

STUDY ON *In-vitro* FERTILIZATION OF ORANGE MUD CRAB,
Scylla olivacea (Herbst, 1796)

AMIN SAFWAN BIN ADNAN

LP
2
PPSMS
1
2014

SCHOOL OF MARINE SCIENCE AND ENVIRONMENT
UNIVERSITI MALAYSIA TERENGGANU

2014

Study on *In-vitro* Fertilization of Orange Mud Crab, *Scylla olivacea*

(Herbst, 1796)

By

Amin Safwan bin Adnan

**Research Report submitted in partial fulfillment of the requirement for the degree
of Bachelor of Science (Marine Biology)**

School of Marine Science and Environment

UNIVERSITI MALAYSIA TERENGGANU

2014

S.A. Amin. (2014). Study on *In-vitro* Fertilization of Orange Mud Crab, *Scylla olivacea* (Herbst, 1796). Undergraduate thesis, Bachelor of Science in Marine Biology, School of Marine Science and Environment, Universiti Malaysia Terengganu, Terengganu, 55p.


No part of this project report may be produced by any mechanical, photographic, or electronic process, or in the form of phonographic recording, nor may it be stored in a retrieval system, transmitted, or otherwise copied for public or private use, without written permission from the author and the supervisor(s) of the project.



**SCHOOL OF MARINE SCIENCE AND ENVIRONMENT
UNIVERSITI MALAYSIA TERENGGANU**

**DECLARATION AND VERIFICATION REPORT
FINAL YEAR RESEARCH PROJECT**

It is hereby declared and verified that this research report entitled Study on *In-vitro* Fertilization of Orange Mud Crab, *Scylla olivacea* (Herbst, 1796) by Amin Safwan bin Adnan, Matric No. UK 25525 have been examined and all errors identified have been corrected. This report is submitted to the School of Marine Science and Environment as partial fulfillment towards obtaining the Degree in Bachelor of Science (Marine Biology), School of Marine Science and Environment, Universiti Malaysia Terengganu.

Verified by: 

First Supervisor
Name: *Mhd. Ikhwanuddin*

15/6/14

Official stamp: **PROF. MADYA DR. MHD. IKHWANUDDIN ABDULLAH** Date:
Pensyarah
Institut Akuakultur Tropika
Universiti Malaysia Terengganu (UMT)
21030 Kuala Terengganu, Terengganu

Christine R. Desser

Second Supervisor

Name:

Official stamp: **ASSOC. PROF DR. SITI AISHAH ABDULLAH** Date: *16 June 2014*
Lecturer
School of Marine Science and Environment
Universiti Malaysia Terengganu
21030 Kuala Terengganu

ACKNOWLEDGEMENT

Foremost, I would like to express my sincere gratitude to my supervisor, Assoc. Prof. Dr. Mhd. Ikhwanuddin bin Abdullah for the continuous support of my final year project, for his patience, motivation, enthusiasm, and immense knowledge. His guidance helped me in all the time of research and writing of this thesis. I could not have imagined having a better advisor and mentor for my final year project research besides him. Besides that, I would like to thank my co-supervisor, Assoc. Prof. Dr. Siti Aishah binti Abdullah for her caring, loves and enthusiasm that help me to complete my thesis writing. My sincere thanks also goes to Miss Baiduri binti Shaabani for offering me a special help and guidance that lead me working on diverse exciting projects. I thank my fellow classmates: Irma Wahida binti Shakri, Zulaikha binti Hamzah and Nor Diana binti Pilus for the stimulating discussions, for the sleepless nights we were working together before deadlines, and for all the fun we have had in the laboratory together. Special thanks also to all staffs of AKUATROP and hatchery that always help and cooperate with me easily. Last but not the least, I would like to dedicate my special thanks to my family especially my parents Mr. Adnan bin Kasim and Madam Safizon binti Saad, for giving birth to me at the first place and supporting me spiritually throughout my life.

TABLE OF CONTENTS

	Page
ACKNOWLEDGEMENTS	ii
LIST OF TABLES	vi
LIST OF FIGURES	vii
LIST OF ABBREVIATIONS	viii
ABSTRACT	ix
ABSTRAK	x
CHAPTER 1: INTRODUCTION	
1.1 Research background	1
1.2 Problem Statement	4
1.3 Significant of Study	5
1.4 Objectives of Study	5
CHAPTER 2: LITERATURE REVIEW	
2.1 Mud crab	6
2.1.1 Taxonomy status	7
2.1.2 <i>Scylla olivacea</i>	9
2.1.3 Species distribution	10

2.1.4	Natural distribution	10
2.1.5	Lifecycle	12
2.2	Reproductive biology	13
2.2.1	Male reproductive system and sperm	14
2.2.2	Female reproductive system and ovary	15
2.2.3	Size at maturity	16
2.2.4	Mating	16
2.2.5	Spawning and hatching	17
2.2.6	Embryonic development	18
2.3	Breeding technology of mud crab	19
2.3.1	Artificial insemination	19
2.3.2	<i>In-vitro</i> fertilization	20

CHAPTER 3: METHODOLOGY

3.1	Study site	21
3.2	Crab sample	22
3.3	Experimental design	22
3.4	Induce ovarian development	24

3.5	Preparation of artificial seawater	25
3.6	<i>In-vitro</i> trials	25
3.7	Data collection and analysis	28
CHAPTER 4: RESULT		
4.1	<i>In-vitro</i> trials	29
4.2	Early embryonic stage development	34
CHAPTER 5: DISCUSSION		
5.1	<i>In-vitro</i> fertilization of <i>S. olivacea</i>	37
5.2	Development of early embryonic stage of <i>S. olivacea</i>	40
CHAPTER 6: CONCLUSION AND RECOMMENDATION		43
REFERENCES		45
APPENDICES		52
CURICULUM VITAE		55

