3.3. The abundance of the LOS cells	126
7.4. Discussion	132
CHAPTER 8: GENERAL DISCUSSION	137
REFERENCES	147
APPENDICES	164

## LIST OF TABLES

	Page
<b>Table 2.1.</b> Differentiation of haemocyte types in crustaceans (adapted from Van de Braak <i>et al.</i> , 1996).	8
<b>Table 2.2.</b> Differentiation of haemocyte types involved in immune reactivity in crustaceans.	10
<b>Table 2.3.</b> Criteria for the moult staging of <i>P. esculentus</i> based on setal development (Smith & Dall, 1985).	41
<b>Table 4.1.</b> The spheroid to total tissue (STT) ratio of <i>P. merguensis</i> (n = 10), using every division and every tenth division of the scale of the graduated eyepiece.	55
<b>Table 4.2.</b> Chi-square test of randomly selected slides ( $\chi^2_{0.05,9} = 16.919$ ) of <i>P. merguensis</i> . The red values represent when the Chi-squared was significantly different.	56
<b>Table 5.1.</b> Moulting (ecdysis) of three control <i>P. monodon</i> at four consecutive lunar phases throughout the experiments. Different colours represents the time of the ecdysis of different animals and number in the same column is the moulting period of prawn from one period to another period. There were three prawns in third control in the first experiment due to replacement of prawns that jumped out of the tanks. FM, full moon; LQM, last quarter moon; NM, new moon; FQM, first quarter moon.	68
<b>Table 5.2.</b> The percentage of moulting (ecdysis) of <i>P. monodon</i> in the first and second experiments at four lunar phases. NM, new moon; FQM, first quarter moon; FM, full moon; LQM, last quarter moon.	69



<b>Table 5.3.</b> The percentage of <i>P. monodon</i> sampled at different moult stages at four lunar phases in the first experiment. NM, new moon; FQM, first quarter moon; FM, full moon; LQM, last quarter moon.	70
<b>Table 5.4.</b> The percentage of <i>P. monodon</i> sampled at different moult stages at four lunar phases in the second experiment. NM, new moon; FQM, first quarter moon; FM, full moon; LQM, last quarter moon.	70
<b>Table 5.5.</b> F values of main effects on the STT ratio of <i>P. monodon</i> in the first and second experiments.	72
<b>Table 5.6.</b> Moult related changes in the immunological and haematological components of prawns.	90
<b>Table 6.1.</b> The number and the moult stages of <i>P. monodon</i> collected at different lunar phases in the first experiment.	98
<b>Table 6.2.</b> The number and the moult stages of <i>P. monodon</i> collected at different lunar phases in the second experiment.	98
<b>Table 6.3.</b> Significant differences (P values) of the STT ratio of <i>P. monodon</i> at different lunar phases in the first experiment.	108
<b>Table 6.4.</b> Significant differences (P values) of the number of vacuoles in spheroids of <i>P. monodon</i> at different lunar phases in the second experiment.	108
<b>Table 7.1.</b> The percentage of moulting (ecdysis) of <i>P. monodon</i> at four lunar phases. NM, new moon; FQM, first quarter moon; FM, full moon; LQM, last quarter moon.	125
<b>Table 7.2.</b> The percentage of <i>P. monodon</i> sampled at different moult stages at four lunar phases. NM, new moon; FQM, first quarter moon; FM, full moon; LQM, last quarter moon.	125

**Table 8.1.** Summary of significant effect (F values) of lunar phases and its interaction with the other variables on the STT ratio of *P. monodon* throughout the experiments.

