DESCRIPITION OF EARLY DEVELOPMENT IN GIANT CLAM, TRIDACNA MAXIMA (RODING, 1798) AND THE DETERMINATION OF PHYSIOLOGICAL BEHAVIOUR OF ADULT AS A RESPONSE TO TEMPERATURE

HENG WEI KHANG

FACULTY OF MARITIME STUDIES AND MARINE SCIENCE UNIVERSITI MALAYSIA TERENGGANU

2011

LP 12 FMSM 3 2011



1100088802

Description of early development in giant clam, tridacna maxima (roding, 1798) and the determination of physiological behaviour of adult as a response to temperature / Heng Wei Khang



PERPUSTAKAAN SULTANAH NUR ZABIRAH UNIVERSITI MALAYSIA TERENGGANU (UMT) 21830 SHALA TERENGGANU

	11100000	chi
	rsiti malaysia terend 21839 kuala terend 1100088	るにん
	-	
	· .	
*: E		
··		
: :		
	1	1
		- 1
		1
	-	
	<u> </u>	
	0.000	
	1	-
- A	_	
	1.	1
****	1	<u> </u>
6.9		
	 	
5	es.de	1
***************************************		1
		1
	-	

Lihat sebelah

HAK MILIK PERPUSTAKAAN SULTANAH NUR ZAHIRAH UNT

DESCRIPITION OF EARLY DEVELOPMENT IN GIANT CLAM, TRIDACNA MAXIMA (RÖDING, 1798) AND THE DETERMINATION OF PHYSIOLOGICAL BEHAVIOUR OF ADULT AS A RESPONSE TO TEMPERATURE

By

Heng Wei Khang

Research report submitted in partial fulfillment of the requirements for the degree of Bachelor of Science (Marine Biology)

Department of Marine Science
Faculty of Maritime Studies and Marine Science
UNIVERSITI MALAYSIA TERENGGANU
2011

Heng, W. K. 2011. Description of early larval development in the giant clam, *Tridacna maxima* (Röding, 1798) and the determination of physiological behaviour of adults as a response to temperature. Undergraduate thesis, Bachelor of Science in Marine Biology, Faculty of Maritime Studies and Marine Science, Universiti Malaysia Terengganu, Terengganu. 132p.

No part of this project report may be reproduced by any mechanical, photographic, or electronic process, or in the form of phonographic recording, nor may it 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.





DECLARATION AND VERIFICATION REPORT

FINAL YEAR RESEARCH PROJECT

It is hereby declared and verified that this research report entitled:

Description of Early Larval Development in the Giant Clam, *Tridacna maxima* (Röding, 1798) and the Determination of Physiological Behaviour of Adults as a Response to Temperature by Heng Wei Khang, Matric No. UK 17087 have been examined and all errors identified have been corrected. This report is submitted to the Department of Marine Science as partial fulfillment towards obtaining the Degree Bachelor of Science (Marine Biology), Faculty of Maritime Studies and Marine Science, Universiti Malaysia Terengganu.

Verified by:	
Principal Supervisor	
Name: Mdm. Norainy bt. Mohd. Husin	
Official stamp:	Date:
Second Supervisor Name: Dr. Siti Aishah Abdullah@ Christine Abellana Orosco Official stamp: DR. RAZAK ZAKARIYA Ketua Jabatan Sains Marin Universiti Malaysia Terengganu (UMT) Head of Description of Marine Science	Date: 26/4/2011
Head of Department of Marine Science	
Name: Dr. Razak bin Zakariya	Date: 27/4/(1
Official stamp:	Date:

ACKNOWLEDGEMENTS

First and foremost, I would like to give my greatest thanks to Department of Marine Science, Faculty of Maritime Studies and Marine Science, Universiti Malaysia Terengganu (UMT) and PETRONAS Carigali Kerteh, Terengganu for sponsoring the sampling trips to Bidong Island.

Next, I would like to express my sincere gratitude to my first supervisor, Madam Norainy bt. Mohd. Husin for all her guidance, advice and support along the way from drafting proposal until the earlier version of the manuscript writing. Her supervision and support had helped me a lot to design my project title regarding of giant clam cultivation.

My deepest gratefulness also dedicated to my second supervisor, Dr. Siti Aishah Abdullah who had contributed a promising guidance during the whole process especially by suggesting a new field to my title and guiding me in the examination of physiology part in the giant clam. Her thoughtful ideas had helped me in completion of my thesis.

