UNIVERSITI PUTRA MALAYSIA

FEMALE BROODSTOCK REPRODUCTIVE OUTPUT, LARVAL REARING AND SPAT SURVIVAL OF BLACK LIPPED OYSTER (*Pinctada margaritifera*)

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By

FARIBORZ EHTESHAMI

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

July 2010

I would like to dedicate this thesis

with love to the memory of my father

Heshmat Ehteshami and my mother

Jamileh yagmaeian to keep their

spirits alive

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

FEMALE BROODSTOCK REPRODUCTIVE OUTPUT, LARVAL REARING AND SPAT SURVIVAL OF BLACL LIPPED OYSTER (*Pinctada margaritifera*)

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Chairman: Dr Annie Christianus, PhD

Faculty: Agriculture

Overexploitation of *Pinctada margaritifera* as one of the natural resource was leading to a dramatic loss of its population in the north coast of Persian Gulf. Low abundance and density existing in the natural beds and poor larval recruitment prompted research on hatchery propagation of this species. Further research should be carried out to improve the survival and growth of larvae produced through artificial propagation.

This study addressed important issues in relation to the supplementation of polyunsaturated fatty acids (PUFA) in diet and their role in egg quality and biochemical composition, and larval growth and survival; microalgae biochemical

composition and its role in larvae culture; and spat settlement and transfer time to the sea farm.

In the first experiment, the effects of supplementary PUFA on oogenesis and hatching rate of *P. margaritifera* broodstock were compared with naturally fed oysters and those fed only microalgae. Supplementary food was effective ($P < 0.05$) on producing larger sized eggs (57.6 µm) and larvae (80.1 µm), and higher percentage of *P. margaritifera* D shape larvae (31.3%). Palmitic (16:0) and stearic (18:0) acid were the major saturated fatty acids in neutral and polar parts of gonad lipid. Oysters fed with supplementary PUFA had more docosahexaenoic acid (DHA) and less monounsaturated fatty acids (MUFA) in their gonad. The ratio of n-3/n-6 fatty acids in neutral lipid was the best representative of differences in conditioning of oysters for spawning and interpretation of the results of egg size and hatching performance.

In the second experiment, the effects of partial supplementation of the diet with PUFA on growth and survival of *P. margaritifera* D-shape and umbo larvae were investigated. PUFA supplemented in droplet form did not increase the growth and survival of D-shape and umbo *P. margaritifera* larvae compared to those fed fresh algae of T. Iso ($P > 0.05$). Considering results of size range of larvae, it can be concluded that through the grading process, a great number of larvae would be lost in treatments with supplementary diet compared to those fed with T. Iso only. D-shape and umbo larvae showed a similar performance in survival, with the highest related to larvae fed with fresh algae followed by 10, 30 and 100% of diet replaced with PUFA emulsions. While the lowest survival attributed to the unfed larvae.

The nutritional value of the three microalgal species used for the feeding *P. margaritifera* D-shape and umbo larvae: T. Iso, *Chaetoceros muelleri* and *C. calcitrans* in mono, binary and ternary species diets were evaluated. D-shape and umbo larval growth and survival were found to be the greatest with diet T. Iso mono and ternary species, respectively. *C. calcitrans* showed the lowest nutritional value for both larval stages. Growth of D-shape larvae was positively correlated with levels of MUFA, DHA, DHA/EPA and majority of the unsaturated fatty acids with 18 C including: 18:1n-9, 18:2n-6, 18:3n-3, 18:4n-3 and negatively correlated to 16:3n-4, 20:4n-6 (arachidonic cid) and EPA contents of microalgae.

The third experiment was conducted to investigate the effect of collector materials and position on *P. margaritifera* spat attachment. Settlement on polyethylene pipes (54.6%) was significantly higher ($P < 0.05$) than on plastic baskets (25%). Significantly higher ($P < 0.05$) spat catch was recorded from collectors installed close to the bottom as compared to the top part of the settlement tank. Numbers of dead spat after settlement on pipe (1.4%) and basket (1.5%) were not significantly different ($P > 0.05$). Possible factors causing the pattern of settlement reveals that it could be related to the material used for collector, light sensitivity and gravity force. These results indicated that polyethylene pipes positioned horizontally in the lower part of the tank are suitable for the settlement of *P. margaritifera* pediveliger larvae in hatchery.

