TAXONOMY, ECOLOGY AND CONTROL OF THALASSINA MUD LOBSTERS ON CAREY ISLAND AND KELANANG SHORE (PENINSULAR MALAYSIA)

MOH HENG HING

INSTITUTE OF BIOLOGICAL SCIENCES FACULTY OF SCIENCE UNIVERSITY OF MALAYA KUALA LUMPUR

2016

TAXONOMY, ECOLOGY AND CONTROL OF THALASSINA MUD LOBSTERS ON CAREY ISLAND AND KELANANG SHORE (PENINSULAR MALAYSIA)

MOH HENG HING

THESIS SUBMITTED IN FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

INSTITUTE OF BIOLOGICAL SCIENCES FACULTY OF SCIENCE UNIVERSITY OF MALAYA KUALA LUMPUR

2016

UNIVERSITY OF MALAYA ORIGINAL LITERARY WORK DECLARATION

Name of Candidate: Moh Heng Hing I.C/Passport No:

Registration/Matric No: SHC080029

Name of Degree: Doctor of Philosophy

Title of Project Paper/Research Report/Dissertation/Thesis ("this Work"): TAXONOMY, ECOLOGY AND CONTROL OF *THALASSINA* MUD LOBSTERS ON CAREY ISLAND AND KELANANG SHORE (PENINSULAR MALAYSIA)

Field of Study:

I do solemnly and sincerely declare that:

- (1) I am the sole author/writer of this Work;
- (2) This Work is original;
- (3) Any use of any work in which copyright exists was done by way of fair dealing and for permitted purposes and any excerpt or extract from, or reference to or reproduction of any copyright work has been disclosed expressly and sufficiently and the title of the Work and its authorship have been acknowledged in this Work;
- (4) I do not have any actual knowledge nor do I ought reasonably to know that the making of this work constitutes an infringement of any copyright work;
- (5) I hereby assign all and every rights in the copyright to this Work to the University of Malaya ("UM"), who henceforth shall be owner of the copyright in this Work and that any reproduction or use in any form or by any means whatsoever is prohibited without the written consent of UM having been first had and obtained;
- (6) I am fully aware that if in the course of making this Work I have infringed any copyright whether intentionally or otherwise, I may be subject to legal action or any other action as may be determined by UM.

Candidate's Signature

Date: 8 September 2016

Subscribed and solemnly declared before,

Witness's Signature

Date:

Name:

Designation:

ABSTRACT

Thalassinid mud lobsters are large burrowing crustaceans commonly found on mangrove shores. They are not well studied due to their cryptic nature and subterranean habit. Mangrove land reclamation puts this enigmatic creature at odds with humans where their burrowing activities wreck coastal dykes and culture ponds. This study investigated the species of mud lobsters in Malaysia, and their abundance and distribution in relation to environmental factors on Kelanang shore and particularly, Carey Island, where coastal dykes are built to protect largely oil palm estates. Thus, a goal of this study was to find environmental-friendly ways to control mud lobster infestations on earthen dykes. Three species of Thalassina, namely, Thalassina anomala Herbst, 1804, Thalasssina gracilis Dana, 1852 and Thalassina kelanang Moh & Chong, 2009 were described and compared to the Australian species, Thalassina squamifera De Man, 1915. Morphological features of the carapace, rostrum, scaphocerite, abdominal pleura and sternites, and petasma was used to distinguish between the newly described species, T. kelanang from T. squamifera which closely resembles it morphologically, as well as the other species. These four species of Thalassina were further verified as distinct by discriminant analysis of their morphological traits and by three molecular gene markers (PEPCK, NaK, and COI). Both approaches agree that T. anomala and T. gracilis are the most distant pair among the four species. Molecular analysis of the combined markers shows that the four species of *Thalassina* belong to a monophyletic clade, and that *T. squamifera* and *T.* kelanang are two closely similar, but distinct, species. Three sympatric species of mud lobsters were spatially distributed along the mangrove shore, occurring in combinations of T. anomala with T. kelanang (Kelanang) or T. gracilis (Carey Island). Spatial partitioning of these species was strongly driven by environmental factors such as tidal inundation, salinity and substrate characteristics. Competitive exclusion was hypothesized with the more aggressive T. kelanang living on the lower shore and T. anomala on the upper shore. T. gracilis (genetically closest to T. kelanang) was spatially partitioned from T. kelanang by its greater tolerance to salinity fluctuations in the mid-estuary where it occupied a similar elevation in tidal height as T. kelanang. The distribution of mud lobster (T. anomala) mounds along the entire dyke perimeter of Carey Island was not random with high densities (up to 70 mounds/100m bund length) occurring at the northeastern horn of the island and near Air Hitam village south of the island. T. anomala first invaded the coastal dyke on the river side of the bund at the highest spring tide level. The animal then tunneled down to the bund bottom at the

opposite or landward side, before venturing farther (up to 10m) into the oil palm plantation, as long as they could access water. Mud lobster burrows on the bund or plantation could reach 60cm in height and 1.2m in depth. Experimental planting of different species of grass vegetation on the dyke to obstruct the passage (vertical barrier) or exit (horizontal barrier) of the mud lobster showed that a combination of *Chrysopogon zizanioides* (Vetiver) and *Cyanodon dactylon* grasses is the most effective way to control mud lobster infestations without the use of toxic chemicals. The study shows the mangrove shore as the endemic habitat for three species of *Thalassina* in Malaysia. Coastal reclamation of *T. anomala* from sea to the landside of mangrove forests. This results in *Thalassina* larvae settling and colonizing coastal dykes constructed by humans to prevent sea water intrusion.

ABSTRAK

Udang Ketak, Thalassinid merupakan krustasea besar yang menggali dan biasanya dijumpai di persisiran hutan bakau. Ianya tidak dikaji dengan baik kerana sifatnya yang misteri dan tabiatnya hidup di bawah tanah. Penebusgunaan tanah hutan bakau meletakkan makhluk misteri ini ganjil dengan manusia di mana aktiviti menggali lubang mereka menghancurkan banteng di persisiran pantai dan kolam perternakan. Kajian ini mengkaji spesies udang ketak di Malaysia, dan kelimpahan dan taburannya berhubungkait dengan faktor persekitaran di Pantai Kelanang dan terutamanya, Pulau Carey, di mana benteng pantai dibina untuk melindungi sebahagian besarnya ladang kelapa sawit. Oleh itu, matlamat kajian ini adalah untuk mencari cara-cara yang mesra alam untuk mengawal serangan udang ketak di benteng tanah. Tiga spesies Thalassina, iaitu Thalassina anomala Herbst, 1804, Thalassina gracilis Dana, 1852 dan Thalassina Kelanang Moh & Chong, 2009 telah diterangkan dan dibandingkan dengan spesies dari Australia, Thalassina squamifera De Man, 1915. Ciri-ciri morfologi bahagian karapas, "rostrum", "scaphocerite", "abdominal pleura" dan "sternites" serta "petasma" boleh digunakan untuk membezakan antara spesies yang baru diterangkan, T. Kelanang dari T. squamifera yang hampir menyerupai ia, serta spesies yang lain. Keempat-empat spesies *Thalassina* selanjutnya telah disahkan berbezaannya dengan analisis diskriminan bagi ciri-ciri morfologinya dan dengan tiga penanda gen molekul (PEPCK, Nak, dan COI). Kedua-dua pendekatan tersebut bersetuju bahawa T. anomala dan T. gracilis adalah pasangan yang paling jauh antara empat spesies. Analisis molekul bagi penanda gabungan menunjukkan bahawa keempat-empat spesies Thalassina tergolong dalam klad monophyletic, dan T. squamifera dan T. Kelanang adalah dua spesies yang paling seiras, tetapi kedua-duanya adalah species yang sangat berbeza. Tiga simpatrik spesis udang ketak adalah ditaburkan atau mendiami kawasan yang berbeza di sepanjang pantai hutan bakau, ia berlaku dalam kombinasi T. anomala dengan T. Kelanang (Kelanang) atau T. gracilis (Pulau Carey). Pembahagian kawasan bagi spesis ini amat didorong oleh faktor-faktor persekitaran seperti paras air pasang surut, kemasinan dan ciri-ciri sedimen. Pengecualian kompetitif telah menghipotesis dengan spesis yang lebih agresif T. Kelanang di kawasan pantai yang lebih rendah dan T. anomala di kawasan pantai yang lebih tinggi. T. gracilis (yang mempunyai genetik paling dekat dengan T. Kelanang) menduduki kawasan yang berbeza dengan T. kelanang oleh toleransi yang lebih tinggi kepada turun naik kemasinan yang tinggi pada pertengahan muara di mana ia menduduki ketinggian kawasan yang sama seperti T. Kelanang. Taburan busut udang

ketak sepanjang benteng di Pulau Carey tidak berlaku secara rawak dengan kepadatan yang tinggi (sehingga 70 busut/ 100m panjang benteng) yang berlaku di kawasan hujung timur laut dan berhampiran Kampung Air Hitam selatan pulau ini. Pencerobohan T. anomala ke dalam benteng bermula di sebelah sungai pada banteng di kawasan tertinggi paras air pasang laut purnama. Haiwan itu kemudian menggali turun ke bahagian bawah benteng di seberang atau kawasan darat, sebelum meneroka lebih jauh (sehingga 10m) ke dalam ladang kelapa sawit, selagi ia boleh mengakses air. Lumpur busut udang ketak pada banteng atau kawasan ladang boleh mencecah 60 cm tinggi dan 1.2m dalam. Eksperimen penanaman spesis rumput berlainan jenis di banteng bagi menghalang laluan (halangan menegak) atau keluar (halangan melintang) udang ketak itu menunjukkan bahawa gabungan rumput Chrysopogon zizanioides (Vetiver) dan *Cyanodon* dactylon adalah cara yang paling berkesan untuk mengawal pencerobohan udang ketak tanpa penggunaan bahan kimia toksik. Kajian ini menunjukkan hutan bakau pantai sebagai habitat endemik untuk tiga spesies Thalassina di Malaysia. Penebusgunaan kawasan hutan bakau pantai memusnahkan habitat semula jadi mereka dan menutup jalan penghijrahan T. anomala dari laut ke darat. Ini menyebabkan Thalassina larva menetap dan menjajah banteng pantai yang dibina oleh manusia untuk mencegah pencerobohan air laut.

ACKNOWLEDGEMENTS

I would like express my gratitude to many remarkable people, who have helped and gave strong positive support to me, without which I could never have finished this thesis. First of all, I would like to express my greatest gratitude to my supervisor Professor Dr. Chong Ving Ching for patiently guiding me throughout my PhD research and study. His persistent positive support and care to me, every time I feeling miserable have inspired me to finish my thesis. His guiding and constructive criticisms throughout my research have significantly improved the outcome and the completion of this thesis. A special appreciation to Dr. A. Sasekumar for his help and support for my work throughout these years.

I'm also grateful to University of Malaya for providing the research facilities and financial support through a Postgraduate Research Fund (PPP) and University of Malaya Graduate Research Assistance Scheme (GRAS). This study was also supported by a research grant from Golden Hope Plantations Bhd (now Sime Darby Plantation Private Limited). The company had also provided field assistance and accommodation to support this research. I would like to acknowledge the Natural Sciences, Museum and Art Gallery of the Northern Territory, Australia, and Raffles Museum of Biological Research (ZMU), National University of Singapore, for loan of their mud lobster specimens for this research. I am grateful to Prof. Dr. Baki Bakar for identifying the vegetation grass.

The fieldwork would not have been possible without help from the Sime Darby Plantation management staff Arnina, Suhailan, Abdul Muhsin, Kharuniza and also all the estate managers. Many thanks to the field assistants from the Sime Darby Plantation, including Osman, Zainudin, Azimi, Faiza, Daud, Lan, Mohd Nor, Sukhri, Roslan Saiful and others. Special thanks to Sime Darby Plantation Academy for providing accommodation and food, staff Norliza for room reservation and Macik Aminah from the kitchen who always reserved the good food for me when I came back late from the field. Many thanks to ISB staff Rosli, Nagapan, Rizan, Ponniah, Zulkulfi, Fauzi, Zaidee and Faris for the assistance and drive me during the field samplings.

Many thanks go to my senior colleagues in Lab B201, IPS, for their advice and sharing of knowledge, experience and support; John, Ai Lin, Li Lee, Amy and Ng. To my other colleagues/ ex-colleagues in Lab B201; Loo, Teoh, Dagoo, Chai Ming, Raven, Tueanta, Dr. Loh, Jin Yung, Raymond, Adam, Soon Loong, Yana, Jun Jie, Kean Chong, Yu Lin, Cindy and others, thanks for all your kind help, support, companionship and great times in the lab.

My utmost appreciation goes to my beloved parents and siblings for their care and endless support and encouragement. Lastly, my deepest gratitude goes to my dearest wife, Bee Sym. To my lovely wife, thank you for your unconditional love, immense understanding and unlimited support for all the time I was not there, without which I would not have been able to complete my PhD.

TABLE OF CONTENTS

Abstract	iv-v
Abstrak	vi-vii
Acknowledgements	viii
Table of Contents	ix-xiii
List of Figures	xiv-xix
List of Tables	xx-xxi

CHAPTER 1: INTRODUCTION

1.1	An overview taxonomy of mud lobster, their distribution and habitat1
1.2	Research problems and objectives
1.3	Objectives of study

CHAPTER 2: MORPHOLOGICAL DIFFERENTIATION OF THREE SPECIES OF MALAYSIAN THALASSINA MUD LOBSTERS, T. ANOMALA, T. GRACILIS AND T. KELANANG (SP. NOVA), AND THE AUSTRALIAN SPECIES, T. SQUAMIFERA

2.1	Introduc	ction	9
2.2	Material	l and methods	11
	2.2.1	Study sites	11
	2.2.2	Sampling	12
	2.2.3	Comparative material examined	12
2.3	Result.		13
	2.3.1	Taxonomy	13
	2.3.2	Description of holotype	14
	2.3.3	Variation	23

	2.3.4	Etymology	24
	2.3.5	Colour	24
	2.3.6	Biological notes	24
2.4	Discus	ssion	25

CHAPTER 3: VERIFICATION OF THE FOUR SPECIES OF MUD LOBSTERS, THALASSINA ANOMALA, T. KELANANG, T. GRACILIS AND T. SQUAMIFERA USING MOLECULAR, MORPHOMETRIC AND MERISTIC CHARACTERS

3.1	Introduc	ction	33
3.2	Material	and methods	36
	3.2.1	Material examined	36
	3.2.2	Meristics and morphometrics	36
	3.2.3	Statistical analysis	
	3.2.4	Molecular analysis	38
		3.2.4.1 DNA extraction, polymerase chain reaction (PCR) and	
		sequencing	
		3.2.4.2 Sequence alignment and molecular analysis	40
3.3	Results		41
	3.3.1	Analysis of morphometric and meristic data	41
	3.3.2	DNA sequences	44
	3.3.3	Molecular analysis	44
3.4	Discus	ssion	49

CHAPTER 4: DISTRIBUTION AND BURROW MORPHOLOGY OF THREE SYMPATRIC SPECIES OF *THALASSINA* MUD LOBSTERS IN RELATION TO ENVIRONMENTAL PARAMETERS ON KELANANG SHORE

4.1	Introduction	.50	6
-----	--------------	-----	---

4.2	Materia	s and methods60
	4.2.1	Study area60
	4.2.2	Sampling methods
	4.2.3	Casting of mud lobster burrows65
	4.2.4	Analysis of particle size of sediment
	4.2.5	Determination of organic matter and root mass
	4.2.6	Data Analysis67
4.3	Result	s
4.3	Result 4.3.1	s
4.3	Result 4.3.1 4.3.2	s
4.3	Result 4.3.1 4.3.2 4.3.3	s
4.3	Result 4.3.1 4.3.2 4.3.3 4.3.4	s
4.3	Result 4.3.1 4.3.2 4.3.3 4.3.4 Thalas	s

CHAPTER 5: MOUND DISTRIBUTION AND DENSITY OF THALASSINA

MUD LOBSTERS ON THE COASTAL DYKES OF CAREY ISLAND

5.1	Introdu	ction	
5.2	Materia	Is and methods	85
	5.2.1	Study area	85
	5.2.2	Distribution of mud lobster in whole Carey Island	86
	5.2.3	Colonization dynamics of mud lobster on the dyke	89
	5.2.4	Sediment analysis	90
	5.2.5	Statistical analysis	91
5.3	Result	S	92
	5.3.1	Distribution and habitation of mud lobster in Carey Island	92

		5.3.1.1 Mud lobster mound distribution and abundance
		5.3.1.2 Classification of mud lobster mound activity95
		5.3.1.3 Mud lobster mound height
		5.3.1.4 Mud lobster mound burrow diameter
	5.3.2	Dyke Colonization by mud lobster at Site A1100
	5.3.3	Colonization of mud lobster mounds at Site B1103
	5.3.4	Colonization of mud lobster mounds at Site C1108
	5.3.5	Spatial and temporal variation of environmental parameters in/ near mud
		lobster mounds
		5.3.5.1 Ditch water parameters
		5.3.5.2 Soil parameters
		5.3.5.3 Sediment particle size
		5.3.5.4 Mud lobster infestation in relation to sediment and water
		parameters121
5.4	Discus	ssion

CHAPTER 6: CONTROL OF MUD LOBTERS (THALASSINA ANOMALA) INFESTATIONS ON COASTAL DYKES

6.1	Introduc	ction	128
6.2	Material	ls and methods	131
	6.2.1	Study area	131
	6.2.2	Vegetation planting	
	6.2.3	Statistical analysis	133
6.3	Result	ts	
	6.3.1	Effectiveness of vegetation covers study to control the	mud lobster
		population on the dyke	134

Discussion	141
	Discussion

CHAPTER 7: GENERAL DISCUSSION AND CONCLUSION

7.1	Taxonomic status of <i>Thalassina</i> : an update of extant species	145
7.2	Colonization strategies and adaptation of <i>T. anomala</i> on the dyke	147
7.3	Control of <i>Thalassina anomala</i> on the dyke	148
	7.3.1 Planting method of the vegetation on the dyke	149
7.4	Limitations and future work	150
7.5	Conclusions	151
Refe	erences	153

LIST OF FIGURES

- Figure 2.1 Dorsal view of carapace. A, *Thalassina kelanang*, holotype-13 male, (TL=150 mm, CL= 54.1 mm)
- Figure 2.2 Dorsal view of carapace. A, *Thalassina kelanang* (holotype, 15 150.0, 54.1 mm); B, *T. anomala*-Male (151.4, 51.1 mm). Zoological Museum, University of Malaya (ZMUM). Scale bar = 10 mm.
- Figure 2.3 Dorsal view of anterior region of cephalothorax. A, *T. kelanang* 16 (holotype, 150.0, 54.1 mm); B, *T. anomala*-Male (151.4, 51.1 mm); C, *T. squamifera*-Male (132.5, 47.8 mm); D, *T. gracilis*-Male (69.8, 25.8 mm). Zoological Museum, University of Malaya (ZMUM). Scale bar = 5 mm.
- Figure 2.4 Dorsal view of right antennal peduncle showing movable 17 scaphocerite. A, *T. kelanang* (holotype, 150.0, 54.1 mm); B, *T. anomala*, (absent)-Male (151.4, 51.1 mm); C, *T. squamifera*-Male (132.5, 47.8 mm). Zoological Museum, University of Malaya (ZMUM). Scale bars: a-b = 20 mm; c = 10 mm.
- Figure 2.5 Right chela of first pereiopod. A-B, outer and inner aspects, *T*. 18 *kelanang* (holotype, 150.0, 54.1 mm); C-D, outer and inner aspects, *T. anomala*-Male (151.4, 51.1 mm). Zoological Museum, University of Malaya (ZMUM). Scale bar = 10 mm.
- Figure 2.6 Dorsal view of first abdominal somite. A, *T. kelanang* 20 (holotype, 150.0, 54.1 mm); B, *T. anomala*-Male (151.4, 51.1 mm). Zoological Museum, University of Malaya (ZMUM). Scale bar = 10 mm.
- Figure 2.7 Second abdominal somite. A-B, dorsal and ventral aspects, *T*. 21 *kelanang* (holotype, 150.0, 54.1 mm); C-D, dorsal and ventral aspects, *T. anomala*-Male (151.4, 51.1 mm). Zoological Museum, University Malaya (ZMUM). Scale bar = 10 mm.
- Figure 2.8 Ventral view of petasma. A, *T. kelanang* (holotype, 150.0, 54.1 23 mm); B, *T. anomala*-Male (193, 61.4 mm); C, *T. squamifera*-Male (132.5, 47.8 mm); D, *T. gracilis*-Male (69.8, 25.8 mm). Zoological Museum, University Malaya (ZMUM). Scale bar = 5 mm.
- Figure 3.1 SFDA ordination diagram of morphological and meristic 43 characters for four species of *Thalassina*.
- Figure 3.2 The 50% majority-rule consensus tree resulting from maximum 45 likelihood analysis of (a) partial PEPCK sequences (substitution

xiv

rate parameters: TC = 0.5206, TA = 0.1254, TG = 0.0125, CA = 0.1254, CG = 0.0125, AG = 0.2036), - Ln likelihood 1935.877; (b) partial NaK sequences (TC = 0.5183, TA = 0.1061, TG = 0.0727, CA = 0.0972, CG = 0.0223, AG = 0.1834), - Ln likelihood 2117.352; (c) partial COI sequences (TC = 0.7231, TA = 0.1315, TG = 1.4301e⁻⁵, CA = 0.0153, CG = 0.0311, AG = 0.0991), - Ln likelihood 2729.365; (d) combined PEPCK, NaK and COI DNA sequences (TC = 0.5687, TA = 0.1190, TG = 0.0211, CA = 0.1190, CG = 0.0211, AG = 0.1511), - Ln likelihood 6914.207.

The bootstrap values (ML/MP/BI) are shown at the branches. Bar indicates substitutions per site.

- Figure 3.3 Locations of examined specimens of *T. squamifera* (circles) and 52 *T. kelanang* (triangles) by various authors. Museum catalog numbers at each site are indicated.

 (I) = Poore & Griffin, 1979, = Ngoc-Ho & de Saint Laurent, 2009, = Moh & Chong, 2009, = Sakai & Türkay, 2012, ▲ = Moh & Chong, 2009, this study. Dotted line indicates Wallace's Line. Modified base map from http://commons.wikimedia.org.
- Figure 4.1 Klang Islands and its estuaries showing study site 1 at Kelanang 62 shore (Kelanang Beach) and sites 2 and 3 on Carey Island.
- Figure 4.2 (a) A schematic profile of the Kelanang mangrove shore 63 showing sampling site 1. Site 1a contained Zone A, Zone B and Zone C; Site 1b contained Zone C, Zone D, Zone E and Zone F. Zones E and F were set 150 m off the set transect through Zones C and D to avoid the disturbed forests below. (b) Composite horizontal profile of the mangrove high shore at Kelanang shore (site 1) and Carey Island (sites 2 & 3), showing the various zones, tidal heights and general distribution of mangroves and mud lobsters. Numerals below demarcated zones indicate the number of days per year shore sites were exposed to air. EHWS= Extreme High Water Spring; MHWS= Mean High Water Neap; MLWN= Mean Low Water Neap.
- Figure 4.3 Distribution of mud lobster species in relation to daily tidal 70 height at Carey Island. Zones A, B (site 1a) and site 2 lie within the spring high tidal range (habitat of *T. anomala*), while site 3 lies within the high neap tidal range (habitat of *T. gracilis*). Zones C, D, E & F lie within the high neap tidal range to low neap tide (habitat of *T. kelanang*). Right vertical arrow bars indicate range of species in term of shore elevation. Horizontal bar indicates size scale (1 cm) of animals.
- Figure 4.4 Schematic drawings of the burrow morphology of the three 74 species of *Thalassina* based on a particular burrow cast. a-*Thalassina anomala*; b- *Thalassina kelanang*; c- *Thalassina gracilis*. Ordering of burrow branches using the Shreve method

XV

is illustrated in a (numerals).

Figure 4.5	RDA triplots of three species of mud lobsters (<i>T. anomala, T. gracilis, T. kelanang</i> , blue arrows) as influenced by environmental factors (red arrows) and sampled from three sites: circles (site 1, zone A to F, sample plots 1-8), squares (site 2, sample plots a-d), diamonds (site 3, sample plots a-e).	76
Figure 4.6	Conceptual model of niche differentiation and competitive interactions among three sympatric species of <i>Thalassina</i> in the Klang-Langat delta (Selangor).	78
Figure 5.1	Map shows study sites for spatial-temporal colonization of newly repaired bund on Carey Island and the surrounding estuaries.	86
Figure 5.2	Schematic diagram of study zones of surveyed area across dyke (bund). *Diagram not in scale.	89
Figure 5.3	Mud lobster mound and burrow measurements. Dm= diameter, H= height, Db= diameter at entrance of burrow.	88
Figure 5.4	Mud lobsters mound distribution in Carey Island (*Black doted). (census from May 2005 – August 2005). Topographic map extracted from Department Survey and Mapping, Malaysia (JUPEM).	94
Figure 5.5	 Distribution of mud lobster mounds across dyke zones for the whole of Carey Island, Peninsular Malaysia. Numerals above bars indicate % contribution of transect zones to mound numbers. For zones (x-axis): Plantation-PL, Plantation canal-PC, Road-RD, Bund ditch-DB, Bottom bund-BB, Top bund slope-TB, Landside bund top-TL and waterside bund top-TW. (see Figure 5.2 for detail of the zones). 	95
Figure 5.6	Distribution of "Active" (A), "Abandoned" (O) and "Inactive" (InA) mounds by zones across bund perimeter for whole of Carey Island. For zones (x-axis): Plantation-PL, Plantation canal-PC, Road- RD, Bund ditch-DB, Bottom bund-BB, Top bund slope-TB, Landside bund top-TL and waterside bund top-TW. (see Figure 5.2 for detail of the zones).	96
Figure 5.7	Frequency distribution of <i>Thalassina</i> mound heights for whole of Carey Island. Numerals above bars indicate % contribution of each size class of mud lobster mound height.	97
Figure 5.8	Mound height (cm) in relation to location (transect across bund from sea to plantation) for the whole length of dykes in Carey Island.	97

For zones (x-axis): Plantation-PL, Plantation canal-PC, Road-RD, Bund ditch-DB, Bottom bund-BB, Top bund slope-TB, Landside bund top-TL and waterside bund top-TW. (see Figure 5.2 for detail of the zones).

- Figure 5.9 Frequency distribution of all burrow mouth diameter for whole 98 of Carey Island (X = mouth diameter, cm).
- Figure 5.10 Burrow/ mouth diameter of mud lobster burrow in relation to 99 position of zones for whole length of dykes in Carey Island. For zones (x-axis): Plantation-PL, Plantation canal-PC, Road-RD, Bund ditch-DB, Bottom bund-BB, Top bund slope-TB, Landside bund top-TL and waterside bund top-TW. (see Figure 5.2 for detail of the zones).
- Figure 5.11 Mound building in newly-repaired bund (Site A1; 100x 25 m) 101 over 12 months. A= full moon (spring tide) B= new moon (spring tide).
- Figure 5.12 Cumulative abundance of newly established mud lobster 101 mounds on transect across dyke at Site A1 From Jan Dec 2006, Carey Island.
 Numerals above bars indicate % contribution of transect zones to mound numbers.
 For zones (x-axis): Road-RD, Bund ditch-DB, Bottom bund-BB, Top bund slope-TB, Landside bund top-TL and waterside bund top-TW. (see Figure 5.2 for detail of the zones).
- Figure 5.13 Monthly distribution of newly colonized mud lobster mounds by 102 zones from January to December 2006 (Site A1).
 A= full moon (spring tide) B= new moon (spring tide).
 For zones (x-axis): Road-RD, Bund ditch-DB, Bottom bund-BB, Top bund slope-TB, Landside bund top-TL and waterside bund top-TW. (see Figure 5.2 for detail of the zones).
- Figure 5.14 Distribution of new "Active" (A), "Abandoned" (O) and 103 "Inactive" (InA) mounds by zones across A1 site (most abundant) over 12 months.
 A= full moon (spring tide) B= new moon (spring tide). For zones (x-axis): Road-RD, Bund ditch-DB, Bottom bund-BB, Top bund slope-TB, Landside bund top-TL and waterside bund top-TW. (see Figure 5.2 for detail of the zones).
- Figure 5.15 Total number of new mud lobster mounds in newly repaired 105 dyke (Site B1) from January to December 2006. A= full moon (spring tide) B= new moon (spring tide).
- Figure 5.16 Distribution and abundance of new mud lobster mounds for each 105 zones at Site B1 on the dyke.
 Numerals above bars indicate % contribution of transect zones to mound numbers.
 For zones (x-axis): Road-RD, Bund ditch-DB, Bottom bund-BB,

Top bund slope-TB, Landside bund top-TL and waterside bund top-TW. (see Figure 5.2 for detail of the zones).

- Figure 5.17 Distribution of new mud lobster mounds by zones from January 106 to December 2006 for Site B1. A= full moon (spring tide) B= new moon (spring tide).
 For zones (x-axis): Road-RD, Bund ditch-DB, Bottom bund-BB, Top bund slope-TB, Landside bund top-TL and waterside bund top-TW. (see Figure 5.2 for detail of the zones).
- Figure 5.18 Monthly distribution of newly colonized mud lobster mounds by 107 zones from January to December 2006 (Site B1).
 A= full moon (spring tide) B= new moon (spring tide). For zones (x-axis): Road-RD, Bund ditch-DB, Bottom bund-BB, Top bund slope-TB, Landside bund top-TL and waterside bund top-TW. (see Figure 5.2 for detail of the zones).
- Figure 5.19 Total number of new mud lobster mounds in newly repaired 109 dyke (Site C1) over 12 months. A= full moon (spring tide) B= new moon (spring tide).
- Figure 5.20 Distribution and abundance of new mud lobster mounds for each 109 zones at Site C1 on the dyke.
 Numerals above bars indicate % contribution of transect zones to mound numbers.
 For zones (x-axis): Road-RD, Bund ditch-DB, Bottom bund-BB, Top bund slope-TB, Landside bund top-TL and waterside bund top-TW. (see Figure 5.2 for detail of the zones).
- Figure 5.21 Distribution of monthly new mud lobster mounds by zones from 110 January to December 2006 for Site C1. A= full moon (spring tide) B= new moon (spring tide).
 For of zones (x-axis): Road-RD, Bund ditch-DB, Bottom bund-BB, Top bund slope-TB, Landside bund top-TL and waterside bund top-TW. (see Figure 5.2 for detail of the zones).
- Figure 5.22 Monthly distribution of newly colonized mud lobster mounds by 111 zones from Jan Dec 2006 (Site C1).
 A= full moon (spring tide) B= new moon (spring tide). For zones (x-axis): Road-RD, Bund ditch-DB, Bottom bund-BB, Top bund slope-TB, Landside bund top-TL and waterside bund top-TW. (see Figure 5.2 for detail of the zones).
- Figure 5.23 Monthly mean values of water parameters and sediment organic 117 contents and soil moisture at three sampling sites in Carey Island. (* standard deviation omitted for clarity). A= full moon (spring tide) B= new moon (spring tide). A1= heavy infestation, B1= moderate infestation, C1 = low infestation.
- Figure 5.24 PCA biplot showing the water and soil characteristics of three 121 habitat sites with different levels of mud lobster infestation. A1= heavy infestation, B1= moderate infestation, C1 = low

infestation.

- Figure 5.25 Diagram shows the hypothesized path of invasion by mud 124 lobsters from the mangrove/ sea side.
- Figure 6.1 Schematic diagrams of study zones and planting method of the 131 vegetation across the dyke to prevent mud lobster intrusion. *Diagram drawn not to scale.
- Figure 6.2 Total number of the new mud lobster mound and percentage 138 cover (%) for *Chrysopogon zizanioides*.
- Figure 6.3 Total number of the new mud lobster mound and percentage 138 cover (%) for *Cynodon dactylon*.
- Figure 6.4 Total number of the new mud lobster mound and percentage 138 cover (%) for *Paspalum distichium*.
- Figure 6.5 Total number of the new mud lobster mound and percentage 139 cover (%) for *Chrysopogon zizanioides* + *Paspalum distichium*.
- Figure 6.6 Total number of the new mud lobster mound and percentage 139 cover (%) for *Chrysopogon zizanioides* + *Cynodon dactylon*.
- Figure 6.7 Total number of the new mud lobster mound for control. 139
- Figure 6.8 Combination treatments on cumulative mounds at month 16, 140 where all vegetation growth achieved 100%.cover.