In addition, I would like to give special thanks to Assoc. Prof. Liew Hock Chark who has helped me a lot in statistical analysis, Dr. Juanita Joseph for willingly gave permission to conduct my experiment in Terrapin Hatchery and Dr. Hii Yi Siang for provided me a hitch to done my sampling. Besides, my special appreciation also goes to Ms. Chong Yee Kuen (Master student) and Mr. Lim Peng Chia (Bachelor student), who had helped me during my sampling and experiment work-out.

Without forgetting all the science officers and lab assistants of Laboratory of Biodiversity, Laboratory of Oceanography, Institute of Tropical Aquaculture (AQUATROP), Saltwater Hatchery, Laboratory of Anatomy and Physiology,

Laboratory of SCUBA, Institute of Marine Biotechnology (IMB), Laboratory of Fluid and Freshwater Hatchery, I would also like to thank all of them for their assistance and willingness to borrow me sampling and experimental equipments.

Last but not least, special gratitude is extended to my family and friends who had support me physically and morally. Thanks for their love and encouragement, without it I would not finish my project successfully.

TABLE OF CONTENTS

		P	age
ACK	NOWLEDGEMENTS		ii
LIST	T OF TABLES		vii
LIST	OF FIGURES	,	viii
LIST	OF ABBREVIATIONS		xii
LIST	T OF APPENDICES		xiv
ABS'	TRACT		xvi
ABS	TRAK	2	xviii
CHA	APTER 1: INTRODUCTION		
1.1	Background of Study		1
1.2	Problem Statement		1
1.3	Significance of Study		3
1.4	Objectives		6
CHA	APTER 2: LITERATURE REVIEW		
2.1	General Information of Tridacna maxima		7
2.2	Taxonomic Classification		8
2.3	Ecological Role and Status		9
2.4	Length of Larval Life Span		9
2.5	Cultivation of Giant Clam		10
2.6	Reproductive Biology of Giant Clam		11
2.7	Life Cycle of Giant Clam		12
2.8	Spawning Induction		13
2.9	Zooxanthellae		16

2.10	Photosynthesis	17
CHAI	PTER 3: METHODOLOGY	
PART	I: DESCRIPTION OF EARLY DEVELOPMENT	
3.1	Sampling Site and Experimental Location	19
3.2	Broodstock Selection	20
3.3	Spawning Induction Using Serotonin	22
3.4	Sperm and Egg Released and Fertilization	24
3.5	Larval Culture	26
PART BEH	I II: DETERMINATION OF PHYSIOLOGICAL AVIOUR OF ADULT AS A RESPONSE TO TEMPERATURE	
3.1	Origin of Experimental Animals	30
3.2	Experimental Design	30
3.3	Physiological Measurement	32
3.4	Data Analysis	35
CHA	PTER 4: RESULTS	
PAR	Γ I: DESCRIPTION OF EARLY DEVELOPMENT	
4.1	Size of T. maxima Broodstocks	36
4.2	Spawning Induction of T. maxima	37
4.3	Larval Culture	40
4.4	Early Development of T. maxima	42
4.5	Early Larval Morphologic Development of T. maxima	46
	4.5.1 Fertilized & Unfertilized Eggs	47
	4.5.2 Dividing Embryo	48
	4.5.3 Trochophore	50
	4.5.4 Veliger	52
	4.5.5 Regressive Ova	55

BEHAVIOUR OF ADULT AS A RESPONSE TO TEMPERATURE			
4.1	Diel Gross Primary Production of Zooxanthellae	57	
4.2	Photosynthetic-Irradiance Relationship	60	
4.3	Production and Respiration	64	
4.4	Production to Respiration Ratios	67	
CHA	PTER 5: DISCUSSION		
PAR	Γ I: DESCRIPTION OF EARLY DEVELOPMENT		
5.1	Spawning Induction of T. maxima	69	
5.2	Larval Culture	73	
5.3	Early Development of T. maxima	75	
5.4	Early Larval Morphologic Development of T. maxima	78	
5.5	Overall Description of Early Development	80	
PAR' BEH	T II: DETERMINATION OF PHYSIOLOGICAL AVIOUR OF ADULT AS A RESPONSE TO TEMPERATURE		
5.1	Diel Gross Primary Production of Zooxanthellae	82	
5.2	Photosynthetic-Irradiance Relationship	83	
5.3	Production and Respiration	86	
5.4	Production to Respiration Ratios	87	
5.5	Overall Physiological Behaviour as a Response to Temperature	88	
СНА	APTER 6: CONCLUSION		
PAR	T I: DESCRIPTION OF EARLY DEVELOPMENT	90	
	T II: DETERMINATION OF PHYSIOLOGICAL BEHAVIOUR ADULT AS A RESPONSE TO TEMPERATURE	92	
REF	ERENCES	93	
APPENDICES		100	
VITAE		133	