In the fourth experiment, *P. margaritifera* spat were transferred to the sea farms in Hendurabi and Lavan Islands at 25, 50, and 65 days post settlement, while one group was maintained in the hatchery. Retaining the spat in the hatchery for more than 25 days did not improve the growth and survival ($P > 0.05$). Spat grown in Hendurabi

were twice the size of those grown in hatchery and were at least 1 cm longer than those grown in Lavan. Spat from two propagation trials upon reaching 55 days old, were deployed to the Hendurabi on 5th September and 7th October 2008, respectively. Culture was carried out for five months. Spat from the first deployment were significantly larger in size than the second one ($P < 0.05$). Daily growth rate (DGR) was observed to be higher in September (warmer month) as compared to February (winter). Statistical analyses showed highly positive correlation between DGR and water temperature, whereas food abundance had a negligible effect.

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

MEMPERBAIKI HASIL PEMBIAKAN INDUK BETINA, TUMBESARAN DAN KEMANDIRIAN LARVA BENTUK-D, UMBO DAN SPAT *Pinctada margaritifera*

Oleh

FARIBORZ EHTESHAMI

Julai 2010

Pengerusi: Dr. Annie Christianus, PhD

Fakulti: Pertanian

Ekploitasi terhadap sumber semulajadi *Pinctada margaritifera* dengan dramatiknya telah mengurangkan populasinya di utara pantai Teluk Farsi. Taburan dan densiti yang rendah di tapak semulajadi dan kekurangan sumber untuk larva telah menggalakkan kajian ke atas pengeluaran spesis ini di hatcheri. Kajian lanjutan adalah perlu untuk meningkatkan kadar kemandirian dan tumbesaran larva yang dihasilkan melalui pengeluaran artifisial.

Kajian ini menekankan isu penting berkaitan dengan penambahan asid lemak poli tidak tepu (PUFA) dalam gizi dan peranannya ke atas kualiti telur dan komposisi biokimia, dan tumbesaran dan kemandirian larva; komposisi biokimia alga mikro

dan peranannya dalam kultur larvi; dan pelekatan dan jangkamasa perpindahan spat ke kultur di laut.

Pada ekperimen pertama, kesan ke atas oogenesis dan kadar penetasan hasil dari induk *P. margaritifera* dibandingkan diantara induk yang diberi makanan dengan penambahan PUFA dengan makanan semulajadi dan alga mikro. Makanan tambahan berjaya ($P < 0.05$) menghasilkan telur (57.6 µm) dan larva yang bersaiz lebih besar (80.1 µm), dan peratusan larva-D yang lebih tinggi (31.3%). Asid palmitik (16:0) dan stearik (18:0) merupakan asid lemak tepu yang utama terdapat di bahagian neutral dan kutub pada lipid gonad. Tiram yang diberi PUFA tambahan mempunyai asid dokosahekaenoik (DHA) yang lebih tinggi dan asid lemak mono tak tepu (MUFA) yang rendah dalam komposisi gonadnya. Nisbah n-3/n-6 pada lipid neutral merupakan penunjuk yang sangat sesuai untuk pembezaan dalam penyesuaian tiram untuk pembiakan dan menerangkan keputusan untuk saiz dan hasil penetasan telur.

Pada eksperimen kedua, kesan penambahan separa PUFA ke dalam gizi larva ke atas tumbesaran dan kemandirian larva bentuk-D dan umbo *P. margaritifera* telah dikaji. Penambahan PUFA dalam bentuk titisan tidak meningkatkan tumbesaran dan kemandirian larva bentuk-D dan umbo berbanding dengan larva yang diberi T. Iso ($P > 0.05$). Berdasarkan julat untuk saiz larva, kesimpulan dapat dibuat bahawa semasa proses pengredan, kehilangan sejumlah besar larva akan berlaku untuk larva yang diberi gizi dengan penambahan PUFA berbanding dengan hanya diberi T. Iso. Larva bentuk-D dan umbo menunjukkan kadar kemandirian yang sama, dengan kadar tertinggi pada larva yang diberi alga segar diikuti dengan gizi yang diberi penggantian emulsi PUFA sebanyak 10, 30 and 100%. Manakala kadar kemandirian yang paling rendah adalah pada larva yang tidak diberi sebarang makanan.