List of Tables

		Page
Table 2.1	Comparative morphology of <i>Thalassina anomala</i> , <i>T. squamifera</i> and <i>T. kelanang</i> (new species).	29
Table 3.1	Definitions of 13 morphometric and six meristic characters of four species of <i>Thalassina</i> (<i>T. anomala, T. gracilis, T. kelanang</i> and <i>T. squamifera</i>).	37
Table 3.2	GenBank accession numbers for the four species of thalassinids and outgroups.	41
Table 3.3	Standardized coefficients for canonical variables derived by SFDA of morphometric and meristic characters.	42
Table 3.4	Uncorrected "p" distance measures (%) among four species of <i>Thalassina</i> based on PEPCK, NaK, COI and combined molecular markers.	48
Table 4.1	Site characteristics, number of above-ground mangrove roots (no. m^{-2}) and mean wet mound density (no. m^{-2}) of three species of <i>Thalassina</i> at Kelanang Beach (Site 1) and Carey Island (Site 2 and 3).	71
Table 4.2	Mean basal diameter, height and burrow mouth diameter of wet mud lobster mounds according to species and site (zone).	73
Table 5.1	Categories of sediment based on particle size.	90
Table 5.2	Frequency-class distribution of all the mud lobster mounds in Carey Island.	93
Table 5.3	Correlations among three mound characteristics (all significant P< 0.05). *Red value indicate significant different at P< 0.05	99
Table 5.4	Mean water parameters, standard deviations (SD) and summary of Kruskal-Wallis test results for difference among three different sites (A1, B1, C1) on the dykes of Carey Island. Similar superscript alphabets indicate homogenous groups tested at 5% significance level using Kruskal-Wallis test. A1= heavy infestation, B1= moderate infestation, C1 = low infestation.	114
Table 5.5	Mean soil composition (%), standard deviations (SD) and summary of Kruskal-Wallis test results between three different sites in the dykes of Carey Island. A1= heavy infestation, B1= moderate infestation, C1 = low infestation.	120

- Table 6.1The random assignment of vegetation species and control plots on133the dyke.
- Table 6.2Comparison total number of accumulative mounds per 3 plots,
mean no. accumulative mounds per plot (90m²) and mean
percentage cover (%) for five different treatments of grass and
control plot over 8 months and last month (16 months).137
- Table 6.3Dunnett test for Post Hoc Tests for new cumulative mounds for141five different treatments for the last month (16 months).*Red value indicates significant different at P<0.05</td>
- Table 7.1The current extant species of *Thalassina* based on morphology145with or without molecular validation.

Chapter 1

Introduction

1.1 An overview taxonomy of mud lobster, their distribution and habitat

Mud lobsters are decapod crustaceans previously described under the family Thalassinidae Latreille, 1831. Thalassinidea is a former infraorder of decapod crustaceans that is now recognized as belonging to two separate lineages now recognized as infraorders; Gebiidea and Axiidea (Sakai, 2004; De Grave et al., 2009). The current the classification of the mud lobsters is now as follows:

Order: Decapoda Latreille, 1802

Suborder: Pleocyemata Burkenroad, 1963

Infraorder: Gebiidea de Saint Laurent, 1979

Family: Thalassinidae Latreille, 1831

Genus: Thalassina Latreille, 1806

The family Thalassinidea is monogeneric. *Thalassina anomala* (Herbst, 1804), was first described as *Cancer* (*Astacus*) *anomalus* Herbst, 1804. The genus *Thalassina* Latreille, 1806, was at one time considered monotypic (Glaessner, 1969), but Holthuis (1991) speculated that there may be more than one species. This genus has recently been revised show to contin more than one species (see below).

Ngoc-Ho & de Saint Laurent (2009) published on seven species of *Thalassina*; *T. anomala* (Herbst, 1804), *T. squamifera* De Man, 1915, *T. gracilis* Dana. 1852 [sensu Ngoc-

Ho & de Saint Laurent, 2009], T. emerii Bell, 1844, T. spinirostris Ngoc-Ho & de Saint Laurent, 2009 (new species from Singapore) and another two new species related to T. anomala, T. spinosa Ngoc-Ho & de Saint Laurent, 2009 (from India and Indonesia) and T. krempfi Ngoc-Ho & de Saint Laurent, 2009 (from Vietnam, Singapore and Indonesia). In the same year, Moh & Chong (2009) reported additionally one new species, Thalassina kelanang Moh & Chong, 2009 from Malaysia. Sakai & Türkay (2012) reviewed the genus Thalassina Latreille, 1806 with a new classification key for the identification of Thalassina. They suggested that T. emerii Bell, 1844 is a nomen dubium, and that the material identified by Ngoc-Ho & de Saint Laurent, 2009 as T. emerii Bell, 1844 were in fact new species; T. australiensis Sakai & Türkay, 2012 and T. saetichelis Sakai & Türkay, 2012. Thus far, the number of *Thalassina* mud lobster species world-wide may be at least nine extant species. The nine species are T. anomala (Herbst, 1804), T. squamifera De Man, 1915, T. gracilis Dana. 1852 [sensu Ngoc-Ho & de Saint Laurent, 2009], T. spinirostris Ngoc-Ho & de Saint Laurent, 2009, T. spinosa Ngoc-Ho & de Saint Laurent, 2009, T. krempfi Ngoc-Ho & de Saint Laurent, 2009, T. kelanang Moh & Chong, 2009, T. australiensis Sakai & Türkay, 2012 and T. saetichelis Sakai & Türkay, 2012.

The most commonly reported mud lobster, *T. anomala* occurs in the southern and south–eastern region of Asia from Bombay in the west to Morton Bay in Queensland, Australia where the shore soils are heavy and clayey rather than sandy. *T. anomala* was reported to be an extremely abundant animal in the mangrove swamps of Singapore and Malaya in the early 1960s (Chuang, 1961; Johnson, 1961). The mud lobster is abundant in water-logged soil with a distribution that extends up the shore to above the extreme high water-mark (Macnae, 1968). From a study by Varuges (1978) one species was found on Carey Island, namely *T. anomala* (Herbst). De Man (1928) recognized only two races of

the one species, *T. anomala gracilis* and *T. anomala squamifera*, and conceded that all those which had been described earlier might prove to be identical.

Thalassina anomala inhabits the upper intertidal zone of estuarine tropical shores. Its distribution within the deposits of this shore zone is influenced by an array of environmental parameters, of which the interplay of salinity, organic matter content and sediment size seem to be the chief determinants (Pillai, 1989). The species has been described from Queensland (Bennet, 1968), the countries of Southeast Asia and the islands of the Indo-West Pacific (Macnae, 1968), and on the subcontinent of India, especially along the western shores (Sankolli, 1963), and in the estuaries of the Ganges (Gayen and Choudhary, 1973).

Verwey (1930) reported that the burrows of *T. anomala* are single, with a long horizontal or near horizontal tunnel and a pool at the bottom. These shy creatures are responsible for the volcano-shaped mounds that are commonly seen in the back mangroves. Their burrows are U-shaped and can be up to 2m deep even below the waterline. During the day, mud lobsters plug up their entrance with mud. In the evening, these are opened and the mud lobsters may emerge. Mud lobsters prefer to dig in the intertidal regions (Ng & Kang, 1988). Macintosh (1988) reported *T. anomala* can stay deep below surface in noxious anaerobic conditions.

According to Marshall & Orr (1960) *T. anomala* feeds on vegetation. Johnson (1961), Sankolli (1963) made detailed observations on the feeding habits and concluded that *Thalassina anomala* is not an herbivore, but a mud-feeder. Mud lobsters are believed to eat tiny organic titbits in mud. To get enough nutrition, they have to process huge

amounts of mud and sand. Processed mud is piled around their burrows as they eat-and-dig through the mud. Soil moisture content, pH and the presence of *T. anomala* activity are important factors in the distribution of the mangrove vegetation (Ng & Sivasothi, 1999). Its behavior of tunneling through coastal and pond dykes can interfere with paddy cultivation and aquaculture, so it is considered a pest in most countries, but in parts of Fiji, it constitutes an important food resource (Pillai 1985).

The destructive nature of *T. anomala* in the man-made environment was noted by (Sankolli, 1963), who stated that the animal is notorious for causing severe damage to coastal bunds by its burrowing habits. Holthuis (1991) summarise that *T. anomala* is considered a pest in many areas. The animal is notorious for causing severe damage to bunds of prawn ponds by its burrowing activities (Macintosh, 1988). The paddy fields and backyards of houses in the proximity of the creeks are also subject to this sort of damage (Sankolli, 1963). This is due to *T. anomala* naturally occurring on the landward margins of mangrove forests beyond the extreme high-water mark.

Several other decapod crustaceans displayed similar destructiveness in man-made (human modified) or new environments in other tropical and temperate regions. Invasive species that are brought into new environments via aquaculture and the aquarium trade can cause biogeomorphic impacts such as bioturbation, bioerosion and bioconstruction (Fei et al., 2014). Faller et al. (2016) reported several species of invasive non-native crayfish in UK that are known to dig burrows into river banks such as the spiny cheek crayfish (*Orconectes limosus*), virile crayfish (*Orconectes virilis*) and signal crayfish (*Pacifastacus leniusculus*). The red swamp crayfish *Procambarus clarkii* in Zambia burrows into the sides or banks of dams, rivers and lakes to make their nests thus damaging the local

infrastructure, causing the water canals to leak, earthern dams to collapse, and the banks of rivers and lakes to erode (Moonga & Musuka, 2014). The activities of other crayfish species (*Pacifastacus leniusculus*; *Procambarus clarkii*) can be extensive and cause complete riverbank collapse (Guan 1994, Barbaresi et al. 2004). The burrowing activities of the mitten crab (*Eriocheir sinensis*) threaten unprotected human earthworks such as digs and levees (Rudnick et al., 2000). Mitten crabs are extensive burrowers and their burrows have been observed to undermine stream banks in Germany, causing banks to collapse (Panning 1939). Since their introduction into San Francisco Bay, there has been much concern on the impact of the burrowing activity of mitten crabs on the bay and delta notably on the stream banks, especially those used for flood control (Cohen & Carlton 1995, Rudnick et al., 2000).

1.2 Research problems and objectives

Both the reviews by Ngoc Ho & de Saint Laurent (2009) and Sakai & Türkay (2012) and all re-descriptions of the *Thalassina* species as well as the newly described species including Moh & Chong (2009)'s work on *T. kelanang*, are based entirely on morphology. The veracity of the new species status has not been validated by molecular studies. It may be possible that the so-called 'new' species are actually morphs as commonly encountered in arthropods, and this issue could only be settled by molecular analysis.

Preliminary studies have indicated populations of mud lobsters from the lower to upper shore. Besides the question of whether they all belonged to the same species or are of different species, there has been no work done on how possible sympatric species of mud lobsters are distributed in the same general area. If the same species is found in the entire intertidal shore, this would indicate a species tolerant of the wide gradient in environmental factors (e.g. moisture, heat), whereas if they are different species, it would be interesting to know how these sympatric species coexist in the intertidal shore.

In Carey Island (2°52' 18.38''N, 101°22' 41.15'E, state of Selangor), extensive areas of mangrove forest were cleared for agricultural development in the early 1900s, coastal bunds, drainage and tidal gates were built around the entire perimeter of the island to protect the crops from seawater intrusion. The impact of coastal dyke in the mangrove area is that the mud lobsters now make their homes in the coastal bunds and on their landward side. Extensive tunneling by the mud lobster weakens and undermines the bund integrity. Bund collapse occurs quite regularly incurring considerable costs to the estate due to bund repair and maintenance. Each year millions of ringgit for bund repair and maintenance are being spent by Sime Darby Plantation Bhd, the company which owns and manages the plantation. It has been estimated that dyke damage and maintenance in Carey Island incurs an annual cost of between RM1.5-2.0 million. An estimated average of about RM15, 000 per kilometer of bund is needed for maintenance and repair cost.

As a result, the mud lobster population in the plantation is being controlled by the regular use of chemical poisons such as Furadan 3G (Carbofuran). The use of such poisons is not recommended as this class of poisons (Class II) is not ecologically friendly. This insecticide is known to be hazardous to fish, wildlife and invertebrates (Eisler, 1985). Nevertheless, chemical control however provides a temporary solution only, as their numbers built up again after a few months. To effectively control the mud lobster

population on the coastal bunds, an ecological study is needed to fully understand their habitat requirements, behavior and other biological aspects.

Thus, the overall aim of this study was to verify whether the different populations of mud lobsters belong to a single species (T. anomala), and to study its ecology and biology so that ecologically-sound and cost effective methods could be used to control their populations in the man-made environment such as in a coastal plantation. It is hoped that from this study, biological controls, rather than chemical controls, could be applied to avoid damage to the environment. The biological control methods will have similar economic and ecological benefits to coastal aquaculture farms that are also afflicted by mud lobster infestations.

1.3 Objectives of study

The objectives of this study are:

- Taxonomy of the mud lobster species from Peninsular Malaysia using both morphological and molecular analysis.
- To study the distributional pattern of mud lobster in relation to the abiotic factors (water parameter, sediment characteristics and tidal inundation) on the mangrove shore.
- 3) To determine the mud lobster distribution and abundance in an oil palm plantation.
- To investigate the spatial-temporal colonization of newly-repaired dykes by mud lobster.
- 5) To determine the biological or ecologically-sound methods to control dyke infestations by mud lobsters in Carey Island.

Hypotheses that were tested in this study are as follows:

- 1) Sympatric species of mud lobsters are spatially partitioned across mangrove shore as a result of niche differentiation (Chapter 4).
- 2) The mode or pathway the *T. anomala* invading into the dyke began at the dyke top on the mangrove side during spring tide, then tunneling diagonally down to the dyke bottom on the plantation side (Chapter 5).

The following investigations were carried out to address the above objectives and/ or hypotheses:

- 1. Morphological differentiation of three species of Malaysian *Thalassina* mud lobsters, *T. anomala*, *T. gracilis* and *T. kelanang* (sp. nova), and the Australian species, *T. squamifera* (Chapter 2).
- Verification of the four species of mud lobsters, *T. anomala*, *T. kelanang*, *T. gracilis* and *T. squamifera* using molecular, morphometric and meristic characters (Chapter 3).
- 3. Distribution and burrow morphology of three sympatric species of *Thalassina* mud lobsters in relation to environmental parameters on Kelanang shore (Chapter 4).
- 4. Mound distribution and density of *Thalassina* mud lobsters on the coastal dykes of Carey Island (Chapter 5).
- 5. Control of mud lobter (T. anomala) infestations on coastal dykes (Chapter 6).

Chapter 2

Morphological differentiation of three species of Malaysian *Thalassina* mud lobsters, *T. anomala*, *T. gracilis* and *T. kelanang* (sp. nova), and the Australian species, *T. squamifera*

Work done in this chapter has been published in:

Moh, H. H., & Chong, V. C. (2009). A new species of *Thalassina* (Crustacea: Decapoda: Thalassinidea) from Malaysia. *Raffles Bulletin of Zoology*, 57(2), 465–473.

2.1 Introduction

The family Thalassinidae Latreille, 1831, contains the single genus *Thalassina* Latreille, 1806, which had at one time been considered monotypic (Glaessner, 1969), but Holthuis (1991) suggested that there may be more than one species. *Thalassina anomala* (Herbst, 1804) is the type species, first described as *Cancer* (*Astacus*) anomalus Herbst, 1804, based on a female type specimen collected from an unknown locality. The species was subsequently redescribed under the following synonyms: *Thalassina scorpionides* Latreille, 1806 (type locality: unknown); *Thalassina scabra* Leach, 1814 (type locality: unknown); *Thalassina talpa* White, 1847 (type locality: Philippines); *Thalassina gracilis* Dana, 1852 (type locality: Telegraph Island, near Singapore); and *Thalassina maxima* Hess, 1865 (type locality: New South Wales, Australia) (in Miers, 1880; De Man, 1928).

De Man (1928) described his specimens of *T. anomala* var. *squamifera* (type locality: Karakelang-islands, Indonesia) as quite different from the typical, widespread *T. anomala*, in that the former had a small movable scaphocerite on its antennal peduncle while the typical *anomala* did not have it. In addition, he also recognized that the shape of the ridge between the second to fifth pleopods is different between the two varieties. Campbell & Woods (1970) elevated *T. anomala* var. *squamifera* to species rank, hence *T. squamifera* De Man, 1915, after examination of the Australian specimens. Support for this assignment came from Poore & Griffin (1979) who examined 32 samples from Australia and one from the Philippines. They further added that *T. squamifera* lacks the oblique tuberculate ridge starting near the base of the fixed finger and running back on the lateral surface of the large cheliped in *T. anomala*. This character was found to be most useful in separating fossil materials of the two species (Campbell & Woods, 1970).

Thalassina emerii Bell, 1844, was first described based on a fossil specimen believed to be likely from the mouth of the Daly River, Northern Territory, Australia, but its recognition in museum specimens from N.W. Australia suggests that this species may be extant (see Davie, 2002, p. 475). Another species, *Thalassina chilensis* Steenstrup & Lütken, 1862, from the coast of Chile believed to be distinct from *T. anomala*, but De Man (1928) suspected that *T. chilensis* is likely *T. gracilis* Dana, 1852 which he thought was synonymous with *T. anomala*. Holthuis (1991) surmised that the type locality of *T. chilensis* is likely to be incorrect given that it has since never been found in Chile. Ngoc-Ho & de Saint Laurent (2009) reviewed *Thalassina* Latreille, 1806 and recognised have seven valid species; *T. anomala* (Herbst, 1804); *T. squamifera* De Man, 1915, *T. gracilis* Dana. 1852, *T. emerii* Bell, 1844, and two new species, *Thalassina krempfi* Ngoc-Ho & de Saint Laurent, 2009, and *Thalassina spinirostris* Ngoc-Ho & de Saint Laurent, 2009. Sakai & Türkay (2012) review the genus with a new classification key for the identification of *Thalassina* and added in two more new species. The new species are *Thalassina australiensis* Sakai & Türkay, 2012, and *Thalassina saetichelis* Sakai & Türkay, 2012. Thus so far, world-wide there appears to be at least nine extant species of mud lobsters belonging to the genus *Thalassina*.

In Malaysia, *Thalassina anomala* was first recorded from the states of Penang and Sarawak (in De Man, 1928), but the species appears quite widespread, appearing also in the states of Selangor (Sasekumar, 1974) and Johor (Ng & Kang, 1988). However, there has been no attempt to examine closely these shy and difficult to catch creatures, to see if there are more than one species. This chapter describes two main species found in Selangor, Malaysia; one the typical *T. anomala*, and the other a new species. We compared their morphology to specimens of *T. squamifera* obtained from Natural Sciences Museum & Art Gallery of the Northern Territory (NSMAG), Australia and *T. gracilis* from the Zoological Reference Collection (ZRC) of the Raffles Museum of Biological Research, National University of Singapore, and our collection.

2.2 Material and methods

2.2.1 Study sites. - Mud lobsters were sampled from two study sites at Kelanang Beach and Carey Island, Selangor, Malaysia. The mud lobster mounds were located inside or near to mangrove forests in the intertidal shore. The sandy-mud beach of Kelanang has a narrow (0.6 km) and degrading fringe of mangrove forests where on the landward side a coastal bund runs along the entire coastline. All specimens of the new species were sampled from Kelanang Beach.

In Carey Island, which is located just north of Kelanang, the mud lobster mounds of only *T*. *anomala* are found on the landward side of perimeter bunds which are built around the entire island to protect the oil palm plantations that occupy former mangrove forests from seawater intrusion.

2.2.2 Sampling. - Mud lobsters were trapped by using a 50 cm –long wire with a tethered piece of fish netting (1.5" or 2.5" mesh size) on one end and a T-handle at the opposite end. The wire was inserted into the burrow with the T-handle resting on top of the mound. The traps were laid during dusk and recovered after 15-18 hours. Trapped animals were entangled in the gill netting as they crawled up from the muddy bottom. The animals were also caught by digging below their mounds using a spade. All specimens were identified and measured using a pair of digimatic calipers, with a precision of 0.01mm. Measurements provided are of the total length (measured along the midline from tip of rostrum to tip of telson) and the carapace length (measured along the midline from tip of rostrum to posterior edge of carapace). Identified specimens are deposited in the Zoological Museum, University Malaya (ZMUM). Two specimens of the new species (male and female) are also deposited in ZRC, Singapore, and Natural Sciences Museum & Art Gallery of the Northern Territory (NSMAG), Australia.

2.2.3 Comparative material examined.

T. anomala – 13 males and 5 females (TL; 114.6-241.1 mm) (ZMUM), Carey Island, Selangor, Malaysia, coll. H.H.Moh, Jun.-Sep.2006.

T. squamifera – 1 male (132.5, 47.8 mm) (NSMAG, Cr.004775) (figured), Micket Creek, Darwin, N.T., Australia, coll. D. Percival, 26 Sep.1983; 1 male (146, 51.7 mm) (NSMAG,

Cr.000517), Ludmilla Creek, Darwin, N.T., Australia, coll. J.R.Hanley, 12 Oct.1983; 1 male (139.6, 51.3 mm) (NSMAG, Cr.001475), Ludmilla Creek, Darwin, N.T., Australia, coll. D. Percival, 13 Oct. 1983; 1 female (113.0, 41.9 mm) (NSMAG, Cr.009965), near bridge Channel Island, N.T., Australia; coll. M. Burke, 19 Feb. 1992; 1 female (70.3, 26.1mm) (NSMAG, Cr. 013891), Port Keats, N.T., Australia, coll. K. Metcalfe & party, undated.

T. gracilis – 1 male (69.8, 25.8 mm) and 1 female (57.3, 21.8 mm) (ZRC 2007.0512), Ranong mangroves, South Thailand, coll. P. Naiyanetr; 1 male (69.8, 25.5 mm) (ZMUM), Carey Island, Kuala Langat [=District], Selangor, Malaysia, coll. H.H.Moh, 19 Sep.2008.

2.3 Result

2.3.1 Taxonomy

Thalassina kelanang, new species (Figure 2.1).



Figure 2.1. Dorsal view of carapace. A, *Thalassina kelanang*, holotype-male, (TL=150 mm, CL= 54.1 mm)

Holotype – male (150.0, 54.1 mm) (ZMUM), from mound in mangrove forest, Kelanang Beach, Kuala Langat, Selangor, Malaysia; coll. H.H. Moh, Apr.2007.

Paratypes - 1 female (141, 50.3 mm) (ZMUM), Kelanang Beach, Kuala Langat, Selangor, Malaysia; coll. H.H.Moh, Oct. 2006; 1 male, (140, 52.0 mm), 4 females (120, 44.6 mm), (162, 58.9 mm), (167, 60.8 mm), (150, 55.4 mm), (ZMUM), Kelanang Beach, Kuala Langat, Selangor, Malaysia; coll. H.H.Moh, Nov. 2006; 1 male, (114, 42.9 mm), 2 females (163, 58.4 mm), (140, 51.5 mm), (ZMUM), Kelanang Beach, Kuala Langat, Selangor, Malaysia; coll. H.H.Moh, Mac. 2007; 2 males (143, 51.9 mm), (133, 48.8 mm), 2 females (120, 43.7 mm), (119, 45.4 mm), (ZMUM), Kelanang Beach, Kuala Langat, Selangor, Malaysia; coll. H.H.Moh, Apr. 2007; 1 female (161, 59.1 mm), (ZMUM), Carey Island, Kuala Langat, Selangor, Malaysia; coll. H.H.Moh, Nov. 2006; 1 male (183, 63.6 mm), (ZMUM), Carey Island, Kuala Langat, Selangor, Malaysia; coll. H.H.Moh, Nov. 2006; 1 male (183, 63.6 mm), (ZMUM), Carey Island, Kuala Langat, Selangor, Malaysia; coll. H.H.Moh, Nov. 2006.

2.3.2 Description of holotype. - Carapace elongate oval in dorsal aspect; sculptured by circular depressions or punctae, largest pair (gastric pits) associated with post-cervical groove; upper lateral sides heavily covered with short anteriorly-directed spines; short posteriorly-directed spine on dorsal median margin not reaching first abdominal tergite (Fig. 2.2A). Rostrum flat, narrowly triangular, waisted near base (Figure 3.3A), with lateral margins that continue posteriorly as short divergent ridges (adrostral carina) extending half the length of gastro-orbital carina; median sulcus or groove deep, extending beyond adrostral carina; 3-11 blunt marginal spinules or tubercles on adrostral carina. Supra-orbital, antennal and branchiostegal spines strong, sharp; orbital and sub-orbital spines short; 4-8 sharp spines at curved anterior end of branchiocardiac groove. Oblique groove with 10-14 spines on dorsal margin, anterior-most spine largest, thinly setose.

Anterior margin of antennal region armed with series of short spines. Numerous tubercles on anterior margin of branchiostegite.



Figure 2.2. Dorsal view of carapace. A, *Thalassina kelanang* (holotype, 150.0, 54.1 mm); B, *T. anomala*-Male (151.4, 51.1 mm). Zoological Museum, University of Malaya (ZMUM). Scale bar = 10 mm.


Figure 3.3. Dorsal view of anterior region of cephalothorax. A, *T. kelanang* (holotype, 150.0, 54.1 mm); B, *T. anomala*-Male (151.4, 51.1 mm); C, *T. squamifera*-Male (132.5, 47.8 mm); D, *T. gracilis*-Male (69.8, 25.8 mm). Zoological Museum, University of Malaya (ZMUM). Scale bar = 5 mm.

Antenna with highly reduced antennal scale or scaphocerite on the outer side, scaphocerite large, setose on inner margin (Figure 2.4A). Antennal flagellum when stretched backwards reached first or second abdominal somite, length more than 5 times length of antennular flagellum.



Figure 2.4. Dorsal view of right antennal peduncle showing movable scaphocerite. A, *T. kelanang* (holotype, 150.0, 54.1 mm); B, *T. anomala*, (absent)-Male (151.4, 51.1 mm); C, *T. squamifera*-Male (132.5, 47.8 mm). Zoological Museum, University of Malaya (ZMUM). Scale bars: a-b = 20 mm; c = 10 mm.

Pereopods 1 (chelipeds) asymmetrical, subchelate, left chela larger than right (but see Paratypes below). Meri large, flattened laterally on dorsal surface, but broad or triangular on ventral surface; right merus with 17 dorsal spines, left with 18 dorsal spines, anteriormost 4 spines large and decurved, inner and outer ventral margins serrulate with numerous subequal denticles on ventral surface. Carpi relatively small, inner dorsal margin with row of 7-8 strong spines, outer ventral margin armed with a row of spines, anterior-most being most prominent. Propodi granulated on entire surface, granules on the posterior half of inner ventral surface comparatively larger than those on anterior half, dorsal surface with two ridges: inner ridge armed with row of 10 strong spines (Figure 2.5A, 2.5B), outer ridge on proximal three fourths distance, armed with row of small tubercles, outer surface with long fine setae occurring in tufts along rows, ventral surface with 2 serrated ridges. Dactyli twice as long as fixed finger, narrow, laterally flattened, right dorsal margin armed with row of 13-15 spines and numerous long fine stae, ventral surface with 2 serrated ridges and 2 rows of fine setae, a few punctae present along dorsal surface.



Figure 2.5. Right chela of first pereiopod. A-B, outer and inner aspects, *T. kelanang* (holotype, 150.0, 54.1 mm); C-D, outer and inner aspects, *T. anomala*-Male (151.4, 51.1 mm). Zoological Museum, University of Malaya (ZMUM). Scale bar = 10 mm.

Pereopod 2 subchelate, smaller than pereopod 1; basi-ischium laterally flattened, with ventral margin armed with 6-9 strong spines and a row of setae; merus laterally flattened, dorsal margin with row of 4-6 strong decurved spines, ventral surface densely setose;

carpus setose on dorsal and ventral margins, dorsal margin with 2 or 3 strong, decurved spines; propodus length slightly longer than width, dorsal margin of fixed finger armed with longitudinal row of blunt teeth; dactylus with 2 rows of setae on dorsal margin, outer surface with medial longitudinal row of setae, ventral margin serrated with row of blunt teeth, postero-ventral margin setose.

Pereopod 3 narrow, flattened laterally; coxa armed with 4-6 spines on inner surface; ischium armed with 4-6 spines on ventral margin; merus with row of 6-10 decurved spines on dorsal margin, ventral margin serrated with 2 rows of 10-12 spines; carpus armed with 4 or 5 strong spines on dorsal margin, ventral margin spineless; propodus small, laterally compressed, with setae on dorsal and ventral surfaces; dactylus slender as long as propodus, ventral margin with row of setae, dorsal margin with 7 short spines and row of setae.

Pereopod 4 similar but slightly smaller than pereopod 3; dactylus slender, longer than propodus.

Pereopod 5 smaller than pereopod 4, not compressed; coxae with gonopores facing each other on either side of mid-ventral line; outer margin armed with row of spines.

Male abdomen elongate and narrow; somite width as wide as length; first abdominal somite smallest and narrowest; third and fourth somites largest. Dorsal tergite of first abdominal somite raised as a distinct rectangular piece, with inverted Y groove (Figure 2.6A).



Figure 2.6. Dorsal view of first abdominal somite. A, *T. kelanang* (holotype, 150.0, 54.1 mm); B, *T. anomala*-Male (151.4, 51.1 mm). Zoological Museum, University of Malaya (ZMUM). Scale bar = 10 mm.

Base of pleuron of second and third abdominal somites, each with 2 longitudinal, serrated ribs or carinae occupying anterior 3/4 distance to posterior end of segment (Figure 2.7A). Abdominal sternite of second to fifth somites with distinct bicuspid sternal ridge between opposite pleopods; each cusp bearing 3-6 teeth (Figure 2.7B).



Figure 2.7. Second abdominal somite. A-B, dorsal and ventral aspects, *T. kelanang* (holotype, 150.0, 54.1 mm); C-D, dorsal and ventral aspects, *T. anomala*-Male (151.4, 51.1 mm). Zoological Museum, University of Malaya (ZMUM). Scale bar = 10 mm.

Mature male pleopod 1 uniramus, with opposing endopods modified and united to form petasma or intromittent organ; distal end of petasma narrowly oval to pointed, setal row on outer and inner margins reaching or almost reaching tip; inner distal setae modified to form fine interlocking hooks on disc-like, subterminal keel of petasma; outer proximal end of petasma armed with 3 or 4 strong spines (Figure 2.8A). Pleopods 2-5 biramous; pleopods 3-5 of equal size, but shorter than pleopod 2.

Uropods styliform. Telson broadly triangular, about as long as previous somite.





Figure 2.8. Ventral view of petasma. A, *T. kelanang* (holotype, 150.0, 54.1 mm); B, *T. anomala*-Male (193, 61.4 mm); C, *T. squamifera*-Male (132.5, 47.8 mm); D, *T. gracilis*-Male (69.8, 25.8 mm). Zoological Museum, University Malaya (ZMUM). Scale bar = 5 mm.

2.3.3 Variation. – Although our holotype male has a larger right cheliped than the left, the left cheliped can be larger than the right for both male and female, but in some of our specimens the difference was not obvious. Our specimens had 14 of the former type and 11 of the latter type. Merus of right cheliped with 12-17 dorsal spines, left 15-18, anterior 3-5 spines large and decurved. In female, gonopores on inner ventral surface of coxa of

pereopod 3; pleopod 1 uniramus; pleopods 2-5 larger and biramous, bearing long setae which are particularly well-developed for carrying eggs during breeding season.

2.3.4 Etymology. – The species is named after its type locality, Kelanang, in the district of Kuala Langat of the state of Selangor, Malaysia. The name is used as a noun in opposition.

2.3.5 Colour. – Carapace orange to brown on dorsal aspect, becoming grey ventrolaterally. Abdomen red to orange on dorsal aspect, pleura grey. Dorsal aspect of pereopods brownish orange, ventral aspect grey.

2.3.6 Biological notes. – *Thalassina kelanang* construct sandy mud mounds of generally less than 0.5 m in height. At Kelanang Beach, while the animal inhabits the fringing *Rhizophora* forest, there were more mounds on open sandy mud substrate without vegetation. In contrast, *T. anomala* were found in muddy substrate particularly in Carey Island, although few *T. kelanang* were found on the island. Thus, the two species appear to occupy their own microhabitats in the same general area. Male *T. kelanang*, unlike *T. anomala*, are very aggressive inflicting painful pinches on handlers; males often ended up with broken legs if placed together in an aquarium.