LIST OF TABLES

Table		Page
3.1	Determination of ripeness by observation (Jameson, 1976).	25
4.1	Spawning record of <i>T. maxima</i> broodstocks.	39
4.2	Number and density of eggs/ larvae in alternate days for third trial.	40
4.3	Chronology of developmental stages in T. maxima.	42
4.4	Production data for three individuals of <i>T. maxima</i> at different temperatures.	61
4.5	Production and respiration of three individuals of giant clam at three temperatures.	65
4.6	Production, respiration and gross production to respiration ratio of three individuals of giant clam at three temperatures within the irradiance range of 1000 to 2000 $\mu mol/\ m^2$ s.	67
5.1	Comparison of induced spawning reactions of <i>T. maxima</i> between present and other studies.	70
5.2	Comparison of time development in early life chronology of <i>T. maxima</i> between the present study and other studies.	76

LIST OF FIGURES

Figure	*	Page
1.1	Upper view, lateral view and dorsal view of <i>T. maxima</i> shell (Braley, 1987).	8
1.2	The basic life stages of giant clam in clam culture (Calumpong, 1992).	12
2.2a	Flow diagram describing sequence and events of induced spawning in giant clams (Ellis, 1992).	14
2.2b	Flow diagram describing sequence and events of intensive larval rearing of giant clams (Ellis, 1992).	15
3.1	Original habitat location of T. maxima broodstocks.	19
3.2	Measuring of shell length using measuring tape in the field for broodstock selection.	20
3.3	Concrete rearing tank with 10-µm filtered natural seawater provided with aeration to accommodate clams in hatchery.	21
3.4	Injection of serotonin hormone into the gonad from the byssal opening in 45°.	23
3.5a	T. maxima (left viewed after removal of valve and mantle lobe showing major organs in mantle cavity peripheral areas). Arrow pointing towards gonad is the direction for injection of serotonin (modified from Yonge, 1981)	23
3.5b	T. maxima, intact animal viewed from under surface showing byssal gape with elongate byssal mass surrounded by middle mantle folds with numerous blunt tentacles. Arrow pointing is the direction for injection of serotonin. (modified from Yonge, 1981)	24
3.6	Two 200 L black tanks used to culture giant clam larvae and provided with aeration. Picture showed tanks which lids have been removed.	26
3.7	Culture of <i>Chaetoceros</i> sp. and <i>Chlorella vagina</i> phytoplankton in Erlenmeyer flasks provided with Conway medium nutrient, artificial light and aeration.	27
3.8	Dimensions of measurement used for larvae.	28

3.9 Top left: mantle fragments in vial; Top right: zooxanthellal brown 29 suspension: Bottom: zooxanthellae cells observed under light compound microscope. 3.10 30 L aquaria which water-jacketed in 200 L tank with temperature 31 maintained by thermostat and heaters. 3.11 Left: side view display of the experimental clam which placed 31 above a PVC scaffold; Right: aerial view display of the clam. 4.1 Four T. maxima's broodstocks. Size (length of shell valve): Ind. A 36 -34 cm; Ind. B -27 cm; Ind. C -23 cm; Ind. D -19 cm. 4.2 Spawning reactions of broodstocks. A: broodstock releasing 37 sperms (first spawning); B: broodstock releasing eggs (second spawning). 4.3 38 Squirting of sperm ("milky smoke") through exhalent siphon by a series of mantle contractions, with ever increasing number of gametes being released with each contraction. Arrow showing expulsion of gonadal debris together with the milt. 4.4 Expulsion of egg cells (granular substances) through exhalent 38 siphons following release of sperm. 4.5 Percentage survival of T. maxima's larvae. 41 4.6 Life cycle of T. maxima 43 4.7 45 Whole shell length of T. maxima's larvae (mean \pm SD) from day 0 until larval mortality. 4.8 Whole shell width of T. maxima's larvae (mean \pm SD) from day 0 45 until larval mortality. 4.9 Morphology of giant clam veliger larvae. 46 4.10 48 Developing to ripe and fertilized ova (Bar: 100 µm). A-B, developing egg; C, unfertilized egg (without chorion); D, unfertilized egg (with chorion); E-F, fertilized egg 4.11 49 Cell divides and undergoes spiral cleavage (Bar: 100 µm). A, single fertilized egg; B, first cleavage (2-celled embryo); C, second cleavage (4-celled embryo); D, third cleavage (8-celled embryo); E, fourth cleavage (16-celled embryo); F, blastula. 4.12 Early trochophore to trochopre-veliger transition state (Bar: 100 51 μm). A-B, late gastrula entering early trochophore; C, trochophore; D, late trochophore entering early veliger.

