UNIVERSITI PUTRA MALAYSIA

PREVALENCE OF GREGARINE PARASITES IN Anadara cornea (Reeve, 1844) AND ITS POTENTIAL INFECTIVITY TO Scylla sp. AND Macrobrachium rosenbergii (De Man, 1879)

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The prevalence of gregarine parasites in wild bivalves is an important indicator for the sustainability of ecosystem health due to interdependence to survive. The data or information of the health status of wild bivalves is important for commercial crustacean culture in the future. All prominent species in the Setiu Lagoon of Terengganu state, are economically important due to the use for both local consumption and commercial in the region. This study will be an important report that can be explored regarding this parasite for the future research, tackling problems and future perspectives. The study of this parasite in the wild population has not been previously reported This undertaken study is important to ensure that the infection of this parasite will not cause a problem for the commercial bivalve's aquaculture in Malaysia. This data obtained can be used to develop a better treatment and quarantine procedure for aquaculture. Ecosystem health status is very important to secure the future of the crustacean culture industry. This study was done to prove the existence of parasites species that commonly infect the bivalves and crustacean species in Malaysia. The detailed correlation between the prevalence of the parasites and environmental factors such as season and water quality that contribute to the intensity of the parasites are of important concern. It is important to know this information to elaborate on the complete lifecycle of this gregarine. Moreover, diagnosis of this parasite is also important to safeguard our aquaculture industry for our national future food security. The furbished information of the occurrence of the gregarine parasite will be a platform for future study in many perspectives such as development of vaccines and treatment to cure the infection. Moreover, the gregarine parasites species identification in the natural host environment is the main undertaking to be clarified. Furthermore, the study of proper prophylaxis and treatment has yet to not reported for this parasite. Importantly, results of the current study recorded that wild marine bivalves *Anadara cornea* (Reeve1844) is a new host for gregarine species-genus *Ascogregarina*. However, further study needs to be explored to detail out the specific information for this species as to elucidate a comprehensive overview of

described species of the genus *Ascogregarina* from wild bivalves, *Anadara cornea* (Reeve 1844), including taxonomy, lifecycle, pathogenicity and host specificity features. The study of outbreak of this parasites infection during the rainy season should include the sedimentation analysis for better correlation of significant factor of infection. The selection of intermediate host candidates should be varied i.e., shrimp, prawn, tubeworm etc. to confirm the specific host for this parasite. Samples were collected monthly throughout 2018 in the seagrass bed area at Setiu lagoon, Terengganu, Malaysia. Forty samples were collected each month to determine the occurrence and prevalence of the parasites. The correlation between the prevalence, water quality and the seasonal factor were critically observed. The general objective of this study are to record the occurrence of gregarine parasites in wild marine bivalves *Anadara cornea* (Reeve, 1844) from Setiu lagoon, Terengganu, Malaysia. From the study, the total number of parasites count was n = 33,239 phagocytes from n = 480 of host samples. The total yearly prevalence of the parasites was 97.5% with a 95% confidence interval value which was CI=96.1% to 98.9%. The prevalence of the parasites was significantly high during the rainy season compared to other season (Dry and Moderate). The findings also proved that the intensity of the parasites decreased at every transition of season. The higher the level of ammonia, the lower number of parasites found. The morphological characteristic of the species was also described and the species was been confirmed by DNA analysis and found to be closely related to *Ascogregarina* sp. The histopathological study proved that the infections of this parasite did not induced any serious effect in the host body. The *in-vitro* infectivity test between intermediate host, *A. cornea* (Reeve, 1844) and definitive host, *Scylla* sp. showed a negative result as no transmission occurred between both target species. It was assumed that *Scylla* sp. was not a definitive host for this bivalve gregarine. This study is the first report of gregarine infection in wild marine bivalves, *A. cornea* (Reeve, 1844) from Setiu Lagoon, Terengganu, Malaysia. This study provided novel information on the prevalence of gregarine parasites and seasonal infection in host species in the Setiu Lagoon, and will help to optimize sustainable management strategies for use in their native environment and wildlife conservation.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Doktor Falsafah

PREVALEN PARASIT GREGARIN PADA *Anadara cornea* (Reeve, 1844) DAN POTENSI KEBOLEHJANGKITAN TERHADAP *Scylla* sp. DAN *Macrobrachium rosenbergii* (De Man, 1879).

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Prevalen parasit gregarin dalam kerang dwicenkerang liar adalah petunjuk penting untuk kelestarian kesihatan ekosistem kerana saling berkait untuk terus hidup. Data atau maklumat untuk status kesihatan dwicenkerang liar adalah penting bagi menjamin penghasilan produk komersial pada masa akan datang. Kesemua spesies kerang-kerangan yang biasa ditemui di Laguna Setiu, Terengganu, adalah penting dari segi ekonomi kerana penggunaan untuk kegunaan tempatan dan komersial di rantau ini. Kajian ini akan menjadi data penting yang berpotensi untuk diterokai dalam mendalami ilmu mengenai parasit ini berdasarkan asas penyelidikan masa lalu dari aspek permasalahan dan perspektif di masa depan. Sehingga kini belum ada satu kajian mengenai parasit ini dalam populasi dwicenkerang liar. Kajian ini penting bagi memastikan jangkitan parasit ini tidak menimbulkan masalah kepada industri akuakultur komersial di Malaysia. Data ini boleh digunakan untuk membangunkan prosedur rawatan dan kuarantin yang lebih baik untuk akuakultur. Status kesihatan ekosistem adalah sangat penting untuk menjamin masa depan industri akuakultur negara. Kajian ini dilakukan untuk membuktikan spesies parasit yang biasanya menjangkiti spesies dwicenkerang dan krustasia di Malaysia. Dalam kajian ini, hubungkait yang terperinci antara kelaziman parasit dan faktor persekitaran seperti musim dan kualiti air yang menyumbang yang kepada kemandirian parasit adalah dititikberatkan. Adalah penting untuk mengetahui maklumat terperinci dalam menghuraikan tentang kitaran hidup lengkap parasit gregarin ini. Selain itu, pendiagnosan parasit ini penting untuk menjamin kelestarian industri akuakultur untuk sekuriti makanan masa depan negara. Maklumat terperinci mengenai kehadiran parasit gregarin boleh menjadi platform untuk kajian masa depan dalam perspektif yang berbeza seperti pembangunan vaksin dan rawatan untuk menyembuhkan jangkitan. Selain itu, perumah spesies parasit gregarin perlu dikenalpasti dalam persekitaran semulajadi perlu diperjelaskan. Kajian profilaksis dan rawatan yang tepat belum pernah dilaporkan lagi terutamanya untuk parasit ini. Oleh itu, berdasarkan hasil kajian semasa kajian ini merekodkan bahawa dwicenkerang liar *Anadara cornea*