2.4 Discussion

Thalassina kelanang shows similarities with the sympatric species, T. anomala in terms of size, coloration and general morphology. However, their morphological differences warrant the separation of the two species. In all our 65 T. anomala specimens, the posterior median spine of the carapace, often decurved, always overhangs the articulation with the first abdominal tergite (Fig. 2.2B). In contrast, the median spine of T. kelanang is short, blunt and never overhangs the first abdominal tergite. However, this character is similar to that of T. squamifera. The oblique groove of the carapace of T. anomala is covered with a thick row of setae, whereas in the new species and in T. squamifera it is sparsely covered by setae. Thalassina kelanang has an acute tip and waisted base of the rostrum with deep median sulcus (groove) that extends up to the posterior pair of dorsal punctae behind the posterior end of adrostrals; the latter reaches only distal half of the gastro-orbital carina (see Figure 2.3A). In contrast, both T. anomala and T. squamifera have a triangular rostrum with a shallow median sulcus that does not reach beyond the adrostrals; the latter extends to three fourths or full length of the gastroorbital carina (Figure 2.3B, 2.3C). In addition, both species have rather smooth or at the most three tubercles on their adrostral and gastro-orbital carinae, posterior to the orbital margin. These features of the rostrum appear to be important in separating the different species of *Thalassina*. In fact, the examined *T. gracilis* specimens show a distinctive depressed and acute rostrum unlike that of *T. kelanang* and the others (Figure 2.3D).

De Man (1928) pointed out the differences between *T. anomala* and *T. squamifera* (as a variety of *T. anomala*), in that only the latter has a scaphocerite on the antennal peduncle as well as a tuberculate ridge on the abdominal sternites (see below). Sankolli

(1970) reported that *T. anomala* specimens in India, except for one female, generally did not have the scaphocerite. From a total of 65 specimens of *T. anomala* examined in this study, 29 of them have much reduced scaphocerites, while 36 specimens do not have scaphocerites (Figure 2.4B). In contrast, all specimens of *T. kelanang* have distinctly large, triangular scaphocerites with a row of long setae on their inner margin (see Figure 2.4A). The five specimens of *T. squamifera* also have scaphocerites but only one specimen has a row of marginal setae on the right scaphocerite (Figure 2.4C). *Thalassina kelanang* has a long antennal flagellum that when stretched backwards reached more than half the length of the first abdominal somite; the flagellum of *T. anomala* is much shorter, reaching less than three fourths the carapace length. While the examined males of *T. squamifera* all had broken antennae, both females have antennal flagella that reached the posterior margins of the carapace.

The merus of the cheliped in *T. kelanang* has a row of 3-5 strong spines on its dorsal margin, whereas in *T. anomala* there are only two strong spines. There are also two or three strong spines in *T. squamifera*. Three other distinguishing features of *T. kelanang* are (i) the fixed finger of its chela is half the length of the movable finger, whereas in *T. anomala* the fixed finger is one-quarter to one-third the length of the movable finger (Figure 2.5C, D), (ii) 8-12 strong dorsal spines on the inner dorsal ridge of the palm, whereas *T. anomala* has 14-20 blunt spines or tubercles, and (iii) lateral dorsal ridge of outer surface of palm extends at least proximal three-fourths but not to the distal end, whereas in *T. anomala*, the ridge extends right up to the distal end (Figure 2.5C). The same characters for *T. squamifera* are respectively fixed finger half the length of movable finger, 9-11 inner dorsal spines and a lateral dorsal ridge that extends more than proximal half but not exceeding three fourths the distance to the distal end.

Several distinctive differences between the three species are also observed in the abdomen and its appendages. *Thalassina kelanang* and *T. squamifera* have an inverted Y-groove on the dorsal tergite of the first abdominal somite (see Figure 2.6A), whereas *T. anomala* has two petaloid depressions in the form of an inverted V (Figure 2.6B). In *T. kelanang* and *T. squamifera*, the longitudinal 'three-quarters long' carina runs across the base of the pleuron of the second and third abdominal somites only, whereas in *T. anomala* the carina runs the entire somite length and is present on the second to sixth abdominal somites (Figure 2.7C). A sternal ridge (second to fifth somites) bearing two cusps each with 3-6 teeth/ tubercles is present in *T. kelanang* as well as in *T. squamifera*, whereas in *T. anomala*, only one median tubercle is present (Figure 2.7D). This is the other feature distinguishing the two varieties of mud lobster (*sensu* De Man, 1928).

The male holotype and paratypes of *T. kelanang* (see Figure 2.8A) have a petasma or intromittent organ that is quite different from that of *T. anomala* in that both outer and inner marginal setal rows of *T. kelanang* are reaching its distal tip, whereas the latter's petasmal tip is broadly rounded without setae (Figure 2.8B). In T. squamifera, inner marginal setae but no terminal setae are present on the petasma tip (Figure 2.8C). Another important difference between the petasma of the three species is the presence of 3-4 proximal spines in *T. kelanang* but which are absent in both *T. anomala* and *T. squamifera*. All our nine male specimens of *T. kelanang* have these proximal spines. The petasma of *T. squamifera* is stouter than that of its congeners. We also examined the petasma of *T. squamifera* (Figure 2.8D). Therefore, despite the close similarities between *T. kelanang* and *T. squamifera* (Table 2.1), based on mainly differences of their petasma and the rostrum, we conclude that they are two different species.

The new species of *T. kelanang* is more similar to *T. squamifera* than to *T. anomala* in general morphology, but can be distinguished from *T. squamifera* based on the morphology of the petasma and rostrum. Thus, the characteristics of both rostrum and petasma are very useful in distinguishing the species of *Thalassina*.

28

Table 2.1. Comparative morphology of *Thalassina anomala*, *T. squamifera* and *T. kelanang* (new species).

T. anomala (Herbst, 1804)	T. squamifera De Man, 1915	T. kelanang, new species			
Dorsal median margin of carapace	Dorsal median margin of carapace projected as	Similar to <i>T. squamifera</i>			
projected as long, hooked spine resting	short spine not resting on the first abdominal				
on first abdominal tergite.	tergite.				
Rostrum triangular. Adrostral carina of	Generally similar to T. anomala except rostrum	Rostrum waisted. Adrostral carina			
carapace extending three fourths or entire	more acute.	extending to about half the distance of			
distance of gastro-orbital carina, both		gastro-orbital carina, both carinae with 3-11			
carinae smooth or bearing maximum of		blunt tubercles postorbitally. Median sulcus			
three blunt tubercles postorbitally.		of rostrum deep, extending behind			
Median sulcus of rostrum shallow, not		adrostrals.			
extending to behind adrostrals.					

Scaphocerite absent or if present, veryScaphocerite present, with or withoutmarginalScaphocerite distinctly large, with marginalsmall.setae.row of long setae.

Oblique tuberculate ridge present on Oblique tuberculate ridge on first cheliped Oblique tuberculate ridge on first cheliped inner surface of first cheliped propodus, propodus absent. propodus absent. starting near the base of the fixed finger

and running posteriorly.

Pereopod 1 with a row of 13-20 blunt Pereopod 1 with a row of 9-11 blunt spines or Pereopod 1 with a row of 8-12 strong dorsal spines or tubercles on the inner propodal tubercles on the inner propodal ridge. Lateral spines on the inner propodal ridge extends to more than half but never dorsal ridge extends to three fourths or more the propodal length. the propodal length.

Merus of pereopod 1 with 2 strong dorsalMerus of pereopod 1 with 2 or 3 large dorsalMerus of pereopod 1 with 3-5 large dorsalspines.spines.spines.

Tergite of first abdominal somite has two Tergite of first abdominal somite raised up as a Similar to *T. squamifera*. petaloid depressions in the form of an distinct rectangular piece, with inverted Y groove.

Pleura of second to sixth abdominal Pleura of second and third abdominal somites Similar to *T. squamifera* somites with single longitudinal carina with 2 longitudinal, serrated carinae occupying occupying entire length of somite.

Abdominal sternites of second to fifth Abdominal sternites of second to fifth somites Abdominal sternites of second to fifth somites with only one median tubercle. with distinct bicuspid sternal ridge, each cusp bearing 3-5 teeth for male while adult female each cusp bearing 3-6 teeth in both male and female.

broadly rounded without setae.

Petasma without proximal spines, tip Petasma without proximal spines; outer and Petasma with 3-4 strong proximal spines on inner marginal setal rows extending almost to outer margins; outer and inner marginal setal pointed tip. "Neck" behind keel broad, width rows extending to pointed tip. "Neck" half the length of inner keel surface. behind keel slender, width one-quarters the

length of inner keel surface.



Chapter 3

Verification of the four species of mud lobsters, *Thalassina anomala*, *T. kelanang*, *T. gracilis* and *T. squamifera* using molecular, morphometric and meristic characters

Work done in this chapter is published in:

Moh, H.H., Chong, V.C., Lim, P.E., Tan, J., & Dally, G. (2013). Verification of four species of the mud lobster Genus *Thalassina* (Crustacea: Decapoda: Gebiidea: Thalassinidae) using molecular and morphological characters. Raffles Bulletin of Zoology, 61(2), 579-588.

3.1 Introduction

The family Thalassinidae Latreille, 1831, contains one genus *Thalassina* Latreille, 1806, with members, commonly called mud lobsters, widely distributed across the Indo-West Pacific region. This genus had, for some time, been considered monotypic (Glaessner, 1969), but a recent work and review by Ngoc-Ho & de Saint Laurent (2009) recognized seven species. In the latter's study, *Thalassina gracilis* Dana, 1852, collected from Telegraph Island near Singapore and synonymised with *Thalassina anomala* (Herbst, 1804) by De Man (1928), was -redescribed and a neotype, collected from Lim Chu Kang mangroves, Singapore, was designated. Ngoc-Ho & de Saint Laurent (2009) further described three new species *—Thalassina spinirostris* (type locality: Lim Chu Kang mangroves, Singapore), *T. spinosa* (type locality: Mentawi Island, Indonesia) and *T*.

krempfi (type locality: Saigon, Vietnam). At almost the same time, another new species, *T. kelanang*, collected from Kelanang Beach, Selangor, western Peninsular Malaysia, was described by Moh & Chong (2009).

De Man (1915) described certain odd specimens of T. anomala, collected off Beo, Karakelong Island (northern Sulawesi) during the Siboga Expedition, which had some notable morphological differences, in particular, a distinct, movable scaphocerite, and recognized them as a distinct variety, T. anomala var. squamifera. Later, Poore & Griffin (1979) redescribed and formally elevated the Australian specimens of T. anomala var. squamifera De Man, 1915, as a valid species. The extant Australian mud lobsters may bear another species, T. emerii Bell, 1844, a fossil species considered extant by Ngoc-Ho & de Saint Laurent (2009) based on their examination of recent specimens (MNHN Th 1524, MNHN Th 1523 and RMNH D 51758) collected from Australia and Indonesia. However, in the most recent review of the genus, Sakai & Türkay (2012) argue that T. emerii is a nomen dubium based on their examination of the recent specimens which, instead, comprised of two new species - T. australiensis Sakai & Türkay, 2012 (RMNH D 51758, type locality: Aru Islands, Indonesia; MNHN Th 1523, type locality: NE of Port Hedland, N.W. Australia) and T. saetichelis Sakai & Türkay, 2012; NHN-IU-2011-5615 (=MNHN Th1524), type locality: Roebourne, N.W. Australia). Hence, these authors have updated the present number of mud lobster species to nine.

The identification keys by Ngoc-Ho & de Saint Laurent (2009) and Sakai & Türkay (2012) both largely rest on the adult morphologies of the carapace, rostrum, cheliped and the abdominal sternites, which nonetheless overlap among species or are variable within species to some degree. Moh & Chong (2009), however, distinguished four outwardly

similar species, *Thalassina kelanang*, *T. anomala*, *T. gracilis* and *T. squamifera*, based on their distinct male gonopods. The importance of this character for species diagnosis is also recognized by Sakai & Türkay (2012) in their figures.

The work of Ngoc-Ho & de Saint Laurent (2009) has however cast doubt on the geographical distribution of T. squamifera which, according to them, was found from Australia to Thailand, including the Solomon Islands, Vanuatu, Fiji, New Caledonia, Papua New Guinea, Philippines, Indonesia, Singapore and Malaysia. They rested their argument on purported T. squamifera specimens collected from these countries (except Malaysia). This wide distribution could be an oversight because of the closely similar morphological characters shared by T. squamifera and T. kelanang. There is also some uncertainty in the species distinction between T. squamifera and T. gracilis. Since Dana's holotype of T. gracilis was considered lost and the lectotype is poorly illustrated, Ngoc-Ho & de Saint Laurent (2009) had selected a male neotype (TL=91.5mm) from Singapore for a redescription of the species. However, Sakai & Türkay (2012) argued that the selection of this neotype is inappropriate because the lectotype is a small female (TL = 2.5" or 63.5 mm) which could be morphologically different. They pointed out that the number of spinules/denticles on the dorsal margin of the cheliped, as figured by Dana (1852: 514, pl. 32, fig.5a-g), did not match that of the neotype. Based on similar cheliped armature, they argue that T. gracilis Dana, 1852 should be synonymous with T. squamifera.

It is apparent that population and species distinctness of the outwardly similar *Thalassina anomala*, *T. kelanang* and *T. gracilis* from the Malay Peninsular, including *T. squamifera* from Australia, need verification. In this chapter, we report on (1) the

population distinctiveness among the three sympatric species, and between *T. kelanang* and *T. squamifera* based on their meristic and morphometric features, and (2) species differentiation among *T. kelanang*, *T. squamifera*, *T. anomala* and *T. gracilis* based on three molecular markers comprising two nuclear protein-coding genes, phosphoenolpyruvate carboxykinase (PEPCK) and sodium–potassium ATPase a-subunit (NaK), and one mitrochondrial-coding gene, cytochrome *c* oxidase subunit I (COI).

3.2 Materials and methods

3.2.1 Material examined. – Mud lobsters were sampled from two nearby sites at Kelanang Beach and Carey Island (< 50 km apart) in Selangor, Malaysia. A total of 58 mud lobsters comprising *T. anomala* (*n*=24), *T. kelanang* (*n*=25) and *T. gracilis* (*n*=9) were collected inside or near to the mangrove forest for meristic and morphological studies. All specimens were deposited in the Zoological Museum University of Malaya (ZMUM). Eleven specimens of *T. squamifera* were loaned out from the National Sciences Museum and Art Gallery (NSMAG), Darwin, Australia. A further two specimens of each species were collected and prepared for molecular analysis. Tissues from the chelae were removed from fresh specimens killed by freezing and immediately preserved in absolute ethanol (99.90%).

3.2.2 Meristics and morphometrics. – A total of 13 morphometric and six meristic characters were used for discriminant analysis (see Table 3.1). The morphometric measurements were made using a pair of digimatic calipers, with a precision of 0.01mm. The meristic characters were counted under a dissecting binocular microscope.

	Abbreviations	Character	Description			
	TL	Total length	Tip of the rostrum to the end of the telson.			
measurements	CL	Carapace length	Tip of the rostrum to the posterior edge of the carapace.			
	ABL	Abdomen length	Anterior edge of the first tergite to the tip of the telson.			
	CW	Carapace width	Straight line measurement between lateral surfaces across the linea thalassinica.			
	ABW	Abdomen width	Straight line measurement between lateral surfaces of third abdominal segment at midregion.			
	ATUL	Antennule length	Base to tip of the antennule.			
	LPL	Propodus length, large chela	Proximal to distal edge of propodus along the mesial dorsal carina.			
	LPW	Propodus width, large chela	Straight line measurement between lateral surfaces at the midregion of propodus.			
	LPH	Propodus height, large chela	Dorsal to ventral edge measured at the midregion of propodus.			
	SPL	Propodus length, small chela	Proximal to distal edge of propodus along the mesial dorsal carina.			
	SPW	Propodus width, small chela	Straight line measurement between lateral surfaces at the midregion of propodus.			
ometric	SPH	Propodus height, small chela	Dorsal to ventral edge measured at the midregion of propodus.			
Morph	RL	Rostral length	Tip of rostrum to postorbital edge of carapace.			
	LMDS	No of dorsal spines of	n the merus of large chela.			
	SMDS	No of dorsal spines on the merus of small chela.				
	LGP	No. of spines/ tubercles on the mesial dorsal carina of propodus of large chela.				
ts	SGP	No. of spines/ tubercles on the mesial dorsal carina of propodus of small chela.				
c coun	LMLS	No. of large dorsal spines on anteriormost margin of the merus of large chela.				
Meristi	SMLS	No. of large dorsal s small chela.	lo. of large dorsal spines on anteriormost margin of the merus of mall chela.			

Table 3.1. Definitions of 13 morphometric and six meristic characters of four species of *Thalassina* (*T. anomala, T. gracilis, T. kelanang* and *T. squamifera*).

3.2.3 Statistical analysis

Data on 11 morphometric (ABL, CW, ABW, ATUL, LPL, LPW, LPH, SPL, SPW, SPH, RL) and six meristic (LMDS, LGP, LMLS, SMDS, SGP, SMLS) characters were used for discriminant analysis. To approximate multivariate normality and linear relationships, all data were first transformed to base 10 logarithms (Pimentel, 1979). Because of the variation in size of mud lobsters, all body part measurements were corrected for differences in body size. Carapace length (CL) was used to indicate body size and as the covariate. Analysis of covariance was used to adjust each morphometric character to the overall mean total length (Misra & Ni, 1983). This adjustment used the following formula: $M'_{ij} = \log M_{ij} - [RC_{ij} (\log CL_i - \log \overline{CL})]$

where M'_{ij} is the measurement adjusted for character *j* of individual *i*, M_{ij} is the original value, RC_{ij} is the pooled regression coefficient of logM on logCL, CL_i is the carapace length of individual *i*, and \overline{CL} is the overall mean carapace length.

The meristic and morphometric variations among species were analyzed using forward stepwise discriminant function analysis (SDFA). All statistical analyses were performed using the software Statistica Version 10. Default settings were retained as the following:-tolerance level at 0.010, F to enter at 3 and F to remove at 2.

3.2.4 Molecular analysis

3.2.4.1 DNA extraction, polymerase chain reaction (PCR) and sequencing.

The genomic DNA were isolated from approximately 100 mg of *Thalassina* cheliped tissues preserved in absolute ethanol (99.90%) using i-genomic CTB DNA Extraction Mini Kit (iNtRON Biotechnology, Inc, Korea). The sequences of nuclear

encoded phosphoenolpyruvate carboxykinase (PEPCK) and sodium–potassium ATPase asubunit (NaK) were amplified using the following primer sets: (1) PEPCK for2: 5'-GCA AGA CCA ACC TGG CCA TGA TGA C-3' and PEPCK rev3: 5'- CGG GYC TCC ATG CTS AGC CAR TG-3' and (2) NaK for-a: '5- GTG TTC CTC ATT GGT ATC ATT GT-3' and NaK rev2: 5'- ATG ACA GTT GCT CAT ATG TGG TT-3' (Tsang et al., 2008). The partial sequences of mitochondrial encoded markers, namely, cytochrome *c* oxidase subunit I (COI) were amplified using primer sets LCO1490: 5'- GGT CAA CAA ATC ATA AAG ATA TTG G-3' and HCO2198: 5'-TAA ACT TCA GGG TGA CCA AAA AAT CA-3' (Folmer et al., 1994).

PCR amplification of all the molecular markers was carried out using MultiGene Gradient Thermal Cycler (Labnet, USA). The PCR amplification was carried out as in Lim et al. (2012) except that the annealing temperature was varied for PECK and NaK at 60°C and for COI at 50°C. PCR products were assayed by electrophoresis on 1.0% agarose mini gel stained with *SYBR®Safe DNA gel stain* (Invitrogen, USA) and visualized under UV light. The target DNA fragments were isolated and purified by the LaboPassTM PCR purification kit (Cosmo Genetech, South Korea). The purified PCR products were sent to a commercial company, Lucigen (Taiwan) for sequencing. The same primers set for PCR amplifications were used for DNA sequencing. Two decapod shrimp species, one a penaeid, *Metapenaeus brevicornis* (H. Milne Edwards, 1837) and the other a palaemonid, *Exopalaemon styliferus* (H. Milne Edwards, 1840) we sequenced in the analysis, were used as outgroups in this study. The palaemonid belongs to the Caridea, a sister group to the Gebiidea within the Pleocyemata, while the penaeid represents the Dendrobranchiata, sister group to the Pleocyemata (Bracken et al., 2009; Lin et al., 2012).

3.2.4.2 Sequence alignment and molecular analysis.

The generated sequences were initially aligned using the CLUSTAL X program (Thompson et al., 1997) and subsequently aligned manually. Additional *T. anomala* (from Singapore) sequences, for PEPCK (EU427241) and NaK (EU427172), from GenBank were used in the analysis. The aligned sequences were subjected to maximum-parsimony (MP). The MP tree was constructed using the heuristic search option, 100 random sequence additions, tree bisection reconnection (TBR) branch swapping, and unordered and unweighted characters. Bootstrap percentage (BP) was computed with 1000 replications. Maximum Likelihood (ML) analysis was performed by Treefinder version October 2008 (Jobb et al., 2004). Bayesian inference (BI) analysis was performed using MrBayes 3.1.1 (Huelsenbeck & Ronquist, 2001). Best-fit nucleotide substitution model was determined using KAKUSAN v.3 (Tanabe, 2007), which also generated the input files for ML and BI.

Best-fit models were evaluated using the corrected Akaike Information Criterion (AICc) (Akaike, 1973; Shono, 2000) for ML and the Bayesian Information Criterion (BIC) for BI. ML analysis was performed with 1,000 bootstrap replicates. Two parallel runs were performed in MrBayes, each consisting of four chains, two "cold" and two incrementally heated. Four million Markov chain Monte Carlo (MCMC) generations were run, with convergence diagnostics calculated every 1000th generation for monitoring the stabilization of log-likelihood scores. Trees in each chain were sampled every 100th generation. A 50% majority rule consensus tree was generated from the sampled trees after discarding the first 20%. The likelihood scores stabilized before 800,000 generations (20%) for all three individual molecular marker analyses and also the combined markers analysis.

To assess the level of variation in PEPCK, NaK and COI among the selected samples of different taxa, uncorrected "p" pairwise genetic distances were estimated using PAUP* 4.0b10 software (Swofford, 2002). The DNA sequences used in this study were deposited in GenBank and their accession numbers are given in Table 3.2.

Crasica	Vouchernumber	GenBank accession number				
species	voucher number	PEPCK	NaK	COI		
Thalassina anomala 1	ZMUMCTA19	JX100440	JX100454	JX100447		
Thalassina anomala 2	ZMUMCTA20	JX100441	JX100455	JX100448		
Thalassina gracilis 1	ZMUMCTG02	JX100442	JX100456	JX100449		
Thalassina gracilis 2	ZMUMCTG03	JX512419	JX512423	JX512421		
Thalassina kelanang 1	ZMUMCTK17	JX100443	JX100457	JX100450		
Thalassina kelanang 2	ZMUMCTK18	JX512420	JX512424	JX512422		
Thalassina squamifera 1	Cr014928	JX100444	JX100458	JX100451		
Metapenaeus brevicornis	ZMUMCMB01	JX100445	JX100459	JX100452		
Exopalaemon styliferus	ZMUMCES01	JX100446	JX100460	JX100453		

Table 3.2. GenBank accession numbers for the four species of thalassinids and outgroups.

3.3 Results

3.3.1 Analysis of morphometric and meristic data.

Of the 17 characters used in the discriminant analysis (SDFA), seven characters (ABL, SMLS, SGP, CW, RL, LMLS, LGP) were adopted by the SDFA model that best distinguished the four species of *Thalassina*, while the remaining 10 characters were removed (Wilks' $\lambda = 0.00022$; F value (21, 169) = 143.20, P<0.001). The classification matrix which compares the known membership with the predicted membership, based on the model's classification functions, showed 100% correctly predicted membership for all species.

The SDFA generated three canonical functions (roots), with the first root contributing to 79% and the second root 17% of the total variance. Hence, the first two roots captured most of the discriminatory power of the SDFA model and were used to interpret the contribution of the measured characters to discrimination of the four species.

The first root was loaded highest by the abdominal length, ABL (0.683), rostral length, RL (0.503) and carapace width, CW (0.452) (Table 3.3). The second root was loaded highest by the number of large dorsal spines on the anteriormost margin of the merus of the small chela, SMLS (0.609), number of large dorsal spines on the anteriormost margin of the merus of the large chela, LMLS (0.424) and number of spines or tubercles on the inner ridge of the propodus of the small chela, SGP (-0.361).

Variable	Standardized Coefficients for Canonical Variables				
variable	Root 1	Root 2	Root 3		
ABL	0.6827	-0.2218	0.1755		
SMLS	-0.1709	0.6094	-0.5403		
SGP	0.0618	-0.3607	-0.3778		
CW	0.4518	0.2088	-0.4275		
RL	0.5026	0.2392	0.1471		
LMLS	-0.1244	0.4241	-0.5837		
LGP	-0.1483	-0.1739	-0.5837		
Eigen	67.3980	14.9089	3.1199		
Cum. P	0.7890	0.9635	1.0000		

Table 3.3. Standardized coefficients for canonical variables derived by SFDA of morphometric and meristic characters.

Eigen = eigenvalue, Cum. P = cumulative proportion of total variance. Variable, see Table 3.1 for explanation.

Plots of the canonical scores of all specimens show clear separation of the four population samples of *Thalassina* species (Figure 3.1). On the first root, *T. anomala* with the longest ABL, RL and CW was farthest from *T. gracilis* which had the shortest measurements of these characters. The second root separates *T. kelanang* with the highest SMLS (3-5) from *T. anomala* (2) and *T. gracilis* (2-3). On the other hand, SGP was highest in *T. gracilis* (17-22) and *T. anomala* (12-17) as compared to *T. squamifera* (8-15) and *T. kelanang* (8-14).



Figure 3.1. SFDA ordination diagram of morphological and meristic characters for four species of *Thalassina*.

The squared Mahalanobis distance between the group centroids shows the farthest distance between *T. anomala* and *T. gracilis* (516.8) and the shortest distance between *T.*

squamifera and *T. gracilis* (84.3). *T. kelanang* was not the closest to *T. squamifera* (154.0), as it was with *T. anomala* (103.9).

3.3.2 DNA sequences.

The aligned sequences of PEPCK consisted of 607 sites, of which 425 characters were constant, 79 characters were parsimony informative and 103 characters were parsimony uninformative. The aligned sequences of NaK consisted of 770 sites, of which 565 characters were constant, 73 characters were parsimony informative and 132 characters were parsimony uninformative. The aligned sequences of COI consisted of 710 sites, of which 459 characters were constant, 184 characters were parsimony informative and 67 characters were parsimony uninformative.

3.3.3 Molecular analysis.

The phylogenetic trees constructed using the three methods (ML, MP and BI) for molecular markers PEPCK, NaK and COI, and combined markers, had similar topology except for variation in the bootstrap support values (Figure 3.2,a, b, c, d). Hence only ML trees are presented here with support from all the analyses. Only results from one specimen of *T. squamifera* were usable because the DNA of the second sample was not able to be extracted due to its poor condition.



Figure 3.2. The 50% majority-rule consensus tree resulting from maximum likelihood analysis of (a) partial PEPCK sequences (substitution rate parameters: TC = 0.5206, TA = 0.1254, TG = 0.0125, CA = 0.1254, CG = 0.0125, AG = 0.2036), - Ln likelihood 1935.877; (b) partial NaK sequences (TC = 0.5183, TA = 0.1061, TG = 0.0727, CA = 0.0972, CG = 0.0223, AG = 0.1834), - Ln likelihood 2117.352; (c) partial COI sequences (TC = 0.7231, TA = 0.1315, $TG = 1.4301e^{-5}$, CA = 0.0153, CG = 0.0311, AG = 0.0991), - Ln likelihood 2729.365; (d) combined PEPCK, NaK and COI DNA sequences (TC = 0.5687, TA = 0.1190, TG = 0.0211, CA = 0.1190, CG = 0.0211, AG = 0.1511), - Ln likelihood 6914.207. The bootstrap values (ML/MP/BI) are shown at the branches. Bar indicates substitutions per site.

PEPCK.

The phylogenetic tree of PEPCK (Figure 3.2a) showed that all the four species *T*. *anomala*, *T. squamifera*, *T. gracilis* and *T. kelanang* are grouped in a monophyletic clade with full support for all analyses. *Thalassina kelanang* (*T. kelanang* 1 and *T. kelanang* 2) was the most basal species among the four species. The three *T. anomala* (*T. anomala* 1, *T. anomala* 2 and *T. anomala* EU427241) were grouped in a monophyletic clade with full to

moderate support values (ML= 100%, MP= 100%, BI=0.79). *Thalassina squamifera* was included in the same clade with *T. anomala* and *T. gracilis*.

NaK.

The phylogenetic tree of NaK (Figure 3.2b) showed all four species grouped in a monophyletic clade with full support for all analyses. *T. squamifera* was the most basal species and showed a sister relationship with the clade containing the other three species (*T. anomala*, *T. gracilis* and *T. kelanang*) which has moderate support from the various analyses (ML=72%, MP=74%, BI=0.65).

COI.

The phylogenetic tree of COI (Figure 3.2c) showed the four species grouped in a monophyletic clade, with bootstrap support values varying from low to high (ML=55%, MP=61%, BI=1.00). *T. squamifera* was the most basal species. *Thalassina kelanang* and *T. gracilis* were in the same clade, supported by variable bootstrap values (ML=67%, MP=76%, BI=0.96).

Combined markers. – The combined phylogenetic tree of PEPCK, NaK and COI (Figure 3.2d) also grouped the four species *T. anomala*, *T. squamifera*, *T. gracilis* and *T. kelanang* in a monophyletic clade with full support from all analyses. *T. squamifera* was shown to be the most basal species among the four species. *T. squamifera* showed a sister relationship to the other three species.

Uncorrected "p" distance. – The uncorrected "p" distances of the three markers (PEPCK, NaK, COI) and their combined markers are shown in Table 3.4. The uncorrected "p"

distances between *T. anomala* 1 and *T. anomala* 2 were close, which ranged from 0.5% for NaK to 0.7% for PEPCK; the combined markers was 0.6%.

The uncorrected "p" distance between *T. gracilis* and *T. kelanang* ranged from 0.5% for NaK to 13.1% for COI; the combined markers gave 5.2–5.4 %. The distance between *T. gracilis* and *T. squamifera* ranged from 1.3% for NaK to 16.5% for COI, with 6.6–6.7 % for combined markers. Between *T. kelanang* and *T. squamifera*, the distance ranged from 2.5% for PEPCK to 14.1% for COI, with the combined markers giving 5.9–6.0%. In summary, the uncorrected "p" distance among species varied according to the type of molecular markers used. The COI gave the highest distance among species ranging from 13.1% (*T. kelanang* 1 and *T. gracilis* 1) to 17.1% (*T. anomala* 1 and *T. gracilis* 2), while NaK gave the lowest distance among species of 0.5% (*T. kelanang* 2 and *T. gracilis* 1 & 2) to 3.3 % (*T. anomala* 2 and *T. squamifera*). Overall, based on the combined markers, *T. anomala* 2 was the most distant from *T. gracilis* 2 (8.2%), while the closest pair was *T. kelanang* 1 and *T. gracilis* 1 (5.2%).

Species (site)		Species					
		1	2	3	4	5	6
1. T. anomala 1							
2. T. anomala 2	PEPCK	0.7					
	NAK	0.5					
	COI	0.8					
	COMBINED	0.7					
3. T. gracilis 1	PEPCK	5.0	4.6				
	NAK	2.5	3.0				
	COI	16.8	16.9				
	COMBINED	7.9	8.0				
4. T. gracilis 2	PEPCK	5.1	4.8	0.5			
	NAK	2.5	3.0	0.0			
	COI	17.1	16.9	0.0			
	COMBINED	8.0	8.2	0.2			
5. T. kelanang 1	PEPCK	4.6	4.5	2.3	2.3		
	NAK	2.6	2.9	0.7	0.7		
	COI	15.2	14.7	13.1	13.1		
	COMBINED	7.3	7.4	5.2	5.3		
6. T. kelanang 2	PEPCK	4.9	5.1	3.2	3.2	1.0	
	NAK	2.5	3.0	0.5	0.5	0.1	
	COI	15.5	15.1	12.8	12.7	0.6	
	COMBINED	7.4	7.7	5.3	5.4	0.5	
7. T. squamifera	PEPCK	3.6	3.3	2.7	2.0	2.5	2.7
	NAK	2.7	3.3	1.3	1.3	1.2	1.0
	COI	15.8	15.1	16.4	16.5	13.8	14.1
	COMBINED	7.3	7.3	6.7	6.6	5.9	6.0

Table 3.4. Uncorrected "p" distance measures (%) among four species of *Thalassina* based on PEPCK, NaK, COI and combined molecular markers.