4.13 Development from early veliger to straight-hinge veliger (Bar: 53 100 um). A, newly formed veliger where the valve enclosed its body; B, early veliger slowly becoming D-shaped; C, side view of early veliger; D, fully developed D-hinge veliger; E, protruded velum with cilia from the valves of D-hinge veliger; F, "winged larvae" glochidia. 4.14 54 Morphological comparison of veliger during day 1 and day 4 in terms of shell length. A, Day 1 veliger was oval circular in shape; B, Day 4 veliger elongated at anteroposterior part of the shell. Angle refers to angle between shoulder and hingle line. 4.15 4 day old larvae showing prodissoconch I and II. 55 4.16 Regressive ova, showing phagocytic amoebocytes (phg) on 56 surface of regressive ova (Bar: 100 μm). A-C, regressive ova; D, regressive ovum showing excessive sperm entering extracellular matrix (ECM). 4.17a Gross production (mg O₂/ h per kg wwt including shell) over time 58 in a day for Ind. L. Wet weight (wwt): 2.05 kg. 59 4.17b Gross production (mg O₂/ h per kg wwt including shell) over time in a day for Ind. M. Wet weight (wwt): 0.75 kg. 4.17c Gross production (mg O₂/ h per kg wwt including shell) over time 59 in a day for Ind. S. Wet weight (wwt): 0.60 kg. 4.18a 61 Gross production (mg O₂/ h per kg wwt) versus irradiance (µmol/ m² s) at 26 °C for three individuals of giant clams. Key: R, respiration. 4.18b 62 Gross production (mg O₂/ h per kg wwt) versus irradiance (µmol/ m² s) at 28 °C for three individuals of giant clams. Key: R, respiration. 4.18c Gross production (mg O₂/ h per kg wwt) versus irradiance (µmol/ 63 m² s) at 30 °C for three individuals of giant clams. Key: R, respiration. 4.19a 65 Changes (Δ) in dissolved oxygen (mg O₂/ h per kg wwt) for Ind. L at three different temperatures. 4.19b Changes (Δ) in dissolved oxygen (mg O₂/ h per kg wwt) for Ind. 66 M at three different temperatures. 4.19c 66 Changes (Δ) in dissolved oxygen (mg O₂/ h per kg wwt) for Ind. S at three different temperatures.

4.20a	Gross production to respiration ratios, P_g/R , calculated based on highest light intensity range of $1000 - 2000 \mu mol/m^2$ s for three individuals of giant clams at three different temperatures.	68
4.20b	Gross production to respiration ratios, P_g/R , calculated based on highest light intensity range of $1000-2000~\mu\text{mol/m}^2$ s for three individuals of giant clams at different temperatures.	68
5.1	Day 4 veliger larvae of T. maxima. Bar: 100 um.	79