(Reeve1844) adalah perumah baharu bagi spesies gregarin genus *Ascogregarina*. Walau bagaimanapun, kajian lanjut perlu diterokai untuk memperincikan maklumat khusus untuk spesies ini mengenai tahap keupayaannya untuk membawa gambaran komprehensif spesis genus *Ascogregarina* dari dwicenkerang liar, *Anadara cornea* (Reeve 1844) termasuk taksonomi, kitaran hidup, patogenik dan ciri khusus perumah. Wabak jangkitan parasit ini semasa musim hujan perlu perlu dikaji memandangkan parasit ini mungkin mendiamkan diri semasa musim tersebut. Pemilihan perumah sementara perlu diperluaskan kepada udang, ketam, cacing tiub dan sebagainya untuk melengkapkan kitaran hidup khusus untuk parasit ini. Sampel kajian ini dikumpulkan setiap bulan sepanjang tahun 2018 di kawasan rumput laut di Laguna Setiu, Terengganu, Malaysia. Empat puluh sampel dikumpulkan setiap bulan untuk melihat kehadiran dan prevalen parasit. Hubungkait antara prevalen, kualiti air dan faktor bermusim diperhatikan secara kritikal. Objektif umum kajian ini adalah untuk melaporkan kehadiran parasit gregarin dalam dwicankerang liar *Anadara cornea* (Reeve, 1844) di Laguna Setiu, Terengganu, Malaysia. Daripada kajian, jumlah kiraan parasit adalah *n* = 33,239 fagosit daripada *n* = 480 sampel perumah. Jumlah kelaziman setahun parasit adalah 97.5% dengan nilai selang keyakinan 95% iaitu CI=96.1% hingga 98.9%. Kelaziman parasit adalah jauh tinggi semasa musim hujan berbanding musim lain (kering dan sederhana). Penemuan itu juga membuktikan bahawa keamatan parasit menurun pada setiap peralihan musim. Apabila paras ammonia meningkat, bilangan parasit ditemui dalam kadar lebih rendah. Ciri-ciri morfologi spesies ini juga diterangkan dan spesies ini telah disahkan melalui analisis DNA dan berkait rapat dengan *Ascogregarina* sp. Kajian histopatologologi membuktikan bahawa jangkitan parasit ini tidak menunjukkan apa-apa kesan serius pada badan perumah. Ujian kebolehjangkitan *in-vitro* antara perumah sementara *A. cornea* (Reeve, 1844) dan hos muktamad *Scylla* sp. menunjukkan hasil negatif kerana tiada penularan berlaku antara kedua-dua spesies sasaran.Oleh itu, *Scylla* sp. dianggap bukan perumah muktamad untuk gregarin ini. Kajian ini adalah laporan pertama jangkitan gregarin dalam dwicenkerang liar*, A. cornea* (Reeve, 1844) dari Laguna Setiu, Terengganu, Malaysia. Kajian ini menyediakan maklumat asli mengenai kelaziman parasit gregarin dan jangkitan bermusim dalam spesis perumah, yang akan membantu mengoptimumkan strategi pengurusan mampan untuk digunakan dalam persekitaran asal dan pemuliharaan hidupan liar.

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Thank you very much!

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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Date: 11 November 2021

Declaration by Members of Supervisory Committee

This is to confirm that:

- the research conducted and the writing of this thesis was under our supervision;
- Supervision responsibilities as stated in the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) are adhered to.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

NAP3	Third National Agriculture policy
N	Number of host samples
N	Number of parasites found
DNA	Deoxyribonucleic Acid
PCR	Polymerase chain reaction
rDNA	ribosomal DNA
H&E	Hematoxylin and Eosin
Pha	Phagocyte
Oc	Oocysts
PV	Parasitophorous Vacoule
Sz	Sporozoites
SEM	Scanning Electron Microscope
ROS	Reactive Oxygen Species
GS	Gill Sample
IS	Intestine Sample
CI	Cumulative Index
OIE	Office for International Epizoootics
ROS	Reactive Oxygen Species
TR	General Time Reversable
HKY	Hasegawa Kishino Yano
TN93	Tamura Nei
T92	Tamura 3 Parameter
K2	Kimura 2 parameter
JC	Jukes Cantor

BIC	Bayesian Information Criterion
AICc	Akaike Information Criterion corrected
1nL	Maximum Likelihood
UniEP	Universal Eukaryotic Primer
MEGA X	Software for genetic study

CHAPTER 1

INTRODUCTION

Farming of bivalves nowadays are become important activities throughout Malaysia (Poutiers, 1998; Hamli et al., 2012). This form of aquaculture contributes to Malaysia's economy and provides a sustainable, efficient source of seafood in an era when over-exploitations and over-fishing threatens the world's marine ecosystems (Kripa et al., 2003; Laxmilatha et al., 2007; Ihwan et al., 2017). In the estuarine/marine environment the production systems of commercial bivalve culture are basically in raft system especially for mussel and oyster, and commonly bottom culture for cockle (Nakao et al., 1989; Nateewathana, 1995; Sallih, 2005; Page, 2011).