(1) *T. anomala* 1 (Kelanang Beach), (2) *T. anomala* 2 (Kelanang Beach), (3) *T. gracilis* 1 (Carey Island), (4) *T. gracilis* 2 (Carey Island), (5) *T. kelanang* 1 (Kelanang Beach), (6) *T. kelanang* 2 (Kelanang Beach), (7) *T. squamifera* (northern Australia).

3.4 Discussion

Meristic, morphometric and molecular evidence has verified that the sampled populations of *Thalassina* in Malaysia belong to four distinct groups, i.e., four species, which form a monophyletic clade. The molecular evidence justifies the recognition of T. squamifera and T. anomala as distinct species. Also, T. kelanang (from Malaysia) is a distinct species from T. squamifera (from Australia), supporting the assertion of Moh & Chong (2009) based on its distinctive rostrum and male gonopod. Based on the figures and descriptions of Ngoc-Ho & de Saint Laurent (2009: 149, fig. 12A, B), the identity of their T. squamifera specimen from Thailand (MNHN Th 438) is doubtful since it very closely resembles T. kelanang (cf. Moh & Chong, 2009: 466, 468, figs. 1, 3A). Moh & Chong (2009) described T. kelanang as having a waisted rostrum, adrostral carina extending $\frac{1}{2}$ the distance of the gastro-orbital carina and median sulcus extending behind the adrostrals (clearly seen in MNHN Th 438, fig. 12A in Ngoc-Ho & de Saint Laurent, 2009). Moh & Chong (2009) also described the chela as having a dorso-lateral carina extending ³/₄ the propodal length and merus bearing 3–5 large dorsal spines (clearly seen in MNHN Th 438, fig. 12B in Ngoc-Ho & de Saint Laurent, 2009). However, the Australian materials of Ngoc-Ho & de Saint Laurent (2009: 149, fig. 12C, D) are clearly T. squamifera (MNHN Th 1518, bearing 2 or 3 large dorsal spines on the merus). Therefore, it is more likely that T. kelanang has a widespread distribution in the Southeast Asian region, rather than T. squamifera spreading out from Australia to Thailand. Our recent examination of one Indonesian specimen of a male T. squamifera (MZB.Cru.211, coll. C. Boden Kloss, 1924), collected from Sipora, W. Sumatra, now in the Wet Biological Collection, Indonesian Institute of Sciences (LIPI), turned out to be a female T. kelanang (Citra Dewi, LIPI, pers. comm.). Another specimen from LIPI (MZB.Cru. 2252, coll. W.T. Laksono & D.C.

Murniati, 2008), a male collected from Legon Cibariang, Panaitan Island, Java, is also identified as *T. kelanang*. Two other locations, Semakau Island, Singapore (Ron Yeo, Raffles Museum of Biodiversity Research, Singapore, pers. comm., 2009), and Lahat Datu, Sabah (Tungku Beach Resort, pers. comm., 2011), have also recorded *T. kelanang*, based on confirmed photo-specimens including rostrum and male gonopod, outside of Peninsular Malaysia.

Results from morphometric and molecular data concur in that *T. anomala* and *T. gracilis* form the most distant pair in terms of morphology and phylogenetics, respectively, while *T. anomala* and *T. squamifera* form the third most distant pair (Table 3.4). Except for these agreements, the conclusions regarding the affinities among other species pairs did not match. For instance, *T. kelanang* and *T. gracilis* were considered to be the closest pair (uncorrected "p" distance = 5.2) based on molecular evidence, but were the second most distant pair based on meristics and morphometrics (Mahalanobis distance = 348.67). In fact, meristics and morphometrics have placed *T. gracilis* as morphologically closest to *T. squamifera*. The incongruence is not unexpected since the meristic and morphometric characters used were selected while the molecular gene markers do not necessarily reflect these expressions.

The molecular evidence suggests that *T. kelanang* or *T. squamifera* are basal species depending on the molecular marker used. In fact, NaK, COI, and combined markers indicate the affinity-between the two species based on their uncorrected 'p' distance (Table 3.4). The combined gene markers, however, indicate *T. squamifera* to be the most basal species (Figure 3.2d). Also, only these two species consistently retain a distinct, movable, and setose scaphocerite: one of the caridoid facies of primitive eumalacostracans. Thus, *T.*

squamifera and *T. kelanang* are likely two basal sister species retaining most of their ancestral characters, and the other species are possibly derived from their common or shared ancestor. One such species, *T. anomala*, the most distant from all other species, is likely derived from a shared ancestor with *T. kelanang*. *T. anomala* is reported to have a wide distribution from west India to Fiji, and as far north as southwest Japan but it is not known in Australia (Davie, 2002; Ngo-Ho & de Saint Laurent, 2009). Some of the derived traits of *T. anomala* may include the distinctively long hooked spine on the posterior dorsomedian margin of the carapace, absence of (or rudimentary) movable scaphocerite on the antennal peduncle, and sexually dimorphic 3^{rd} maxilliped (dactylus bearing stiff setae in males).

Since the molecular gene markers have also conclusively resolved the distinction between the species *T. squamifera* and *T. kelanang*, we hypothesize that these species represent two sister groups in their present biogeographical regions. The records of their collections, especially *T. squamifera* (see Ngoc-Ho & de Saint Laurent, 2009: 148; Sakai & Türkay, 2012: 1371–1373), attest to their distribution east and west of Wallace's line, respectively (Figure 3.3). This hypothesis of two sister groups is also supported by the molecular evidence and by the retention of closely similar morphological traits in the two 'basal' sister species (see Moh & Chong, 2009). These morphological traits have caused confusion in the work of Ngoc-Ho & de Saint Laurent (2009) regarding the biogeographical occurrence of *T. squamifera*. The identity of the lone specimen of *T. squamifera* from Ranong, Thailand, in Sakai & Türkay (2012) is similarly doubtful. Like Ngoc Ho & de Saint Laurent (2009), they did not examine-material of *T. kelanang*. It is not likely that *T. squamifera* is found in Singapore and Thailand, and, yet, escaped detection in Peninsular Malaysia. Hence, by implication, *T. squamifera* cannot be regarded as
synonymous to *T. gracilis* Dana, 1852, as suggested by Sakai & Türkay (2012), even if the neotype for the latter is indeed a misdesignation. From an ecological point of view, it is unlikely that two very similar species are found together (co-exist) in a similar habitat. For instance, in the Langat estuary, Selangor, either *T. kelanang* or *T. gracilis* live sympatrically on the lower intertidal shore with *T. anomala* on the upper and supratidal shore of mangrove forests (Moh, unpublished data). From the molecular data, *T. kelanang* is the species closest to *T. gracilis*, but the indication from extensive samplings in Selangor is that they are spatially separated – the latter occupying the upper estuary while the former is on the coast.



Figure 3.3. Locations of examined specimens of *T. squamifera* (circles) and *T. kelanang* (triangles) by various authors. Museum catalog numbers at each site are indicated.

S = Poore & Griffin, 1979, ● = Ngoc-Ho & de Saint Laurent, 2009, $\textcircled{\bullet}$ = Moh & Chong, 2009, ○ = Sakai & Türkay, 2012, ▲ = Moh & Chong, 2009, this study. Dotted line indicates Wallace's Line. Modified base map from <u>http://commons.wikimedia.org</u>.

As for *T. gracilis* Dana, 1852 being a *nomen dubium*, we have examined further our material of *T. gracilis*, in particular two small females slightly larger than Dana's lectotype: (1) TL=77.50 mm/CL=28.15 mm, (ZMUM CTG010), coll. H.H.Moh, 10 Nov. 2010, number of dorsomesial denticles on cheliped: right = 15, left = 16; and (2) TL=99.19 mm/CL=32.34 mm (ZMUM CTG011), coll. H.H.Moh, 10 Nov. 2010, number of dorsomesial denticles on cheliped: right =18, left =17. These results may support Sakai & Türkay (2012) for a case of nomen dubium if indeed the small drawing as provided by Dana (1852: 514, pl. 32, fig. 5d) accurately portrays the spination of the cheliped. Nonetheless, his description and illustration of a short acute rostrum (Dana, 1852: 515, pl. 32, fig. 5c) are clearly not indicative of T. anomala, T. squamifera nor T. kelanang, including our collection of three young individuals (TL 37.80, 47.14 and 61.63 mm) of T. kelanang. Dana's description that on "either side of the beak there is a slight ridge running longitudinally for a short distance from the front edge" clearly meant the rostrum of T. gracilis. None of the other species show this feature since the ridge on their rostrum extends anteriorly to the rostral tip. Even if the description meant extending posteriorly, the ridge in T. gracilis only extends about the same length as its short rostrum, whereas in the others it extends a distance farther than the length of their (longer) rostrum (see Moh & Chong, 2009: 468, fig. 3). Thus, it is likely that Dana's drawings of the denticles on the cheliped of *T. gracilis* were done in perspective, and lacked the remaining hidden denticles. We therefore, conclude that Ngoc-Ho & de Saint Laurent's assignment of the neotype for T. gracilis is correct.

Fossil specimens of *Thalassina emerii* Bell, 1844 are known from Australia, but living specimens with rudimentary scaphocerites, thought to be of this species, have been redescribed by Ngo-Hoc & de Saint Laurent (2009). Notwithstanding the assertion of Sakai & Türkay (2012) that *T. emerii* is a species *inquirenda* without further status, the living specimens of two new species (Sakai & Türkay, 2012) instead may suggest a similarly derived condition from *T. squamifera*. Three further thalassinid species not treated in the present study (*Thalassina spinosa*, *T. krempfi* and *T. spinirostris*) are unlikely to be basal species based on their described morphological traits. Unlike the basal species with movable developed scaphocerite, *Thalassina spinosa*, *T. krempfi* and *T. spinirostris* have either a rudimentary scaphocerite or none at all. *Thalassina spinosa* and *T. krempfi* are described as morphologically similar to *T. anomala*, while *T. spinirostris* is similar to *T. gracilis* (Sakai & Türkay, 2012).

The molecular evidence based on NaK, COI and combined markers suggests a close phylogenetic relationship between *T. gracilis* and *T. kelanang*, probably reflecting a more recent speciation of the former due to its small scaphocerite, short dorsomedian process, and a third maxilliped that is not sexually dimorphic. Nonetheless, the depressed, spiny-tipped rostrum of *T. gracilis* is likely a derived feature not observed in the basal species. On the other hand, the examined morphological traits suggest a close relationship between *T. gracilis* and *T. squamifera*, which is supported by their similar morphologies with *T. kelanang*. The *T. kelanang* + *T. gracilis* pairing is however more plausible based on the molecular evidence (Figs. 2b, c, d), and the fact that more specimens of *T. gracilis* have been collected in the Asian region including Thailand, Malaysia, Singapore and Indonesia (Sumatra), whereas there was only one broken specimen of dubious identity recorded from northwest Australia (Ngoc-Ho & de Saint Laurent, 2009). Our hypotheses and

speculations, however, require further substantiation from molecular work on the remaining extant species, *T. krempfi, T. spinosa, T. spinirostris, T. australiensis* and *T. saetichelis*, in order to validate their species identities and to fully elucidate the phylogenetic relationships of the thalassinid mud lobsters.

Chapter 4

Distribution and burrow morphology of three sympatric species of *Thalassina* mud lobsters in relation to environmental parameters on Kelanang shore

Work done in this chapter is published in:

Moh, H. H., Chong, V.C., & A. Sasekumar (2015). Distribution and burrow morphology of three sympatric species of *Thalassina* mud lobsters in relation to environmental parameters on a Malayan mangrove shore. Journal of Sea Research, 95, 75-83.

4.1 Introduction

Distribution and abundance of intertidal decapods have been shown to vary greatly across the continent, intertidal zone and tidal creek, (Smith III et al., 1991; Frusher et al., 1994; Salgado-Kent & McGuiness, 2010). Such variability has been attributed to various environmental variables including elevation and vegetation (Mouton & Felder, 1996; Salgado-Kent & McGuiness, 2010), sediment characteristics (Snelgrove & Butman, 1994), temperature and salinity (Bliss, 1968), and competition (Diamond, 1975). Crab distribution and abundance also depend on their ability to make and maintain their burrows in a given habitat (Pandya, 2010). Crabs burrow in different habitats to overcome harsh estuarine conditions, for protection from predation, and for feeding, moulting and mating (Leme, 2002). Their activities as well as those of other decapods have been shown to influence mangrove forest vegetation structure and ecological processes (Lee, 1999; Cannicci et al.,

2008). Sesarmid crabs remove mangrove plant litter by ingestion or burial (Ashton, 2002; Thongtham, 2008), thus, contributing to nutrient cycling; their burrowing habits also modify the mangrove substrate and facilitate soil oxygenation (Robertson & Daniel, 1989). Fiddler crabs (Ocypodidae) not only retexture sediments but also produce pseudofecal pellets that significantly increase the nitrogen and carbon content of mud (Hogarth, 1999). Crab activity also stimulates the growth of *Avicennia* mangrove, while both crabs and tree roots have complementary effects on sediment microbial processes (Alongi, 2009). Hydrodynamic studies of tidal flow over mangrove swamp flats suggest that animal burrows provide an efficient pathway for the transfer of nutrients and oxygen across the swamp-bed interface (Ridd, 1996; Stieglitz et al., 2000).

Among the decapods that colonize soft-sediment habitats, thalassinideans which are common decapods found in abundance in the littoral and sublittoral shores of coastal environments (Griffis & Suchanek, 1991), are the least studied due to their cryptic habits, deep burrows and the harsh environments they occupy. Yet, the mounds of *Thalassina* mud lobsters are a ubiquitous feature of high shore mangroves in the Indo-West Pacific Region. The complexity and extensiveness of their burrows are even more dramatic than those of crabs (Hogarth, 1999). *Thalassina* mounds can reach heights of one meter above the ground, forming depressions between them and completely altering the substrate topography (Macnae, 1968). On the other hand, their burrows can reach 1.6 metre depth, and the excavated materials surrounding the mouth of the burrow is an indication of the large amounts of sediments excavated. In the process of burrowing and extracting organic matter from the sediment, *Thalassina* excavates and aerates otherwise anoxic subterranean soil (Johnson, 1961). Like large sesarmid crabs, mud lobsters can excavate large branching tunnels thus playing a major role in reducing sediment toxicity by allowing water

circulation during high tides (Stieglitz et al., 2000) especially on the upper shore where tidal inundation is irregular.

In fact, due to their active burrowing activity, *Thalassina* mud lobsters have been described as "ecosystem engineers" that not only alter the topography of high mangrove shores but also cause physical changes in the abiotic and biotic materials (Macintosh, 1988; Hogarth, 1999). As a result, such ecosystem engineers modify, maintain and create new habitats (Jones et al., 1994). The burrows of mud lobsters provide them protection from exposure to extreme environmental conditions, predators and perturbations, as well as sites for feeding, moulting and breeding (Atkinson & Taylor, 1988; Bromley, 1990). Their burrows are used by other fauna as habitat and refugia (Dubey et al., 2012). The waterlogged depressions between mud lobster mounds are colonized by the bivalve *Geloina erosa* (Morton, 1976).

Sympatric occurrence of thalassinidean shrimps is interesting but poorly understood since several species can live close to each other often in a homogenous mud environment (Dworschak, 2003). This has also been observed for bivalve assemblages living in intertidal soft sediment systems where coexistence between species is not associated with increased sediment heterogeneity (Compton et al., 2008), contrary to expectation (e.g. Whitlatch, 1981; Snelgrove & Butman, 1994). It is possible that niche differentiation has enabled each thalassinidean species to become highly specialized or adapt to their occupied area. For example, *Neotrypaea* and *Upogebia* can live together in the same intertidal mud flat but they differ in their seasonal reproductive cycle and timing of postlarval recruitment (Dumbauld et al., 1996). Previous studies of the relatively unknown mud lobsters had all named these creatures as belonging to *Thalassina anomala* (e.g. Watson, 1928; Ferguson,

1951; Johnson, 1961, Chuang, 1961; Macnae, 1968; Sasekumar, 1974; Macintosh, 1988), based on their rather similar features first described by Herbst (1804) and subsequently by De Man (1928). In fact, thalassinid mud lobsters were then thought to be monotypic (Glaessner, 1969). Holthuis (1991) later suggested that there may be more than one species. In a recent review by Ngoc-Ho & de Saint Laurent (2009), seven species of *Thalassina* have been identified across the Indo-West Pacific region to as far as Fiji in the east, and to southern Japan and northern Australia. In the same general area of the coastal and estuarine environment of Klang-Langat delta in southern Selangor, Malaysia, three sympatric species of *Thalassina (T. kelanang, T. gracilis, T. anomala)* have been described for the first time (Moh & Chong, 2009; Moh et al., 2015). These species had never being reported as co-occurring species in other geographical regions (see Ngoc-Ho & de Saint Laurent, 2009).

This hypothesis is that the three species of mud lobsters are spatially partitioned across the mangrove shore as a result of niche differentiation. This chapter investigates how the mud lobsters are horizontally and vertically distributed on the mangrove shore and the possible environment factors driving their distribution. We examined sediment texture, organic matter, interstitial salinity, surface root bulk or mass, shore elevation and tidal inundation as influencing factors.

4.2 Materials and methods

4.2.1 Study area

The area of study in the state of Selangor (west coast of Peninsular Malaysia) was heavily vegetated by mangrove forests largely extirpated for agriculture, industry, settlement and a major port. As a result, the current mangroves are restricted to narrow fringes that line the Langat River estuary and parts of the coast that faces the Straits of Malacca (Figure 4.1). Environmental and biotic samplings were carried out at three sites where mud lobsters were known to occur in reasonable abundance.

The three sites were separate sites located from the seashore to the upper estuary in the same general area, but either in the mangrove reserve or within the coastal buffer zone of mangrove forest. Site 1 was located by the shore at Kelanang where the buffer mangrove forest stretched from the low to high shore. Site 1a consisted of three zones (Zones A to C). The lower region of site 1 (site 1b) comprised of mangroves as well as an open area close to the lower dyke. Site 1b consisted of four zones (Zones C to F). Zone C was divided into two sections by the lower dyke; an upper section of 35 m above the dyke in site 1a and a lower section of 5 m below the dyke in site 1b (Figure 4.2a). Site 2 and site 3 on Carey Island were respectively located about 6 km and 20 km to the north of site 1 (Figure 4.1). Site 2 was located on the upper reaches of a small coastal inlet (henceforth called the Air Hitam River) that drains the western half of the Jugra mangrove forest reserve. Site 3 was located on the mangrove buffer zone situated 26 km upstream in the upper estuary of the large Langat River, north of Carey Island.

The mangrove *Rhizophora apiculata* Blume dominated site 1 from zone A to zone D, while *Avicennia alba* Blume and *Sonneratia alba* Griffith became the main species in zone E and zone F (Figure 4.2b). In site 2, the mangrove was mostly *R. apiculata* with a few *Bruguiera* spp., *Xylocarpus granatum* Koenig and *Nypa fruticans* Wurmb. The remnant mangrove forest at site 3 consisted mostly of *Bruguiera* spp. and a few *R. apiculata*, and *Avicennia marina* (Firsk.)Vierh.

We used belt transect sampling across the shore or river bank to investigate mud lobster distribution from low to high shore. To enable equidistant samplings of environmental parameters and fauna along the shore and across dykes, three parallel transects along Kelanang shore (Figure 4.2a) were established at the lower dyke and extended 200 m landward via Zones C, B and A towards the high mangrove shore (site 1a). Another set of four seaward transects, also started from the lower dyke and extended 160 m via Zones C to F to the low shore mudflat (site 1b). These transect lines did not follow a straight line all through, but were broken after 60m (Zones C to D) to avoid disturbed forests, and shifted 150 m away towards the south before extending a further 100 m through Zone E and F. All transects at site 1 were marked with reference sampling points at every 20 m intervals. The landward transects of 200 m distance consisted of 33 sampling points while the seaward transects of 160 m distance consisted of 36 sampling points. At site 2, four parallel 60-m transects were established from the dyke towards the Air Hitam River. A total of 16 reference sampling points were established along thetransects, each point marked at every 20- m intervals. At site 3, four parallel 40-m transects were established from the dyke beside the oil palm plantation to the Langat River. Each transect was marked every 20-m intervals giving a total of 12 reference sampling points for all transects. The study was carried out from October 2010 to March 2012.



Figure 4.1. Klang Islands and its estuaries showing study site 1 at Kelanang shore (Kelanang Beach) and sites 2 and 3 on Carey Island.

Oil palm plantation



Figure 4.2. (a) A schematic profile of the Kelanang mangrove shore showing sampling site 1. Site 1a contained Zone A, Zone B and Zone C; Site 1b contained Zone C, Zone D, Zone E and Zone F. Zones E and F were set 150 m off the set transect through Zones C and D to avoid the disturbed forests below. (b) Composite horizontal profile of the mangrove high shore at Kelanang shore (site 1) and Carey Island (sites 2 & 3), showing the various zones, tidal heights and general distribution of mangroves and mud lobsters. Numerals below demarcated zones indicate the number of days per year shore sites were exposed to air. EHWS= Extreme High Water Spring; MHWS= Mean High Water Spring; MHWN= Mean High Water Neap; MLWN= Mean Low Water Neap.

4.2.2. Sampling methods

The height of the sites above Chart Datum (C.D.) was determined using a pole with test tubes tied on it at 2 cm intervals. The height of the site was determined from the height where sea water filled the top most test tube on the pole. Height of the water was measured from the ground surface. Tidal heights were determined by reference to the Tide Tables Malaysia (2011). The number of days the tides wetted or inundated the sampling stations in a year was estimated from the tidal movements for the year 2011 (Tide Tables Malaysia, 2011).

More than 30% of the total sampling points for each zone were sampled based on random sequence numbers. Mud lobster samplings were carried out within 5 x 5 m sampling plots established as feasibly close to the sampling points as possible. The final number of sampling plots randomly selected, or at least 30% of the total plots, for each zone is listed in Table 4.1. For the identification of species, mud lobsters were trapped from their burrows in every sampling plot using a self-devised contraption, comprising of a stiff steel wire of 50cm length, with a tethered piece of fish gill net (2.5 inch mesh size) on one end, and a T-handle on the other. The net end was pushed into the burrow with the Thandle resting across the opening of the mound. The traps were left overnight. All caught specimens were preserved in 80% alcohol for identification in the laboratory. All or a maximum of five mounds of identified species per sampling plot were randomly selected for mound measurements.

Although traps were deployed on every mound, the total animal captures represented about 30% of the total deployment. This is because a single individual could

construct burrows that open up into several mounds (see Figure 4.4), and animals could have escaped from their traps. Thus, animal captures could not be reliably used as estimates of the total population. The density of mud lobster was better estimated from the number of active mound per sampling plot (25 m^2) for each zone. An active mound is capped fresh/ wet mud dug out by the mud lobster the night before.

Soil samples and root counts were taken from smaller sampling units obtained by subdividing the 25 m² sampling plot into 25 subplots or quadrats of 1 m² each. The quadrats were numbered and randomly sampled according to the random sequence numbers generated. The samples were collected using a hand scoop to a depth of 12 cm. Sediment was individually kept in labeled plastic bags for determination of water content, organic matter content and particle size. Salinity was determined using a hand held refractometer (Milwaukee, USA) for water that seeped into a dugout shallow pit. Three quadrats were sampled for counting the number of above-ground pneumatophores and/ or prop roots.

4.2.3. Casting of mud lobster burrows

The burrow of each species of mud lobster was identified by first trapping the animal using the method described above. A cast of the internal burrow morphology was then made by pouring in and filling up the burrow with synthetic epoxy resin (Asasin 142TM and Asahard 142TM) purchased from ASACHEM (M) Private Limited, Malaysia. The filling agent was prepared by mixing one kg of Asasin 142TM with 0.42 kg of Asahard 142TM. After allowing 24 hours for the resin to completely solidify, the burrow cast was completely dug out by hand and spade. The burrow casts of *Thalassina anomala* were

obtained from Site 1a, *Thalassina kelanang* from Site 1b and *Thalassina gracilis* from Site 3. Five burrow casts were made for each species, but four perfect casts were ultimately used for analysis.

4.2.4. Analysis of particle size of sediment

Oven dried sediment were treated with 10% hydrogen peroxide and left overnight to allow digestion of the organic matter. The treated samples were then washed with distilled water to remove soluble salts before analysis by a Coulter 230 L Particle Size Analyzer. The method showed the volume-based fractions of the clay, silt and various sand grain sizes. Particle sizes were then categorized according to the Wentworth grade scale (Wentworth, 1922).

4.2.5. Determination of organic matter and root mass

A portion of air-dried sediment was weighed before combustion in a muffle furnace at 550°C for five hours, after which it was weighed again (Buchanan, 1984). The percentage of organic matter was calculated based on the weight lost. The subterranean 'root mass' of mangrove plants in the sampling plots was estimated by proxy based on the density (no. per m²) of above-ground roots including pneumatophores and stilt roots. This assumption was based on (i) the allometric equations (in logarithms) of stilt biomass and below-ground root biomass with stem girth of *Rhizophora apiculata* derived by Ong et al. (2004) (their Figure 4.4 and 6), which yielded a well fitted regression line of below-ground root biomass against stilt biomass (r²= 0.9331, p<0.01), and (ii) the fact that the pneumatophores of *Avicennia* and *Sonneratia* give rise to the underground fibrous network of nutrition roots, while the pneumatophores themselves arise from underground cable roots that radiate from the tree trunk (Tomlinson, 1986).

4.2.6. Data Analysis

We attempt to index the network complexity of the casted burrows of each species by using the Shreve (stream) ordering method (Shreve, 1966). The method assigns a number to each river stretch based on the hierarchy of its tributaries or branches, thus giving an indication of its relative size within the drainage system. The higher the number the more complex is the branching network. Stream ordering methods have been applied to biological systems such as tree branching (Borchert & Slade, 1981) and animal respiratory and circulatory systems (Horsfield, 1976). The burrow casts were stream ordered from the top (ground level) to the main shaft excluding the bottom resting chambers. All outer branches or segments were assigned an order of 1, and tunnel order or magnitude increased when two segments intersected below them. Increase in tunnelling magnitude was additive, i.e. two first-order links created a second-order link, while the intersection of a second- and third-order link created a fifth-order link, and so on until the bottom of the main shaft was reached.

All variables including basal diameter, height, burrow (mouth) diameter (cm) of the mounds, wet mound density and number of roots (per m²) were tested for normality and homogeneity of variances prior to ANOVA (Zar, 2010). If the ANOVA yielded significant results, significant difference among means was further tested by Tukey test. All statistical analyses were performed using the software Statistica Version 10.

Redundancy analysis (RDA), a constrained ordination technique, was used to analyze the relationships between mud lobster abundance with the measured water and sediment characteristics. RDA uses a direct gradient analysis approach useful for short gradient analysis assuming that animal abundance increases or decreases along the shore transect due to the environmental conditions and species interactions (Ter Braak & Smilauer, 2002). To justify the use of RDA, the detrended correspondence analysis (DCA) was first used to derive the gradient length expressed in standard deviation of the species turnover units (SD value) which should not be over 4 (Leps & Smilauer, 2003). The test gave the highest SD value or longest gradient of 2.345, hence justifying the use of RDA. In RDA, the species data comprised of the abundance of active mounds of known mud lobster species (T. anomala, T. kelanang and T. gracilis). The sample data set comprised of sampling plots from all zones (site 1 only) and sites, whereas the environmental data set comprised of shore elevation (height above CD), number of tidal inundations per year, water parameters (salinity), sediment organic content, root mat density and sediment texture (clay, silt, sand). DCA and RDA analysis was performed using CANOCO 4.5 software (Ter Braak & Smilauer, 2002). To avoid the paradox problem in computing distance between samples in the Euclidean space due to double zeros in the species abundance data, the chord distance was used (Legendre & Legendre, 1998). To implement this in CANOCO, standardization by sample norm was selected.

4.3 Results

4.3.1 Distribution of *Thalassina* species along the mangrove shore

Three species of *Thalassina* were sampled from Kelanang shore and Carey Island. In no sampling plot for all zones were more than one species of *Thalassina* found at any one time. *Thalassina anomala* was found in Zone A and Zone B of site 1a on the high shore at Kelanang, and at site 2 in Carey Island; these zones/ sites were situated 330 to 370 cm above Chart Datum (C.D.) (Figure 4.2b). *Thalassina kelanang* was found at mid-shore from Zone C (site 1a) to Zone F (site 1b), that is, from 140 to 330 cm C.D. *Thalassina gracilis* was found in site 3 on the high shore of the Langat estuary in Carey Island from 250 to 300 cm C.D. (Table 4.1; Figure 4.2b).

Semi-diurnal tides prevailed in the study area. Zone A to Zone E at Kelanang shore were variably exposed to air, while Zone F was always inundated by sea water (Table 4.1). The highest point inhabited by mud lobsters (only *T. anomala*) at zone A was inundated by 159-303 tides/ year, while the lowest point at Zone F was nearly always inundated, being covered by 707-839 tides per year. All sampling stations were wetted by spring tides, while neap tides only wetted Zones C, D, E, F and site 3 (Figure 4.3). Hence, only the mounds of *T. anomala* were not wetted during neap tides.



Figure 4.3. Distribution of mud lobster species in relation to daily tidal height at Carey Island. Zones A, B (site 1a) and site 2 lie within the spring high tidal range (habitat of *T. anomala*), while site 3 lies within the high neap tidal range (habitat of *T. gracilis*). Zones C, D, E & F lie within the high neap tidal range to low neap tide (habitat of *T. kelanang*). Right vertical arrow bars indicate range of species in term of shore elevation. Horizontal bar indicates size scale (1 cm) of animals.

4.3.2. Active mound density of *Thalassina*

On Kelanang shore, *Thalassina anomala* showed the highest mean active mound density (no. m⁻²) in Zone A (0.46 ± 0.08) at site 1a, but declined slightly (p>0.05) in Zone B (0.39 ± 0.08). In Carey Island (Site 3), the species showed almost similar mean mound density (0.45 ± 0.12) as in Zone A at Kelanang shore (Table 4.1).

There was a general decline in the active mound densities of *Thalassina kelanang* with increase in tidal height on the mangrove shore (Table 4.1). Mean mound densities (0.96) were similar at the open area station (Zone C, Site 1b) and *Rhizophora* area (Zone D, Site 1b) but declined towards Zone E (0.30) where there were mainly young *Avicennia alba* and few *Bruguiera* sp. and *Sonneratia alba* trees. The lowest mean mound density of *T. kelanang* (0.09) was found at the lowest shore in Zone F which consisted of mature *Sonneratia alba*. Mean active mound density of *T. gracilis* (0.46) was almost similar to that of *T. anomala* at Site 2, Carey Island.

Station &	Salinity (ppt)	Height	Days exposed	Species	Plot	Mean total	Mean wet	No. of roots/ m ²
Zone		above Chart	per year			mound/ plot	mound density/	
		Datum (cm)					m^2	
Site 1a (A)	15.40 ± 1.67	370-350	254-181	Thalassina anomala	5	30.80 ± 3.35	0.46 ± 0.08^{a}	30.28 ± 5.69^{a}
Site 1a (B)	22.33 ± 0.58	350-330	181-126	Thalassina anomala	3	24.00 ± 4.58	0.39 ± 0.08^{a}	34.80 ± 8.64^{a}
Site 1a (C)	23.25 ± 1.26	330-310	126-82	Thalassina kelanang 🧹	4	40.75 ± 4.57	$0.96 \pm 0.16^{\rm b}$	11.70 ± 4.38 ^c
Site 1b (C)	21.50 ± 1.29	310-300	82-61	Thalassina kelanang	4	41.00 ± 4.32	$0.96 \pm 0.14^{\rm b}$	2.70 ± 1.43 ^c
Site 1b (D)	23.50 ± 1.29	300-250	61-3	Thalassina kelanang	4	37.75 ± 3.00	$0.96 \pm 0.16^{\rm b}$	$67.55 \pm 18.77^{\text{ b, d}}$
Site 1b (E)	24.00 ± 1.41	250-200	3-0	Thalassina kelanang	4	10.00 ± 1.83	$0.30 \pm 0.10^{a,c}$	79.40 ± 12.70^{b}
Site 1b (F)	24.20 ± 0.84	200-140	0	Thalassina kelanang	5	3.60 ± 2.61	$0.09 \pm 0.09^{\circ}$	74.88 ± 6.01 ^b
Site 2	14.20 ± 3.49	370-350	254-181	Thalassina anomala	4	26.25 ± 5.00	0.45 ± 0.12^{a}	29.52 ± 2.51 ^a
Site 3	15.20 ± 2.17	300-250	61-3	Thalassina gracilis	5	24.4 ± 5.03	0.46 ± 0.10^{a}	$57.68 \pm 7.57^{\rm d}$

Table 4.1. Site characteristics, number of above-ground mangrove roots (no. m^{-2}) and mean wet mound density (no. m^{-2}) of three species of *Thalassina* at Kelanang Beach (Site 1) and Carey Island (Site 2 and 3).

