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DEVELOPING OF PROTOCOL FOR EGG INCUBATION AND LARVAL CULTURE OF Tachypleus gigas Muller

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By

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

DEVELOPING OF PROTOCOL FOR EGG INCUBATION AND LARVAL CULTURE OF *Tachypleus gigas* Muller

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July 2013

Chairman : Annie Christianus, PhD

Institute : Bioscience

Culture of horseshoe crab in laboratory for future restocking most likely is the best solution so far in order to reduce the possibility of extinction of this species. Establishment of suitable protocols is crucial to successfully hatch the eggs and culture the larvae of *Tachypleus gigas*. Studies were carried out at MTDC Laboratory, Putra Science Park, Universiti Putra Malaysia, Serdang, Selangor. The objectives of this study were divided into two main parts. First, to determine the effects of salinity, watering frequency and incubation medium on the hatching of *T. gigas* eggs. Second, to determine the effects of salinity, temperature, culture medium and stocking density on growth and survival of *T. gigas* larvae. Eggs, larvae and sand were collected from three natural spawning sites in Sitiawan (Perak), Banting (Selangor) and Muar (Johor), Malaysia. This research consists of seven experimental studies. Three experiments were carried out on eggs and four experiments on larvae. In the first, second and third experiment, effects of

water salinities (15, 20, 25, and 30 ppt), watering frequencies (once in 1, 3 and 6 day/s), and different incubation medium (water and sand) on the incubated eggs were investigated. As for the fourth, fifth, sixth and seventh experiment, effect of salinities (15, 20, 25, 30 ppt), temperatures (ambient, 30 and 32˚C), using sand and water medium, and stocking densities on horseshoe crab larvae was studied.

Results from experiment 1, showed that at the end of the incubation period, watering with salinity of 25-30 ppt produced significantly larger eggs diameter ($P<0.05$) while highest percentages of hatching at 30 ppt salinity. In experiment 2, it was found that percentages of hatching were significantly higher ($P<0.05$) when watered once a day and three days. As for experiment 3, at the end of the incubation period, there was no significant different ($P>0.05$) in the eggs diameter and percentage of hatching between sand and water medium.

Results for the fourth experiment (salinity) on instars 1 to 4 (1^{st} to 6^{th} month) and instars 4 to 7 (6^{th} to 12^{th} month) only showed significant different ($P<0.05$) in percentage of survival at 4^{th} instar stage while all parameters (prosomal width, weight and survival) were significantly different ($P<0.05$) from 4 to 7^{th} instar stages. In the fifth experiment, percentage of survival was highest ($P<0.05$) at 30˚C. As for the sixth experiment, significant increments ($P<0.05$) were observed for prosomal width, weight and survival when cultured in sand at the end of the 12 month period. In the seventh experiment, no significant different ($P>0.05$) for the

prosomal width, weight and survival of *T. gigas* larvae were observed when cultured at stocking densities of 20, 40 and 60 larvae/L.

Based on the findings of this study, it can be concluded that the most suitable salinity and watering frequency for the incubation of horseshoe crab eggs are between 25 to 30 ppt and once in 3 days, respectively. Both sand and water are suitable media to incubate *T. gigas* eggs. As for the experiments on larvae, the best salinity for optimum survival is between 20 to 30 ppt at temperature of 30˚C. Meanwhile stocking density and culture media does not affect the growth and survival of the larvae. Observation on the growth of *T. gigas* larvae throughout the study period showed that prosomal width may not be a reliable indicator of growth since the size will increase significantly whenever larvae molt. Therefore it is better to measure the growth of *T. gigas* larvae through weight increment.

Abstrak tesis dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan ijazah Master Sains

MEMBANGUNKAN PROTOKOL UNTUK PENGERAMAN TELUR DAN KULTUR LARVA *Tachypleus gigas* Muller