## LIST OF FIGURES

	Page
<p><b>Figure 1.1.</b> The hypothetical association between the lymphoid organ spheroid cells and moulting stages in penaeid prawns. The ratio of the spheroids to total tissue (STT) increases with progression through the moulting stages. During the ecdysis, the lymphoid organ spheroid (LOS) cells are disposed of.</p>	5
<p><b>Figure 2.1.</b> Open circulatory system of prawn. The vascular system (blue) was continuous with the haemolymph sinuses and irrigated the body cavity and the sites of haematopoiesis. The hematopoietic tissue consisted of densely packed lobules (grey), haematopoietic antennal lobules (Hal), haematopoietic dorsal lobules (Hdl), haematopoietic ventral lobules (Hvl) throughout the prawn's anterior region. LO, lymphoid organ; H, heart; Vs, vascular system; Hp, hepatopancreas (Bachere <i>et al.</i>, 2004).</p>	12
<p><b>Figure 2.2.</b> Cross-sectional view of the lymphoid organ (LO) and surrounding tissue of <i>P. monodon</i>. The LO consists of two lobes located ventro-lateral of the gastric sieve and dorsal of antennal gland. H &amp; E stain. Scale bar = 100 <math>\mu</math>m. Ag, antennal gland; Cut, cuticle; Gan, ganglion; Gs, gastric sieve, Hdl, haematopoietic dorsal lobules; Hvl, haematopoietic ventral lobules; Mus, muscle; LO, lymphoid organ; Ova, ovary.</p>	32
<p><b>Figure 2.3.</b> Light micrograph of H &amp; E stained tissue section of the LO of <i>P. monodon</i>. (a) Overall longitudinal view of the lymphoid organ and surrounding tissue; (b) normal lymphoid organ tubules without LOS cells; (c) lymphoid organ with LOS cells. Scale bar = 200 <math>\mu</math>m (a), 100 <math>\mu</math>m (b and c). Ag, antennal gland; Gs, gastric sieve; Hp: hepatopancreas; LT: lymphoid tubule (Lum, lumen; Smc and stromal matrix cells); Mus, muscle; Ova, ovary; Sin, haemal sinuses.</p>	33

**Figure 2.4.** Longitudinal section of the LO of *P. monodon*.

(a) Lymphoid organ spheroid (LOS) cells with karyolytic nuclei (arrow), H & E stain, scale bar = 50  $\mu\text{m}$ ; (b) phloxine & tartrazine stain of the LO showing intranuclear eosinophilic inclusion body resembling LPV inclusion body of Owens *et al.* (1991), scale bar = 50  $\mu\text{m}$ ; (c) three distinct morphotypes of LOS cells of *P. merguensis* representing a developmental series: Type A (tA), Type B (tB) and Type C (tC) of Hasson *et al.* (1999b). H & E stain, scale bar = 20  $\mu\text{m}$ . LT, lymphoid tubule; Sin, haemal sinuses.

36

**Figure 3.1.** Experimental *P. monodon* were kept individually in recirculation aquaria to determine their moult stages. There were two modules which consisted of 8 aquaria and 1 recirculation tank with filter and each aquarium had an air lift corner filter. Salinity was maintained at 35 ‰ and temperature around 28 – 30 °C.

47

**Figure 4.1.** Transect lines for measurement of the LOS cells of *P. merguensis*. (a) Three transect lines as previously developed by Littik (2003); (b) one transect along the longest diagonal of the lymphoid organ in the present study. H & E stain. Scale bar = 500  $\mu\text{m}$ . Ag, antennal gland; Gs, gastric sieve; Hp, hepatopancreas; LO, lymphoid organ, and Ov, ovary.

53

**Figure 4.2.** Spheroid to total tissue (STT) ratio of *P. merguensis* (n = 30) by using one transect line in 10 divisions. SD, standard deviation; CV, coefficient of variation.

55

**Figure 5.1.** Moulting staging of *P. monodon*. Median part of inner uropod near the telson tip was examined to stage the moult and photographed under a light microscope (Olympus BH-2) connected to digital camera (Olympus Camedia C-5050 ZOOM). Scale bar = 100  $\mu\text{m}$ . E, epidermis; S, setal shaft; Sc, setal cone; Sn, setal node; Ss, new setal shaft, Nc, new cuticle; Pp, pinpoints of light where new setal node will develop.

65

**Figure 5.2.** Abnormal setal development in the median part of the inner uropod near the telson tip of *P. monodon*. (a) Further retraction of epidermis from the setal bases suggested stage D1 while the constriction of cellular matrix in the proximal end of setae where the setal cones would be formed pointed to stage B; (b) late stage D2 was marked by the colour change of the setal shaft to red orange while setal cones have not yet formed in the setae (stage B); (c) apolysis in stage D2 with some setae already having setal cones (stage B), some setae were not symmetrical (swollen like) in deformity of uropod; (d) one setum (arrow) just grew like the final stage before moulting (stage D4) while the epidermis had already retracted from the setal bases (stage D1). Scale bar = 200  $\mu$ m. E, epidermis; S, setal shaft; Ss, new setal shaft, Sc, setal cone; Sn, setal node; Nc, new cuticle; Cm, cellular matrix.