Homogenous groups indicating no significant difference (p>0.05) by Tukey test are shown by similar superscript alphabets.

4.3.3 Burrow morphology and mound size

Thalassina anomala- All four burrow casts examined were each constructed by a single animal. Each cast revealed a similar system of one to three Y-shape burrows, all joined at their bases, and continued downwards in a single vertical shaft (Figure 4a). The number of surface burrow openings varied from 2 to 6, rarely 7. Mound openings are plugged by mud caps. Below the Y-burrows, paired horizontal burrows or chambers each of 30-40 cm length connect to the main shaft where at its bottom, the horizontal burrows bifurcate into several chambers serving as the animal's main resting area. The number of paired horizontal burrows varied from 3 to 4. These horizontal chambers probably act as places for the animal to rest and turn. The walls of the burrows are smooth. The distance between two mouth openings ranged from 0.5 to 1.50 m. Burrow depths ranged from 1.5-3.0 m. Mean burrow diameter at the mouth was 7.0 cm (\pm 1.00) at Kelanang shore (Site 1) and 8.4 cm (\pm 1.14) at Carey Island (Site 2). The burrow index of network complexity based on the Shreve method for four casts ranged from 7-10.

Thalassina kelanang- The total of four burrow casts studied all showed an upper section of 2-5 short burrow branches, some with blind-ends while others (numbering 3-6) open at the ground surface (Figure 4b). The upper anastomosing burrows finally join together in a single vertical shaft, sinous and not linear as in *T. anomala*. As in the latter, similar short horizontal chambers join the main vertical shaft, but these chambers are not paired but alternate with each other. The lower part or end of the burrow has 3-5 extended resting chambers. The mound's surface openings are spaced 0.1-0.5 m apart. Average depth of burrow ranged from 1.0-1.5 m with mean burrow mouth diameter of 5.4 cm (\pm 0.82). The burrow index of network complexity for four casts ranged from 8-16.

Thalassina gracilis- Four good burrow casts extracted from site 3 showed a complex, upper anatomosing network of blind ending, branching and non-branching burrows that open to the ground surface, similar to that of *T. kelanang*. However, unlike the burrow of *T. kelanang* and *T. anomala*, the main vertical shaft with short blind-ending branches twirls around in a loop before descending vertically (Figure 4c). The lower end of the burrow has many resting chambers (4-8). There were 3 to 5 mound openings, each separated by a distance of 0.1-0.6 m. The average depth of the main burrow ranged from 1.0-1.5 m with mean burrow mouth diameter of 4.2 cm (\pm 1.11). The burrow index of network complexity ranged from 13-17.

The largest mounds were made by *T. anomala* in Site 2, with mean basal diameter of 92.4 cm and mound height of 83.6 cm (Table 4.2). Both mound measurements compared to smaller measurements made in Site 1 were however not significantly different (P<0.05). Mounds of *T. gracilis* were the smallest in terms of basal diameter (29.2 cm) and height (9.0 cm). *T. kelanang* constructed mounds that were intermediate in size. The burrow mouth diameters of the three species ranged from 4.2 to 8.4 cm, with magnitude corresponding to the size of their mounds.

Table 4.2. Mean basal diameter, height and burrow mouth diameter of wet mud lobster mounds according to species and site (zone).

Species/ site	Mean n	nound Me	an mound	Mean burrow	
	basal dia	meter hei	ght (cm)	mouth	diameter
	(cm)			(cm)	
T. anomala (Site 1, A-B)	$81.00^{a} (\pm 7.9)$	94) 56.	$00^{a} (\pm 5.57)$	$7.00^{a,c} (\pm 1.$	00)
T. kelanang (Site 1, C-F)	$35.20^{b} (\pm 4.3)$	32) 16.	$40^{a,b}(\pm 2.07)$	$5.40^{a,b}$ (± 0.	.82)
T. anomala (Site 2)	$92.40^{a} (\pm 26)$	5.2) 83.	$60^{\circ} (\pm 17.66)$	$8.40^{\circ} (\pm 1.1)$	4)
T. gracilis (Site 3)	29.15 ^b (±5.5	9.0	$0^{b} (\pm 1.41)$	$4.23^{b} (\pm 1.1)$	1)

Homogenous groups in columns indicating no significant difference (p>0.05) by Tukey test are shown by similar superscript alphabets



Figure 4.4. Schematic drawings of the burrow morphology of the three species of *Thalassina* based on a particular burrow cast. a- *Thalassina anomala*; b- *Thalassina kelanang*; c- *Thalassina gracilis*. Ordering of burrow branches using the Shreve method is illustrated in a (numerals).

4.3.4 Effect of sediment parameters on the distribution of the three species of *Thalassina*

Result of RDA of the active mound density of the three species of mud lobsters by zones or/ and sites in relation to various environmental factors is illustrated in Figure 4.5. The first two axes accounted for 82.9% (first 58%) of the total variability of the species data. Further, the first and second axis accounted for 69.2% (eigen value = 0.580) and 29.8% (0.249) of the species-environment relation, respectively. The Monte Carlo permutation test of the significance of the relation between species and whole set of environmental variables based on the first (F ratio = 40.025) and all canonical axes (F =18.685) indicated that both were highly significant (p <0.002).

Zones A and B in Site 1 (circles) and Site 2 (filled squares) on higher shore elevation had similar soil conditions of relatively higher composition of silt and highest organic matter content. Zone C, D and E in Site 1 had similar conditions of higher composition of sand, interstitial water of higher salinity and higher frequency of tidal inundation. Zone F (Site 1) and Site 3 (filled diamonds) had relatively higher root mass than all other zones/ sites.

The distribution of *T. anomala* was correlated to silt and high organic matter, whereas *T. kelanang* appeared to colonize relatively sandy substrate inundated by seawater of higher salinity (23.5 ppt); higher abundance of *T. kelanang* was however at the middle shore (Site 1, zones C&D). While *T. gracilis* occurred in clayey-fine sand substrate, its only habitat at Site 3 was thickly covered by mangrove roots and flooded by freshwater and seawater (15.2 ppt). Interstitial water where *T. anomala* colonized in the upper sea shore was also low in salinity (14.2 ppt in Site 2 and 15.4 ppt in Site 1a).

Species interactions among the three species of mud lobsters were predicted to be low (low or negative correlation) since the species arrows produced obtuse angles with one another suggesting that the three species were well partitioned in their habitats.



Figure 4.5. RDA triplots of three species of mud lobsters (*T. anomala, T. gracilis, T. kelanang*, blue arrows) as influenced by environmental factors (red arrows) and sampled from three sites: circles (site 1, zone A to F, sample plots 1-8), squares (site 2, sample plots a-d), diamonds (site 3, sample plots a-e).

4.4 Discussion

The three sympatric species of *Thalassina* are spatially distributed on the mangrove shore, with no overlap in their distribution. There was no site where all three species occurred together, but each species did occur with one of the others. They occur in combinations of *T. anomala* with either *T. kelanang* or *T. gracilis*. *Thalassina anomala* occurs at the high intertidal near the landward side of the mangrove shore where tides inundate the ground for 30-65% of the time in a year (Figure 4.2b & 4.3). *Thalassina kelanang* inhabits the middle to lower mangrove shore which is subjected to daily tidal

inundation (Figure 4.2b). *Thalassina gracilis* also occurs at the mid-tidal zone indicating that it shares a similar microhabitat as *T. kelanang* in terms of tidal inundation and frequency. However, *T. gracilis* was observed at the upper estuary and never found together with *T. kelanang* at the lower estuary or sea shore. It is hypothesized that *T. kelanang* is less tolerant of large salinity fluctuations.

The mud lobster distribution pattern appears not to be solely influenced by sediment characteristics (Fig.4.5) but also by tidal inundation and salinity. Only more silty, organically rich substrates may be favoured by T. anomala on the high shore. This could be due to the presence of rather similar mud substrates in the mangrove area, whereas tidal inundation and salinity are a function of the elevation of the shore where the animals are distributed. Interestingly, both species have been reported to be genetically closest to each other as compared to either of them with T. anomala (Moh et al., 2013). Laboratory experiments also showed that T. kelanang is more aggresive than T. anomala (Moh & Chong, 2009). Hence, interference competition leading to species exclusion is a potential mechanism that explains separation of T. kelanang from T. anomala, as well as from T. gracilis. The Thalassina mud lobster distribution model differs from that of other thalassinideans. For example, the distribution of three species of *Callianassa* species in Victoria, Australia, interestingly shows a rather similar situation as in the present study; only two species were found together, except that the mud shrimps co-occurred with considerable overlap in distribution (Coleman & Poore, 1980). Apparently, the mechanism of competitive interaction differs to allow for spatial co-existence.

A conceptual model of niche differentiation and competitive interactions among the three *Thalassina* species is depicted in Figure 4.6. With interference competition, mud

lobsters are expected to compete for habitat space, which in the mangroves are restricted to the unvegetated space of the forest floor. A successful 'stake-out' of the forest floor and its defense also guarantee the food resources within and presumably the opportunity for mating. On the forest floor, total mound densities can reach a maximum of $1.52/m^2$ depending on species (Table 4.1), while in unvegetated areas such as coastal dykes or landward plantation, mound densities could reach 2.40 /m² (Moh, unpublished).



Figure 4.6. Conceptual model of niche differentiation and competitive interactions among three sympatric species of *Thalassina* in the Klang-Langat delta (Selangor).

Ellipse denotes the distributional range of each species. Niche differentiation is achieved by animal's preference or tolerance to salinity (horizontal axis) and duration/frequency of tidal inundation, a function of shore elevation (vertical axis). Strength of competitive interactions is denoted by the weight of broken arrow; lesser competition allows for a closer area of distribution of *T. anomala* with either *T. kelanang* (fringing mangrove, shore area) or *T. gracilis* (riverine mangrove, upper estuary), while intense competition widely separates *T. gracilis* from *T. kelanang*.

Shore animals have evolved a variety of morphological, physiological and behavioural mechanisms to withstand the rigours of the shore, particularly aerial exposure and variation in salinity (Raffaelli & Hawkins, 1999). Mud lobsters make mud-capped burrows not only to avoid exposure but also as refugia space. It has also been reported that they dig while simultaneously feeding on the organic matter in the substrate (Johnson, 1961; Dubey et al., 2012) which are eventually pushed out to form their mounds. Thus, active mounds which indicate their recent feeding habits can be used to indicate the animal's presence. Nevertheless, we caution the absolute use of active mounds to estimate population size since a single animal may deposit the freshly excavated mud onto one (39%), two (35%) or three (17%) mounds on the same night (Moh, unpublished). Thalassina mounds are very prominent on the upper shore but not so on the lower shore as tidal currents and wave action re-distributes or removes freshly excavated sediments. Hence, surface accumulation of excavated sediment on Thalassina anomala mounds increases when frequency of tidal inundation decreases in the upper shore. Therefore, T. anomala have mounds higher above ground level than Thalassina kelanang and T. gracilis which occur on the lower shore.

Burrow morphology of thalassinidean shrimps and shore crabs can be affected by environmental factors including sediment type, tidal height and climate, or biological factors such as population density, age and sex (Nash et al., 1984; Griffis & Chavez, 1988; Lim & Diong, 2003; Chan et al., 2006). The present study shows that the three species of

Thalassina constructed burrows of different morphologies and complexity (Figure 4.4). The Shreve method provides an indication that the burrows of T. gracilis and T. kelanang are more extensive and complex than those of T. anomala as well as brachyuran crabs. Burrows of fiddler crabs (Uca) may have single (L or J types) or/and double (or triple) openings (U, V or Y types) (Lim, 2006; Qureshi & Saher, 2012). The portunid, Scylla serrata, digs J, U or Y typed burrows (Nandi & Dev Roy, 1991), while the ghost crab Ocypode ceratophthalma digs burrows that vary from J type in juveniles to Y type in adults (Chan et al., 2006). The burrows of Thalassina mud lobsters are also more complex compared to those of their close relatives, the mud shrimps. Upogebia (Upogebiidae) constructs U- or usually Y-shaped burrows (Dworschak, 1983; Coelho et al., 2000). However, the burrow architecture of callianassid shrimps, a group that is almost completely confined to marine soft sediments, is equally complex consisting of an interconnected tubesystem with several surface openings often in volcano-like mounds (Ziebis et al., 1996; Stamhuis et al., 1997). Griffis & Suchanek (1991) distinguished six burrow types among the three major feeding guilds of thalassinideans: deposit feeders (complex or branched burrows), drift catchers (simple straight burrows) and filter/ suspension feeders (U- or Yshaped burrows). Based on this classification, *Thalassina* mud lobsters are deposit feeders.

A link between burrow morphology and organic content of the sediment has been suggested for the subtidal mud shrimp *Callianassa subterranea*, which appears to construct burrows that are more shallow and complex in organically poor than in organically rich sediments (Nickell & Atkinson, 1995; Rowden & Jones, 1995). This may meet the requirements of *Thalassina gracilis* and *T. kelanang* as mud feeders need to process high amounts of sediment in organically poor substrates, resulting in more complex and shallow burrows. In contrast, *T. anomala* living on the higher shore with sediment of higher

organic content, digs deeper but less complex burrows. Stamhuis et al., (1997) suggested that higher competition for food and space induces mud shrimps to construct deeper burrows in organic rich sediments. Several studies have observed changes in burrow characteristics with changes in tidal height. Dworschak (1987) observed that the high intertidal burrows of *Upogebia pusilla* reached greater depths than burrows from subtidal areas. A review by Griffis & Suchanek (1991) concluded that changes in thalassinidean burrow architecture along tidal gradients are primarily related to burrow depth, and that the basic morphology of the burrow is fairly constant within species across tidal and latitudinal gradients.

Finer sediments can provide a more stable substrate for extensive, more complex burrows owing to their higher cohesive nature (Takeda & Kurihara, 1987; Rudnick et al., 2005). Sediment type, below-ground roots and other plant matter in the sediment can interfere with burrow construction and increase the complexity of crab burrows (Katrak et al., 2008). Seagrass roots and shell debris in sediments reduced the population density and frequency of occurrence of three species of the burrowing mud shrimp *Callianassa* (Coleman & Poore, 1980). Pillai (1989) reported the cable roots of Fijian mangroves that extensively ramify the ground can limit the space for *T. anomala* to construct its burrows. In the present study, the mangrove species (*R. apiculata* and *A. marina*) present at Site 3 in Carey Island have more numerous and wider cover of roots. Thus, the presence of root mat and fine sediments may explain the more complex burrow morphology including the circular bend in burrows of *T. gracilis*. Bertness & Miller (1984) suggest that intermediate root mat density may be necessary to provide the hard structural elements for burrow support and longevity.

While sediment characteristics may explain the depth of burrows, the topography and tidal regime of the animal's locality vis-a-vis its physiological requirements may be just as important. Berry (1972) reported that the burrow of *Thalassina anomala* leads down to the water table, where the animal can occasionally wet its gills. Therefore, T. anomala residing at the upper shore digs deeper burrows (1.5-3.0 m) as compared to T. kelanang and T. gracilis on the lower shore (1.0-1.5 m) where tidal inundation is more frequent. The deep burrows of T. anomala may also be a way to maintain lower burrow temperature in the higher shore similar to crab burrows (Powers & Cole, 1976; Lim & Diong, 2003). The morphology of *T. anomala*'s mound maintains an interior temperature $(29.1 \pm 1.1^{\circ}C)$ of about 3°C lower than the exterior temperature $(32.1 \pm 1.9^{\circ}C)$ (Moh, unpublished). Macintosh (1988) concluded that T. anomala must be highly tolerant of the hypoxic condition prevaling at the bottom of its deep burrow. A study of the oxygen transporting properties of the haemocyanim of five species of thalassinidean mud-shrimps shows that all have a high oxygen affinity, but Calianassa subterranea, a deposit feeder living in severely hypoxic burrows have significantly higher oxygen carrying capacity of the haemocyanin (Taylor et al., 2000).

Niche differentiation allows the three *Thalassina* species to live sympatrically in slightly different mangrove habitats within the same deltaic system. There are however no major morphological differences among the three species that would suggest their adaptive differences as a result of niche differentiation. Therefore, these species are likely to have adopted different physiological and/or behavioral adaptations to maintain survival. For instance, *T. anomala* on the high shore, would need to have a higher ability to tolerate dessication and occassional low-salinity stress from episodic rainwater; these animals can be found in rather dry parts of oil palm plantations and in freshwater ditches (Moh et al.,

2006). Also, berried females were more often observed during spring tides when strong tides are needed to export their larvae off shore. On the other hand, *T. gracilis*, at the upper estuary, will need to tolerate a wide range of fluctuating salinity. Its small size (about half of the size of the other two species) may be an adaption to live amongst mangrove roots. Nevertheless, future studies, particularly mesocosm and laboratory experiments, are needed to test the physiological and behavioral responses of this group of poorly studied thalassideans.

Chapter 5

Mound distribution and density of *Thalassina* mud lobsters on the coastal dykes of Carey Island

5.1 Introduction

Among all the known species of *Thalassina* mud lobsters, *T. anomala* is the most common and most widely distributed mud lobster species in the Indo-West Pacific region (Moh et al., 2015). According to Johnson (1961) *T. anomala* is an extremely abundant animal in the mangrove swamps of Singapore and Malaya. This species inhabits the higher region of the intertidal shore with a distribution that extends above the extreme high water mark of the mangrove shore (Moh et al., 2015). However, mangrove deforestation and land-claim for agriculture and development since the early 1960s have made the mud lobster no longer the ubiquitous creature they once were other than in some places.

The burrowing behavior of mud lobster, *T. anomala* can have profound impact on the topography and vegetation structure of mangrove shores (Macintosh, 2002) including reclaimed mangrove areas. Extensive land reclamations of high shore mangroves during the 60s for agriculture and aquaculture have resulted in the loss of the natural habitat of *T. anomala* which now has made coastal dykes its new home. Macintosh (1988) reported the burrowing activities of *Thalassina* caused extensive damage to man-made dykes and embankments of aquaculture ponds to collapse. The burrowing activities of the mud-lobster on the dyke has weakened it and now *Thalassina* is considered a pest to human (Holthuis,

1991). *T. anomala* seems to have developed a remarkable tolerance to the harsh environment of the dyke (Ng & Kang, 1988).

In this chapter the distribution and colonization pattern of *Thalassina* anomala on the coastal dyke in Carey Island is investigated, including the influence of environmental factors on its distribution. In this study it is assumed that *T. anomala* is the only *Thalassina* species that inhabit the coastal dykes given that it is the only species found on the high shore mangroves. Initial investigations using traps had also recorded *T. anomala* as the only species captured.

5.2 Materials and methods

5.2.1 Study area

Carey Island (2°52' 18.38''N, 101°22' 41.15'E) was chosen as the study site since the island is completed surrounded and protected by coastal dykes. The island is located in the state of Selangor, on the west coast of Peninsular Malaysia (Figure 5.1). It is the largest single island separated from the mainland by the bifurcated Langat River at its lower estuary. The island is separated from Lumut (=Indah) Island by one of the estuarine channels of the Klang River system, the Lumut Strait, to the north. Carey Island has a land area of approximately 16,187 hectare and is approximately 1.5-2.0 meters below sea level. The island is presently an oil palm plantation of about 10,521 hectares. The entire perimeter of Carey Island is surrounded by 101.8 km of earthen dykes of 2.5 to 3.5 m high and 3.0 to 4.0 m wide to protect the mainly oil palm plantation from seawater intrusion. Thirty-seven tidal gates are built interspersed along the dykes to control the outflow of fresh water and inflow of sea water via a system of canals inside the plantation. The plantation is built on reclaimed mangroves, and now surrounded by a narrow fringe of mangroves mostly of *Avicennia*, *Bruguiera* and *Rhizophora* species (Figure 5.1).



Figure 5.1. Map shows study sites for spatial-temporal colonization of newly repaired bund on Carey Island and the surrounding estuaries.

- A1 Sites with the most abundant mud lobster mounds
- B1 Sites with moderate number of mud lobster mounds
- C1 Sites with very few mud lobster mounds

5.2.2 Distribution of mud lobster in whole Carey Island

Locations of all mud lobster mounds along the entire 91.9 km length of the dyke were determined by belt transect sampling from May 2005 to August 2005. The belt consisted of 919 sampling plots, each measuring 100 m length (along the length of the dyke) by 25 m width (including both slopes of the dyke). Census began at the first sampling plot at the first tidal gate, TG 1 ($2^{\circ}90219^{\circ}N$, $101^{\circ}38350^{\circ}E$) and continued over a period of 105 days until the last sampling plot (TG 1) was reached. Every mud lobster mound found in each surveyed plot was recorded and counted on the day of observation. Individual positions of all mud lobster mounds were recorded by a Global Positioning System (GPS) device (Garmin e-Trex Venture, CX model). Characteristics of each observed mound, namely, height, diameter and burrow mouth diameter were measured (see Figure 5.3). Mound activity, which is a measure of how likely the mound is occupied by an animal, is evaluated based on the presence of freshly excavated or moist mud at the burrow opening. An active mound is one that has freshly capped moist mud (labelled A= active mound). An inactive mound is still occupied by an animal but without recent excavations since the burrow exit is capped by dry mud (labelled In A = inactive mound). An abandoned or old mound is one that is without animal and outwardly appeared worn-out, eroded or collapsed (labelled O= old mound).

At three selected sites (A1, B1, C1), mound distribution across or perpendicular to the dyke was further studied to investigate *Thalassina anomala*'s preference for specific microhabitats. The location of all mud lobster mounds were recorded according to the following demarcated zones: waterside bund top (TW), landside bund top (TL), top bund slope (TB), bottom bund slope (BB), bund ditch (DB), road zone (RD), plantation canal (CB), and plantation zone (PL) (Figure 5.2).






Figure 5.3. Mud lobster mound and burrow measurements. Dm= diameter, H= height, Db= diameter at entrance of burrow.

5.2.3 Colonization dynamics of mud lobster on the dyke

This study was conducted to investigate mud lobster colonization of a newly built dyke or repaired section (first week after repair) of the dyke (i.e. with no mounds) at selected sites of mud lobster infestation (see Figure 5.1). Two sites for each condition (level) of infestation by mud lobster mounds, namely, high (> 30 mounds/ 100m, Site A1), moderate (20-30 mounds/ 100m, Site B1) and low infestation (< 20 mounds/ 100m, Site C1), were selected on the dyke. Each plot of the experimental quadrat was 100 m long by 25m wide (across), i.e. on the bund from the waterside bund top (TW) zone to the plantation zone (PL). New mud lobster mounds were observed during spring tides at full moon and new moon every fortnight from January to June 2006, and monthly at full moon from July 2006 to July 2007. Location of each new mound was marked with pegs driven into the ground and recorded with a Global Positioning System (GPS) device. The methodology of measurements for each location, zone, mound activity and mound measurements is to section 5.2.2 above.

Water parameters at ditch water between the dyke and road such as water salinity (ppt), ditch water pH and redox potential (Mv) were measured at all sites using Cyberscan pH 310 (Eutech Instruments). Salinity of the ditch water was measured using a handheld refractometer (Milwaukee MR100ATC). Reading of soil parameters for pH and temperature (°C) were taken at the mud lobster mound at bottom bund slope (BB) of the dyke. Soil from the mud lobster mound (wet or dry) mixed with some distill water to soften the soil and soil pH were measured using Cyberscan pH 310 (Eutech Instruments). Soil thermometer was poke into the soil for measurement of the soil temperature (°C). A soil auger was used to sample the dyke soil at zone (BB). The soil auger was repeatedly

bored into the ground by twisting its handle, taking care to empty the dug soil between successive bores until a depth of 1 metre) was reached. A portion of the collected sediment at each 50 cm depth was kept in a labeled plastic bag for subsequent assessment of the organic matter content (%), soil moisture (%) and particle size of the sediment in the laboratory. All sediment prior to analysis was dried in the oven at 70 °C for a week.

5.2.4 Sediment analysis

A. Particle size analysis

Large particles of the sediment such as stones and leaves were removed manually from the samples. The sediment was then treated with 10% hydrogen peroxide (H_2O_2) in a beaker and left for 24 hours for the digestion of the organic matter (English et al., 1994). The treated samples were then washed with distilled water to remove soluble salts before particle size analysis using a Beckman Coulter Particle Size Analyzer (Model LS230). This method gave the volume-based fractions of the clay, silt and sand of various grain sizes. The soil particle groups were then categorized according to the Wentworth grade scale as described in Table 5.1.

Particle size class (µm)	Categories
0-<3.9	Clay
3.9-<15.6	Fine silt
15.6-<62.0	Coarse silt
62.0-<125	Very fine sand
125-<250	Fine sand
250-<500	Medium sand
500-<1000	Coarse sand
1000-<2000	Very coarse sand

Table 5.1. Categories of sediment based on particle size.

B. Organic matter content

A small portion of the air-dried sediment in triplicates was placed in individual preweighed soil cups. The weight of sediment with its cup was taken using a 4-decimal point digital balance (Model GR-200) before combustion inside a muffle furnace (Model: JSMF-30T) at 550°C for 6 hours. The sediment with its cup was then weighed again. The percentage of organic matter was calculated based on the weight lost during combustion based on the following equation:

Percentage composition of organic matter= $[(B-C) \times 100]$

B-A

Where, A = weight of soil cup

B = weight of soil cup + dried sediment before combustion

C = weight of soil cup + sediment after combustion

5.2.5 Statistical analysis

All data were tested at the 5% significance level for homogeneity of variance using Levene's test and normality using Kolmogorov- Smirnov's test before analysis of variance (ANOVA) (Zar, 2010). If the homogeneity of variance or normality requirement for parametric testings was not fulfilled, the data were either logarithmically transformed [log(x+1)] or arcsine transformed (percentage data) before analysis. The non-parametric Kruskal-Wallis test was used for data analysis if data transformation still could not fulfil the parametric requirements. All statistical analysis was performed using Statistica Version 10 Software Package.

Principal component analysis (PCA) was carried out to visualize the spatial distribution of the mud lobster with the on-site water and soil parameters. The sample data comprised of the different sampling sites (A1, B1 and C1) whereas the environmental data set comprised of ditch water pH, salinity, redox potential (Mv), soil pH, soil temperature, sediment organic content, soil moisture content, sediment grain size (clay, fine silt, coarse silt, very fine sand, fine sand, medium sand, coarse sand and very coarse sand). PCA analysis was performed using CANOCO 4.5 software (Ter Braak & Smilauer, 2002).

5.3 Results

5.3.1 Distribution and habitation of mud lobster in Carey Island

5.3.1.1 Mud lobster mound distribution and abundance

The total number of 100-m quadrats established around the whole island was 919, in which 3073 mud lobster mounds were enumerated. Thus, the mean number of mud lobster mounds was $3.35 (\pm 10.38)$ per 100m length of dyke (quadrat). Mound numbers ranged from 0 – 121 per quadrats. Approximately 67% of the 100m-quadrats (618) did not contain any mud lobster mounds. Twenty five percent (25%) of the quadrats contained less than 10 mud lobster mounds per quadrat, while less than 5% contained between 10-20 mounds. Less than 3.5% of the quadrats contained more than 30 mounds (Table 5.2). Most of the mounds were observed on the northeastern of the island (9.19 ± 13.70/ 100m) around the meander of the lower estuary of the Langat River, and the southern coast of the island at the upper tidal creeks (6.69 ± 17.01/ 100m) that fed the coastal inlet of Sungai Air Hitam (Figure 5.4). The across-dyke distribution showed the highest number of mud lobster mounds at the road or zone RD (38%), followed by the dyke bottom or zone B (28%). The dyke top (TL and TW) recorded 10% of the total number of mounds enumerated along the transect (Figure 5.5).

No. per quadrat	Count	Cumulative Percent		Cumulative	
		Count	(%)	percent (%)	
-10.0 <x<=0.0< td=""><td>618</td><td>618</td><td>67.24</td><td>67.24</td></x<=0.0<>	618	618	67.24	67.24	
0.0 <x<=10.0< td=""><td>226</td><td>844</td><td>24.59</td><td>91.83</td></x<=10.0<>	226	844	24.59	91.83	
10.0 <x<20.0< td=""><td>39</td><td>883</td><td>4.24</td><td>96.07</td></x<20.0<>	39	883	4.24	96.07	
20.0 <x<30.0< td=""><td>6</td><td>889</td><td>0.65</td><td>96.72</td></x<30.0<>	6	889	0.65	96.72	
30.0 <x<40.0< td=""><td>11</td><td>900</td><td>1.20</td><td>97.92</td></x<40.0<>	11	900	1.20	97.92	
40.0 <x<50.0< td=""><td>9</td><td>909</td><td>0.98</td><td>98.90</td></x<50.0<>	9	909	0.98	98.90	
50.0 <x<60.0< td=""><td>3</td><td>912</td><td>0.33</td><td>99.23</td></x<60.0<>	3	912	0.33	99.23	
60.0 <x<70.0< td=""><td>3</td><td>915</td><td>0.33</td><td>99.56</td></x<70.0<>	3	915	0.33	99.56	
70.0 <x<80.0< td=""><td>2</td><td>917</td><td>0.22</td><td>99.78</td></x<80.0<>	2	917	0.22	99.78	
80.0 <x<90.0< td=""><td>0</td><td>917</td><td>0.00</td><td>99.78</td></x<90.0<>	0	917	0.00	99.78	
90.0 <x<100.0< td=""><td>0</td><td>917</td><td>0.00</td><td>99.78</td></x<100.0<>	0	917	0.00	99.78	
100.0 <x<110.0< td=""><td>0</td><td>917</td><td>0.00</td><td>99.78</td></x<110.0<>	0	917	0.00	99.78	
110.0 <x<120.0< td=""><td>1</td><td>918</td><td>0.11</td><td>99.89</td></x<120.0<>	1	918	0.11	99.89	
120.0 <x<130.0< td=""><td>1</td><td>919</td><td>0.11</td><td>100.00</td></x<130.0<>	1	919	0.11	100.00	
130.0 <x<140.0< td=""><td>0</td><td>919</td><td>0.00</td><td>100.00</td></x<140.0<>	0	919	0.00	100.00	

Table 5.2. Frequency-class distribution of all the mud lobster mounds in Carey Island.



Figure 5.4. Mud lobsters mound distribution in Carey Island (*Black doted). (census from May 2005 – August 2005). Topographic map extracted from Department Survey and Mapping, Malaysia (JUPEM).



Figure 5.5. Distribution of mud lobster mounds across dyke zones for the whole of Carey Island, Peninsular Malaysia.

Numerals above bars indicate % contribution of transect zones to mound numbers. For zones (x-axis): Plantation-PL, Plantation canal-PC, Road-RD, Bund ditch-DB, Bottom bund-BB, Top bund slope-TB, Landside bund top-TL and waterside bund top-TW. (see Figure 5.2 for detail of the zones).

5.3.1.2 Classification of mud lobster mound activity

A total of 3073 mounds were recorded between May to August 2005, of which 1330 were old or abandoned mounds (43.3%), 1233 were active mounds (40.1%) and 510 were inactive mounds (16.6%). Most of the old mounds were found in the road zone (RD) while occupied mounds (active and inactive mounds) were largely found in the road zone (RD) and bottom bund slope (BB) (Figure 5.6).



Figure 5.6. Distribution of "Active" (A), "Abandoned" (O) and "Inactive" (InA) mounds by zones across bund perimeter for whole of Carey Island. For zones (x-axis): Plantation-PL, Plantation canal-PC, Road-RD, Bund ditch-DB, Bottom bund-BB, Top bund slope-TB, Landside bund top-TL and waterside bund top-TW.

5.3.1.3 Mud lobster mound height

Analysis of the mound height measurements, which ranged from <5 cm to about 105cm, showed that most of the mounds (41%) had heights of between 10 to 20 cm, but the mound height distribution was positively skewed (Figure 5.7). There were approximately 1260 mounds (41%) with heights of this size range, and almost all the mounds observed had heights of less than 80 cm. Very few mounds had heights of over 100 cm. Investigation on the mean mound height across the bund revealed that it was the highest at the canal (C) zone and the lowest at the bund top (TL). Mound heights of the latter also showed the smallest standard deviations (Figure 5.8). The tallest mean mound height and mound occurred at the banks of the oil palm plantation canal (PC) about 8-13 m from the dyke.