LIST OF ABBREVIATIONS

CITES - Convention on International Trade in Endangered Species

IUCN - International Union for Conservation of Nature

SST - Sea Surface Temperature

°C - Degree Celsius

m - Metre

cm - Centimetre

e.g. - exempli gratia

mM - Millimole

ml - Millilitre

et al. et alia

% - Percentage

UMT - Universiti Malaysia Terengganu

N - North

E - East

mm - Millimetre

l - Litre

μm - Micrometre

ppt part per thousand

° - Degree

min - Minute

NaHCO₃ - Sodium Bicarbonate

FSW - Filtered seawater

rpm - Revolutions per minute

SL - Shell length

wwt - Wet weight

PVC - Polyvinyl Chloride

DO - Dissolved oxygen

h - Hour

s Second

μmol - Micromole

m² - Meter square

PAR Photosynthetic Active Radiation

P_n Net primary production

P_g Gross primary production

R - Respiration

RLC - Rapid Light Curve

mg – Milligram

ANOVA - Analysis of Variance

Ind. - Individual

SD - Standard deviation

ECM - Extracellular matrix

kg - Kilogram

ca. - circa

P_{max} - Maximal photosynthesis production

 Δ - Delta

i.e. - id est

g - gram

LIST OF APPENDICES

Apper	ndix	Page
1	Egg/ larvae count (individual) at alternate days.	99
2	Density of egg/larvae (ind/L) at alternate days.	99
3	Percentage survival (%) of larvae for third trial.	100
4A	Measurement of shell length, shell width and hinge length of Batch 1 larvae from day 0 until day 6.	101
4B	Measurement of shell length, shell width and hinge length of Batch 2 larvae from day 0 until day 2.	103
4C	Measurement of shell length, shell width and hinge length of Batch 3 larvae from day 0 until day 4.	104
5	Mean growth of clam larvae in terms of shell length, width and hinge length.	106
6A	DO and light intensity of Ind. L at 26 °C.	107
6B	DO and light intensity of Ind. L at 28 °C.	108
6C	DO and light intensity of Ind. L at 30 °C.	109
7A	DO and light intensity of Ind. M at 26 °C.	110
7B	DO and light intensity of Ind. M at 28 °C.	111
7C	DO and light intensity of Ind. M at 30 °C.	112
8A	DO and light intensity of Ind. S at 26 °C.	113
8b	DO and light intensity of Ind. S at 28 °C.	114
8C	DO and light intensity of Ind. S at 30 °C.	115
9A	Net DO production and mean light intensity of Ind. L at 26, 28 and 30 °C.	116
9B	Net DO production and mean light intensity of Ind. M at 26, 28 and 30 °C.	118
9C	Net DO production and mean light intensity of Ind. S at 26, 28 and 30 °C.	120

10	P_g of three clam individuals at different temperatures within irradiance range of 1000- 2000 μ mol/m ² s.	122
11A	Net DO production and gross production (Pg) in one day for Ind. L.	123
11B	Net DO production and gross production (P_g) in one day for Ind. M.	125
11C	Net DO production and gross production (Pg) in one day for Ind. S.	127
12	Comparison of gross production in three different sizes of clams at three different temperatures.	128
13	Respiration (R) of each clam individuals at different temperatures.	129
14	Comparison of respiration in three different sizes of clams at three different temperatures.	129
15	P_{g}/R ratios of three clam individuals at three different temperatures.	130
16	Comparison of P_g/R ratios in three different sizes of clams at three different temperatures.	130
17	Average light intensity measured at Redang Island at the mantle	131
	level of Tridacna maxima	

ABSTRACT

Population of giant clam has been decreasing severely in many regions due to harvesting and stress-induced bleaching. Mariculture studies have been directed to focus on artificial spawning induction, rearing of larvae and relationship of zooxanthellae to host nutrition. However, data for this purpose is specifically lacking in Malaysia especially for Tridacna maxima. This study was done to describe the artificial spawning induction events in broodstocks using Serotonin and the early life development from embryo to larvae morphologically. Furthermore, was to determine the physiological behaviour of adults as a response to temperature in terms of photosynthesis and respiration. Three trials were conducted using 1 ml of 2 mM serotonin hormone to induce spawning in four different broodstocks. Sperm were released after 1-2 min after injection and lasted for about 10 min while eggs released took place after about 13 min and lasts about 45- 75 min. After fertilization, the survival and growth of larvae collected were followed until pre-metamorphosis stage. Larval survival was 21.62 % at day 2 and 0.11 % at day 4. On day 6 whole batch of culture collapsed because of bacterial contamination. Size of unfertilized eggs was $102.78 \pm 2.78 \,\mu m$ and reached larvae size of $145.56 \pm 8.08 \,\mu m$ on day 6. Increase in total shell length and width length is more noticeable compared to hinge length. Morphological study was carried out using light compound microscope. Description of eggs, embryo, trochophore, veliger and regressive ova were discussed. On the other hand, three different sizes of adult giant clams were maintained in 26 °C, 28 °C and 30 °C water, and exposed to natural sunlight. Gross primary production as obtained by measuring the changes in dissolved oxygen (DO) during start and end of every 30 min for 24 h. Respiration measurements were taken at night. All physiological measurements were related to wet weight, including shell. Temperature range of 26 to 30 °C has no significant effect on the primary production of zooxanthellae. However, primary production at 28 °C was relatively higher. All clams regardless of size had higher stress at 30 °C but lower stress at 26 °C. This study provided description for identification of *T. maxima* larvae and proved the feasibility of 26- 30 °C as the range of culture temperature. Small clams are optimally reared under high irradiance at 28 °C to avoid higher stress at 30 °C. For adult higher irradiance is better.