67

**Figure 5.3.** Light micrograph of longitudinal section of the LO of *P. monodon*. LOS cells accumulated in the haemal sinuses (Sin) and appeared to have a more basophilic cytoplasm and lack of a central lumen (Lum) compare to the normal lymphoid tubule (LT). Some spheroids demonstrated cytoplasmic vacuoles (arrow). H & E stain. Scale bar: 50  $\mu$ m. Cnf, connective tissue fibre; Smc, stromal matrix cells.

71

**Figure 5.4.** Mean ( $\pm$  SE) the spheroid to total tissue (STT) ratio (a), the prevalence of vacuolated spheroids (b) and the number of vacuoles in spheroids (c) of *P. monodon* at different time after moulting. The duration of the moult cycle was between 8 and 12 days in the first experiment and from 8 to 22 days in the second experiment. sam, soon after moulting; dom, day of moulting.

73

**Figure 5.5.** The spheroid to total tissue (STT) ratio of *P. monodon* at time after moulting at different lunar phases (FM, full moon; LQM, last quarter moon; NM, new moon, FQM, first quarter moon) in the second experiment.

74

**Figure 5.6.** Mean ( $\pm$  SE) the spheroid to total tissue (STT) ratio (a and b), the prevalence of vacuolated spheroids (c and d) and the number of vacuoles in spheroids (e and f) of *P. monodon* at different moult stages (a, c and e) and three main moult stages (b, d, and f) in the first and second experiments.

76

**Figure 5.7.** Mean ( $\pm$  SE) the spheroid to total tissue (STT) ratio (a), the prevalence of vacuolated spheroids (b) and the number of vacuoles in spheroids (c) of *P. monodon* at different lunar phases in the first and second experiments. NM, new moon; FQM, first quarter moon; FM, full moon and LQM, last quarter moon.

78

**Figure 5.8.** Light micrograph of longitudinal section of the LO of *P. monodon* from one slide demonstrated eosinophilic foci (Ef) and LOS cell in one tubule within the LO (a) suggesting eosinophilic foci (b) developed from LOS cell. Circulating haemocytes (arrow) were observed in the interstitial space between tubules (Sin). H & E stain. Scale bar = 50  $\mu$ m. LT, lymphoid tubule (Smc, stromal matrix cells and Lum, lumen); Sin, haemal sinuses.

80

**Figure 5.9.** Light micrograph of longitudinal section of the LO of *P. monodon* showing bacterial granuloma (arrow) in the interstitial space between tubules (Sin). Multiple layers of haemocytes encapsulated the melanized nodule (a and b) or elongated flattened cells surrounded the nodule (c). H & E stain. Scale bar = 50  $\mu$ m (a and b) and 20  $\mu$ m (c). Cnf, fibrous connective tissue; Lum, lumen; Smc, stromal matrix cells; Sin, haemal sinuses.

81

**Figure 5.10.** Light micrograph of longitudinal section of various tissues of *P. monodon* showing bacterial granulomas (arrow) in hepatopancreas (a), the heart (b) and antennal gland (c). H & E stain (a and c). Gram stain (b). Scale bar = 50  $\mu$ m.

82

**Figure 6.1.** The mean error bar ( $\pm 1$  SE) of the STT ratio (a and b), the prevalence of vacuolated spheroids (c and d) and the number of vacuoles in spheroids (e and f) of *P. monodon* with moult stages in the first (a, c and e) and second experiments (b, d and f). 100

**Figure 6.2.** The spheroid to total tissue (STT) ratio of *P. monodon* with moult stages at different lunar phases (NM, new moon; FQM, first quarter moon; FM, full moon and LQM, last quarter moon) in the first experiment. 101

**Figure 6.3.** The mean error bar ( $\pm 1$  SE) of the STT ratio (a and b), the prevalence of vacuolated spheroids (c and d) and the number of vacuoles in spheroids (e and f) of *P. monodon* with three main moult stages in the first (a, c and e) and second experiments (b, d and f). 103

**Figure 6.4.** The spheroid to total tissue (STT) ratio of *P. monodon* with three main moult stages at different lunar phases (NM, new moon; FQM, first quarter moon; FM, full moon and LQM, last quarter moon) in the first experiment. 104

**Figure 6.5.** The mean error bar ( $\pm 1$  SE) of the STT ratio (a and b), the prevalence of vacuolated spheroids (c and d) and the number of vacuoles in spheroids (e and f) of *P. monodon* in the first (a, c and e) and second experiments (b, d and f) with lunar phases. NM, new moon; FQM, first quarter moon; FM, full moon; LQM, last quarter moon. 106