Figure 5.7. Frequency distribution of *Thalassina* mound heights for whole of Carey Island. Numerals above bars indicate % contribution of each size class of mud lobster mound height.



Figure 5.8. Mound height (cm) in relation to location (transect across bund from sea to plantation) for the whole length of dykes in Carey Island.

For zones (x-axis): Plantation-PL, Plantation canal-PC, Road-RD, Bund ditch-DB, Bottom bund-BB, Top bund slope-TB, Landside bund top-TL and waterside bund top-TW. (see Figure 5.2 for detail of the zones).

5.3.1.4 Mud lobster mound burrow diameter

Figure 5.9 shows the frequency distribution of burrow mouth diameter which indicates that mouth diameters generally fell between 3 to 5 cm, and were slightly positively skewed in distribution. The diameter of the burrow mouths ranged from 1 to 12 cm. The mean mouth diameter of mounds located on low ground between BB to PL (plantation) were generally larger (>5cm) than those on higher ground (dyke). Figure 5.10 shows the mean of burrow diameters in relation to position of zones which indicated that the lowest mean burrow diameter (4-5cm) was found at the bund top (TL) and followed by TW zone. The mean burrow diameter from BB to PL zone generally fell between 5- 6cm. The correlations among three mound characteristics showed that all were significant with each other (P<0.05); the highest was between mound height and mouth diameter (r=0.52), and the lowest was between burrow diameter and mound height (r = 0.36) (Table 5.3).



Figure 5.9. Frequency distribution of all burrow mouth diameter for whole of Carey Island. (X = mouth diameter, cm).



Figure 5.10. Burrow/ mouth diameter of mud lobster burrow in relation to position of zones for whole length of dykes in Carey Island.

For zones (x-axis): Plantation-PL, Plantation canal-PC, Road-RD, Bund ditch-DB, Bottom bund-BB, Top bund slope-TB, Landside bund top-TL and waterside bund top-TW. (see Figure 5.2 for detail of the zones).

Table 5.3.	Correlations	among three	mound charac	cteristics (all	significant	P<0.05).
		U			U	

		Spearman R	ons (new data)				
		MD pairwise deleted					
		Marked correlations are significant at p <.05000					
		Height (cm) Diameter (cm) Burrow Diameter					
Variable				(cm)			
Height (cm)		1.00000	0.52278	0.36418			
Diameter(cm)		0.52278	1.00000	0.47691			
Burrow Diameter	(cm)	0.36418	0.47691	1.00000			

*Red value indicates significant different at P<0.05

5.3.2 Dyke Colonization by mud lobster at Site A1

From January to December 2006, a total of 245 new mud lobster mounds were established in the 100 x 25m experimental plot at Site A1, an area where the dyke had been just repaired with excavated sediment from the ditch and mangrove area close by. Colonization frequency by mud lobsters was very high during the first 1-2 months, but rapidly dropped off over the following 2 months (Figure 5.11). Over the 12 months of observations, the rate of establishment of new mounds by zone was highest at the waterside dyke top (TW) (9.08 mounds/ month), followed by the bottom bund slope (BB) (5.75 mounds/ month), dyke ditch (DB) (2.25 mounds/ month), landside dyke top (TL) (1.75 mound/ month), top dyke slope (TB) (1.5 mounds/ month), and road (RD) (0.25 mounds/ month). Locations in the plantation canal (PC) and plantation proper (PL) did not indicate the presence of lobster mounds.

The distribution of new mounds was found to be the highest at the bund top (water side, TW) (44%), followed by the bottom bund slope (BB) (28%), (Figure 5.12). The zones of TL, TB, DB and RD recorded 9%, 7%, 11% and 1% respectively. Figure 5.16 shows that the number of new mounds at zone (TW) was relatively higher during the full moon or bright lunar phase (Month "A") as compared to new moon or dark phase (Month "B"), although the numbers progressively decreased with time. This moon-phase pattern was not so obvious on the dry side of the bottom bund slope (BB). The (TW) zone also showed the number slightly increased from month of September to November (Figure 5.13).



Figure 5.11. Mound building in newly-repaired bund (Site A1; 100x 25 m) over 12 months. $A = full \mod (spring tide) B = new \mod (spring tide).$



Figure 5.12. Cumulative abundance of newly established mud lobster mounds on transect across dyke at Site A1 From Jan – Dec 2006, Carey Island.

Numerals above bars indicate % contribution of transect zones to mound numbers. For zones (x-axis): Road-RD, Bund ditch-DB, Bottom bund-BB, Top bund slope-TB, Landside bund top-TL and waterside bund top-TW. (see Figure 5.2 for detail of the zones).



Figure 5.13. Monthly distribution of newly colonized mud lobster mounds by zones from January to December 2006 (Site A1).

A= full moon (spring tide) B= new moon (spring tide).

For zones (x-axis): Road-RD, Bund ditch-DB, Bottom bund-BB, Top bund slope-TB, Landside bund top-TL and waterside bund top-TW. (see Figure 5.2 for detail of the zones).

From the total of new mounds recorded, it was found that 77.6% (190) were active mounds (A), followed by 15.1% (37) "Inactive" mounds (InA) and 7.3% (18) abandoned mounds (O). Figure 5.14 shows the "activity" of the mounds by zones across site A1 over 12 months. Most of the active new mounds were found on the water side zone (TW; n=98), followed by bottom bund slope (BB; n=56), TB (; n=14) and bund ditch (DB; n=21). From January to April, the number of new active mounds was high at water side zone (TW) and the number of new active mounds increased again from August to November at zone (TW). The bottom bund slope (BB) also exhibited the same activity

pattern with high number of new active mounds from January to March but this activity pattern was not seen from August to September at zone (BB) (Figure 5.14).



Figure 5.14. Distribution of new "Active" (A), "Abandoned" (O) and "Inactive" (InA) mounds by zones across A1 site (most abundant) over 12 months. A= full moon (spring tide) B= new moon (spring tide). For zones (x-axis): Road-RD, Bund

ditch-DB, Bottom bund-BB, Top bund slope-TB, Landside bund top-TL and waterside bund top-TW. (see Figure 5.2 for details of the zones).

5.3.3 Colonization of mud lobster mounds at Site B1

The total number of new mud lobster mounds enumerated was 305 mounds in an area of 100 X 25 m. The total number of new mounds at site B1 was higher compare to the most abundant site A1 due to the mud lobster actively creating new mound at the first two month of the experiment, which comprised (37.4% or n=114) for month (January-

February). Most of the mounds emerged on the first and second month after the bund had been repaired and slowly decreased in the subsequence months (March to Jun). The total number of mounds started to increased again after August to November (Figure 5.15). The distribution of mounds was found to be highest at the bund top zone (TW) (8.17 mounds/ month; n=99; 32%). This was followed by the bottom bund slope (BB) (7.67 mounds/ month; n=91; 30%) (Figure 5.16). The zones of TL, TB, DB and RD recorded 21 (7%), 20 (6.6%), (43; 14%) and (31; 10%) new mounds, respectively (Figure 5.16).

Figure 5.17 shows that the number of the new mounds at zone TW was comparatively higher during the full moon or bright phase (Month "A") as compared to new moon or dark phase (Month "B") and the numbers of mounds progressively decreased the following month. The numbers of the new mounds at zone (TW) started to increase again from the month of August to October and decreased again from November to December (Figure 5.17). This moon-phase activity pattern also showed on the dry side of the bottom bund slope (BB) but with higher new mounds at new moon (Month "B") compared to full moon (Month "A") (Figure 5.17). Apart from the first peak during month January-February, zone TW and BB also clearly showed a second peak during month October-November (Figure 5.17).



Figure 5.15. Total number of new mud lobster mounds in newly repaired dyke (Site B1) from January to December 2006. A= full moon (spring tide) B= new moon (spring tide).



Figure 5.16. Distribution and abundance of new mud lobster mounds for each zones at Site B1 on the dyke.

Numerals above bars indicate % contribution of transect zones to mound numbers. For zones (x-axis): Road-RD, Bund ditch-DB, Bottom bund-BB, Top bund slope-TB, Landside bund top-TL and waterside bund top-TW. (see Figure 5.2 for detail of the zones).



Figure 5.17. Distribution of new mud lobster mounds by zones from January to December 2006 for Site B1. A= full moon (spring tide) B= new moon (spring tide). For zones (x-axis): Road-RD, Bund ditch-DB, Bottom bund-BB, Top bund slope-TB, Landside bund top-TL and waterside bund top-TW. (see Figure 5.2 for detail of the zones).

From the total new mounds recorded, 76.4% (233) were active mounds (A), followed by 17.4% (53) "Inactive" mounds (InA) and 6.2% (19) abandoned mounds (O). Monthly colonization and distribution of new active mud lobster mounds by zones showed most of the active mounds (30.0 %) were found on the bottom bund slope (BB), followed by 29.2% at the water side zone (TW) and 15.9% was at the dyke ditch (DB) zone (Figure 5.18). From January to April, the number of new mounds was high at water side zone (TW) and the number of new mounds increased again from August to November. The bottom zone also exhibited the same activity pattern with high number of new mounds from January to March and increased again from September to November (Figure 5.18).



Figure 5.18. Monthly distribution of newly colonized mud lobster mounds by zones from January to December 2006 (Site B1). A= full moon (spring tide) B= new moon (spring tide). For zones (x-axis): Road-RD, Bund ditch-DB, Bottom bund-BB, Top bund slope-TB, Landside bund top-TL and waterside bund top-TW. (see Figure 5.2 for detail of the zones).

5.3.4 Colonization of mud lobster mounds at Site C1

Site C1 recorded the lowest total numbers of new mud lobster mounds with 53 (n) mounds in an area of 100 x 25 m from January to December 2006 compared to site A1 and B1. Temporally, colonization of new mud lobster mound at site C1 did not show clear pattern compared to sites A1 and B1 (Figure 5.19). Month of April showed the highest number of new mounds (n=7) compared to other months for the site C1 (Figure 5.19).

The distribution of new mud lobster mound colonization on the dyke zones was found to be highest at the road zone (RD) (1.58 mounds/ month; n=19; 35.9%). This was followed by the bund ditch zone (DB) (1.41 mounds/ month; n=17; 32%). The other zones of BB, TW, TL and TB recorded of (9; 17%), (4; 7.5%), (3; 5.7%) and (1; 1.9%) respectively (Figure 5.20).

Monthly Distribution of new mud lobster mounds by zones did not show any clear pattern on thedyke. Most of the new mounds were found on the road zone (RD) and ditch of the dyke (DB) from month January to April 2006 (Figure 5.21).



Figure 5.19. Total number of new mud lobster mounds in newly repaired dyke (Site C1) over 12 months. A= full moon (spring tide) B= new moon (spring tide).



Figure 5.20. Distribution and abundance of new mud lobster mounds for each zones at Site C1 on the dyke.

Numerals above bars indicate % contribution of transect zones to mound numbers. For zones (x-axis): Road-RD, Bund ditch-DB, Bottom bund-BB, Top bund slope-TB, Landside bund top-TL and waterside bund top-TW. (see Figure 5.2 for detail of the zones).



Figure 5.21. Distribution of monthly new mud lobster mounds by zones from January to December 2006 for Site C1. A= full moon (spring tide) B= new moon (spring tide). For zones (x-axis): Road-RD, Bund ditch-DB, Bottom bund-BB, Top bund slope-TB, Landside bund top-TL and waterside bund top-TW. (see Figure 5.2 for detail of the zones).

From the total of new mud lobster mounds recorded at site C1 (n=53), it was found that 73.6% (n=39) were active mounds (A), followed by 18.9% (n=10) "Inactive" mounds (InA) and 7.5% (n=4) abandoned mounds (O). Monthly colonization and distribution of new active mud lobster mounds (A) by zones showed most of the active mounds were found on the ditch at the dyke zone (DB) (n=13) and road zone (RD) (n=13) with 33.3%. This was followed by 18.0% at the bottom bund slope (BB) (n=7), water side zone (TW) (n=3; 7.7%), top dyke toward landside zone (TL) (n=2; 5.1%) and top of the dyke (TB) (n=1; 2.6%) (see Figure 5.22). However, activities of the mud lobster mounds did not showed any consistent pattern.



Figure 5.22. Monthly distribution of newly colonized mud lobster mounds by zones from Jan – Dec 2006 (Site C1). A= full moon (spring tide) B= new moon (spring tide). For zones (x-axis): Road-RD, Bund ditch-DB, Bottom bund-BB, Top bund slope-TB, Landside bund top-TL and waterside bund top-TW. (see Figure 5.2 for detail of the zones).

5.3.5 Spatial and temporal variation of environmental parameters in/ near mud lobster mounds

5.3.5.1 Ditch water parameters

The mean ditch water pH at the dyke ranged from 4.1 to 5.3 in the entire three sites, with the most acidic water recorded at site C1 (Table 5.4). Kruskal-Wallis test showed that there was no significant differences (H=5.60, P>0.05) in water pH among the three sites (Table 5.4). The mean monthly ditch water pH shows general increase of the pH reading starting from the month of April 2006 to June-August when the pH reached the highest at around neutral. Ditch water pH then sharply dropped to the lowest (pH 2-3) in October or November at all three sites (Figure 5.23a).

The mean salinity of the ditch water ranged from 9.7 to 16.5 ppt (Table 5.4). Kruskal-Wallis test showed that salinity at site A1 (16.5 ± 7.2) was significantly (p<0.05) higher than site B1 (9.7±5.4) (Table 5.4). However, site A1 showed two similar high peaks in May and November and both recorded salinities fell after one month indicating the highly fluctuating salinity of the ditch water (Figure 5.23b).

Kruskal-Wallis test showed that the redox potential (mv) of the ditch water at site A1 (149.8 \pm 100.6) and site C1 (252.6 \pm 119.7) were significantly different (P<0.05) (Table 5.4). However, like salinity, the redox potential fluctuated greatly and the lowest values near to zero or below occurred from June to September (Figure 5.23c).

5.3.5.2 Soil parameters

The mean soil pH was acidic ranging from 4.2 to 5.4 (Table 5.4). Kruskal-Wallis test showed significant differences (P<0.05) in soil pH between site A1 (5.4 ± 1.2) and site C1 (4.2 ± 1.0). Mean monthly soil pH shows an almost similar pattern as the ditch water pH for all three sites (Figure 5.23d).

Mean temperature at all three sites similarly ranged from 28.7°C to 29.7°C. Soil temperatures among the three site were not significantly different (P>0.05; Table 5.4). Mean monthly temperatures did not show any clear temporal pattern (Figure 5.23e).

Mean sediment moisture (%) recorded the highest value at Site A1 with mean of $36.5\pm7.0\%$ and ranged from 25.4 to 47.0%. Site C1 recorded the lowest value with mean of $30.1\pm5.4\%$ (Table 5.4). Kruskal-Wallis test revealed that sediment moisture (%) at Site A1 was significantly higher (P<0.05) than Site C1 (Table 5.4). The temporal trend of the mean monthly sediment moisture (%) at all sites indicated that moisture content at the highest in February/ March gradually decreased to the lowest between May-July before it gradually increased to November (Figure 5.23f).

Highest organic content was recorded at Site A1 with mean of $16.8\pm2.1\%$ and ranged from 14.1 to 19.2%, while the lowest was recorded at Site B1 with mean of $14.3\pm1.9\%$. Site C1 recorded almost similar organic content as Site B1 with mean of $14.7\pm1.5\%$. Kruskal-Wallis test revealed that sediment organic content at Site A1 was significantly higher (P<0.001) than the other two sites (Table 5.4). The monthly mean sediment organic content (%) over the sampling period showed no clear temporal pattern (Figure 5.23g).

Table 5.4. Mean water parameters, standard deviations (SD) and summary of Kruskal-Wallis test results for difference among three different sites (A1, B1, C1) on the dykes of Carey Island. Similar superscript alphabets indicate homogenous groups tested at 5% significance level using Kruskal-Wallis test. A1= heavy infestation, B1= moderate infestation, C1 = low infestation.

Parameter		A1	B1	C1	p- level
Ditch water pH	Mean Sd Range n	5.3 ^a 1.4 2.8-7.3 72.0	4.6 ^a 1.5 2.5-6.7 72.0	4.1 ^a 1.1 2.7-6.2 71.0	0.0607
Ditch water Salinity (ppt)	Mean Sd Range n	16.5 ^a 7.2 4.0-26 72.0	9.7 ^b 5.4 0-23 72.0	12.4 ^{a,b} 5.1 4.0-22 71.0	<0.05
Ditch water redox potential (Mv)	Mean Sd Range n	149.8 ^a 100.6 387-(-51.1) 72.0	203.9 ^{a,b} 85.4 334-16.2 72.0	252.6 ^b 119.7 492-44.6 71.0	<0.05
Soil pH	Mean Sd Range n	5.4 ^a 1.2 2.9-7.3 72.0	5.1 ^a 1.2 3.5-7.0 72.0	4.2 ^b 1.0 2.6-6.1 71.0	<0.05
Soil temperature (°C)	Mean Sd Range n	29.7 ^a 2.12 27.1-33.8 72.0	29.0 ^a 1.2 27.3-31.8 72.0	28.7 ^a 0.8 27.5-30.7 71.0	0.4240
Sediment moisture (%)	Mean Sd Range n	36.5 ^a 7.0 25.4-47.0 32	32.7 ^{a,b} 7.1 21.2-44.8 30	30.1 ^b 5.4 19.2-39.7 20	<0.05
Organic matter (%)	Mean Sd Range n	16.8 ^a 2.1 14.1-19.2 36	14.3 ^b 1.9 10.8-17.5 36	14.7 ^b 1.5 12.8-18.3 36	<0.001









Figure 5.23. Monthly mean values of water parameters and sediment organic contents and soil moisture at three sampling sites in Carey Island. (* standard deviation omitted for clarity). A= full moon (spring tide) B= new moon (spring tide). A1= heavy infestation, B1= moderate infestation, C1 = low infestation.

5.3.5.3 Sediment particle size

Site A1

a) Clay

Clay showed highest mean (%) value at site A1 with mean of $32.3\pm8.5\%$, followed by site C1 with mean of $32.1\pm8.4\%$. Site B1 recorded the lowest mean value of $25.0\pm4.8\%$ (Table 5.5). Kruskal-Wallis test showed that composition of clay was significantly higher (P<0.01) at site A1 & C1 compared to site B1 (Table 5.5).

b) Fine silt

Site A1 recorded the highest mean of fine silt $(36.9\pm8.9\%)$ with the range 25.2 to 48.8 % compared to other site (Figure 5.12). Kruskal-Wallis test showed that composition of fine sand was significantly higher (P<0.01) at site A1 (36.9\pm8.9\%) and C1 (36.0\pm8.9\%) compared to site B1 (28.2\pm6.5\%) (P<0.01) (Table 5.5).

c) Coarse silt

Site B1 recorded the highest mean composition (%) of coarse silt with the value $(35.5\pm5.6\%)$, followed by the lowest at site A1 (23.1±15.4%) (Table 5.5). Kurskal-Wallis test showed the significant difference between site A1 with site B1 (p<0.05) (Table 5.5).

d) Very fine sand

Highest mean (%) of very fine sand recorded at site B1 with mean of $24.2\pm9.1\%$ and range between 0- 32.3% (Table 5.5). Kruskal-Wallis test revealed site A1 ($10.1\pm11.2\%$) and C1 ($11.5\pm13.8\%$) was significantly different with site B1 (Table 5.5).

e) Fine sand

Kruskal-Wallis test revealed that site B1 ($10.7\pm7.5\%$) was significantly different with site C1 ($2.0\pm3.3\%$) (P<0.001) (Table 5.4).

f) Medium sand

Site A1 recorded the highest mean of medium sand $(3.2\pm5.5\%)$, followed by site B1 $(2.4\pm4.1\%)$ and the lowest recorded at site C1 $(0.6\pm2.5\%)$ (Table 5.5). There was no significant difference of medium sand among site (P>0.01).

g) Coarse sand

Site A1 and B1 are the two sites which recorded the sediment content composition of coarse sand with the mean of $(0.2\pm0.7\%)$ and $(0.8\pm3.6\%)$ (Table 5.5).

h) Very coarse sand

Site B1 was the only site recorded with the sediment has very coarse sand with mean of $0.15\pm0.63\%$ (Table 5.5).

Soil composition (%)		A1	B1	C1	p- level
		2	h		
Clay	Mean	32.3ª	25.0 [°]	32.1ª	<0.01
	Sd	8.5	4.8	8.4	
	Range	20.7-43.9	19.3-37.1	21.8-44.1	
	n	36	36	36	
Fine silt	Mean	36.9 ^a	28.2 ^b	36.0 ^a	<0.01
	Sd	8.9	6.5	8.9	
	Range	25.2-48.8	21.7-46.8	24.0-45.7	
	n	36	36	36	
Coarso silt	Moon	23 1 ^a	35 5 ^b	20 1 ^{a,b}	<0.05
Coalse shi	Sd	23.1 15 <i>1</i>	5.5	12.6	<0.00
	Banga	25422	10 / /1 7	20429	
	Range	3.5-42.2	19.4-41.7	3.9-42.0	
	11	30	30	30	
Very fine sand	Mean	10.1 ^a	24.2 ^b	11.5 ^a	<0.001
-	Sd	11.2	9.1	13.8	
	Range	0-26.1	0-32.3	0-34.3	
	n	36	36	36	
Fine sand	Mean	7 2 ^{a,b}	10 7 ^b	2 0 ^a	<0.001
	Sd	8.9	75	2.0	
	Range	0.21 2	0-22 1	0-9 1	
	n	36	36	36	
		30	30	50	
Medium sand	Mean	3.2	2.4	0.6	0.0242
	Sd	5.5	4.1	2.5	
	Range	0-15.9	0-13.7	0-10.8	
	n	36	36	36	
Coarse sand	Mean	0.2	0.8	0	0.3675
	Sd	0.7	36	0	
	Range	0-2.6	0-15.5	0	
	n	36	36	36	
Voru opprog cond	Moon	0	0.15	0	0 3670
very coarse sand	NIEGII Sa	0	0.10	0	0.3073
	Su	0	0.03	0	
	Range	0	0-2.8	0	
	n	36	36	36	

Table 5.5. Mean soil composition (%), standard deviations (SD) and summary of Kruskal-Wallis test results between three different sites in the dykes of Carey Island. A1= heavy infestation, B1= moderate infestation, C1 = low infestation.

5.3.5.4 Mud lobster infestation in relation to sediment and water parameters

The biplot results from principal components analysis (PCA) show that the highly infested site (A1) contained mainly clayey-fine silt, medium sand substrate of high organic and moisture content, with interstitial water that recorded the highest salinity, pH and temperature as compared to other sites (Figure 5.24).



Figure 5.24. PCA biplot showing the water and soil characteristics of three habitat sites with different levels of mud lobster infestation. A1= heavy infestation, B1= moderate infestation, C1 = low infestation.

5.4 Discussion

The distribution pattern of *T. anomala* along the perimeter dyke of Carey Island was not random. The results from the distribution and abundance of *T. anomala* mounds along the entire dyke length (91.9 km) showed that 618 quadrats or 61.8 km of the dyke did not show any mud lobster mounds (Table 5.2). These results indicate that the infestation occurs only in areas that likely favor the animal's habitat requirements or physiological needs. Based on the number of the active mounds and on the assumption that one active mound is occupied by a single individual, the mud lobster population on the dyke (to about 25 m inland) was estimated to be 1233, or a mean population density of 3.34 ± 10.38 mud lobsters per 100 m of dyke length (or 2500 m²).

The mound distribution pattern shows that most of the mounds occurred at two major areas, one on the northern ear of the island at the lower estuary of the Langat River and the other, on the southern coast of the island at the Sungai Hitam estuary which is located next to the mangrove reserve (see Figure 5.4).

Most of the active mounds were found in the road zone (RD) and the bottom bund slope (BB); hence, these two zones as the most preferred area in the plantation beyond the dyke. It is likely that these two zones are the most preferred area since they are located next to the bund ditch where the sediment water content is high and the ground water table is just below (see Figure 5.2). Ng & Kang (1988) reported that *T. anomala* burrows lead down to the water table so that the animal can wet its gills.

The mean heights of mounds built on the TL (11.6 ± 5.70 cm) and TW (13.3 ± 7.05 cm) zones were the lowest on the top dyke slope compared to other zones (Figure 5.8). The result also showed that the mean mouth diameter of the burrow under the mound at the top bund slope between TL and TW zones were smaller than those on low ground between B to PL zones (Figure 5.10). Thus, this suggests that the mounds at TL and TW zones were occupied by smaller mud lobsters which arrived from the mangrove side of the bund and therefore, these two zones are the first place of invasion by the mud lobsters.

The colonization frequency and distribution of new mud lobster mounds on the newly repaired bunds was found to be highest at the bund top on the water side zone (TW) followed by the bottom bund slope (BB) (Figure 5.12 & 5.16). On the water side of the bund, there was no mud lobster mounds found below the mean high water neap level (see Figure 5.25). The results also show that there were relatively much fewer mounds in the intervening zones between TW and BB zones. Thus, it is hypothesized that water side (TW) zone presents the first line of invasion by mud lobsters which penetrate through the bund before invading the landward side of the bund, and subsequently staying at the bottom bund slope (BB) near to the bund ditch (Figure 5.25). This shows that the mud lobster prefers proximity to water.


Figure 5.25. Diagram shows the hypothesized path of invasion by mud lobsters from the mangrove/ estuary side.

The pH levels of the ditch water at the dyke indicated that the pH was highly acidic, ranging from 2.5-7.3 for the all three sampling sites (A1, B1, C1). The pH levels showed it start to decrease from July to November 2006 and the extremely low pH values were recorded in the month of October and November with the range of 2.5 to 3.5 pH (see Figure 5.23a). Andriesse et al. (1973) working in the mangrove swamps of Sarawak (East Malaysia) revealed that the mud lobster's burrowing activity brings up sulfide-rich subsoils that will develop extreme acidity upon aeration and oxidation at the surface. In addition, the carbon dioxide from respiration of the mud lobster and associated fauna inside the mounds, as well as decomposition of organic matter, could decrease the pH values in the soil (Sasekumar 1974; Ashton et. at., 2002).

Organic matter could be an important factor influencing the area of infestation by *T*. *anomala* on the dyke. According to Johnson (1961), Sankolli (1963) and Ng & Kang (1988), *T. anomala* is a mud feeder obtaining and seiving the organic matter in the mud. Therefore, *T. anomala* likely favours dykes with high organic matter for their nutrition. Moh et al. (2015) showed that the distribution of *T. anomala* on the high mangrove shore is correlated to high sediment organic matter. Organic matter such as from plankton and detritus is presumed to be brought into the dyke by the daily incoming tides via the mud lobster burrow. Nevertheless, the coastal bunds are regularly shored up and maintained by plantation soils every two years.

The distribution of *Thalassina* on the dyke shows that fine sediment of less than 250 um with high moisture is the preferred kind of substrate (Table 5.4 & 5.5). Webb (1958) reported finer sediment size hold more water than coarse grains and the water table tends to stay higher because of greater capillarity. High soil moisture content can either be due to frequent tidal inundation or freshwater input. Pillai (1989) reported *T. anomala* prefers fine-grained sediment in the mangroves of Laucala Island, Fiji.

Sankolli (1963) conducted experiments to test the tolerance of *T. anomala* to salinity by placing the animal separately in fresh water and sea water for 15 days, and found both lived well. Therefore he concluded *T. anomala* can tolerate wide changes in salinity. Moh et. al. (2006) reported that since *T. anomala* live on the high shore, it would need to be better adapted to tolerate lower salinity stress from episodic rainwater. In the present study, the salinity measured at all the three sampling sites fluctuated widely each month. Such fluctuations could be due to the weather of the day the measurements were taken, e.g. rainfall has the effect of diluting the saline ditch water at the dyke. According to

local people, mud lobsters could be seen emerging from their mounds during freshwater floods after they had fully covered (capped) their mounds for more than a day. Most of the mud lobsters would die after emerging from their mound. This strange behavior seems to indicate they cannot tolerate living in fresh water for long periods.

During high spring tide there appears to be a higher number of invasions or recruitment of young mud lobsters through the dyke, from the mangrove area to the plantation, at full moon compared to new moon. Nonetheless, the burrowing activities of *Thalassina* on the dyke are very much dependent on tidal fluctuations or height of the tide, rainfall and the frequency of wettings on the dyke. All these factors have the effect of wetting and softening the dyke soil which make it easier for the animal to dig and feed on the mud.

Higher saline water from the estuary can flow into the dyke ditch either via the mud lobster burrow during high spring tide or through the tidal gate if opened. However the tidal gate is only opened during heavy downpour or to drain excessive rain water from the plantation. It had been observed that large amount of the saline water from the estuary flowed through the dyke from TW to BB zone inside the dyke via mud lobster burrows during high spring tide. It is also hypothesized that the mud lobster also uses the same burrow to move out of the plantation from the BB (or DB) zone to the TW zone during the breeding season when they release their mature eggs into the water during high spring tides. *Thalassina anomala* came to the surface during nocturnal high tides to disperse the freshly hatched larvae. Dispersal is presumably facilitated by vigorous flexion of the abdomen (Pillai, 1982). According to Pillai 1985 there is a spawining peak between September and November which is 14 berried females been trapped for the larval lide

studies. Thus, the result also shows more activities of the mud lobster, where both sites A1 and B1 clearly showed two frequency peaks of active mounds (A) at zone BB and zone TW during spring tide. From the mud lobster catches during March to May and September to November at the TW zone (or dyke slope facing mangrove area, it was found that more berried females occurred during these periods as compared to the other months. Berried females were more often observed during spring tides when strong tides are needed to export their larvae off shore (Moh et. al., 2015). The results indicate that *T. anomala* living inside the bund has two breeding periods in a year as attested by two major recruitment periods (Fig. 5.13 and 5.17). Nevertheless, further studies are required, particularly the occurrence of larvae of mud lobsters in the estuaries and off shore waters needs to be substantiated in order to confirm the spawning season of *Thalassina* in the study area.

Chapter 6

Control of mud lobters (Thalassina anomala) infestations on coastal dykes

6.1 Introduction

The most common species of mud lobster, *Thalassina anomala* is distributed widely across the Indo-West Pacific region, and are found wherever there are mangroves up to the extreme high water mark several kilometers inland from the water margin (Macnae, 1968). The mud lobster burrows down to a depth of 1.5 meter. The dug sediment is moved up to the surface resulting in mounds that often reach up to 2 meter height. Their excavating activities contribute to recycling of material from deep in the mud, loosening the mud and allowing air and oxygenated water to penetrate the otherwise oxygen-poor sediment (Ng & Sivasothi, 1999).

Thalassina anomala is however destructive to man-made coastal structures, as for example in reclaimed mangrove land. Sankolli (1963) noted that the animal is notorious for causing severe damage to coastal bunds by its burrowing habits. The bunds of paddy fields and aquaculture ponds located in coastal lands are also subject to this sort of damage by *Thalassina* mud lobsters, often interfering with paddy cultivation and aquaculture and thus have been regarded as pests in India, Bangladesh and Southeast Asian countries (Pillai, 1985; Macintosh, 1988; Holthuis, 1991). In the early 1950s, when much of the coastal mangrove forests were reclaimed for agriculture in Malaysia, Ferguson (1951) reported that this creature wrecked damage to coastal dykes incurring high costs of repair and maintenance.

Carey Island located on the west coast of peninsular Malaysia (Figure 5.1; Chapter 5) was formerly mangrove forests which had been cleared felled for agricultural development in the early 1900s, and earthen dykes were constructed around the entire island to protect it (plantation) from seawater intrusion. These dykes have since existed and been maintained by the plantation owners. Interestingly, the plantation practices began with the planting of rubber followed by coconut, tea, coffee and finally to oil palm in 1955, as a result of successive crop failures and changing market demand. Coastal dykes not only block the intrusion of seawater during high tide, but also cut off the natural ingression of marine animals into the reclaimed land. However, these coastal dykes do not deter T. anomala from searching their preferred natural habitat on the upper mangrove forest including dykes where the substrate is suitable. It has been estimated that dyke damage and maintenance in Carey Island incurs an annual cost of between RM1.5 to 2million. An estimated average cost of about RM15, 000.00 per kilometer is spent on maintenance and repair each year (Mr. Gunasekaran Uthiradam, Senior Manager for East estate at Carey Island from Sime Darby plantation, personal communication).