DESKRIPSI PERKEMBANGAN AWAL KIMA, TRIDACNA MAXIMA (RÖDING, 1798) DAN PENENTUAN PERILAKU FISIOLOGI OLEH KIMA DEWASA SEBAGAI TINDAK BALAS TERHADAP SUHU

ABSTRAK

Kerang gergasi (kima) populasi telah semakin berkurangan secara mendadak di banyak lokasi akibat daripada exploitasi dan pemutihan disebabkan oleh tekanan suhu. Kajian marikultur telah bertumpu kepada pembiakan aruhan, pembelaan larva dan hubungan antara "zooxanthellae" dengan nutrisi kima. Namun, data bagi tujuan tersebut adalah kurang di Malaysia terutamanya pada Tridacna maxima. Kajian ini dilakukan untuk menggambarkan tindak balas scenario induk terhadap rangsangan suntikan Serotonin dan perkembangan morfologi daripada embrio ke peringkat larva. Selanjutnya, untuk menentukan perilaku fisiologi oleh kima dewasa sebagai tindak balas terhadap suhu dari segi fotosintesis dan respirasi. Tiga percubaan telah dijalankan dengan menggunakan 1 ml serotonin dengan kepekatan 2mM bagi mengaruh pembiakan ke atas empat induk yang berlainan. Sperma dilepaskan 1-2 min selepas suntikan dan berterusan selama kira-kira 10 min manakala telur dilepaskan lebih kurang 13 min selepas suntikan dan berterusan selama kira-kira 45- 75 min. Selepas persenyawaan, kemandirian and pertumbuhan larva diikuti sehingga tahap pra-metamorphosis. Peratus kemandirian larva adalah 21.62 % dan 0.11 % masingmasing pada hari ke-2 dan hari ke-4. Pada hari ke-6, kesemua larva telah mati akibat daripada pencemaran bakteria. Saiz telur yang belum disenyawakan adalah 102.78 ± 2.78 µm dan kemudian mencapai saiz larva iaitu 145.56 ± 8.08 µm pada hari ke-6. Peningkatan panjang keseluruhan dan lebar cangkerang larva adalah lebih ketara berbanding panjang garisan engsel. Kajian morfologi telah dilaksanakan dengan menggunakan mikroskop cahaya. Penerangan tentang telur, embrio, trokofor, veliger dan telur merosot turut dibincangkan. Di samping itu, tiga individu kima dewasa yang berlainan saiz didedahkan dalam air yang bersuhu 26 °C, 28 °C serta 30 °C di bawah pancaran matahari. Pengeluaran primer kasar diperolehi daripada perubahan kandungan oxygen terlarut dalam 30 min untuk seharian. Pengukuran respirasi dilakukan pada waktu malam. Kesemua pengukuran fisiologi adalah berkait dengan berat basah, termasuk cangkerang. Lingkungan suhu daripada 26 hingga 30 °C mempunyai kesan yang tidak ketara ke atas pengeluaran primer oleh "zooxanthellae". Namun, pengeluaran primer adalah lebih tinggi pada 28 °C secara perbandingan. Kesemua kima menghadapi tekanan yang lebih tinggi pada 30 °C tetapi tekanan yang lebih rendah pada 26 °C. Kajian ini menyediakan penerangan bagi pengenalan larva T. maxima serta membuktikan kebolehan dan kesesuaian 26-30 °C sebagai lingkungan suhu untuk pengkulturan. Kima saiz kecil adalah sesuai untuk dibela bawah pancaran cahaya yang tinggi pada 28 °C bagi mengelakkan tekanan tinggi pada 30 °C. Manakala bagi kima saiz besar, dedahan pancaran cahaya yang tinggi adalah digalakkan.