Most of mollusk, especially bivalves species are fully utilized as seafood in Malaysia, Thailand, Indonesia and Philippines and other Asian countries. In Malaysia, total production of edible brackish water bivalves was 54% followed by shrimp and prawn at 17.3% and marine fish at 6.3% (Hamli et al., 2012). Since bivalve culture was categorized as one of aquaculture sector, its becomes a priority sector under the Third National Agriculture Policy (NAP3). Total production of crustaceans and mollusks, fresh, chilled, frozen, salted, dried including bivalves exported from aquaculture was 76397.81 metric tonnes (RM1,299,642,901), recorded in 2016 (DOF, 2016). However, the number of published documents regarding to bivalve study in Malaysia were not progressively reported (Nakao et al., 1989; Abu-Hena et al., 2004; Sallih, 2005; Abdullah et al., 2007; Idris et al., 2011; Hamli et al., 2012).

Disease problem is one of the serious threats for the aquaculture production (Tuntiwaranuruk et al., 2004; 2008; Ihwan et al., 2016). Diagnosis of parasite and monitoring for disease pathogen presence in farms and natural bivalve populations need to be intensively practice to secure the Malaysian aquaculture industry in future. This kind of activities will ensure the long-term sustainability of the pristine shellfish populations and their aquaculture industry (Poutier, 1998).

Protozoan parasite is one of the important threats in farmed bivalves that is well recognized nowadays especially in the aspect of biology and life-cycle (Levine, 1976; 1977; 1979; 1981; 1988). Potential destructive effect on development of bivalve industry in relation to disease problem is well-recognized. This problem could be one of the factors that can caused a serious impact to the production of bivalve cultured industry. Most of the previous report assumed that the primary factors that attributed to the parasite outbreaks was poor environmental condition.

Gregarine was categorized as one of the protozoan parasites that generally reported not to cause serious effect to their host (Desportes and Schrevel, 2013).

Some of the gregarine parasite hosted in the commercially cultured species such as shrimp, prawns, crabs and bivalves (Setna and Bhatia, 1934; Jones et al., 1994; Clopton, 2002; Jimenez et al., 2002). Most of this cultured species may potentially be infected by this parasite and make them as an intermediate and definitive host (Ball, 1938; 1959; 1963). From previous reports most of the gregarine infection cases indicated that they started from 1800'. In most cases the present of gregarine protozoan parasite infection in wild and cultured populations showed a level of prevalence up to 41% in bivalves and widely spread in shrimp with a prevalence of 10 to 90%. (Humphrey et al., 1998; Humphrey and Norton, 2005; Peter et al., 2010 and Maguregui, 2020).

In Malaysia, detailed study of gregarine parasite was scanty especially in the bivalves. Most of the infections were reported from the imported bivalves. There was a report on this parasite which originated from bivalve species imported from Thailand (Kua and Taha, 2004). Recently, there was a local detection from bivalves cultured in Penang, Johor, Terengganu and Sabah (Kua et al., 2013; Marina et al., 2015). In Thailand, most of the problem reported regarding to protozoan disease was heavy infestation with *Nematopsis* spp. particularly in *Perna viridis* which had caused heavy annual losses in the green mussel industry (Tuntiwaranuruk et al., 2008). Currently, there was also a report of mortality cases in the shrimp culture that has been associated with this parasite (Jones et al., 1994). Nevertheless, preliminary study made on this parasite showed a high infection prevalence in the Hairy cockle, *Anadara cornea* (Reeve, 1844) and another commercial bivalve from East coast of Peninsular Malaysia (Ihwan et al., 2018a; 2018b).

Furthermore, a previous study done indicated that this parasite probably showed effects in the epithelial cell by the penetration of trophozoites into the gills, muscles and intestinal wall of the infected host (Stephen et al., 2005; Walker et al., 2007; Rueckert and Leander, 2008). This method of cell penetration could make real damage to the host if the number of parasites was in high intensity. It also showed that severely infected in shrimp will manifested yellowish discoloration in the midgut as seen through the cuticle of the host abdomen (Bower, 1996).

Bivalves is main sources of food for crustaceans such as crab and shrimp/prawn in the natural environment. Cockles genus *Anadara* sp., Mud crab genus *Scylla* sp. and giant freshwater prawn genus *Macrobrachium* sp. are commercial species that are highly cultured and produce in Malaysia. Currently, the culture of mud crab and prawn are depending on wild live food as a source of nutrient particularly to give enough supplement to stimulate hormone production for broodstock to breed and enhanced their eggs production (Colin and Alessandro, 2011). If the source of live food for these species cultured is not properly secure and managed, the possibilities of the cultured species can be as intermediate host to the parasites is high.

The aquaculture research nowadays has been focusing more on the diseases of fish and shrimp rather than crab and prawn. Currently, there is an overdemand for mud crabs and prawns that has led to over-exploitation in many areas (Keenan, 1998). Industry of mud crab and prawn culturing needs to apply proper and sustainable strategies in the culture procedures in order to fulfill the high demand of mud crab and prawn as exotic seafood. Few studies on crab disease have been published in Malaysia even though this species was cultured for commercial purposes for sometime. However, no comprehensive studies about gregarine parasites infection and its effect to the aquaculture industry of wild mud crab and prawn has been done. In Malaysia, studies about gregarine parasites in aquatic species are poorly known especially to the important commercial crustacean species. This group of parasites is reported nowadays as indicated by a preliminary study done to determine the occurrence of gregarine parasites in wild bivalves at Setiu Lagoon Terengganu, Malaysia. Even mortality cases have been reported. Thus, if this problem is not promptly control, it will affect the aquaculture sector and seafood trading industries locally and worldwide.

The international organization that controls of the quality of exported seafood species such as OIE will basically banned the countries that break the rules and regulation in unsustainable culture method and disease control. The occurrence of the gregarine parasites will affect Malaysia seafood export industry if the quality of the seafood produced is not safely monitored and control. It is very important to control this problem as to ensure the quality of bivalves and crustacean production always in accordance to an international safety standard especially for Malaysia food safety. Currently, there is no detectable symptom to determine the occurrence of this parasite. So, it is farmer and stakeholder's responsibility to follow export protocols of international standard such as quarantine procedure in order to prevent this problem.