Thalassina anomala has also been reported to build burrows in the bunds of coastal prawn ponds weakening them, causing leakage and eventual collapse if the bunds are not maintained and repaired (Tham, 1967). Fish farmers eliminate mud lobsters by dropping into their burrows a small piece of calcium carbide which reacts with water releasing toxic acetylene gas which kills them, or by pouring quicklime (calcium oxide) into the burrows to generate heat that will kill on contact the moist animal (Tham, 1967). More toxic chemicals used include DDT mixed with kerosene, Sevin and carbofuran employed to kill mud lobsters. Nonetheless, chemical control provides only a temporary solution as their numbers soon build up after a few months. There is thus a need to find a long-term solution

to the problem, and develop biological and safe methods to control mud lobster infestations in man-made coastal dykes.

Initial observations in the distribution study of mud lobster mounds on the dykes in Carey Island showed that areas that were heavily covered with grass had much less mud lobster mounds. It became apparent that the thick covers of suitable grass species with closely-set stolons or deep fibrous roots could obstruct the tunneling activities of the mud lobster. Thus, the main objective of this study was to find environment-friendly ways to control the colonization of these burrowing animals on coastal dykes. The hypothesis tested was that suitable small vegetation such as grasses hinder mud lobster infestations. Based on the earlier findings regarding mud lobster tunneling behavior on encountering a coastal dyke (Chapter 5 on distribution), grass planting of suitable species on it may help to control mud lobster invasions. The idea is to plant a grass species with long vertical roots on the outer bund slope to prevent the first entry of the animal (point A to B), and also to plant another species of grass with net-like stolons to prevent the aerial emergence of the animal when they pile up the dug soil (Figure 6.1).

6.2 Materials and methods

6.2.1 **Study area**

The study sites on Carey Island were the coastal dyke located on the northern branch of the Langat River, Selangor. The selected areas were among the most infested areas by mud lobsters on Carey Island (Chapter 5).

The experimental design used was a completely randomized design of 6 treatments of vegetation, each with three replicates. Three different species of grass and their combinations constituted the treatments including a control or no vegetation for the plot (Table 6.1). Eighteen plots of 5m x 6 m size were prepared for an experimental design that comprised of treatments (with grass) and controls (without grass). All existing vegetation was completed removed from the bund before the experiment.



Figure 6.1 Schematic diagrams of study zones and planting method of the vegetation across the dyke to prevent mud lobster intrusion.

*Diagram drawn not to scale.

6.2.2 Vegetation planting

Three grass species with different root and stem characteristics were selected for the experiment. These were *Chrysopogon zizanioides* (L.) Roberty, *Cynodon dactylon* (L.) Pers. and *Paspalum distichium* (L.). *Chrysopogon zizanioides* commonly know as Vetiver grass is an introduced grass species that forms deep growing roots and commonly used in highway slope protection. *Cynodon dactylon* and *P. distichium* are naturally-occurring grass species on the bund which form horizontal and thick ramifying stolons.

A single row of Vetiver (*Ch. zizanioides*) grass were planted at the edge of the water side zone (TW) on top of the dyke with distances of 25 cm apart for the *Ch. zizanioides* alone, *Ch. Zizanioides* + *Cy. dactylon* and *Ch. zizanioides* + *P. distichium* treatment plot. Slope or BB zone plants were planted "staggered" from bottom to top, with distances of 60 cm between plants (up to approximately 3 meters) on the dyke slope. *Cy. dactylon* and *P. distichium* grass were planted among the *Ch. zizanioides* grass clumps (see Figure 6.1). Every week, the control plots were removed of all vegetation that grew on the plot.

The percentage coverage of the growing grass was recorded using quadrats (plots with grid of $1m^2$) and eye estimation method (Cox, 1990) once every month, from September 2006 to December 2007. Each month, new mounds of mud lobster were enumerated and recorded. The position of each new mound was located using GPS before the mound was marked with a wooden peg driven into the ground.

Plot	Treatment
Plot 1	Chrysopogon zizanioides (L.) Roberty + Paspalum distichium (L.)
Plot 2	Chrysopogon zizanioides (L.) Roberty + Paspalum distichium (L.)
Plot 3	Chrysopogon zizanioides (L.) Roberty + Cynodon dactylon (L.)
Plot 4	Control
Plot 5	Chrysopogon zizanioides (L.) Roberty + Paspalum distichium (L.)
Plot 6	Cynodon dactylon (L.)
Plot 7	Chrysopogon zizanioides (L.) Roberty
Plot 8	<i>Chrysopogon zizanioides</i> (L.) Roberty + <i>Cynodon dactylon</i> (L.)
Plot 9	Cynodon dactylon (L.)
Plot 10	Chrysopogon zizanioides (L.) Roberty
Plot 11	Paspalum distichium (L.)
Plot 12	Paspalum distichium (L.)
Plot 13	Chrysopogon zizanioides (L.) Roberty + Cynodon dactylon (L.)
Plot 14	Control
Plot 15	Chrysopogon zizanioides (L.) Roberty
Plot 16	Control
Plot 17	Cynodon dactylon (L.)
Plot 18	Paspalum distichium (L.)

Table 6.1. The random assignment of vegetation species and control plots on the dyke.

6.2.3 Statistical analysis

One-way ANOVA of cumulated no. of new mounds was used to determine any significant differences among the five different treatments (and control) at the 8th and 16th month from the start of the experiment. If the ANOVA data showed significant results, significant difference among means was further post-tested by Tukey test. Dunnett test was further used to investigate if there was any significant difference in the grass treatments against the control group. All statistical analyses were performed using the software Statistica Version 10.

6.3.1 Effectiveness of vegetation covers study to control the mud lobster population on the dyke.

The treatment with *Ch. zizanioides* alone gave the lowest mean cumulated new mound of 1.00 ± 1.00 and 2.67 ± 0.58 new mound per plot at the 8th month and 16th month of experiment, respectively. Treatment with *Ch. zizanioides* alone also showed the slowest growth in mean vegetation cover after 8 month period (66.7%) and reached 100% cover after 9 month period (Table 6.2). Mean cumulative new mounds in plots of *Ch. zizanioides* alone showed significant difference with plots planted with *Cy. dactylon*, *Ch. zizanioides* + *Cy. dactylon* and control treatment at end of 16 months (Table 6.2). With *Ch. zizanioides* alone treatment, no new mud lobster mounds were built after 12 months of the experiment (Figure 6.2).

Cynodon dactylon alone treatment gave a mean cumulative new mound of 4.33 ± 2.08 and 6.00 ± 1.00 mounds per plot after 8 month and 16 month, respectively. *Cynodon dactylon* alone had a grass cover of 78.33 % after 8 months and over 100% after 9 months. *Cynodon dactylon* alone treatment showed there were new mounds enumerated even after the grass cover achieved 100 % (Figure 6.3). Mean cumulative new mound of *Cy. dactylon* alone showed significant difference with *Ch. zizanioides*, *Ch. zizanioides* + *Cy. dactylon* and control treatment after 16 months (Table 6.2).

Paspalum distichium alone treatment showed mean cumulative new mounds of 3.33 ± 0.58 and 5.67 ± 1.53 mounds per plot for 8 months and 16 months of the experiment. *Paspalum distichium* alone treatment showed there were new mud lobster mounds enumerated in the plot even with a mean cover of 100% after 9 months (Figure 6.4). Mean cumulative new mounds for *P. distichium* alone only showed significant difference with control treatment after 16 months (Table 6.2).

The plots given *Chrysopogon zizanioides* + *Paspalum distichium* treatment gave a mean cumulative new mound of 4.00 ± 0.00 and 6.67 ± 2.08 mound per plot at the 8th and 16th month, respectively. *Chrysopogon zizanioides* + *Paspalum distichium* treatment also showed there were new mud lobster mounds that emerged from the plot even with a cover of 100% at month 10 (Figure 6.5). Mean cumulative new mound of *Ch. zizanioides* + *P. distichium* treatment showed significant difference with *Ch. zizanioides*, *Ch. zizanioides* + *Cy. dactylon* and control treatment at 16th month (Table 6.2).

Chrysopogon zizanioides + *Cynodon dactylon* treatment gave 2.67 ± 0.58 mound per plot at the 16th month. The *Chrysopogon zizanioides* + *Cynodon dactylon* treatment had the fastest vegetation growth reaching 100 % grass cover on the plot the 8th month and there was no more new mud lobster entry after 9 months (Figure 6.6). Mean cumulative new mound of *Ch. zizanioides* + *Cy. dactylon* showed significant difference with *Cy. dactylon, Ch. zizanioides* + *P. distichium* and control treatment the 16th month (Table 6.2).

The control group showed gave the highest infestation rate at 11.33 ± 0.58 mounds per plot at the 16th month. Figure 6.7 showed that the number of the new mounds increased at the control plot after 7th months (when all other treatment plots reached 100% of grass cover). The number of new mounds deceased reaching a peak at 13th month. Mean cumulative new mound of control showed significant difference with all other treatment at 16th month (Table 6.2). However, mean cumulative new mounds for all the treatments including control showed no significant difference among each other at month 8 (Table 6.2).

Table 6.2: Comparison total number of accumulative mounds per 3 plots, mean no. accumulative mounds per plot $(90m^2)$ and mean percentage cover (%) for five different treatments of grass and control plot over 8 months and last month (16 months).

		No. of	cumulative	Mean no.	of cumulative	Mean % to	tal cover of
Plots	Treatments	mounds a	fter	mounds per plot after		vegetation after	
							Cover >
		8 months	16 months	8 months	16 months	8 months	100%
7, 10 and 15	Chrysopogon zizanioides	3	8	1.00 ± 1.00^{a}	2.67 ± 0.58^{a}	66.67 ± 5.78	9 months
6, 9 and 17	Cynodon dactylon	13	18	4.33 ± 2.08^{a}	$6.00 \pm 1.00^{\rm b}$	78.33±10.4	9 months
11, 12 and 18	Paspalum distichium	10	17	3.33 ± 0.58^{a}	$5.67 \pm 1.53^{a,b}$	77.66 ± 6.43	9 months
1, 2 and 5	Chrysopogon zizanioides + Paspalum distichium	12	20	$4.00{\pm}~0.00^{\rm a}$	6.67 ± 2.08^{b}	73.00± 5.00	9 months
3, 8 and 13	Chrysopogon zizanioides + Cynodon dactylon	6	8	2.00 ± 1.00^{a}	$2.67{\pm}0.58^a$	86.67±7.64	8 months
4, 14 and 16	Control	8	34	2.67 ± 1.15^{a}	$11.33 \pm 0.58^{\circ}$	/	/

Homogenous groups indicating no significant difference (P>0.05) by Tukey's test are shown by similar superscript alphabets.



Figure 6.2. Total number of the new mud lobster mounds and percentage cover (%) of *Chrysopogon zizanioides* on experiment plots.



Figure 6.3. Total number of the new mud lobster mounds and percentage cover (%) of *Cynodon dactylon* on experiment plots.



Figure 6.4. Total number of the new mud lobster mounds and percentage cover (%) of *Paspalum distichium* on experiment plots.



Figure 6.5. Total number of the new mud lobster mounds and percentage cover (%) of *Chrysopogon zizanioides* + *Paspalum distichium* on experiment plots.



Figure 6.6. Total number of the new mud lobster mounds and percentage cover (%) of *Chrysopogon zizanioides* + *Cynodon dactylon* on experiment plots.



Figure 6.7. Total number of the new mud lobster mounds of control on experiment plots (no vegetation).



Figure 6.8. Combination treatments on cumulative mounds at month 16, where all vegetation growth achieved 100%.cover.

One way ANOVA on cumulative mounds showed *Ch. zizanioides* alone *and Ch. zizanioides* + *Cy. dactylon* treatment was the most significant different (P < 0.05) compare with control treatment than *Cy. dactylon*, *P. distichium*, *Ch. zizanioides* + *P.* distichium. This result showed that both *Ch. zizanioides* alone and *Ch. zizanioides* + *Cy. dactylon* treatments are the best preventive measures against mud lobster infestation at lowest rate on cumulative new mounds at 16 month (Fig. 6.8). Although both *Cy. dactylon* and *P. distichium* treatments was significantly (P < 0.05) different than control treatments, however, both treatments are not the best protection to lower the infestation rates of the mud lobster (Figure 6.8 and Table 6.3).

Table 6.3. Dunnett test for Post Hoc Tests for new cumulative mounds for five different treatments for the last month (16 months). *Red value indicate significant different at P<0.05

	Dunnett test; variable NewCum (LastMonth in vegcontro2fil Probabilities for Post Hoc Tests (M <control) Error: Between MS = 1.4444, df = 12.000 Include condition: Ref =16</control) 				
	Grass	{1}			
Cell No.		11.333			
1	Con				
2	Cyn	0.00034			
3	Pas	0.00021			
4	Vet	0.00002	\mathbf{A}		
5	Vet + Cyn	0.00002			
6	Vet + Pas	0.00099			

From the result the grass treatment against control showed planting of vegetation method can reduce the mud lobster mound on the dyke (Figure 6.3).

6.4 Discussion

Cynodon dactylon grows by forming extensive ramifying horizontal stolons that form a closely-knit cover. This grass appears to discourage mound building by mud lobsters by preventing them from emerging (horizontal obstruction). The burrowing ability of fiddler crab to create burrows and the distribution and abundance *Uca pugnax* correlated with root mats density (Ringold, 1979). On the hand, the deep growing roots of *Chrysopogon zizanioides* form an effective subterranean net curtain against landward invasions (vertical obstruction) of the mud lobster. The fibrous roots of *Ch. zizanioides* grass could reach down to 3 meter depth (Erskine, 1992; Truong, 1999; Hellin and Haigh, 2002), to obstruct the tunneling movement of the mud lobster through the dyke as it migrates from the mangrove to the plantation. Wang et. al, 2015, reported old *Spartina alterniflora* stands have higher root biomass peaked at soil depth of 10-30 cm and prevent crabs burrow through the thick root layer. Wang et. al, 2015 also indicated that fine roots impacted on crab burrow dimensions and depth, while rhizomes influenced burrow branch number and curved degree. The root system of *Ch. zizanioides* forms a thick underground curtain of tightly bound soil particles and roots that penetrate deeply and expand radially to at least 3 meter (Grimshow, 1994; Chomchalow, 1998). Bertness and Miller (1984) suggest that medium root density provides structural support for crab burrow and heavy root density accumulation limits crab burrowing. Highly dense fine roots of *S. alterniflora* limited crab burrow dimension (Ringold, 1979; Bertness and Miller, 1984). High pneumatophore density limit burrow construction, crab movement, and food harvesting, particulary in the case of large sesarmid crabs; high root density also limits crab density (Van Nedervelde et al., 2015). In salt marshes, vegetation cover influences the morphology and complexity of the burrows of grapsid crabs since roots and other plant matter in the sediment can interfere with burrow construction (Katrak et. al., 2008; Brook et al., 2009).

The combination of the two different grass covers of *Ch. zizanioides* and *Cy. dactylon* appear to best control the mud lobster infestation on the dyke. The *Ch. zizanioides* and *Cy. dactylon* combination retarded new invasions by mud lobsters after 8 months of grass planting which achieved a total grass cover growth of 100%. The grass planting programme of *Cy. dactylon* and *Ch. zizanioides* achieved 100% coverage in 8 months which was also the shortest period among the five treatments (Table 6.2). The combination of *Cy. dactylon* and *Ch. zizanioides* showed no new mud lobster mound entry to the dyke after the cover of the grass reached 100 % after 9 month period. The *Ch. zizanioides* and *Cy. dactylon* combination showing no new mud lobster mound emerged after 9 month. While, *Ch. zizanioides* alone does not stop the new entry of the new mud lobster mound after the cover of the grass reached 100% (9 month) and it only stop the new entry of mud lobster after 12 month period. The

combination of *Chrysopogon zizanioides* + *Paspalum distichium* do not showed the stop of the new entry of the mud lobster mound until the end of the experiment. This could due to the *Paspalum distichium* has shallow rhizomes compared to *Cy. dactylon* which forming a dense mat and deep root system. *Cynodon dactylon* or called Bermuda grass is a creeping grass and root where ever a node touched the ground. It has a deep root system and in the drought or dry condition the root system can grow up to 120-150 cm deep. Most of the root mass of *Cy. dactylon* lies 60 cm under the surface. *Cynodon dactylon* rhizomes are strong, stout, branched and spread rapidly. *Paspalum distichium* or commonly known as knotgrass are the dense mat of stolons grass that has shallow rhizomes and creeping, extensively branched stolons with adventitious roots at nodes (Holm et al., 1997).

The field experiment showed that *Ch. zizanioides* alone is just as suitable for controlling the mud lobster dyke infestation in the long-term (12 month) (Figure 6.2). This result is interesting because at 8 months of grass growth, the *Ch. zizanioides* grass alone was not as effective as the grass combined with *Cy. dactylon*, suggesting that although total grass cover appears to be an important factor in mud lobster control, the 'vertical element' as provided by *Ch. zizanioides* is the vital key factor in the control of the animal. This is because full vegetation cover achieved in 9 months by the shortrooted *Cy. dactylon* and *P. distichium* grasses were not as effective as *Ch. zizanioides* alone. Capehart & Hackney (1989) reporting that dense roots of *S. alterniflora* effectively limit *P. carolzniana* burrowing activities. The overall results clearly show that grass vegetation cover provides good control on mound construction by mud lobsters.

Chrysopogon zizanioides grass is thus the best of all treatment grasses. It is a natural, low cost, and eco-friendly control method to reduce mud lobster invasion of coastal mud dykes. The grass is tolerant to extreme climatic variations, highly adaptable to a wide range of soil types (pH 3.0-10.5) as well as tolerant to acidic and saline conditions (Truong & Baker 1998; Truong 1994; Truong et al., 2003), giving it an advantage over other grasses against mud lobster invasions in coastal dykes including aquaculture pond dykes that are often plagued by mud lobster damage. However, in the case of Carey Island, there is the problem slow-subsidence of mud dykes which requires topping of mud dykes to maintain their height so as to prevent tidal ingressions.

Chapter 7

General Discussion and Conclusion

7.1 Taxonomic status of *Thalassina*: an update of extant species

The family Thalassinidae Latreille, 1831, contains one genus *Thalassina* Latreille, 1806, of decapod crustaceans commonly called mud lobsters. They are widely distributed across the Indo-West Pacific region. Recently, the taxonomic status of the *Thalassina* species has undergone much changes and review. From a status that is first considered monotypic (Glaessner, 1969), the recent discoveries and review suggest a total of nine extant species based on morphology (Table 7.1).

No.	Species name	Species status		
		Morphology	Validated by molecular data	
1.	Thalassina anomala (Herbst, 1804)	Yes	Yes	
2.	Thalassina squamifera De Man, 1915	Yes	Yes	
3.	<i>Thalassina gracilis</i> Dana,1852 [sensu Ngoc-Ho & de Saint Laurent, 2009]	Yes	Yes	
4.	Thalassina kelanang Moh & Chong, 2009	Yes	Yes	
5.	<i>Thalassina krempfi</i> Ngoc-Ho & de Saint Laurent, 2009	Yes	No	
6.	* <i>Thalassina spinirostris</i> Ngoc-Ho & de Saint Laurent, 2009	Yes	No	
7.	<i>Thalassina spinosa</i> Ngoc-Ho & de Saint Laurent, 2009	Yes	No	
8.	Thalassina australensis Sakai & Türkay, 2012	Yes	No	
9.	Thalassina saetichelis Sakai & Türkay, 2012	Yes	No	

Table 7.1. The current extant species of *Thalassina* based on morphology with or without molecular validation.

* Molecular data show this as *species inquirenda*, likely a morphotype of *T. gracilis* (Moh, unpublished)

The present study has revalidated four species of Thalassina using both molecular and morphological characters; these are T. anomala, T. gracilis, T. squamifera and T. kelanang (see Moh & Chong, 2013). The molecular approach is considered a useful tool to validate species distinctness and to verify the phylogenetic relationships between the species of the Thalassina. From the collections of the samples of Thalassina from the Malaysian mangrove area, we found a new species of Thalassina from Malaysia; T. kelanang and one new record, T. gracilis. We also collected 'another species' of Thalassina (Thalassina spinirostris) from the same area as T. gracilis on Carey Island (Site 3) (see Chapter 4). Thalassina spinirostris samples was considered a new species by Ngoc-Ho & de Saint Laurent, (2009) based on two paratypes; 1 female (MNHN Th 1521) and 1 male (NHML 2008.3111), both of which were specimens from Malaysia. They further contended that the species is found only in Singapore and Malaysia. However, molecular analysis of the T. spinirostris showed that it is very likely to be T. gracilis (Moh, unpublished). This species has also been reported to be very similar to T. gracilis in many morphological features by Ngoc-Ho & de Saint Laurent (2009). Thus, based on morphology, T. gracilis is likely to possess two morphotypes; one that possess 1-2 spines at lateral border while the other has 5-6 tubercles at lateral border (as in T. gracilis). The presence of morphotypes in Thalassina makes it imperative that molecular analysis must be carried for the remaining species of *Thalassina* (*T. spinosa*, *T. australensis* and *T. saetichelis*) in order to verify their validity as species (Table 7.1). Hence, we contend that there are currently eight species of Thalassina but four species need to be confirmed by molecular evidence.

7.2 Colonization strategies and adaptation of *T. anomala* on the dyke

This study shows that only T. anomala was found on the man-made dyke and is the sole cause of dyke damage in Malaysia. This is due to the higher tolerance of T. anomala to dry condition where they are found on the high shore of the mangrove area. T. anomala occurs on the high intertidal shore close to the landward side of the mangrove shore where tides inundate the ground for 111-184 days of the time in a year. Thus, it is also possible T. anomala is less tolerant of immersion in water. This is supported by observations made on *T. anomala* which are not common in the mangrove area that is subjected to daily tidal inundation. From the study, T. anomala prefers sediment with high organic content and constructs deeper but less complex burrows to obtain enough nutrients from dyke sediment (Chapter 4) which comprised of mainly clayey-fine silt and medium sand substrate of high organic and moisture content. Here, the interstitial water also recorded the highest salinity, pH and temperature as compared to other sites lower down the shore. The digging frequency of the T. anomala on the dyke is dependent on rainfall, tidal fluctuation, and frequency of tidal wettings. When spring tidal frequency of wetting increases and the top soil becomes soft, animal activity increases. The active mound which indicates tunneling activity below is capped with wet or soft soil. Basically, all these factors (rainfall, tidal wetting) have the effect of softening the mud or soil in the dyke which make it much easier for the *T. anomala* to dig deeper into the dyke or high shore area.

The present study shows that the first invasion of mud lobsters begins at the bund top nearest to the river bank at the high spring tide, and proceeded diagonally downwards to the opposite side of the bund on the plantation side near the bund ditch (Chapter 5), and then spread further landwards. It is to be assumed that mounds are

constructed near to the water table since water moisture is important to the animal. However on the bund top, mounds are constructed on the river side (TW) since incoming tides continually wet the bund slope. Indeed, the bund top on the river side (TW) harbours the most number of mounds, albeit generally smaller ones. This indicates the smaller individuals of mud lobsters occurred at the bund, while the larger individuals occurred more landwards. It appears that the smaller individuals of mud lobster enter the bund during spring tide only, and the reason is to avoid the flooding of the dug tunnel. It is unlikely that the invasion began below the bund top on the river side, i.e. during neap tide, since the tunnels will be filled with seawater during spring tides. The number of active mounds was relatively higher during spring tides compared to neap tides, suggesting more active feeding during the spring tide. This is the important strategy shown by T. anomala living at the man-made bund. The ontogenetic shift or spatial distribution of T. anomala population in the man-made environment likely mirrors what happens in its natural habitat (mangrove).

7.3 Control of *Thalassina anomala* on the dyke

Based on our findings of the entrance and passage route of the mud lobster tunnel on the coastal dyke, we were able to suggest an effective control measure for mud lobster infestations without the use of toxic chemicals. Overall, the study has shown that the combination of *Chrysopogon zizanioides* (Vetiver) and *Cynodon dactylon* was the most effective way to reduce the mud lobster mounds on the manmade earthern dyke. This control method uses both the technique of vertical barrier (long plant roots) and horizontal obstruction (closely set horizontal stolons), respectively. The physical structures of vegetation have been shown to be important hindrances to animals that inhabit burrows. For instance, the dense root mat of Australian mangroves is particularly impenetrable to crabs (Hutchings & Saenger, 1987). Based on the success of the present study, we have proposed a grass planting programme at minimal cost. It is recommended that this grass planting procedure be implemented at the same time the dykes are repaired and maintained. In this way, the entire dyke perimeter will eventually be protected by selected grass cover against mud lobster invasions.

7.3.1 Planting method of the vegetation on the dyke

Vetiver grass should be planted in rows, one row on the top of the bund, and several rows (3-5) on the side facing the plantation up to the mid-height (approximately 0.3m) of the bund (Chapter 5, Fig. 5.2). The top bund plants should be planted 25cm apart along the edge of the bund. The slope rows should be planted "staggered" with distances of 60 cm between plants. Cynodon grass should be planted among the Vetiver grass clumps at the slope bund. When planted in single row, Vetiver grass will form a hedge which can form extremely deep (3-4m) and massively thick root system underground (Truong et al., 2008). Studies have showed that it is very difficult for crabs to cut dense and flexible roots belowground when burrowing (Ringlod, 1979; Bertness & Miller, 1984). Burrow dimension and crab burrowing performance are acutely reduced by dense roots (Lim & Heng, 2007; Wang et al., 2015). From the planting experiment, the combination of Cy. dactylon and Ch. zizanioides showed that the cover of the grass on the surface reached 100 % just after 9 months from planting. From the survey of the rooting depth after one year of the experiment, the root length of Ch. zizanioides could reach 1.0 meter while roots mat of single plant Cy. dactylon could reached to four square feet on the dyke of Carey Island.

7.4 Limitations and future work

In this study, the behavior of the mud lobster in making inter- connected burrows that give rise to several mounds on the surface, gives rise to the problem of making a good estimate of the mud lobster population. The abundance of the mud lobster population on the dyke was estimated from the number of active mound i.e. those covered by wet mud which was assumed to indicate recent occupancy by the mud lobster. It was also assumed that one wet mound is equivalent to a single occupant. Trial diggings of the mound and underground tunnels showed this to be true most of the time, except when the mud lobsters paired during the breeding season. However, it is not known whether one individual could dump wet soil onto two mounds on the same night. Future work should attempt to accurately estimate the population by using digging out mud lobsters from adjacent wet mounds. Such information would enhance the accuracy of the density and population estimation of adult mud lobster on the dyke and plantation.

Sankolli (1963) reported that mud lobsters emerge outside their burrows only during moonlit night, to unload mud and presumably also to search for mates. According to Pillai (1982), the mud lobster probably comes to the surface during nocturnal high tides to disperse the freshly hatched larvae. Based on these reports and on the results of the present study, it is hypothesized that the adult *T. anomala* mud lobster is less tolerant of immersion, preferring the drier conditions as found on the upper intertidal shore, while the larval stages are wholly marine and gradually move landwards with ontogenetic development. Future work should be planned to explain some of the interesting observations made in the present study, for instance, are the two peaks of abundance (Figure 5.15 related to the peak recruitment of juveniles from the adjacent mangrove – thus suggesting two spawning peaks? Also, how do adult mud lobsters in

the plantation close their life cycle, and do they migrate back to the river via their entry burrows? We can speculate that *T. anomala* that stays near the bund migrates back to river via their entry burrow since sea water was observed to flow in and out of their burrows during extreme high spring tides. While, mud lobsters that inside further in the plantation could access the river via the plantation canals and through the tidal gates that control the discharge of freshwater from the plantation. Reproduction and larval studies of the mud lobster could provide further evidence to answer and support the current results and observations. For instance, it would be informative to find out how the mud lobsters living inside the plantation breed and disperse their larvae.

The food habits of *T. anomala* in the mangrove habitat had been studied by Johnson (1961) who found that they feed on organic matter in the mud. However, it is not known whether the food source for plantation-dwelling mud lobsters is derived from the plantation soil itself although it is rich in organic matter (Chapter 5), or they depend on flood tide entry of seawater into the tunnels that bring in plankton and detritus. If indeed they depend on the latter, it appears that mud lobsters may feed only intermittently since food is only replenished during spring tide which floods the initial tunnel entrance at the dyke. There are still many unanswered questions which need further studies on this enigmatic creature.

7.5 Conclusions

The present study is the first detailed taxonomic and ecological study of the mud lobster *Thalassina* on Malaysian mangrove shores. A total of three species of *Thalassina* are found on the mangrove shore of Kelanang and Carey Island, namely, *T. anomala*, *T. gracilis* and *T. kelanang*. The present study has not only contributed to the

existing taxonomic knowledge on the three *Thalassina* species including the Australian species, T. squamifera based on morphological traits, but also to the molecular genetics and phylogenetic relationships of the four species of *Thalassina*. On the mangrove shore, spatial distribution and partition of the three *Thalassina* species is strongly driven by environmental factors such as tidal inundation, salinity and substrate characteristics. T. anomala occurs at the high intertidal near to landward side of the mangrove shore; T. kelanang inhabits the middle to lower mangrove shore and is more tolerant to tidal inundation; and T. gracilis also occurs at the mid-tidal zone sharing a similar microhabitat as T. kelanang in term of tidal inundation and frequency. However, T. gracilis is observed at the upper estuary and never found together with T. kelanang at the lower estuary or at the sea shore. The greater tolerance of T. anomala to the harsher and drier conditions in the upper intertidal shore, explains why it is the only species found inside coastal dykes where they caused dyke damage and seawater intrusion into coastal plantations. T. anomala invasion into dyke began at the dyke top on the mangrove side before settling down at the dyke bottom on the plantation side where there is a dyke ditch. Most of the mounds (73%) were found on either side of the dyke ditch, but mounds could be found as far as 10m into the plantation area from the dyke. Fresh mounds occur throughout the year but appear to occur in two larger peaks (January- February and August- November). The elucidated mud lobster's tunneling route across the dyke and its habit of discarding dug soil to the surface make it possible to experimentally obstruct its movements by planting suitable vegetation. The combination of Chrysopogon zizanioides (Vetiver) and Cynodon dactylon grasses is the most effective method to control and reduce the invasion of mud lobster (*T. anomala*); the first grass with its long vertical roots and the second grass with its dense mat of surface stolons. This study thus contributes to new knowledge and an ecologicallyfriendly method to reduce mud lobster invasions through the man-made dyke.

REFERENCES

Akaike, H. (1973). Information theory and an extension of the maximum likelihood principle. *In*: Petrov, B. N. & F. Csaki (eds.), Second International Symposium on Information Theory. Akademia Kiado, Budapest. 267-281 pp.

Alongi, D.M., (2009). Paradigm shifts in mangrove biology. In (Perillo, G.M.E., Wolanski, E., Cahoon, D.R., Brinson, M.M., (eds.) Coastal Wetlands: An Integrated Ecosystem Approach, Elsevier, Oxford, U.K. 615-640 pp.

Andriesse, J.P., Breemen, N.V. & Blokhuis, W.A. (1973). The influence of mudlobsters (*Thalassina anomala*) on the development of acid sulphate soils in mangrove swamps in Sarawak (East Malaysia). In *Proceedings of an International Symposium on Acid Sulphate soils*. 1972. Wageningen, The Netherlands. 11-13 pp. Figs. 1-3.

Ashton, E.C., (2002). Mangrove sesarmid crab feeding experiments in Peninsular Malaysia. *Journal of Experimental Marine Biology and Ecology*, 272(1), 97-119.

Atkinson, R.J.A., & Taylor, A.C. (1988). Physiological ecology of burrowing Decapods. Symposium of Zoological Society of London, 59, 201–226.

Barbaresi, S., Tricarico, E., Gherardi, F., (2004a). Factors inducing the intense burrowing activity by the red swamp crayfish, *Procambarus clarkii*, an invasive species. *Naturwissenschaften*, 91, 342-345.

Bell, T. (1844). On the *Thalassina emerii*, a fossil crustacean, forwarded by Mr. W. S. MacLeay, from New Holland. Proceedings of the Geological Society of London, 4, 360–362.

Bennett, I. (1968). The mudlobster. Australian Natural History, 16, 122–25.

Berry, A.J. (1972). The Natural History of West Malaysian Mangrove Faunas. *Malayan Nature Journal*, 25, 135-162.

Bertness, M.D., & Miller, T. (1984). The distribution and dynamics of *Uca pugnax* (Smith) burrows in a New England salt marsh. *Journal of Experimental Marine Biology and Ecology*, 83, 211-237.

Bliss, D.E. (1968). Transition from water to land in decapod crustaceans. *American Zoologist*, 8, 355-392.

