UNIVERSITI PUTRA MALAYSIA

LARVAL REARING AND JUVENILE PRODUCTION OF TROPICAL BLACK SEA URCHIN, Diadema setosum (LESKE, 1778)

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By

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Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

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Abstract of thesis presented to the senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

LARVAL REARING AND JUVENILE PRODUCTION OF TROPICAL BLACK SEA URCHIN, *Diadema setosum* (LESKE, 1778)

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March 2018

Chairman : Fatimah Md. Yusoff, PhD
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Long-spined black sea urchin Diadema setosum were successfully cultured within 35 days in closed culture system at a culture temperature of 28ºC. Complete observations of their physical characteristics and skeleton structure were done to determine normal and healthy larvae using this culture method. Trials on environmental effects discovered the critical range for high survival of this sea urchin larva. On salinity, it was shown that D. setosum larvae could not survive and develop when salinity is less than 28 or more than 37 PSU. The best salinity was at 31 PSU. Moreover, fertilization success decreased with increasing or decreasing salinities. The critical tolerance temperature for embryonic development was 16 and 34°C for the minimum and maximum levels, respectively. Among these, 28°C was the best temperature for growth and development rates in all larval stages. Optimization on culture methods was done by determining the optimal food concentrations, larval densities, and food types. Food concentration showed a trade-off relation between arm length and stomach size of the larvae with a value of -0.955 which was likely due to evolution plasticity of feeding structures. The best density for survival and development of larvae culture was 1 larvae/m, leading to a survival of 67.33%. The best microalgae for D. setosum was Chaetoceros calcitrans, leading to metamorphosis after 35 days, followed by mixed algae culture (C. calcitrans and Isochrysis galbana) and lastly Isochrysis galbana. All larvae cultured with Nannochloropsis sp. died before reaching the late 4-arm stage. Addition of coralline red algae induces highest percentange settlement of D. setosum as compared other micro algae. This indicates that potent inducing substances were sufficiently present in red algae. The use of biofilm did not improve the metamorphosis rate (2-5%) compared to the control. In summary, findings from this study can be useful to understanding the life cycle of this sea urchin. Investigations of other environmental factors, such as pH, alkalinity and turbulence, would likely also help customize optimum conditions for culturing the sea urchin in closed or open culture systems. Such information may help the farmer to better develop aquaculture systems for sea urchin culture.

Abstrak tesis yang dikemukan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

PEMELIHARAAN LARVA DAN TEKNIK PEMBENIHAN UNTUK LANDAK LAUT HITAM TROPIKA, *Diadema setosum* (LESKE, 1778)

Oleh

MOHAMMAD SARIFUDIN BIN ABDUL WAHAB

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Landak laut hitam, Diadema setosum telah berjaya dikultur dalam tempoh 35 hari dalam sistem kultur tertutup pada suhu 28º C. Pemerhatian lengkap terhadap ciri fizikal dan struktur kerangka landak laut dilakukan untuk mengenalpasti larva normal dan sihat menggunakan kaedah pengkulturan. Kajian mengenai kesan persekitaran menemui aras kritikal untuk kemandirian tinggi landak laut. Bagi saliniti, didapati larva D. setosum tidak dapat hidup dan berkembang pada saliniti kurang dari 28 PSU dan lebih daripada 37 PSU. Didapati saliniti yang terbaik pada 31 PSU. Selain itu, kejayaan persenyawaan berkurangan dengan peningkatan atau penurunan saliniti. Suhu toleransi kritikal bagi pembangunan embrio adalah masing-masing, 16 dan 34° C untuk tahap minimum dan maksimum. Di dapati suhu 28° C adalah suhu terbaik untuk kadar pertumbuhan dan perkembangan dalam semua peringkat larva. Pengoptimuman kaedah pengkulturan dilakukan dengan menentukan kepekatan makanan yang optimum, kepadatan larva, dan jenis makanan. Kepekatan makanan menunjukkan wujudnya kompromi di antara panjang lengan dan saiz perut larva dengan nilai -0.955 yang kemungkinan disebabkan oleh kelelahan evolusi struktur makan. Kepadatan terbaik untuk kemandirian dan perkembangan kultur larva adalah 1 larva /m, yang membawa kepada kemandirian hidup 67.33%. Mikroalga terbaik untuk D. setosum adalah Chaetoceros calcitrans, yang membawa kepada metamorfosis selepas 35 hari, diikuti oleh campuran alga (C. calcitrans dan Isochrysis galbana) dan terakhir Isochrysis galbana. Semua larva yang diberi Nannochloropsis sp. mati sebelum mencapai peringkat akhir 4-lengan. Penambahan algae coralline merah merangsang penempatan paling tinggi D. Setosum berbanding dengan mikroalga yang lain. Ini menunjukkan terdapatnya bahan perangsang yang kuat pada alga merah. Penggunaan bioflim tidak meningkatkan kadar metamorfosis (2-4%) berbanding kawalan. Secara ringkas, penemuan dari kajian ini boleh digunakan untuk memahami kitaran hidup dari landak laut ini. Ekperimen faktor persekitaran yang lain, seperti pH, kealkalian dan arus (ombak), mungkin dapat membantu mengoptimalan keadaaan untuk kultur landak laut dalam sistem tertutup atau terbuka. Maklumat