The prevalence of gregarine parasites in wild bivalves is important indicator for sustainability of ecosystem health due to interdependence to survive. The data or information for health status of wild bivalves is important for commercial crustacean culture in the future. All prominent species in the Setiu Lagoon of Terengganu state, are economically important due to the utilisation for both local consumption and commercial market in the region. Thus, this undertaken study will be an important report that can be used and explored for future research, problems shooting and other economic perspectives.

Preliminary study of gregarine parasite in the wild bivalves population has yet been reported. Thus, this study is important to ensure that the infestation of this parasite will not cause a problem for the expanding commercial bivalve's aquaculture in Malaysia. The data obtained can be used to develop a better treatment and quarantine procedure for the bivalves aquaculture. Ecosystem health status is very important to secure the overall future of crustacean culture industry.

This study was undertaken in order to determine the species of parasites that commonly infecting species of bivalves and crustacean in Malaysia. Beside that, an in-depth and detailed correlation between the prevalence of the parasites and environmental factors such as season and water quality that might contributed to the intensity of the parasites infestation needs to be clarified. It is important to know detailed information in order to understand the complete lifecycle of this gregarine. Moreover also, rapid and correct diagnosis of the parasite infestation are important to safe guard the national aquaculture industry for sustainable future food supply. The comprehensive information of the gregarine parasite occurrence can be a platform for future study in different perspectives such as development of vaccines and treatment to cure the infection. Importantly, species identification of the gregarine parasites in the natural host and environment needs to be urgently clarified.

Thus, the hypothesis of this study was the occurrence of gregarine parasites is present at a level equal to or greater than found in the bivalve compared to mud crab and prawn. The mean intensity of gregarine parasite occurrence in the bivalves is also increased during the rainy season than a dry season. It also has the potential to infect other crustacean species such as crab and prawn. The bivalve is the intermediate host for gregarine parasites to transmit into the crab as a definitive host. The required level of confidence in the surveillance study is greater than or equal to 95 %.

The objectives of this study were:

1. to investigate the occurrence of gregarine parasite in wild bivalve species from Setiu Lagoon, Terengganu.
2. to identify the species of gregarine parasite from wild Hairy cockle, *Anadara cornea* (Reeve, 1844) found in Setiu Lagoon, Terengganu.
3. to determine the prevalence of gregarine parasites in wild Hairy cockle, *Anadara cornea* (Reeve, 1844), Mud crab, *Scylla* sp. and Giant freshwater prawn, *Macrobrachium rosenbergii* (De Man, 1879) from Setiu lagoon, Terengganu.
4. to correlate the association of gregarine parasite occurrence in the host with seasonal changes and water qualities.
5. to determine the oral transmission of gregarine infection from infected Hairy cockle, *Anadara cornea* (Reeve, 1844) as food to the Mud crab, genus *Scylla*.

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APPENDICES

Appendix A1

MEGA-X Software DNA Analysis

a. MEGA User Mode

MEGA can be used with either a graphical user interface (useful for visual exploration of data and results) or a command-line interface (useful for batch or scripted execution). The graphical user interface (GUI) is run in one of two modes. The first mode is the *Analyze* mode in which all GUI tools in MEGA are enabled and visual results explorers are available for tasks such as editing sequence alignments and viewing phylogenies. This is the mode that most MEGA users are familiar with. The second mode is the *Prototype* mode which is used solely for generating MEGA Analysis Options (.mao) files that specify analysis settings when using MEGA from a command shell. The command-line interface of MEGA is accessed by opening a command shell and executing the megacc command. The megacc command requires several options, including the path to a .mao file and paths to input data file(s) to be analyzed

Appendix A2

MEGA-X Software DNA Analysis

b. Aligning Sequences (using GUI)

MEGA supports sequence alignment using both the ClustalW and MUSCLE programs. Alignment (or refinement) is done in the Analysis Explorer (*Alignment > Open Alignment Explorer* from main menu). Software either can start with a blank alignment (if we are importing sequences from *NCBI*, or don't have a compatible sequence file) or from a compatible sequence file. By key in the sequences in the Alignment Explorer (AE), the select Alignment from the menu can be preceded then either using *ClustalW* or Muscle. Set the alignment parameters to the values that required or leave the options alone to use the defaults. Click Compute/OK. Depending on the length and number of sequences you may see a progress bar while the alignment is running. The aligned sequences will replace the previously unaligned sequences in the Alignment Explorer. The results may now export them to MEGA or Fasta format for analysis.

Appendix A3

MEGA-X Software DNA Analysis

c. Running an Analysis (using GUI)

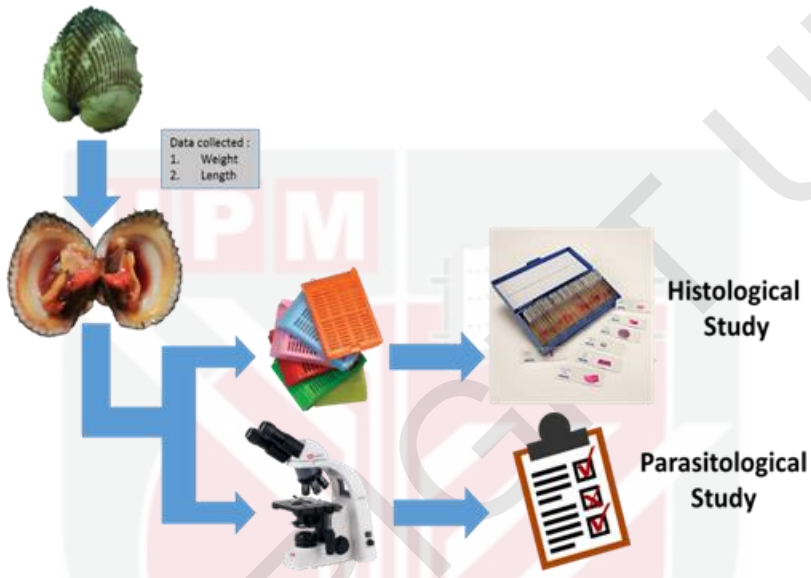
(*Note: Sequences MUST be aligned before analysis can proceed.*)

Select the analysis required to run from the top toolbar in the main window and are shown a list of options for this analysis. It can only change the options which are drawn in a white box. Click Compute. Depending on the length of the analysis you may see a progress bar while the analysis is running. The output will appear as a Tree, Matrix, Text, etc. In most results there will be the option to save your analysis. This usually resides in the File or Data menus of the results window.