Oleh

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Julai 2013

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Kultur belangkas dalam makmal untuk pelepasan stok pada masa akan datang mungkin satu penyelesaian yang terbaik untuk mengelakkan kepupusan spesies ini. Memperkenalkan protokol yang sesuai adalah penting untuk menetaskan telur dan mengkultur larva *Tachypleus gigas*. Kajian dijalankan di Makmal MTDC, Taman Sains Putra, Universiti Putra Malaysia, Serdang, Selangor. Objektif kajian ini dibahagikan kepada dua bahagian utama. Pertama, untuk menentukan kesan saliniti, frekuensi pemberian air dan medium untuk pengeraman telur ke atas penetasan telur *T. gigas*. Kedua, untuk menentukan kesan saliniti, suhu, medium kultur dan densiti stok ke atas tumbesaran dan kemandirian larva *T. gigas*. Telur, larva dan pasir diambil dari tiga lokasi semulajadi pembiakan di Sitiawan (Perak), Banting (Selangor) dan Muar (Johor), Malaysia. Kajian ini merangkumi tujuh eksperimen. Tiga eksperimen dijalankan ke atas telur dan empat ke atas larva. Dalam ekperimen pertama, kedua dan ketiga, kesan saliniti air (15, 20, 25, dan 30

ppt), frekuensi pemberian air (sekali dalam 1, 3, dan 6 hari), dan medium pengeraman berbeza (air dan pasir) ke atas telur dikaji. Untuk eksperimen keempat, kelima, keenam dan ketujuh, kesan saliniti (15, 20, 25, dan 30 ppt), suhu (ambien, 30, DAN 32˚C), penggunaan medium pasir dan air, dan densiti stok larva belangkas dikaji

Keputusan eksperimen pertama menunjukkan bahawa pada akhir tempoh pengeraman, pemberian air dengan saliniti 25 to 30 ppt menghasilkan diameter telur yang lebih besar ($P<0.05$) dengan peratus penetasan yang paling tinggi ($P<0.05$) pada saliniti 30 ppt. Dalam ekperimen kedua, didapati bahawa peratus penetasan ketara lebih tinggi ($P<0.05$) bila pemberian air dijalankan sekali dalam sehari dan tiga hari. Manakala untuk eksperimen ketiga, pada akhir tempoh pengeraman, tidak terdapat perbezaan ketara ($P>0.05$) pada diameter telur dan peratus penetasan apabila menggunakan medium pasir dan air.

Keputusan untuk eksperimen keempat (saliniti) keatas instars 1 hingga 4 (1 hingga 6 bulan) dan instars 4 hingga 7 (6 hingga 12 bulan) hanya menunjukkan perbezaan ketara ($P<0.05$) dalam peratus kemandirian pada instar keempat, manakala semua parameter (saiz prosoma, berat dan kemandirian) adalah ketara berbeza ($P<0.05$) untuk peringkat instar ke 4 hingga 7. Dalam ekperimen kelima, peratus kemandirian adalah paling tinggi ($P<0.05$) pada suhu 30˚C. Sementara untuk eksperimen keenam peningkatan yang ketara ($P<0.05$) diperhatikan untuk saiz prosoma, berat dan kemandirian apabila larva dikultur dalam pasir pada akhir

tempoh 12 bulan. Dalam eksperimen ketujuh, tidak terdapat perbezaan yang ketara ($P>0.05$) pada saiz prosoma, berat dan kemandirian larva *T. gigas* apablia dikultur pada densiti stok 20, 40 dan 60 larva/L.

Berdasarkan hasil kajian ini, kesimpulan dapat dibuat bahawa saliniti dan frekuensi pemberian air untuk pengeraman telur belangkas adalah di antara 25-30 ppt dan sehari sekali dan 3 hari sekali, setiap satunya. Kedua-dua pasir dan air adalah sesuai digunakan untuk mengeramkan telur *T. gigas.* Sementara untuk eksperimen ke atas larva, saliniti terbaik untuk kemandirian optima adalah di antara 20 hingga 30 ppt pada suhu 30˚C. Sementara densiti stok dan kultur media tidak memberi kesan ke atas tumbesaran larva. Pemerhatian ke atas tumbesaran larva *T. gigas* dalam tempoh kajian menunjukkan bahawa saiz prosoma mungkin tidak sesuai digunakan sebagai penunjuk tumbesaran kerana saiz akan meningkat dengan ketara apabila larva bertukar kulit. Oleh itu, adalah lebih baik mengukur tumbesaran larva *T. gigas* melalui peningkatan berat.

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I certify that a Thesis Examination Committee has met on 23rd July 2013 to conduct the final examination of Mohamad Faizul Mat Isa on her thesis entitled "Developing of Protocol for Egg Incubation and Larval Culture of *Tachypleus gigas* (Muller, 1785)" in accordance with the Universities and University College Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The committee recommends that the student be awarded the Master of Science.

Members of the Thesis Examination Committee were as follows:

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

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DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledge. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any institution.

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Date: 23 July 2013

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