sedemikian boleh membantu penternak untuk membangunkan sistem akuakultur yang lebih baik untuk pengkulturan landak laut.

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

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LIST OF ABBREAVIATIONS

PSU	Practical Salinity Unit
PUFAs	Polyunsaturated fatty acids
KCl	Potassium chloride
ml	Mili Liter
L	Liter
cm	Centimetre
m	Meter
m^2	Square Meter
%	Percentage
µm	Micrometer
°C	Degree Celsius
g	Gram
M	Molar
h	Hour
rpm	Revolutions per minute
SFSW	Steril filtered seawater
LL	Larval length
LW	Larval width
BL	Body length
BW	Body Width
POA	Post oral arm length
ALA	Anterolateral arm
POVL	Post oral vibratile lobes
ADL	Adoral lobes
PO	Post oral
VT	Ventral transverse
LSI	Larval stage index
VTD	Vertical test diameter
HTD	Horizontal test diameter
Cc	*Chaetoceros calcitrans*
Iso	*Isochrysis galbana*

CRA	Coralline red algae
CRMA	Coralline and regular mixed algae
RBA	Regular brown algae
RGA	Regular green algae

CHAPTER 1

INTRODUCTION

Echinoids are the high-valued marine invertebrates that have been used as raw material to produce foodstuff, in particular, the product of processed gonads known as "Sea urchin Roe or Uni" (Kaneniwa and Takagi, 1986; Oshima *et al.,* 1986; Ichihiro, 1993). It has also been considered as a prized delicacy in Asia, Mediterranean countries, and Western Hemisphere countries such as Barbados and Chile (Lawrence *et al*., 1997; Yur'eva *et al*., 2003). Peoples in the Asian Pacific Region have used sea urchin gonads for many years as a remedy for improving health condition, treatment for a number of diseases and also for increasing the sexual potency of the middle-aged men (Yer'eva *et al.,* 2003). Gonads of sea urchins have long been considered as one of the luxury food items in Japan (Shimabukuro, 1991). Some study has proven that sea urchin gonads are rich in valuable bioactive compounds, like polyunsaturated fatty acids (PUFAs) and β-carotene (Dincer and Cakli, 2007). Sea urchin fisheries have expanded so greatly in recent years that the populations of sea urchins around the world have been overfished (Andrew *et al*., 2002, 2004). Not surprisingly, the decrease in supply and the continued strong demand have led to a great increase in interest in aquaculture of sea urchins, particularly in those areas where their populations have been depleted (Lawrence *et al*., 1997, 2001; Robinson, 2004).

Aquaculture activities for marine species do not normally establish until the source from wild stocks are depleted. When sources become less, high demand may generally increase the prices up to the point where the investments for establishing a culture capability are necessary. The illegal overfishing activities by several countries have resulted in the fishery industry to collapses the in the future (Robinson, 2004). Even though, the topic of sea urchin culture was discuses only on the periphery of scientific conferences less than 20 years ago but it has now improvement to the point where directed conferences on the subject are regularly held because of the continuing depletion of wild stocks and the relatively high-value product (Robinson, 2004a).