Appendix B1

Experimental Design

Figure 2. Flow of Methodology for Objective.

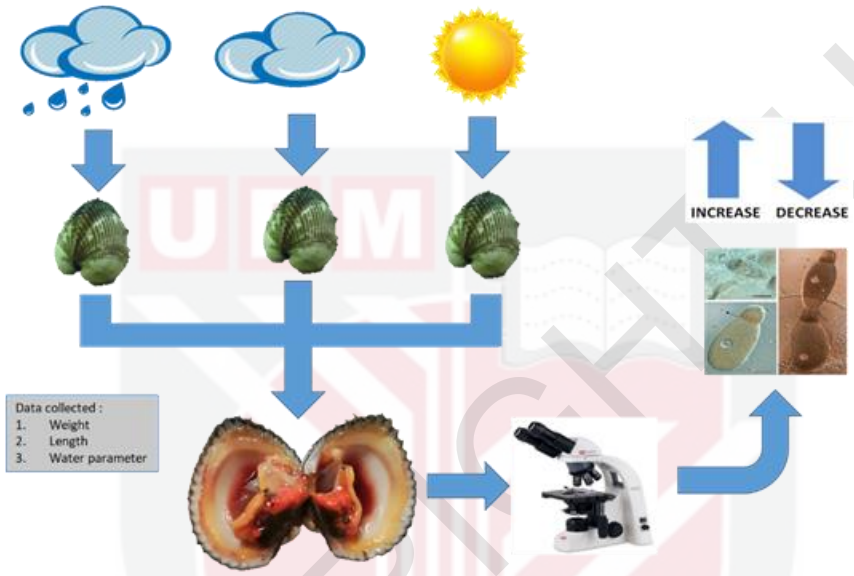


Note: All samples were examined for the species prevalence and mean intensity.

Appendix B2

Experimental Design

Figure 3. Flow of Methodology for Objective.



Note: Sample that collected in objective 2 were group into dry, moderate and rainy seasons to relate the occurrences of the parasites in the respective seasons.

Appendix B3

Experimental Design

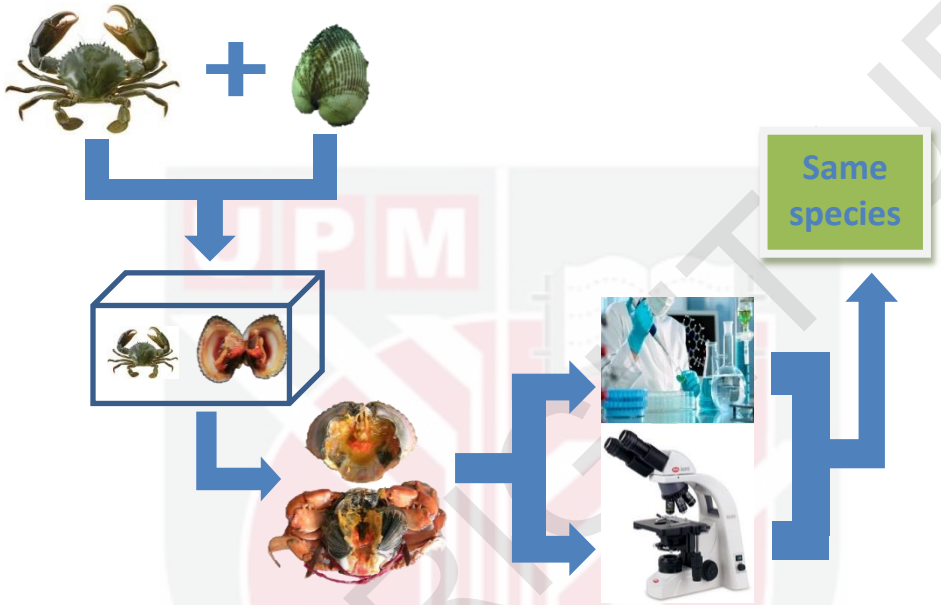
Figure 4. Flow of Methodology for Objective.

Note: Sample that collected were used for morphological characteristic study and DNA application

Appendix B5

Experimental Design

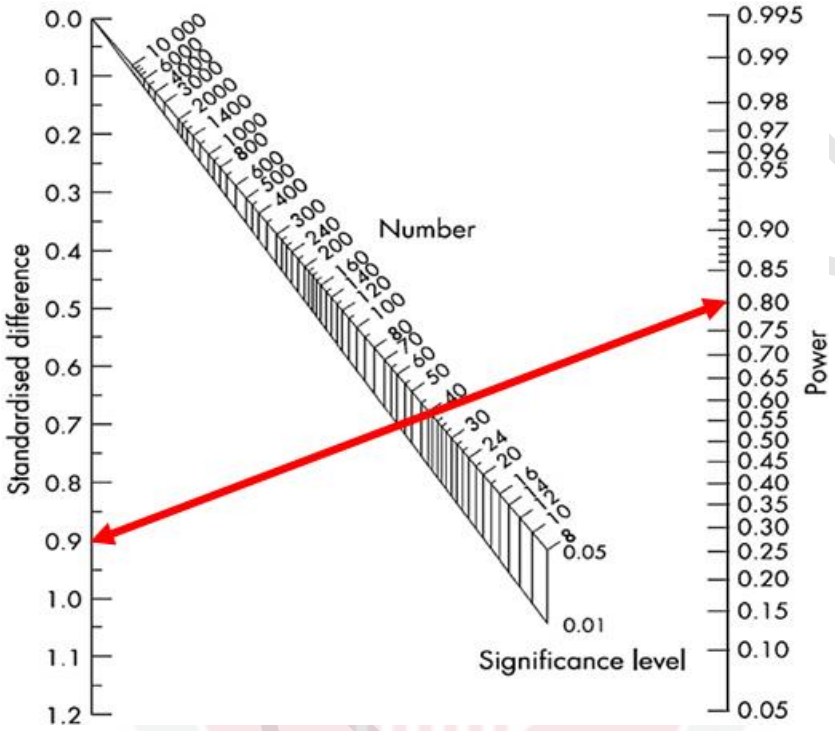
Figure 5. Flow of Methodology for Objective.