LIST OF TABLES

	Table	Page
4.1	Success rate of In-vitro trials on <i>S. olivacea</i>	30
4.2	The fertilization rate of <i>In-vitro</i> trials from female Stage 4 ovary with the sperms from the female spermatheca of <i>S. olivacea</i>	31
4.3	The fertilization rate of <i>In-vitro</i> trials from the female Stage 4 ovary with the sperms from the male of <i>S. olivacea</i>	32
4.4	The development of early embryonic stage of <i>S. olivacea</i> from the successful <i>In-vitro</i> trials	34

LIST OF FIGURES

	Figure	Page
2.1	The differentiation of dorsal view and claws of <i>Scylla</i> sp.	8
2.1 (a)	<i>S. serrata</i>	
2.1 (b)	<i>S. paramamosain</i>	
2.1 (c)	<i>S. olivacea</i>	
2.1 (d)	<i>S. tranquebarica</i>	
3.1	Hatchery of AKUATROP	22
3.2	Experimental design for entire procedures in conducted trials	23
3.3	The vas deferens in male crab	27
3.4	The spermatheca in female crab	27
4.1	Fertilization rate of <i>S. olivacea</i> that utilized spermatheca and vas deferens	33

LIST OF ABBREVIATIONS

L	-	liter
ml	-	milliliter
cm	-	centimeter
mm	-	millimeter
g	-	gram
ppt	-	part per thousand
%	-	percentage
°C	-	degree Celsius

ABSTRACT

Treated with eyestalk ablation and continuous feeding of squids which is *Loligo* spp, female mud crab, *Scylla olivacea* were induced for ovarian maturation till Stage 4 of ovary maturation stage in order to perform *In-vitro* trials. After the female crabs were fully mature enough Stage 4, the crabs were selected and been dissected. There are eight trials that have been done and the trials were divided into two parts which are by using sperms in spermatheca of female crabs and the other one is by using sperms in vas deferens of male crabs. Stages 4 ovary was combined with sperms and were maintained in small aquarium with 30°C of water temperature set by using water heater. Daily observation was done by using compound and advance microscope while the fertilization rate and early stage of embryonic development were calculated and being identified. The fertilization rates were different in each trial. Trial 1, 2 and 3 were failed and starting from Trial 4 until Trial 8, the fertilization rate starting to give favorable result. Trial 4 with 14.00% success of fertilization rate, Trial 5 with 10.00% fertilization rate, Trial 6 with 20.00% of fertilization rate, Trial 7 with 19.78% fertilization rate and Trial 8 with 30.83% fertilization rate. Embryonic development of *S. olivacea* was divided into 10 stages. In this experiment, only five stages were successfully achieved. The stages are cleavage, precleavage, multicell, intermediate multicell-gastrula and gastrula. After gastrula, there are no more embryonic development and the eggs mostly are retarded and dead.

Kajian Terhadap Persenyawaan *In-vitro* ke atas Ketam Bakau, *Scylla olivacea*

(Herbst, 1796)

ABSTRAK

Ketam betina, *Scylla olivacea* telah dirawat dengan mempraktikkan ablasi mata iaitu pemotongan mata dan juga diberi makan sotong iaitu *Loligo* spp. secara berterusan untuk mendorong kepada penghasilan telur ovari pada peringkat keempat untuk dilakukan kaedah ujian secara *In-vitro*. Apabila ketam betina tersebut telah matang ovary peringkat keempat sepenuhnya, ketam tersebut akan dipilih dan akan dibedah. Terdapat dua kaedah ujian *In-vitro* tersebut dilakukan. Pertamanya menerusi penggunaan sperma dalam 'spermatheca' ketam betina, manakala satu kaedah lagi menerusi penggunaan sperma dalam 'vas deferens' ketam jantan. Terdapat lapan ujian yang telah dijalankan. Ovari peringkat keempat telah digabungkan dengan sperma dan dikekalkan dalam akuarium kecil dengan kawalan suhu sebanyak 30°C dengan penggunaan pemanas air. Pemerhatian harian telah dilakukan dengan menggunakan 'compound' mikroskop dan juga 'advance' mikroskop. Selain itu, kadar persenyawaan dan juga peringkat perkembangan embrio telah dikira dan dikenalpasti peringkatnya. Kadar persenyawaan didapati berbeza dalam setiap percubaan. Percubaan 1, 2 dan 3 telah gagal menunjukkan sebarang kadar persenyawaan dan bermula dari percubaan ke 4 sehinggalah percubaan ke 8, kadar persenyawaan menunjukkan hasil yang menggalakkan. Percubaan 4 dengan 14.00% tahap keberjayaannya, Percubaan 5 dengan 10.00% tahap keberjayaan, Percubaan 6 dengan 20.00% tahap keberjayaan, Percubaan 7 dengan 19.83% tahap keberjayaan dan Percubaan 8 dengan 30.83% tahap keberjayaan persenyawaan.

Perkembangan awal embrio *S. olivacea* telah dibahagikan kepada sepuluh peringkat. Dalam kajian ini, hanya lima peringkat pertama sahaja telah berjaya dicapai. Peringkat-peringkat tersebut ialah 'cleavage', 'precleavage', 'multicell', 'intermediate multicell-gastrula' dan 'gastrula'. Selepas 'gastrula' tiada tahap perkembangan embrio yang berjaya dikesan dan kebanyakan telur telah terbantut dan mati selepas tahap ini.