Borchert, R., & Slade Norman, A. (1981). Bifurcation ratios and the adaptive geometry of trees. *Botanical Gazette*, 142(3), 394-401.

Bracken, H.D., Toon, A., Felder, D.L., Martin, J.W., Finley, M., Rasmussen, J., Palero, F., & Crandall, K.A. (2009). The decapod tree of life: compiling the data and moving toward consensus on decapod evolution. *Arthropod Systematics & Phylogeny*, 67, 99-116.

Bromley, R.G. (1990). Trace fossils: Biology and Taphonomy. Special Topics in Paleontology 3. Unwin Hyman, London, 280pp.

Brook, S., Grant, A., & Bell, D. (2009). Can land crabs be used as a rapid ecosystem evaluation tool? A test using distribution and abundance of several genera from the Seychelles. *Acta Oecologica*, 35, 711–719.

Buchanan, J.B. (1984). Sediment Analysis, in: Nolme, N.A., & McIntyre, A.D. (eds.), Methods for the study of Marine Benthos. McIntyre, Blackwell Science, Oxford, 41-65 pp.

Burkenroad, M.D. (1963). "The evolution of the Eucarida (Crustacea, Eumalacostraca), in relation to the fossil record". Tulane Studies in Geology. 2 (1): 1–17.

Campbell, B.M., & Woods, J.T. (1970). Quaternary crustaceans from northern Australia in the collections of the Bureau of Mineral Resources, Canberra. *Bulletin of the Bureau of Mineral Resources*, 108, 41-42.

Cannicci, S., Burrows, D., Fratini, S., Smith III, T.J., Offenberg, J., & Dahdouh-Guebas, F. (2008). Faunal impact on vegetation structure and ecosystem function in mangrove forests: A review. *Aquatic Botany*, 89, 186-200.

Capehart, A. A., & Hackney, C. T. (1989). The potential role of roots and rhizomes in structuring salt-marsh benthic communities. *Estuaries Coast* 12(2), 119-122.

Chan, B.K.K., Chan, K.K.Y., & Leung, P.C.M. (2006). Burrow architecture of the ghost crab *Ocypode ceratophthalma* on a sandy shore in Hong Kong. *Hydrobiologia*, 560, 43-49.

Chuang, S. H., 1961. On Malayan Shores. Muwu Shosa, Singapore, 226 pp, 112 pls.

Coelho, V.R., Cooper, R.A., & Rodrigues, S.R. (2000). Burrow morphology and behavior of the mud shrimp *Upogebia omissa* (Decapoda: Thalassinidea: Upogebiidae). *Marine Ecology Progress Series*, 200, 229-240.

Cohen, A.N., & Carlton, J.T. (1995). Nonindigenous aquatic species in United States estuary: case study of the biological invasions of the San Francisco Bay and Delta. report for the U.S.Fish and Wildlife Service, Washington, D.C.

Coleman, N., & Poore, G.C.B. (1980). The distribution of Callianassa species (Crustacea, Decapoda) in Western Port, Victoria. Proceedings of the Royal Society of Victoria, 91, 73-78.

Compton, T.J., Troost, T., Vander Meer, J., Kraan, C., Honkoop, P., Rogers, D., Pearson, G., de Goeij, P., Bacher, P., Lavaleye, M., Leyrer, J., Yates, M., Dekinga, A., & Pierma, T. (2008). Distributional overlap rather than habitat differentiation characterizes co-occurrence of bivalves in intertidal soft sediment systems. *Marine Ecology Progress Series*, 375, 25-35.

Cox, G. (1990) Laboratory manual of general ecology 6th edition. Dubuque, Iowa: William C. Brown.

Dana, J. D. (1852). Crustacea. Part I. United States Exploring Expedition, during the years 1838, 1839, 1840, 1841, 1842, under the command of Charles Wilkes, U.S.N., 13(2): i–viii, 1–685; Atlas (1855), C. Sherman, Philadelphia.

Davie, P.J.F., 2002. Crustacea: Malacostraca: Phyllocarida, Hoplocarida, Eucarida (Part 1). In: Wells, A. & Houston, W.W.K. (eds) Zoological Catalogue of Australia 19.3A, CSIRO Publishing, Melbourne, 1-151 pp.

De Grave Sammy, N. Dean Pentcheff, Shane T. Ahyong, Tin-Yam Chan, Keith A. Crandall5, Peter C. Dworschak, Darryl L. Felder, Rodney M. Feldmann, Charles H. J. M. Fransen, Laura Y. D. Goulding, Rafael Lemaitre, Martyn E. Y. Low, Joel W. Martin, Peter K. L. Ng, Carrie E. Schweitzer, S. H. Tan, Dale Tshudy, Regina Wetzer (2009). A Classification of Living and Fossil Genera of Decapod Crustaceans. *Raffles Bulletin of Zoology*, 21, 1–109.

De Man, J. G. (1915). Zur Fauna von Nord-Neuguinea. Nach den Sammlungen von Dr. P. N. van Kampen und K. Gjellerup in den Jahren 1910–1911. Macrura. *Zoologisches Jahrbücher Abtheilung für Systematik*, Jena, 38, 385–458, pls. 27–29.

De Man, J. G. (1928). The Decapoda of the Siboga-Expedition. Part 7. The Thalassinidae and Callianassidae collected by the Siboga-Expedition with some remarks on the Laomediidae. *Siboga Expéditie Monographie*, 39a, 1–187.

de Saint Laurent, M. (1979). Vers une nouvelle classification des Crustacés Décapodes Reptantia. *Bulletin de l'Office Nationale de Pêche de Tunisie*. 3, 15-31.

Diamond, J.M. (1975). Assembly of species communities, in: Cody, M.L., & Diamond, J.M. (eds.), Ecology and Evolution of Communities. Cambridge, Massachusetts, Belknap, 342-444 pp.

Dubey, S.K., Choudhury, A., Chand, B.K., & Trivedi, R.K. (2012). Ecobiological study on burrowing mud lobster *Thalassina anomala* (Herbst, 1804) (Decapoda: Thalassinidea) in the intertidal mangrove mudflat of Deltaic Sundarbans. *Exploratory Animal and Medical Research*, 2(1), 70-75.

Dumbauld, B.R., Armstrong, D.A., & Kristine, L.F. (1996). Life-History characteristics of two sympatric Thalassinidean shrimps, *Neotrypaea californiensis* and *Upogebia pugetiensis*, with implications for oyster culture. *Journal of Crustacean Biology*, 16(4), 689-708.

Dworschak, P.C. (1983). The biology of *Upogebia pusilla* (Petagna) (Decapoda: Thalassinidea). I. The burrows. Pubblicazioni della Stazione Zoologica di Napoli I: *Marine Ecology*, 4, 19-43.

Dworschak, P.C. (1987a). Burrows of Mediterranean Decapoda. Investigación Pesquera, Barcelona 51, 264 pp.

Dworschak, P.C. (1992). The Thalassinidea in the Museum of Natural History, Vienna; with some remarks on the biology of the species. *Annalen des Naturhistorischen Museums in Wien*, 93(B), 189-238, figs. 1-18.

Dworschark, P.C. (2003). Biology of Mediterranean and Caribbean Thalassinidea. Proceedings of International Symposium: Ecology of large bioturbators in tidal flats and shallow sublittoral sediments – from individual behavior to their role as ecosystem engineers, Nagasaki University, Japan, 1-22 pp.

Eisler, R. (1985). Carbofuran hazards to fish, wildlife and invertebrates: a synoptic review. U.S. Fish and Wildlife Service Biological Report, 85 (1.3). 36 pp.

English, S. A., Wilkinson, C., & Baker, V. J. (1994). Survey manual for tropical marine resources. Australian Institute of Marine Science, Townsville, Australia, 368 pp.

Erskine, J.M. (1992). Vetiver grass: its potential use in soil and moisture conservation in Southern Africa. *South African Journal of Science*, 88, 298-299.

Faller, M., Harvey, G.L., Henshaw, A.J., Bertoldi, W., Bruno, M.C., & England, J. (2016). River bank burrowing by invasive crayfish: Spatial distribution, biophysical controls and biogeomorphic significance. *Science of the Total Environment*, 569-570, 1190-1200.

Fei, S., Phillips, J., & Shouse, M. (2014). Biogeomorphic impacts of invasive species. *Annual Review of Ecology, Evolution, and Systematics*, 45, 69–87.

Ferguson, D. S., 1951. Control of damages to bunds by burrowing lobster and crabs. *Malaysian Agricultural Journal*, 34(2), 61-64, Figs. 1-9.

Folmer, O., Back, M., Hoeh, W., Lutz, R., & Vrijenhoek, R. (1994). DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology*, 3, 294–299.

Frusher, S.D., Giddins, R.L., Smith III, T.J. (1994). Distribution and abundance of Grapsid crabs (Grapsidae) in a mangrove estuary: effects of sediment characteristics, salinity tolerances, and osmoregulatory ability. *Estuaries*, 17, 647-654.

Gayen, N.K. & Choudhury, A. (1973). On the occurrence of. *Thalassina anomala* (Herbst), a burrowing shrimp of Sagar Island, Sunderbans. Proceedings of Indian Scientific Congress. 517-518 pp.

Glaessner, M. F. (1969). Decapoda: R400-533. *In*: Moore, R. C. (eds.), *Treatise on invertebrate paleontology*, Part R, Arthropoda 4(2). University of Kansas Press and Geological Society of America, Inc., Boulder, Colorado.

Griffis, R.B., Chavez, F.L. (1988). Effects of sediment type on burrows of *Cahanassa californiensis* Dana and *C. gigas* Dana. *Journal of Experimental Marine Biology Ecology*, 117, 239-253.

Griffis, R.B., Suchanek, T.H. (1991). A model of burrow architecture and trophic modes in thalassinidean shrimp (Decapoda: Thalassinidea). *Marine Ecology Progress Series*, 79, 171-183.

Chomchalow, N. & Henle, H.V. (1998). Vetiver: A Miracle Grass. Proc. ICV-1, ORDPB, Bangkok.

Grimshaw, R.G. (1994). Vetiver grass—its use for slope and structure stabilizatioon under tropical and semitropical conditions. In: Vegetation and Slopes. Institution of Civil Engineers, London. 26-35.

Guan, R.Z. (1994). Burrowing behaviour of signal crayfish, *Pacifastacus leniusculus* (Dana), in the River Great Ouse, England. *Freshwater Forum*, 4, 155-168.

Hellin, J, Haigh, M.J. (2002b). Impact of Vetiveria zizanioides (vetiver grass) live barriers on maize production in Honduras. In: Wang, L., Wu, D., Tu, X., Nie, J. (eds.), Proceedings of the 12th International Soil Conservation Organisation Conference on Sustainable Utilization of Global Soil and Water Resources, 26-31 May 2002, Vol, 3, Beijing, China, 277-281 pp.

Herbst, J. F. W. (1804). Versuch einer Naturgeschichte der Krabben und Krebse nebst einer systematischen Beschreibung ihrer verschiedenen Arten, Gottlieb August Lange, Berlin & Stralsund, 3(4), 1–49, pls. 59–62 pp.

Hess, W. (1865). BeitrSge zur Kenntnis der Decapoden-Krebse Ost-Australiens. Archiv für Naturgeschichte, 31, 127-173.

Hogarth, P.J. (1999). The biology of mangroves. Oxford University Press, Oxford, 228 pp.

Holm, L.G., Doll, J., Holm, E., Pancho, J.V., & Herberger, J.P. (1997) World Weeds: Natural Histories and Distribution: John Wiley and Sons, Inc., New York, NY.

Holthuis, L.B. (1991). FAO Species Catalogue, Vol. 13: Marine Lobsters of the World: An Annotated and Illustrated Catalogue of Species of Interest to Fisheries Known To Date. FAO Fisheries Synopsis, 125, 1-292 pp.

Horsfield, K. (1976). Some mathematical properties of branching trees with application to the respiratory system. *Bulletin of Mathematical Biology*, 38(3), 305-315.

Huelsenbeck, J. P. & Ronquist, F. (2001). MrBayes: Bayesian inference of phylogenetic trees. *Bioinformatics*, 17, 754–755.

Hutchings, P. and Saenger, P. (1987) Ecology of Mangroves. University of Queensland Press, Brisbane, Australia, p. 388.

Jobb, G., A. von Haeseler, & Strimmer, K. (2004). Treefinder: a powerful graphical analysis environment for molecular phylogenetics. *BMC Evolutionary Biology*, 4, 18.

Johnson, D.S. (1961). The food and feeding of the mud-lobster *Thalassina anomala* (Herbst). *Crustaceana*, 2, 325-326.

Jones, C.G., Lawton, J.H., & Shachak, M. (1994). Organisms as ecosystem engineers. *Oikos*, 69, 373–386.

Katrak, G., Dittmann, S., & Seuront, L. (2008). Spatial variation in burrow morphology of the mud shore crab, *Helograpsus haswellianus* (Brachyura, Grapsidae), in South Australian Saltmarshes. *Australian Journal of Marine and Freshwater Research*, 59, 902-911.

Latreille, P.A. (1802–1803) Histoire naturelle, générale et particulière des Crustacés et des Insectes; ouvrage faisant suite aux oeuvres de Leclerc de Buffon, et partie du cours

complet d'histoire naturelle rédigé par. C. S. Sonnini. Volume 2. F. Dufart, Paris, 467 pp.

Latreille, P.A. (1806). Genera Crustaceorum et Insectorum secundum ordinem naturalem in familias disposita, iconibus exeplisque plurimis explicata. A. Koenig, Paris, & Argentorati, i-xviii, 302 pp.

Latreille, P.A. (1831). Cours d'entomologie ou de l'histoire naturelle des Crustaces, des Arachnides, des Myriopodes et des Insectes. Exposition methodique des ordres, des families et des genres des trois premieres classes. A l'usage des eleves de l'ecole du Museum d'Histoire Naturelle, Paris, i–xiii, Atlas: pls. 1–26, 1–568 pp.

Leach, W.E. (1814). Crustaceology. In Brewster, D. (eds) *The Edinburgh Encyclopaedia*, Blackwood: Edinburgh, **7**(2), 383-437.

Lee, S.Y. (1999). Tropical mangrove ecology: physical and biotic factors influencing ecosytem structure and function. *Australian Journal of Ecology*, 24(4), 355-366.

Legendre, P., & Legendre, L. (1998). Numerical ecology. 2nd English edition. Elsevier Science BV, Amsterdam. xv, 853 pp.

Leme, M.H.A. (2002). A comparative analysis of the population biology of the mangrove crabs *Aratus pisonii* and *Sesarma rectum* (Brachyura, Grapsidae) from the north coast of Sao Paulo State, Brazil. *Journal of Crustacean Biology*, 22, 553-557.

Lepš, J., & Šmilauer, P. (2003). Multivariate analysis of ecological data using canoco. Cambridge University Press, Cambridge, UK, 269 pp.

Lim, P. E., Tan, J., Suana, I. W., Eamsobhana, P., & Yong, H. S. (2012). Distinct genetic lineages of *Bactrocera caudata* (Insecta: Tephritidae) revealed by COI and 16S DNA sequences. *PLoS ONE*, **7**(5): e37276. doi:10.1371/journal.pone.0037276.

Lim, S.S.L. (2006). Fiddler crab burrow morphology: How do burrow dimensions and bioturbative activities compare in sympatric populations of *Uca vocans* (Linnaeus, 1758) and *U. annulipes* (H. Milne Edwards, 1837). *Crustaceana*, 79, 525-540.

Lim, S.S.L., & Diong, C.H. (2003). Burrow morphological characters of the fiddler crab, *Uca annulipes* (H. Milne Edwards, 1837) and ecological correlates in a lagoonal beach on Pulau Hantu, Singapore. *Crustaceana*, 76, 1055-1069.

Lim, S.S.L., & Heng, M.M.S. (2007). Mangrove micro-habitat influence on bioturbative activities and burrow morphology of the fiddler crab, *Uca annulipes* (H. Milne Edwards, 1837) (Decapoda, Ocypodidae). *Crustaceana*, 80(1),31-45.

Lin, F. J., Liu, Y., Sha, Z. L., Tsang, L. M., Chu, K. H., Chan, T. Y., Liu, R. Y., & Cui, Z. X. (2012). Evolution and phylogeny of the mud shrimps (Crustacea: Decapoda) revealed from complete mitochondrial genomes. *BMC Genomics*, 13, 631

Macintosh, D.J. (1988). The ecology and physiology of decapods of mangrove swamps. *Symposium of the Zoological Society of London*, 59, 315-341.

Macintosh, D.J.; Ashton, E.C.; Havanon, S., (2002). Mangrove rehabilitation and intertidal biodiversity: a Study in the Ranong Mangrove Ecosystem, Thailand. *Estuarine, Coastal and Shelf Science*, 55, 331–345.

Macnae, W. (1968). A general account of the fauna and flora of the mangrove swamps and forests in the Indo-Pacific Region. *Advances in Marine Biology*, 6, 73-270.

Marshall, S.M., & Orr, A.P. (1960). Feeding and Nutrition. In: Waterman, T.H. (ed.). The Physiology of Crustacea 1. Academic Press, New York. 227-258 pp.

Miers, E.J. (1880). On a collection of Crustacea from the Malaysian region.—Part III. Crustacea Anomura and Macrura (except Penaeidea). *Annals and Magazine of Natural History (ser. 5)*, 3, 370-384.

Milne Edwards, H. (1837). *Histoire naturelle des Crustacés, comprenent l'anatomie, la physiologic et la classification de ces animaux*. Paris, Librairie de Roret, II: 1–532pp.

Misra, R. K., & Ni, I-H. (1983). Distinguishing beaked redfishes (deepwater redfish, *Sebastes mentella* and Labrador redfish, *S. fasciatus*) by discriminant analysis (with covariance) and multivariate analysis of covariance. *Canadian Journal of Fisheries and Aquatic Science*, 40, 1507–1511.

Moh, H. H., & Chong, V. C. (2009). A new species of *Thalassina* (Crustacea: Decapoda: Thalassinidea) from Malaysia. *Raffles Bulletin of Zoology*, 57(2), 465–473.

Moh, H.H., Chong, V.C., & Abdul Muhsin, Y. (2006). Ecology and management of Mud lobster in the Golden Hope Oil Palm Plantation, Carey Island. Proceedings Seminar on Biodiversity Conservation through Sustainable Plantation Practices on Carey Island, 51-55 pp.

Moh, H. H., Chong, V.C., & A. Sasekumar (2015). Distribution and burrow morphology of three sympatric species of *Thalassina* mud lobsters in relation to environmental parameters on a Malayan mangrove shore. *Journal of Sea Research*, 95, 75-83.

Moh, H.H., Chong, V.C., Lim, P.E., Tan, J., & Dally, G. (2013). Verification of four species of the mud lobster Genus *Thalassina* (Crustacea: Decapoda: Gebiidea: Thalassinidae) using molecular and morphological characters. *Raffles Bulletin of Zoology*, 61(2), 579-588.

Moonga, K., & Musuka, C.G. (2014). The effect of accidentally introduced red swamp crayfish (*Procambarus clarkia*) in Kafue fishery. *International Journal of Agriculture*, *Forestry and Fisheries*, 2 (1), 8-15.

Morrisey, D.J., Dewitt, T.H., Roper, D.S., & Williamso, R.B. (1999). Variation in the depth and morphology of burrows of the mud crab Helice crassa among different types of intertidal sediments in New Zealand. *Marine Ecology Progress Series*, 182: 231–242.

Morton, B., 1976. The biology and functional morphology of the Southeast Asian mangrove bivalve, Polymesoda (Geloina) erosa (Solander, 1786) (Bivalvia: Corbiclidae). *Canadian Journal of Zoology*, 54, 482–500.
Mouton Jr., E.C., & Felder, D.L. (1996). Burrow distributions and population estimates for the fiddler crabs *Uca spinicarpa* and *Uca langisignalis* in a Gulf of Mexico salt marsh. *Estuaries*, 19(1), 51-61.

Nandi, N.C., & Dev Roy, M.K. (1991). Burrowing activity and distribution of *Scylla serrata* (Forskal) from Hooghly and Malta estuaries, Sundarban, West Bengal, India. *Journal of the Bombay Natural History Society*, 88, 167-171.

Nash, R.D.M., Chapman, C.J., Atkinson, R.J.A., & Morgan, P.J. (1984). Observations on the burrows and burrowing behaviour of *Calocaris macandreae* (Crustacea: Decapoda: Thalassinoidea). *Journal of Zoology*, 202, 425-439.

Ng, P.K.L., & Kang, N. (1988). *Thalassina*: the mud lobster. *Nature Malaysiana*, 13(4), 28-31.

Ng. P.K.L., & Sivasothi, N (1999). A guide to mangroves of Singapore. 1, the ecosystem & plant diversity. Singapore Science Centre, 160 pp.

Ngoc-Ho, N., & de Saint Laurent, M. (2009). The genus *Thalassina* Latreille, 1806 (Crustacea: Thalassinidea: Thalassinidae). *Raffles Bulletin of Zoology*, Supplement No. 20, 121–158.

Nickell, L.A., & Atkinson, R.J.A. (1995). Functional morphology of burrows and trophic modes of three thalassinidean shrimp species, and a new approach to classification of thalassinidean burrow morphology. *Marine Ecology Progress Series*, 128, 181-197.

Ong, J.E., Gong, W.K., & Wong, C.H. (2004). Allometric and partitioning of the mangrove, *Rhizophora apiculata*. *Forest Ecology and Management*, 188, 395-409.

Pandya, P.J., & Vachhrajani, K.D. (2010). Spatial distribution and substratum preference of brachyuran crab *Macrophthalmus depressus* (Decopoda: Ocypodidae) along the lower estuarine mudflats of Mahi River, Gujarat, India. *Crustaceana*, 83(9), 1055-1067.

Panning, A., (1939). The Chinese mitten crab. Report of the board of Regents of the Smithsonian Institution, Washington, 3508: 361- 375.

Pillai, G. (1982). Aspects of the biology of *Thalassina anomala* (Herbst). MSc. Thesis, University of South Pacific, Suva, Fiji. 135pp.

Pillai, G. (1985). The Mana, or Mangrove Lobster. Domodomo, 3, 2-10.

Pillai, G. (1989). Environmental factors limiting the distribution of the mangrove lobster (*Thalassina anomala*). In Haynes, R. J. & Naidu, R. (eds), Agricultural Development in the Pacific Islands in the 90's, University of the South Pacific Library, Suva, Fiji, 305-313 pp.

Pimentel, R.A. (1979). Morphometrics: The multivariate Analysis of Biological Data. Kendall Hunt Publishing Company, Dubuque, Iowa, USA. 276 pp.

Poore, G.C.B., & Griffin, D.J.G. (1979). The Thalassinidea (Crustacea: Decapoda) of Australia. *Records of the Australian Museum*, 32, 217–321.

Powers, L.W., & Cole, J.F. (1976). Temperature variation in fiddler crab microhabitats. *Journal of Experimental Marine Biology and Ecology*, 21, 141-157.

Qureshi, N.A., & Saher, N.U. (2012). Burrow morphology of three species of fiddler crabs (*Uca*) along the coast of Pakistan. *Belgian Journal of Zoology*, 142(2), 114-126.

Raffaelli, D., & Hawkins, S. (1999). Intertidal Ecology. Kluvier Academic Publishes, London, 356 pp.

Ridd, P.V. (1996). Flow through animal burrows in mangrove creeks. *Estuarine, Coastal and Shelf Science*, 43(5), 617-625.

Ringold, P. 1979. Burrowing, root mat density, and the distribution of fiddler crabs in the eastern United States. *Journal of Experimental Marine Biology and Ecology*, 36, 11-21.

Robertson, A.I., & Daniel, P.A. (1989). The influence of crabs on litter processing in high intertidal mangrove forests in tropical Australia. *Oecologia*, 78, 191-198.

Rowden, A.A., & Jones, M.B. (1995). The burrow structure of the mud shrimp *Callianassa* subterranea (Decapoda: Thalassinidea) from the North Sea. *Journal of Natural History*, 29, 1155-1165.

Rudnick, D.A., Chan, V., & Resh, V.H. (2005). Morphology and impacts of the burrows of the Chinese mitten crab, *Eriocheir sinensis* H. Milne Edwards (Decapoda, Grapsoidea), in southern San Francisco Bay, California, USA. *Crustaceana*, 78, 787-807.

Rudnick, D.A., Halat, K.M., & Resh, V.H. (2000). Distribution, ecology and potential impacts of the Chinese mitten crab (*Eriocheir sinensis*) in San Francisco Bay. Water Resources Center, Contribution #206, University of California Water Resources Center, Berkeley, CA.

Sakai, K. (2004). "The diphyletic nature of the Infraorder Thalassinidea (Decapoda, Pleocyemata) as derived from the morphology of the gastric mill". *Crustaceana*, 77 (9): 1117–1129.

Sakai, K., & Türkay, M. (2012). A review of the species of the genus *Thalassina* (Thalassinidea, Thalassinidae). *Crustaceana*, 85(11), 1339–1376.

Salgado-Kent, C., & McGuinness, K. (2010). Spatial and temporal variation in relative numbers of grasid crabs (Decapoda: Grapsidae) in northern Australian mangrove forests. *The Beagle, Record of the Museums and Art Galleries of the Northern Territory*, 26, 79-87.

Sankolli, K.N., (1963). On the occurrence of *Thalassina anomala* (Herbst), a burrowing crustacean, in Bombay waters, and its burrowing methods. *Journal of the Bombay natural History Society*, 60, 600-605.

Sankolli, K.N. (1970). The Thalassinoidea (Crustacea, Anomura) of Maharashtra. *Journal of the Bombay Natural History Society*, 67, 235-249.

Sasekumar, A. (1974). Distribution of macrofauna on a Malayan mangrove shore. *Journal of Animal Ecology*, 43, 51-69.

Shono, H. (2000). Efficiency of the finite correction of Akaike's Information Criteria. *Fisheries Science*, 66, 608–610.

Shreve, R.L. (1966). Statistical law of stream numbers. Journal of Geology, 75, 17-37.

Smith III, T.J., Boto, K.G., Frusher, S.D., & Giddins, R.L. (1991). Keystone species and mangrove forest dynamics: the influence of burrowing by crabs on soil nutrient status and forest productivity. *Estuarine Coastal and Shelf Science*, 33, 419-432.

Snelgrove, P.V.R., & Butman, C.A. (1994). Animal-sediment relationships revisited: cause versus effect. *Oceanography and Marine Biology*: An Annual Review, 32, 111-177.

Stamhuis, E.J., Schreurs, C.E., & Videler, J.J. (1997). Burrow architecture and turbative activity of the thalassinid shrimp *Callianassa subterranea* from the central North Sea. *Marine Ecology Progress Series*, 151, 155-163.

Steenstrup, J. & Lutken, C. (1862). Om *Thalassina anomala* (HERBST). - *Vidensk. Medd. naturhist. Foren., Kjobenhavn*; 1861: pls. 1-7, 267-248 pp.

Stieglitz, T., Ridd, P., & Muller, P. (2000). Passive irrigation and functional morphology of crustacean burrows in tropical mangrove swamp. *Hydrobiologia*, 421, 67–76.

Swofford, D.L. (2002). *PAUP*: Phylogenetic analysis using parsimony (*and other methods)*. Version 4. Sinauer Associates, Sunderland, Massachusetts.

Takeda, S., & Kurihara, Y. (1987). The distribution and abundance of *Helice tridens* (De Haan) burrows and substratum conditions in a north eastern Japan salt marsh. *Journal of Experimental Marine Biology and Ecology*, 107, 919.

Tanabe, A.S. (2007). Kakusan: a computer program to automate the selection of a nucleotide substitution model and the configuration of a mixed model on multilocus data. *Molecular Ecology Notes*, 7: 962–964.

Taylor, A.C., Astall, C.M., & Atkinson, R.J.A. (2000). A comparative study of the oxygen transporting properties of the haemocyanin of five species of thalassinidean mudshrimps. *Journal of Experimental Marine Biology and Ecology*, 244, 265-283.

Ter Braak, C.J.F., & Šmilauer, P. (2002). CANOCO Reference Manual and CanoDraw for Windows User's Guide: Software for Canonical Community Ordination (version 4.5). Ithaca, NY, USA (www.canoco.com): Microcomputer Power, 500 pp.

Tham, A.K. (1967). Prawn Culture in Singapore. Review paper. World Symposium on the biology and culture of shrimps and prawns. Mexico city, 1967.

Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F., & Higgins, D.G. (1997). The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research*, 24, 4876–4882.

Thongtham, N., Kristensen, E., & Puangprasan, S.Y. (2008). Leaf removal by sesarmid crabs in Bangrong mangrove forest, Phuket, Thailand: with emphasis on the feeding ecology of Neoepisesarma versicolor. *Estuarine, Coastal and Shelf Science*, 80, 583-590.

Tide Tables Malaysia 2011. National Hydrography Centre, Royal Malaysian Navy, Malaysia, 221 pp.

Tomlinson, P.B. (1986). The botany of mangroves. Cambridge University Press, Cambridge, United Kingdom, 419 pp.

Truong, P. N, (1999). Vetiver grass technology for land stabilisation, erosion and sediment control in the Asia Pacific region. In Proceedings of First Asia Pacific Conference on Ground and Water Bioengineering for Erosion Control and Slope Stabilisation, Manila, Philippines, April 1999. pp 72–84. International Erosion Control Association, Steamboat Springs, USA.

Truong, P.N. (1994). Vetiver grass, its potential in the stabilisation and rehabilitation of degraded and saline lands. In "Halophytes a resource for livestock and for rehabilitation of degraded land, Squire, V.R. & Ayoub, A.T. (eds.), Kluwer Academics Publisher, Netherlands. 293-296 pp.

Truong, P.N., Carlin, G, Cook, F. (2003b). Vetiver grass hedges for water quality improvement in acid sulfate soils, Queensland, Australia. Proceedings of the Third International Conference on Vetiver and Exhibition, Guangzhou, China.

Truong, P.N. & Baker, B. (1998). Vetiver grass system for environmental protection. In Technical Bulletin No. 1998/1. Pacific Rim Vetiver Network. Office for the Royal Development Projects Board. Bangkok, Thailand.

Truong, P.N., Tran, T.V., & Pinners, E. (2008). Vetiver System Applications: Technical Reference Manual, 2nd Ed. Laity Grimshaw. The Vetiver Network International. Peyron L. 1989. Vetiver in perfumery.Quintessenza

Tsang, L. M., Ma, K. Y., Ahyong, S. T., Chan, T. Y., & Chu, K. H. (2008). Phylogeny of Decapoda using two nuclear protein-coding genes: Origin and evolution of the Reptantia. *Molecular Phylogenetics and Evolution*, 48, 359–368.

Van Nedervelde, F., Cannicci, S., Koedam, N., Bosire J.O., & Dahdouh-Guebas, F. (2015). What regulates crab predation on mangrove propagules? *Acta Oecologica*, 63, 63-70.

Varuges, V.M.A. (1978). Some aspects of ecology of Mud lobster *Thalassina anomala* (Herbst) and their control in Carey Island. MSc. Thesis, University of Malaya, Kuala Lumpur, Malaysia, unpublished.

Verwey, J. (1930). Einiges über die Biologie der ost-indischen Mangrovekrabben. *Treubia*, 12,167–261.

Wang, J.Q., Bertness, M.D., Li, B., Chen, J.K., Lu, W.G. (2015). Plant effects on burrowing crab morphology in a Chinese salt marsh: Native vs. exotic plants. *Ecological Engineering*, 74, 376-384.

Watson, J.G. (1928). Mangrove Forests of the Malay Peninsula. Malayan Forest Records No. 6. Federated Malay States Government, Singapore: 275 pp.

Webb, J.E. (1958). The Ecology of Lagos lagoon. 1: The lagoons of the Guinea Coast. *Philosophical Transaction Royal Society London*. Series B, 241-283

Wentworth, C.K. (1922). A scale of grade and class terms for clastic sediments. *Journal of Geology*, 30, 377-392.

White, A. (1847). List of the specimens of Crustacea in the collection of the British Museum. British Museum Natural History, London. Viii +1-143 pp.

Whitlatch, R.B. (1981). Animal-sediment relationships in intertidal marine benthic habitats: some determinants of deposit-feeding species diversity. *Journal of Experimental Marine Biology and Ecology*, 53, 31–45.

Zar, J.H. (2010). Biostatistical Analysis, fifth edition. New Jersey, Prentice Hall, 944 pp.

Ziebis, W., Forster, S., Huettel, M., & Jorgensen, B.B. (1996). Complex burrows of the mud shrimp *Callianassa truncata* and their geochemical impact in the sea bed. *Nature*, 382, 619-622.