Note: *In-vitro* co-habitation test will be applied to confirm the horizontal transmission from intermediate host to definitive host. Data DNA species from objectives 2 will be used as a reference to detect the present of gregarine in the crab after experiment. The result from both bivalves and crab will be compare either species that was detect similar or different.

Appendix C1

Figure 1. Altman's Nomogram chart for sample size calculation.



Note : Red line showed the suggested number for this sampling study.

Appendix D1

Table 1. Summarized of gregarine reported infecting the aquatic species worldwide

Species	Host species	Group of hosts	Origin	References
Frenzelina delphinia	*Thalorchestia longicorni*	Sand Flea	Oyster Bay, Long Island	Watson, 1916
Frenzelina nigrofugsa	*Uca pugnax* *Uca pugilator*	Fiddler crab		
Frenzelina olivia	*Libinia dubia*	Spider Crab		
Polyplicarium lacrimae *Polyplicarium curvarae* *Polyplicarium translucidae*	*Notomastus tenuis*	Polychaete	Boundary Bay, Tsawwassen	Wakeman & Leander, 2013
Polyplicarium citrusae	*Heteromastus filiformis*		Jericho Beach, Vancouver	
Unknown	*Parapeneopsis sculptilis*	Prawn	Bombay, India	Setna & Bhatia, 1934
Caridohabitans setnai	*Peneus semisulcatus*			
Carcinoecetes etisi	*Etisus laevimanus*	Crabs		Ball, 1959
Carcinoeretes matutae	*Matuta lunaris*			
Cephaloidophora carpilodei	*Carpilodes rugatus*	Xanthid Crab	Coconut Island, Hawaii	Ball, 1963
Cephaloidophora pinguis				
Unknown	*Balanus eburneus*	Barnacles		
Thiriotia pugettiae	*Pugettia gracilis*	kelp crab	Vancouver Island, Canada	Rueckert, et al., 2011
Cephaloidophora communis	*Balanus glandula* *Balanus balanus*	barnacles	Moscow	
Heliospora longissima	*Eulimnogammarus verrucosus* *Eulimnogammarus vittatus*	freshwater amphipods	Lake Baikal	
Heliospora caprellae	*Caprella alaskana*	skeleton shrimp	Vancouver Island	
Nemaptosis marinum	*Litopanaeus vannamei*	Shrimp	Ecuador	Jimenez, et al., 2002
Nematopsis mytella	*Crassostrea rhizophorae*	Oyster	Brazil	Padovan et al., 2003.

Continued Table 1

Nematopsis sp.	*Callista chione*	Clam	Italy	Canestri-Trotti, et al., 2000
	Anadara granosa	Blood cockle	Thailand	Tuntiwaranuruk et al., 2004
			Malaysia	Uddin 2010
	Perna veridis	Green muscle	Thailand	Tuntiwaranuruk et al., 2004
	Perna veridis		Malaysia	Kua and Taha., 2004
	Gerastoderma edulis	Clam	Portugal	Azevedo, 1992
	Ruditapes decusatus			
	Arcuatula arcuatula	Clam	Thailand	Tuntiwaranuruk et al., 2004
	Paphia undulata	Mussel	Thailand	
	Crassostrea irredilai	Tropical oyster	Philippines	Erazo-pagador., 2010
			Malaysia	Kua and Taha., 2004.

Appendix D2

Table 2. List of existing gregarine-host relationships and the known effects of these infections on their hosts.

Host order	Host family	Host (common name)*	Gregarine species*	Effect	Disease	Effect#	References
Odonata	Calopterygidae	*Calopteryx splendens* (Banded demoiselle)	Gregarinasina indet.	Strong selection pressure against host inbreeding and homozygosity.		+	Kaunisto et al. (2013)
		Calopteryx splendens xanthostoma (Yellow-tailed damselfly)	Gregarinasina indet.	Infection during teneral life phase results in reduced fat content, which equals reduced fitness of the host. Reduced ability to fight for and maintain a territory.		-	Siva-Jothy et al. (1999)
		Mnais costalis (Broad-winged damselfly)	*Hoplorhynchus polyhamatus*	Gregarine abundance has relatively small effects on the survival when food availability is high, but lowered longevity for parasitized hosts under low food conditions		-; +/-	Tsubaki and Hooper (2004)
	Coenagrionidae	*Enallagma praevarum* (Arroyo bluet)	*Eugregarine*	More female hosts infected. Reduced egg production, and lower survival in male hosts		-	Canales-Lazcano et al. (2005)
		Enallagma boreale (Boreal bluet)	*Hoplorhynchus* sp.	Increased host survival with gregarine burden		+	Hecker et al. (2002)

Continued Table 2

	Libellulidae	*Libellula pulchella* (Twelve-spotted skimmer)	*Hoplorhynchus* sp.	Negative effect on host fat content and reduced muscle power. Low fat content and muscle power affect territory holding ability, which could result in lower offspring.		-	Marden and Cobb. (2004)
		Libellula pulchella (Twelve-spotted skimmer)	*Hoplorhynchus* sp.	Hosts reduced muscle performance (muscles do not oxidize fatty acids), lipid accumulates in their thorax. Elevated levels of blood carbohydrates that do not respond normally to insulin and signs of chronic systemic inflammation	Metabolic Syndrome	-	Schilder and Marden. (2006)
Orthoptera	Acrididae	*Schistocera gregaria* (Desert locust)	*Gregarina garnhami*	Reduced weight of male locusts, otherwise no effect.		(-)	Harry (1970)

