

**AEROBIC BACTERIA ABUNDANCE AND MINERAL ELEMENTS  
STATUS IN AGED AND NEW CULTURE PONDS OF  
TIGER SHRIMP (*PENAEUS MONODON*)**

**By**

**HAMIDAH BINTI ISMAUN**

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia,  
in Fulfilment of the Requirement for the Degree of Master of Science**

**July 2006**

DEDICATION

To my

Husband, Ahmad bin Mulot  
And Sons, Muhammad Zahid,  
Muhammad Zulhelmi,  
Muhammad Zulhusni

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

**AEROBIC BACTERIA ABUNDANCE AND MINERAL ELEMENTS  
STATUS IN AGED AND NEW CULTURE PONDS OF  
TIGER SHRIMP (*PENAEUS MONODON*)**

By

**HAMIDAH BINTI ISMAUN**

**July 2006**

**Chairman : Hishamuddin bin Omar, PhD**

**Faculty : Science**

The shrimp farming in Malaysia is a rapidly developing industry. However, in recent years, the production of cultured shrimp has decreased due to the unsustainable farming practices that lead to outbreaks of diseases. Thus, a comparative study was carried out to analyze the bacterial abundance in aged and new culture ponds of *Penaeus monodon* situated in Merlimau, Malacca.

The water quality parameters of the pond, the bacterial levels and the mineral element status of water and sediment were examined at intervals of 30 days for two crop cycles. Results showed that the water quality parameters remained within the optimum range for shrimp culture. The total bacterial levels (*Vibrio* and aerobic bacteria) ranged from  $10^3$  to  $10^5$  CFU mL<sup>-1</sup> in water and  $10^3$  to  $10^6$  CFU g<sup>-1</sup> in sediment. The ANOVA analysis showed that the *Vibrio* and aerobic bacteria levels in water and sediment samples were significantly higher ( $p < 0.05$ ) in new pond compared to aged

pond. The sediment of both aged and new ponds recorded higher values of bacterial level than water and demonstrated a general trend of increase bacterial level with the progress of the culture period. The results also showed that there is no clear pattern of bacterial level between crop cycles. Seven genera of bacteria were identified by using API® 20E, API® 50 CHB and Microgen™ GN-ID identification commercial kits, namely *Vibrio*, *Bacillus*, *Moraxella*, *Acinetobacter*, *Aeromonas*, *Pasteurella* and *Staphylococcus*.

Six major elements (Na, Mg, Al, K, Ca, Fe) and 14 trace elements (V, Cr, Co, Cu, Se, As, Pb, Mn, Cd, Sb, Zn, Ti, Ni, Ag) were detected using INAA, ICP-MS methods in both water and sediment from aged and new ponds. Results showed that the major elements concentrations in water and sediment from aged and new ponds were quite similar. The trace element concentrations in water and sediment from aged and new ponds were also quite similar. The results of correlation coefficients showed that water quality parameters and mineral elements concentrations contribute towards the variance of bacterial level. The data collected during this study could provide background information for further research on the mineral elements in penaeid brackish water culture ponds in Malaysia.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

**DENSITI BAKTERIA AEROBIK DAN STATUS UNSUR  
MINERAL DALAM KOLAM LAMA DAN BARU  
UDANG HARIMAU (*PENAEUS MONODON*)**

**Oleh**

**HAMIDAH BINTI ISMAUN**

**Julai 2006**

**Pengerusi : Hishamuddin bin Omar, PhD**

**Fakulti : Sains**

Penternakan udang laut di Malaysia merupakan satu industri yang sedang berkembang pesat. Walau pun begitu, kebelakangan ini pengeluaran udang ternakan telah merosot disebabkan oleh amalan pengurusan kolam yang tidak baik yang menyebabkan timbulnya banyak masalah penyakit. Oleh itu, satu kajian bandingan telah dijalankan untuk menganalisa densiti bakteria di dalam kolam yang lama dan baru *Penaeus monodon* yang terletak di Merlimau, Melaka.

Parameter kualiti air kolam, bilangan bakteria dan status unsur mineral di dalam sampel air dan sedimen kolam udang harimau yang baru dan lama ditentukan pada setiap 30 hari selama dua tempoh pengkulturan. Keputusan menunjukkan bahawa semua parameter kualiti air didapati berada di dalam julat yang sesuai untuk pengkulturan udang.