**Figure 6.6.** Longitudinal section of various tissues of *P. monodon*. (a) Eosinophilic foci (bold arrow) within the lymphoid organ, scale bar = 50  $\mu$ m; (b) bacterial granuloma inside the lymphoid organ spheroid cells (bold arrow), note flattened epithelial cells encapsulating the melanized zone, scale bar = 50  $\mu$ m; (c) bacterial granuloma within the hepatopancreas (arrow), scale bar = 100  $\mu$ m. H & E stain. Lum, lumen; Smc, stromal matrix cells; Sin, haemal sinus, and haemocyte (arrow). 109

**Figure 7.1.** Various tissue section of *P. monodon* with H & E stain.

(a) Numerous highly encapsulated LOS cells with fibrocytes in haemal sinuses (Sin) between lymphoid organ tubules and one eosinophilic focus (arrow), scale bar = 100  $\mu\text{m}$ ; (b) highly degenerated LOS cells (arrow) and necrotic eosinophilic foci (bold arrow), scale bar = 50  $\mu\text{m}$ ; (c) abnormal interstitial space (haemal sinuses)/gapping between tubules, note the LOS cells (arrow), scale bar = 100  $\mu\text{m}$ ; (d) eosinophilic foci (arrow) and one focus inside the LOS cell (bold arrow) suggesting that originally these foci developed from the LOS cells, scale bar = 50  $\mu\text{m}$ ; (e) multiple formation of bacterial granulomas (arrow) in the two lobes of lymphoid organ, cross section, scale bar = 200  $\mu\text{m}$ ; (f) melanized nodule in the gill of the prawn with multiple layer of haemocytes encapsulated the nodule (arrow), note the haemocytic infiltration (bold arrow), scale bar = 100  $\mu\text{m}$ ; (g and h) massive melanized nodules (arrow) in the hepatopancreas surrounded by multiple layer of haemocytes resulted in the inflammation of the tissue and atrophy of hepatopancreatic tubules (bold arrow), scale bar = 100  $\mu\text{m}$  (g) and 200  $\mu\text{m}$  (h); (i) massive haemocytic aggregations in the haemal sinuses between tubules (bold arrow) and apoptotic cells (arrow) in the hepatopancreas tubules, scale bar = 50  $\mu\text{m}$ .

124

**Figure 7.2.** Mean ( $\pm$  SE) the spheroid to total tissue (STT) ratio (a), the prevalence of vacuolated spheroids (b) and the number of vacuoles in spheroids (c) of *P. monodon* at various days post-injection (dpi).

127

**Figure 7.3.** Mean ( $\pm$  SE) the spheroid to total tissue (STT) ratio (a), the prevalence of vacuolated spheroids (b) and the number of vacuoles in spheroids (c) of *P. monodon* at different moult stages.

128

**Figure 7.4.** Mean ( $\pm$  SE) the spheroid to total tissue (STT) ratio (a), the prevalence of vacuolated spheroids (b) and the number of vacuoles in spheroids (c) of *P. monodon* at four lunar phases. NM, new moon; FQM, first quarter moon; FM, full moon and LQM, last quarter moon.

129



**Figure 7.5.** The spheroid to total tissue (STT) ratio of *P. monodon* at four lunar phases with two different treatments. 130

**Figure 7.6.** Mean ( $\pm$  SE) the spheroid to total tissue (STT) ratio(a), the prevalence of vacuolated spheroids (b) and the number of vacuoles in spheroids (c) of *P. monodon* with two different treatments.  
\* Significant difference ( $P < 0.05$ ). 131

**Figure 7.7.** Mean ( $\pm$  SE) the spheroid to total tissue (STT) ratio (a), the prevalence of vacuolated spheroids (b) and the number of vacuoles in spheroids (c) of *P. monodon* in both sexes.  
\* Significant difference ( $P < 0.05$ ). 132

**Figure 8.1.** Light micrograph of longitudinal section of various tissue of *P. monodon* from one slide showing ectopic spheroids (arrow) in hepatopancreas (a), connective tissue (b) and the heart (c). H & E stain.  
Scale bar = 100  $\mu$ m. 144

**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	<i>In situ</i> hybridization
KBr	Potassium bromide
kDa	kiloDalton
LGBP	Lipopolysaccharide and $\beta$ -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 <i>Penaeus japonicus</i>
SDS-PAGE	Sodium dodecyl sulphate polyacrylamide gel electrophoresis
SGH	Small granular haemocyte
SPF	Specific pathogen free
TCID <sub>50</sub>	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