Continued Table 2

	Gryllidae	*Gryllus bimaculatus* (Two-spotted cricket)	Gregarinasina indet.	Increased male guarding behavior with increased gregarine infection. Guarding ability of heavily infected males shorter, which could lead to reduced fertilization effect.	(-)	Simmons (1990)
		Gryllus veletis (Spring field cricket), *G. pennsylvanicus* (Fall field cricket)	Gregarinasina indet.	No differences between infested and non-infested hosts in weight change, longevity and fecundity when hosts fed ad libitum. Reduced longevity and increased weight loss when hosts kept with suboptimal diets. Infected hosts needed longer for development from second to third instar.	-; +/-	Zuk (1987)
	Pyrgomorphidae	*Atractomorpha crenulata* (Tobacco grasshopper)	*Leidyana subramanii, Retractocephalus dhawanii* sp. n.	Reduced food consumption as well as body weight considerably compared to the control	-	Johny et al. (2000)
Blattodea	Ectobiidae	*Blattella germanica* (German cockroach)	*Eugregarine*	Swollen abdomens, slower movement at high incidences of the protozoan, and short antennas – septicemia?	-	Lopes and Alves (2005)
Psocoptera	Psyllipsocidae	*Dorypteryx domestica* (Cave barklouse)	*Enterocystis dorypterygis sp. n.*	No mention	+/-	Rueckert and Devetak. (2017)

Continued Table 2

Hemiptera	Aphididae	*Aphis fabae* (Black bean aphid)	*Hirmocystis theodoridesi*	No mention	+/-	Yumnam and Mohilal. (2017)
	Gerridae	*Gerris buenoi* (Blue-winged water strider)	Gregarinasina indet.	No effect, but blocking the gut passage, gregarines must impede food flow and most likely also nutrient absorption in the midgut.	+/-	Klingenberg et al. (1997)
Coleoptera	Curculionidae	*Ips sexdentatus* (Six-toothed bark beetle)	*Gregarina typographi*	No mention	+/-	Yaman (2007)
	Scarabaeidae	*Aphodius depressus* (Dung beetle)	*Gregarina macrocephalia*	Hypertrophy of gastric caeca, rapture leading to septicemia and death	-	Lipa (1967)
		Melolontha melolontha (Cockchafer)	*Neogregarine*	No mention	+/-	Yaman et al. (2016)
	Tenebrionidae	*Lagria hirta* (Rough-haired lagria beetle)	*Gregarina rostrata*	Destruction of gut epithelial cells	-	Lipa (1967)
		Tenebrio molitor (Mealworm beetle)	*Gregarina* sp.	Enlarged host growth	+	Sumner (1933)
		Tenebrio molitor (Mealworm beetle)	*Gregarina* sp.	Positive impact on host development, fitness and longevity	+	Valigurová (2012)
		Tenebrio molitor (Mealworm beetle)	*Gregarina niphandrodes*	No detectable effect on the transition probabilities between different stages of the life cycle in a population, but lower longevity of highly infected hosts.	-; +/-	Rodriguez et al. (2007)

Continued Table 2

		Tenebrio molitor (Mealworm beetle)	*Gregarina cuneata*	Destruction of gut epithelial cells	-	Lipa (1967)
		Tribolium castaneum (Red flour beetle)	*Gregarina cuneata*	Occlusion of host midgut. Nutrient depletion in host leading to decreased growth, delayed development and high mortality.	-	Gigliolli et al. (2016)
		Tribolium castaneum (Red flour beetle)	*Mattesia dispora*	Potential for increased mortality in some host strains	-	Laird (1959)
	Monotomidae	*Rhizophagus grandis*	*Mattesia sp.*	No mention	+/-	Yaman et al. (2012)
	Chrysomelidae	*Chrysolina fastuosa* (Dead-nettle leaf beetle)	*Gregarinasina indet.*	No mention	+/-	Yaman et al. (2011)
		Chrysolina herbacea (Mint leaf beetle)	*Neogregarine*	Possible cause of tissue malfunction and disorders in host	-	Bekircan et al. (2016)
		Chrysomela populi (Poplar leaf beetle)	*Ophryocystis anatoliensis* sp.	Reddening of Malpighian tubules in host	(-)	Yaman and Radek (2017)
		Diabrotica virgifera virgifera (Western corn rootworm)	*Eugregarine*	Increase adult mortality and inhibit ovarian and fat body development	-	Kuhlmann and Van (1998)
		Diabrotica virgifera virgifera (Western corn rootworm)	*Eugregarine*	No mention	+/-	Brooks and Jackson (1990)
Diptera	Culicidae	*Aedes aegypti* (Yellow fewer mosquito)	*Ascogregarina culicis*	Mortality increases with gregarine dose	-	Sulaiman (1992)
		*Aedes aegypti (*Yellow fewer mosquito)	*Lankesteria culicis*	Stunted / dead larvae – also pupae and adults “damage to the Malpighian tubes in all the infected stages of the insect”	-	Barrett (1968)

Continued Table 2

		Aedes albopictus (Asian tiger mosquito)	*Ascogregarina taiwanensis*	With low nutrients, mosquitos, larvae and pupae had increased mortality	-	Comiskey et al. (1999)
		Aedes geniculatus (Tree Hole mosquito)	*Ascigregarina eniculate*	Although nonpathogenic in normal host in others it may reduce fitness (Walsh and Olson 1976)	(-)	Mustermann and Levine (1983)
		Aedes hendersoni (Tree Hole mosquito)	*Ascogregarina barretti*	Natural parasite of *A. triseriatus* but infected *A hendersoni* larvae competed poorly	-	Copeland and Craig (1992)
		Aedes polynesiensis (Polynesian tiger mosquito)	*Lankesteria culicis*	Minimal effect on host	(-)	Pillai and Sone (1976)
		Aedes triseriatus (Eastern tree hole mosquito)	*Ascogregarina barretti*	Reduction in wing length	-	Siegel et al. (1992)
		Aedes triseriatus (Eastern tree hole mosquito)	*Ascogregarina barretti*	Reduced host cohort mortality. Hosts seeking more refuge, less thrashing leading to reduced predation.	+	Soghigian et al. (2017)
		Ochlerotatus sierrensis (Western tree hole mosquito)	*Ascogregarina clarki*	Destruction of midgut epithelial cells during its trophic stage and Malpighian tubule cells during gametogenesis and sporogony	-	Sanders and Poinar (1973)
	Psychodidae	*Lutzomyia longipalpis* (Sand fly)	*Ascogregarina chagasi*	“Reduced adult longevity of the Brazilian strain of *L. longipalpis*, but it had little effect on fecundity”	-	Wu and Tesh (1989)

