

**STOCK ENHANCEMENT OF *MACROBRACHIUM
ROSENBERGII* (GIANT FRESHWATER PRAWNS)
INFERRED BY MOLECULAR GENETICS
AND ECOLOGICAL STUDIES**

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**FACULTY OF SCIENCE
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AND ECOLOGICAL STUDIES**

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ABSTRAK

Berdasarkan jumlah berat *Macrobrachium rosenbergii* atau dikenali sebagai Udang galah merupakan spesies akuakultur yang ke-enam terbesar di Asia. Permintaan yang tinggi menyebabkan berlakunya lebih penangkapan lalu populasi liar udang galah telah jatuh merundum bilangannya di seluruh sistem sungai di dunia ini termasuklah di Malaysia. Oleh itu, program penambahan stok telah dilaksanakan oleh Jabatan Perikanan untuk meningkatkan semula populasi ini. Tujuan kajian ini dilaksanakan adalah untuk menentukan keberkesanan program ini dengan menggunakan 18 mikrosatelit (Jenis I dan Jenis II) dan Mitokondria DNA penanda molekul. Pelbagai analisis telah dijalankan untuk mengkaji perbezaan genetik di antara populasi liar, populasi induk dan populasi tangkapan semula. Sejalan dengan program penambahan stok, juvenil dari populasi induk yang telah digenotipik telah dilepaskan di Sungai Timun pada tahun 2007. Beberapa populasi telah berjaya ditangkap semula secara berkala (F1, F2 dan F3) untuk diperiksa dengan menggunakan kedua-dua jenis penanda molekul. Analisis variasi molekular (AMOVA) menunjukkan populasi induk dan populasi tangkapan semula mempunyai perbezaan yang paling minimal (0.03%). Keputusan ini disokong dengan Konsensus Pokok UPGMA yang terhasil dengan menggunakan data mikrosatelit. Di samping itu, penanda molekul mitokondria dapat menunjukkan bahawa populasi induk bukan sahaja berjaya hidup malah ada yang berjaya menghasilkan populasi hybrid bersama populasi liar. Ini boleh dibuktikan dengan melihat perkongsian haplotip di antara populasi tersebut. Analisis lain juga menunjukkan terdapat kesan alel berlebihan yang dijangka dilihat di populasi yang sedang berkembang semula dalam populasi liar dan F1. Sementara analisis *pairwise F_{ST}* juga menunjukkan bahawa perbezaan yang kecil di antara populasi liar dan induk (0.01964) tetapi perbezaan semakin ketara di antara populasi liar dengan populasi F2. Kesimpulannya, kajian ini telah menunjukkan bahawa Program penambahan stok di Sungai Timun telah berjaya namun perlu

dikaji semula untuk mengelakkan perkara yang tidak diinginkan terjadi seperti kejadian domestikasi populasi induk.

ABSTRACT

The freshwater prawn *Macrobrachium rosenbergii* or locally known as “Udang galah” in Malaysia is ranked as the sixth largest aquaculture species in Asia based on volume. Overharvesting of this species has caused its depletion in all the river line systems in the whole world including Malaysia. A stock enhancement program has been done by the Malaysian Department of Fisheries to fulfil the demand for this prawn. The objective of this study was to determine the productivity success of stock enhancement using 18 microsatellite (Type I and Type II) and mitochondrial DNA markers (mtDNA). In order to evaluate the productivity success, several tests were used to estimate the genetic divergence between the wild samples, the hatchery population, and the tentative recaptured samples. In relation to the stock enhancement program, hatchery juveniles produced in 2007 at Kampung Acheh, Perak were genotyped and then released to natural waters which is Sungai Timun, Negeri Sembilan. Subsequently, recaptured individuals, designated as F1, F2 and F3, were examined using both markers. An analysis of molecular variance (AMOVA) showed that hatchery population and the recaptured individuals had the most minimal differentiation with a value of 0.03 % variation. This result was supported by the UPGMA consensus tree generated using the microsatellites data. On the other hand, mitochondria result also explained that the hatchery population survived and hybridized with the wild type population. This was proven by looking at the haplotypes shared among the populations. Another analysis showed some excess number of alleles, as would be expected from a recent population expansion or from genetic hitchhiking in the wild type population and F1. Pairwise F_{ST} showed that the wild type population and the hatchery population were most closely related (0.01964) while the wild type population had a little differentiation with the F2 population. In conclusion, the results showed that the stock enhancement of prawn at Sungai Timun was success but needed to be reassess again to prevent any unexpected outcome such as domestication effect.

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LIST OF ABBREVIATIONS

°C	Degree Celsius
ppt	Parts per thousand
µg	Microgram
µl	Microlitre
µM	Micromolar
10X	Ten times
1X	One time
AMOVA	Analyses of molecular variance
amp	Ampere
BLAST	Basic local alignment search tool
bp(s)	Basepair
cDNA	Complementary DNA
D	Genetic Distance
D-Loop	Displacement loop
dATP	deoxyadenosine triphosphate
dCTP	deoxycytidine triphosphate
dGTP	deoxyguanosine triphosphate
dNTP	deoxyribonucleotide triphosphate
dTTP	deoxythymidine triphosphate
ddH ₂ O	Double distilled water
DNA	Deoxyribonuclei acid
<i>E.coli</i>	<i>Escherichia coli</i>
EST	Expressed Sequence Tags
F1	First Generation

F_{is}	Inbreeding Coefficient
F_{st}	Fixation Index
g(S)	Gram(s)
h or hr	Hour
H_e	Expected Heterozygosity
H_o	Observed Heterozygosity
HWE	Hardy Weinberg equilibrium
IAM	Infinite allele mutation model
K	Kilo
LB Agar	Luria Bertani Agar
LD	Linkage disequilibrium
m	Meters
M	Molar
ml	Militre
mg	Milligram
mM	Milimolar
nmole	Nanomole
$MgCl_2$	Magnesium Chloride
mRNA	Messenger RNA
mtDNA	Mitochondrial DNA
NCBI	National Centre for Biotechnology Information
n	Nano
n_a	Observed number of alleles
n_e	Effective number of alleles
PCR	Polymerase Chain Reaction

PHYLIP	Phylogeny Inference Package
PIC	Polymorphic Information Content
RNA	Ribonuclei Acid
rRNA	Ribosomal RNA
T _A	Annealing temperature
TL	Tissue Lysis Buffer (Vivantis)
TB	Tissue Genomic DNA Binding Buffer (Vivantis)
TBE	Tris-borate-EDTA Buffer
tRNA	Transfer RNA
rpm	Rotations per minute
s	Second
SMM	Stepwise mutation model
Std. Dev.	Standard Deviation
UPGMA	Unweighted pair group method with arithmetic averaging
UV	Ultra Violet
V	Volt
X-gal	5-bromo-4-chloro-3-indolyl-b-D-galactopyranoside