Nilai nutrient untuk tiga spesis alga mikro yang diberikan untuk larva bentuk-D dan umbo *P. margaritifera*: T. Iso, *Chaetoceros muelleri* dan *C. calcitrans* dalam bentuk gabungan spesis mono, binari dan ternari telah dinilai. Tumbesaran dan kadar kemandirian larva bentuk-D dan umbo yang paling tinggi adalah dengan gizi T. Iso gabungan spesis mono dan ternari, masing-masingnya. *C. calcitrans* memberikan nilai nutrient paling rendah untuk kedua-dua peringkat larva tersebut. Tumbesaran larva bentuk-D menunjukkan korelasi positif dengan tahap MUFA, DHA, DHA/EPA dan kebanyakan asid lemak tak tepu dengan 18 C termasuk: 18:1n-9, 18:2n-6, 18:3n-3, 18:4n-3, manakala korelasi negatif dengan 16:3n-4, 20:4n-6 (asid arakidonik) dan kandungan EPA alga mikro.

Eksperimen ketiga dijalankan untuk mengkaji kesan bahan dan kedudukan penggutip untuk penggutipan spat *P. margaritifera*. Pelekatan pada paip polietilene (54.6%) ketara lebih tinggi ($P < 0.05$) berbanding dengan bakul plastik (25%). Kutipan spat ketara lebih tinggi ($P < 0.05$) dicatatkan untuk penggutip yang diletakkan dekat dengan dasar berbanding dengan bahagian atas tangki pemendapan. Jumlah spat yang mati selepas pelekatan ke atas paip (1.4%) dan bakul (1.5%) tidak menunjukkan perbezaan yang ketara ($P>0.05$). Faktor yang menyebabkan corak pelekatan kemungkinan mempunyai kaitan dengan bahan yang digunakan untuk penggutip, sensitiviti terhadap cahaya dan tarikan graviti. Keputusan ini menunjukkan bahawa paip polietilene yang diletak secara horizontal di bahagian bawah tangki adalah sesuai untuk pelekatan larva pediveliger *P. margaritifera* pediveliger di hatcheri.

Pada eksperimen keempat, spat *P. margaritifera* dipindahkan ke tapak di laut di Pulau Hendurabi dan Lavan pada 25, 50, dan 65 hari selepas pelekatan, manakala

satu kumpulan lagi diletakkan di hatcheri. Meletakkan spat di hatcheri lebih daripada 25 hari tidak menunjukkan kadar tumbesaran dan kemandirian yang ketara ($P > 0.05$). Spat yang dikultur di Hendurabi bersaiz dua kali ganda berbanding dengan kumpulan yang dikultur di hatcheri dan sekurang-kurangnya 1 sm lebih panjang berbanding dengan kumpulan di Lavan. Spat daripada dua pengeluaran, apabila mencapai 55 hari, dilepaskan di Hendurabi pada 5^{hb} September dan 7^{hb} Oktober 2008, tiap satunya. Kultur dijalankan selama lima bulan. Spat dari perlepasan pertama ketara lebih besar ($P < 0.05$) berbanding dengan kumpulan yang kedua. Kadar tumbesaran harian (DGR) didapati lebih tinggi pada bulan September (musim panas) berbanding dengan bulan Februari (musim sejuk). Analisis statistik menunjukkan korelasi positif yang tinggi di antara DGR dan suhu air, manakala taburan kehadiran makanan tidak memberikan sebarang kesan.

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I certify that an Examination Committee has met on (date of viva voce) to conduct the final examination of Fariborz Ehteshami on his PhD thesis entitled "Female broodstock reproductive output, larval rearing and spat survival of pearl oyster, *Pinctada margaritifera*" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommended that the student be awarded the Doctor of Philosophy degree.

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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 Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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Date: 12 August 2010

DECLARATION

I declare that the thesis is my original work expect for quotations and citations which have been duly acknowledged. 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 other institution.

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Fariborz Ehteshami

Date

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