Continued Table 2

		Lutzomyia longipalpis (Sand fly)	*Gregarinasina indet.*	Reduced longevity and egg production	-	Dougherty and Ward (1991)
		Phlebotomus sergenti (Sand fly)	*Psychodiella sergenti*	Increased the mortality of immature sand fly stages, negatively affected the survival of adult males and females (no effect on blood-fed females)	-	Lantová et al. (2012)
Siphonaptera	Pulicidae	*Ctenocephalides felis* (Cat flea)	*Steinina ctenocephali*	Accelerated host larval development	+	Alarcón et al. (2016)
		Ctenocephalides felis (Cat flea)	*Gregarina ctenocephali*	No mention	+/-	Alarcón et al. (2011)
Lepidoptera	Pyralidae	*Ephestia kuhniella* (Mediterranean flour moth)	*Mattesia dispora*	Destruction of fat body of the host leading to death.	-	Lipa (1967)
		Ephestia kuhniella (Mediterranean flour moth)	*Leidyana ephestiae*	Destruction of gut epithelial cells	-	Lipa (1967)
Hymenoptera	Vespidae	*Polybia occidentalis* (Camoati)	*Eugregarine*	Host individuals foraging rates reduced, colony nests with fewer brood cells and fewer brood mass per capita per, but lower adult mortality (due to lower risk to foragers)	-; (+)	Bouwman, A.M. et al. (2005)
	Formicidae	*Myrmecia pilosula* (Jack's jumper ant)	Gregarinasina indet.	Brown instead of black coloured ant with softer bodies. Higher mortality rate is suggested	(-)	Crosland (1988)
Heteronemertea	Lineidae	*Lineus ruber* (Red ribbon worm)	*Urospora nemertes*	Occasional destruction of host columnar cells, but overall, no harm	+/-	Jennings (1969)

Continued Table 2

Diplopoda	Julidae	*Schizophyllum sabulosum* (Striped millipede)	*Stenophora schizophylli*	Destruction of gut epithelial cells		-	Lipa (1967)
Pseudoscorpiones	Withiidae	*Victorwithius similis* (pseudoscorpion)	Gregarinasina indet.	High parasite intensity levels increased host survival rate. Most results indicate no effect.		(+)	Bollatti and Ceballos (2014)
Mollusca		Marine bivalves	Species of the family Porosporidae, e.g. *Nematopsis ostreum*	Reports concerning the pathogenicity of *Nematopsis* spp. infestations in bivalves are inconclusive.		+/-	Lauckner (1983)
Mollusca, Gastropoda		Marine bivalves and gastropods as intermediate hosts	*Nematopsis legeri*	Minor local lesions on the surfaces of the host's gill lamellae, but only slight general effect on host		+/-	Lauckner (1980)
Enterogona	Cionidae	*Ciona intestinalis* (Sea vase)	*Lankesteria ascidiae*	Clogging of intestine, mortality in hosts often quite high	Long Faeces Syndrome	-	Mita et al. (2012)
Anura	Ranidae, Hylidae	*Rana temporaria* (European common frog), *R. dalmatina* (Agile frog), *Hyla arborea* (European tree frog)	*Nematopsis temporarirae*	Intracellular infection of liver cells. Some livers slightly enlarged and light coloured, but no signs of disease or impairment of fitness/function		-/+	Chambouvet et al. (2016)

*Highlighted in bold are potential gregarine-host systems for future investigations of symbiosis. # **-,** negative effects on host; +, positive effects on hosts; **+/-,** neither positive nor negative effects, or both effects on hosts; (+) or (-), minor effects.

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Mohd Ihwan Bin Zakariah was born on August 8, 1983 in Hospital Besar Melaka. He completed his primary and secondary school at Durian Tunggal Primary School (SKDT) and Durian Tunggal Secondary School (SMKDT) respectively. He continued his study in Diploma of Fisheries at the University College of Science and Technology (KUSTEM), Terengganu. He further his study in Bachelor of Science in Marine Biology (2004-2007) and Master of Science in Aquaculture (2011-2014) at Universiti Malaysia Terengganu (UMT), before he proceeds for master study, he worked as Science Officers at the Institute of Tropical Aquaculture and Fisheries (AKUATROP) at UMT starting from 2007 till 2014. After finish his master, he working as Research Officer at the same institute starting in 2015 till now. Finally, he continued his PhD study in the field of Aquatic Animal Health at the Faculty of Veterinary Medicine, Universiti Putra Malaysia, under the supervision of Assoc. Prof. Dr. Haji Hassan Haji Mohd Daud.

LIST OF PUBLICATION

Mohd Ihwan Zakariah, Hassan Mohd Daud, Reuben Sunil Kumar Sharma, Marina Hassan and Ikhwanuddin, M., (2018). Histological Occurrence Study of *Nematopsis* spp. Oocyst in Wild Common Bivalve from Setiu Lagoon, Malaysia. *8th International Fisheries Symposium 2018*, Hatyai, Thailand. 18-21 November 2018.

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PREVALENCE OF GREGARINE PARASITES IN *Anadara cornea* (Reeve, 1844) AND ITS POTENTIAL INFECTIVITY TO *Scylla* sp. AND *Macrobrachium rosenbergii* (De Man, 1879)

MOHD IHWAN BIN ZAKARIAH

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