Andrew *et al*. (2003) documented that land-based sea urchin culture activities have seen increasing rapidly in recent years. Research into the full-cycle culture of sea urchins has been carry out, however, while all aspects are biologically feasible, several logistic and economic issues due to the feeding behaviours and impacts of the urchins require resolution. Some research about cage culture such study on abalone; researchers have found that the development rates are double compared to abalone that harvests from wild but in sea urchin culture some experiments are needed to prove it. Robinson (2004) also found out that the element that influences the taste of gonad remains the most elusive subject of study. For example, utilize of wild algae as a last-stage feed are still necessary for about the final six months to finish off the product so it has an acceptable taste.

To discover, a better perceptive of echinoids biology and ecology into improved management is tough, because less of information about it and some are complicated. These include some details on the oceanographic conditions (temperature, nutrients, chemistry, feed particle size, predator), currents and weather and also how these are affecting the sea urchin larvae and it influence on the degree of interconnectedness amongst the sub-populations (Andrew *et al*., 2002). Planktonic larvae of sea urchin usually, spend between 14-40 days swimming and then they will be dispersed over considerable areas by ocean currents. Dispersion process permitted the hybridization occurs among the diverse sub-populations within a fishery which gives rise to the concept of a meta-population as the functional ecological unit of the stock. In sea urchin fisheries, to maximize their harvest and their prospects of sustainability over the long-term, they may have to move from large-scale capture fisheries to some form more intensive management. Andrew *et al*. (2002), demonstrated that small-scale management on sea urchin culture has only been implemented in some countries only (e.g. Japan, Mexico, South Korea and parts of Chile also Nova Scotia).

Compared to other countries, information on sea urchin is still lacking in Malaysia. Even though there were eight species of sea urchin have been documented in Malaysia' coral reef community such as *Diadema setosum*, *Echinometra mathaei*, *Astrophyga radiate*, *Toxopneustes pileolus*, *Echinometrix calamaris*, *Echinometrix diadema*, *Parasalaenia gratiosa* and *Salmacis sphaeroides* (Kee, Universiti Kebangsaan Malaysia, Bangi, Selangor, Malaysia, pers. Comm.). Among these sea urchins, *Diadema setosum* is one of the most abundant species in the Straits of Malacca and South China Sea (Effendi, University of Malaya, Kuala Lumpur, Malaysia and Kee, Universiti Kebangsaan Malaysia, Bangi, Selangor, Malaysia, pers. Comm.) This sea urchin can be found on the natural reefs, artificial reefs and marine park jetty in Pulau Tioman of Pahang (Kee, 2003; Wei *et al*., 2008) and Pulau Pangkor (Rahman *et al*., 2012a).

Problem statement

Echinoids study is rather new in Malaysia mostly in molecular and cultures studies. Even though, very few ecology studies have been complete on the distribution patterns and abundance of *D. setosum* in Peninsular Malaysia but less published information on their development pattern are available. Due to commercial values of echinoid gonads, information of the early life history, the range of environmental condition and optimize culture condition for larvae and the late stage is an indispensably important for improving large-scale seed production, culture, and management. Consequently, an attempt was made to revise the detailed embryonic, larval and juvenile development of *D. setosum* in a captive lab-rearing system with full controlled condition (almost similar to their natural habitat).

Thus, this study was carried out with the following hypotheses and objectives which will be paramount to establish a better understanding of *D. setosum* life cycle and fully optimize rearing condition for sea urchin culture from embryonic stage until juvenile with high survival rates

1.1 Objectives

I. To establish the life cycle of *D. setosum* (including complete body shape and skeleton structure).
II. To access the influences of environmental effect (temperature and salinity) in embryonic and early larval development of *D. setosum*.
III. To establish the suitable condition of closed larval rearing method based on food concentration, larval density, and feed types.
IV. To determine the most suitable induction cues and substratum preferences for *D. setosum* larvae settlement.

1.2 Hypotheses

Hypotheses Null

I. Environmental conditions have no influence on *D. setosum* in embryonic and early larval development of *D. setosum*.
II. Food concentration, larval density, and feed types do not affect the growth of sea urchin larvae in a closed larval rearing system.
III. Induction cues and substratum are no importance to the *D. setosum* larvae settlement success.

Hypotheses Alternative

I. Environmental conditions influence the embryonic and early larval development of *D. setosum*.
II. Food concentration, larval density, and feed types experiment affect the growth of sea urchin larvae in closed larval rearing system.
III. Induction cues and substratum are important to the *D. setosum* larvae settlement success.

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