Bilangan mutlak bakteria (*Vibrio* dan bakteria aerobik) di dalam air mempunyai julat di antara  $10^3$  hingga  $10^5$  CFUmL<sup>-1</sup> dan di dalam sedimen di antara  $10^3$  hingga  $10^6$  CFUg<sup>-1</sup>. Analisis ANOVA menunjukkan bahawa bilangan *Vibrio* dan bakteria aerobik mempunyai perbezaan bererti yang tinggi ( $p < 0.05$ ) di dalam kolam baru berbanding kolam lama. Bilangan bakteria di dalam sedimen bagi kedua-dua kolam mencatatkan nilai yang lebih tinggi berbanding bilangan bakteria di dalam air. Keputusan juga menunjukkan tiada corak yang tertentu pada bilangan bakteria di antara tempoh pengkulturan. Kesimpulannya, jumlah bilangan bakteria di dalam kolam baru lebih banyak berbanding kolam lama. Pencaman bakteria juga dilakukan dengan menggunakan kit komersil API® 20E, API® 50 CHB dan Microgen™ GN-ID. Tujuh genera bakteria telah dikenalpasti dari sampel air dan sedimen kolam baru dan lama iaitu *Vibrio*, *Bacillus*, *Moraxella*, *Acinetobacter*, *Aeromonas*, *Pasteurella* dan *Staphylococcus*.

Sebanyak enam unsur major (Na, Mg, Al, K, Ca, Fe) dan 14 unsur surih (V, Cr, Co, Cu, Se, As, Pb, Mn, Cd, Sb, Zn, Ti, Ni, Ag) telah dikesan dengan menggunakan kaedah INAA, ICP-MS di dalam air dan sedimen kedua-dua kolam lama dan baru. Keputusan menunjukkan bahawa kepekatan unsur major di dalam air dan sedimen kedua-dua kolam lama dan baru menunjukkan corak yang serupa. Kepekatan unsur surih di dalam air dan sedimen dari kolam lama dan baru juga mempunyai corak yang sama. Keputusan hubungan korelasi menunjukkan terdapat beberapa faktor parameter kualiti air dan unsur mineral yang menyumbang kepada varian paras bakteria. Data yang terkumpul dari kajian ini boleh digunakan sebagai rujukan bandingan bagi kajian yang akan datang kerana masa kini, kurang atau tiada maklumat mengenai status unsur mineral di dalam kolam udang air payau di Malaysia.

## ACKNOWLEDGEMENTS

All praises to Allah, the Almighty, The Most Gracious, The Most Merciful, for giving me the strength and will to write and complete this thesis.

First and foremost, I wish to express my most sincere gratitude and appreciation to the Chairman of my supervisory committee, Dr. Hishamuddin Omar, for his valuable advice, guidance and encouragement during this study. Also, I wish to express my appreciation to other members of the supervisory committee, Professor Madya Dr. Raja Noor Zaliha Raja Abdul Rahman and Dr. Misri Kusnan for their supervision, support and constructive comments.

I would like to take this opportunity to acknowledge Professor Madya Datin Dr. Khatijah Mohd Yusoff, the Head Department of Biochemistry and Microbiology for allowing me to use the facilities in Microbiology laboratory. I am also greatly indebted to En. Onn Hj. Ahmad for his valuable supervision and kind help rendered for the successful completion of the laboratory work. Also, I wish to thank all staff members of Microbiology laboratory who had helped me in one way or another and for making my time in the laboratory a comfortable one.

In particular, I would like to mention En. Yahya Mohamed, the Project Manager of the tiger shrimp culture ponds in Merlimau, Malacca and his assistant, En. Hamidi for their co-operation and help during field sampling.

I wish to extend my appreciation to my friend, Abu Hena Mustaffa Kamal, a PhD candidate for his help and guidance and also my colleagues in Institut Perguruan Raja Melewar, Seremban for their encouragement and moral support.

Special thanks to Teachers Education Division and the Ministry of Education Malaysia in Putrajaya for the opportunity and financial support given to me.

Finally, I also owe my special thanks to my loving husband and my lovely sons for their moral encouragement, patience and sacrifices along the way.



I certify that an Examination Committee has met on 17 July 2006 to conduct the final examination of Hamidah binti Ismaun on her Master of Science thesis entitled “Aerobic Bacteria Abundance and Mineral Elements Status in Aged and New Culture Ponds of Tiger Shrimp (*Penaeus monodon*)” in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

**Siti Shapor Siraj, PhD**

Associate Professor  
Faculty of Science  
Universiti Putra Malaysia  
(Chairman)

**Hassan Mohd Daud, PhD**

Associate Professor  
Faculty of Medical Veterinary  
Universiti Putra Malaysia  
(Internal Examiner)

**Muskhazli Mustaffa, PhD**

Professor  
Faculty of science  
Universiti Putra Malaysia  
(Internal Examiner)

**Zulfigar Yasin, PhD**

Professor  
Faculty of Biological Science  
Universiti Sains Malaysia  
(External Examiner)

---

**HASANAH MOHD. GHAZALI, PhD**

Professor/Deputy Dean  
School of Graduate Studies  
Universiti Putra Malaysia

Date: 22 NOVEMBER 2006

This thesis submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee are as follows:

**Hishamuddin Omar, PhD**

Lecturer  
Faculty of Science  
Universiti Putra Malaysia  
(Chairman)

**Raja Noor Zaliha Raja Abd. Rahman, PhD**

Professor Madya  
Faculty of Science  
Universiti Putra Malaysia  
(Member)

**Misri Kusnan, PhD**

Lecturer  
Faculty of Science  
Universiti Putra Malaysia  
(Member)

---

**AINI IDERIS, PhD**  
Professor/Dean,  
School of Graduate Studies,  
Universiti Putra Malaysia.

Date: 14 DISEMBER 2006

## DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

---

**HAMIDAH BINTI ISMAUN**

Date: 31 OCTOBER 2006

## TABLE OF CONTENTS

		<b>Page</b>
<b>DEDICATION</b>		ii
<b>ABSTRACT</b>		iii
<b>ABSTRAK</b>		v
<b>ACKNOWLEDGEMENTS</b>		vii
<b>APPROVAL</b>		ix
<b>DECLARATION</b>		xi
<b>LIST OF TABLES</b>		xv
<b>LIST OF FIGURES</b>		xxi
<b>LIST OF PLATES</b>		xxii
<b>GLOSSARY OF TERMS</b>		xxiii
<b>CHAPTER</b>		
<b>1</b>	<b>INTRODUCTION</b>	1
	1.1 Significance of study	4
<b>2</b>	<b>LITERATURE REVIEW</b>	
	2.1 History and Growth of Shrimp Farming	7
	2.2 Problems in Shrimp Culture	8
	2.3 Penaeid Shrimp Diseases	9
	2.4 The Role of Microorganisms in Shrimp Culture Pond	12
	2.5 Decomposing Bacteria in Shrimp Culture Pond	15
	2.6 Effects of Pond Management on Shrimp Culture	17
	2.7 Effects of Probiotics on Shrimp Culture	20
	2.8 Mineral Elements in Water and Sediments/Soil	24
<b>3</b>	<b>METHODOLOGY</b>	
	3.1 Study Site	29
	3.2 Sampling Procedures	29
	3.2.1 Water Quality Analysis	31
	3.3 Determination of Total Suspended Solids (TSS)	31
	3.4 Bacterial Load Analyses on Water Sample	32
	3.4.1 Preparation of the Thiosulphate Citrate Bile Sucrose Agar (TCBS)	32
	3.4.2 Preparation of the Tryptone Soya Agar (TSA)	33
	3.4.3 Preparation of the Mannitol Salt Agar	33
	3.4.4 Preparation of the Methyl-Red and Voges- Proskauer (MRVP) Medium	33
	3.4.5 Bacterial Enumeration	34

3.5	Isolation and Characterization of Isolates	36
3.5.1	Gram-staining	37
3.5.2	Motility	38
3.5.3	Oxidase Test	38
3.5.4	Catalase Test	38
3.5.5	Mannitol	39
3.5.6	Voges-Proskauer Test	39
3.6	Identification of the Non-fastidious Gram-negative Rods	40
3.7	Identification of the Genus <i>Bacillus</i>	40
3.8	Identification of Gram-positive Cocci	41
3.9	Bacterial Load Analyses on Sediment Sample	41
3.10	Mineral Elements Analyses	42
3.10.1	Pretreatment of Sediment Sample	42
3.10.2	Acid Digestion	44
3.11	Statistical Analysis	44
<b>4</b>	<b>RESULTS</b>	
4.1	Water Quality Parameters	46
4.2	Microbial Study in Aged and New Pond	48
4.2.1	<i>Vibrios</i> in Water and Sediment from Aged and New Pond	48
4.2.2	Aerobic Bacteria in Water and Sediment from Aged and New Pond	54
4.3	Species Identification	61
4.4	Mineral Elements Status in Water and Sediment from Aged and New Pond	63
4.4.1	Major Elements in Water and Sediment from Aged and New Pond	65
4.4.2	Trace elements in Water and Sediment from Aged and New Pond	67
4.5	Relationships between Bacteria Level and Water Quality Parameters	70
4.6	Relationship between Bacteria Level and Mineral Element Status	72
<b>5</b>	<b>DISCUSSION</b>	
5.1	Water Quality Parameters	74
5.2	Microbial Study in Aged and New Pond	79
5.2.1	Species Identification	83
5.3	Mineral Elements Status in Water and Sediment from Aged and New Pond	85
5.4	Relationships between Bacteria and Water Quality Parameters; and Bacteria and Mineral Elements	89
<b>6</b>	<b>CONCLUSION</b>	91

<b>REFERENCES</b>	94
<b>APPENDICES</b>	
<b>A</b>	112
<b>B</b>	116
<b>C</b>	133
<b>BIODATA OF THE AUTHOR</b>	135