

**GENETIC DIFFERENTIATION OF THE BROODSTOCKS OF BLACK
EAR CATFISH (*PANGGASius LARNAUDII* BOCOURT, 1866)**

By

THIPSUDA TANGPRAKHON

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia
in Fulfilment of the Requirement for the Degree of Master of Science**

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DEDICATIONS

To...

PIW...PUN...PARN...

Unforgettably to my father and mother...

Thanks for your dedication to give me inspiration and bravery

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

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Black ear catfish, *Pangasius larnaudii* Bocourt, 1866 is one of the dominant food fish and commercially important pangasiid species in Thailand. The Thai Department of Fisheries (DoF) has engaged in breeding programmes of this fish for aquaculture as well as for restocking natural waterways for enhancing fish production. Presently, the *P. larnaudii* fingerlings are produced from ten DoF hatcheries with the broodstocks either from the wild (P_0), F_1 or F_2 progenies some of which were transferred among the fisheries. Little is known about the history of the stock as no record was properly kept. Thus, this study was undertaken to determine the genetic variation, relationships and differentiation among ten of DoF hatchery populations and three natural populations from rivers using microsatellite markers. Four microsatellite primer pairs, namely PSP-G507, PSP-G513, PSP-G576, and PSP-G579 were used in this study. DNA was extracted from individual fin

clips using a single tube extraction procedure for fish blood with some modifications. This procedure was used to extract DNA with good quality for PCR amplification.

A total of 27 primer pairs were used to screen and optimize microsatellite loci for studying genetic variation in *P. larnaudii*, of which only four microsatellite primer pairs were selected for further analysis. The results indicated a wide range of genetic variability among the populations. The average number of alleles per locus ranged from 2.8 to 15.8 and the observed heterozygosity ranged from 0.319 to 0.864. A wide range of allele frequencies were observed over all the loci, with the highest in the Uthaitani population, followed by the Mekong population while the lowest was found in the Chainat population. The average genetic distance (D_T) and genetic differentiation (F_{ST}) between populations ranged from 0.120 to 0.539 and 0.013 to 0.349, respectively.

The UPGMA dendrogram showed that the populations of *P. larnaudii* are clustered into two main groups: the Thai DoF hatchery and the natural river groups. Most of the populations from the DoF hatcheries were distinctly separated from the natural populations. In turn, the populations from the ten DoF hatcheries can be separated into four subgroups: Subgroup I consists of Lopburi and Uthaitani, Subgroup II consists of Ubonratchatani, Srisaket and Nakhonpanom, Subgroup III consists of Yasothon, Surin, Buriram and Chainat while Roi-ed is the only member of Subgroup IV. Besides, the populations of the DoF hatcheries can also be categorized into two separate regions; the middle and the northeastern regions. The Lopburi and Uthaitani populations represented the middle group and the rest represented the northeastern group. The Chainat population was included in the

northeastern group, despite of its actual location, indicating that the stock was transferred from the Yasothon hatchery. The small genetic differentiation among the populations of the northeast group suggested that there were closely related predecessors among their broodstocks. From this study, the occurrence of inbreeding effects was more obvious in the populations of the DoF hatcheries than in the natural rivers. The small genetic differentiation among all the populations indicated that there was migration among the populations of *P. larnaudii* in Thailand. The findings from this study are relevant and useful to the Thai DoF hatchery managers for improving the efficiency of breeding programmes and to maintain the genetic integrity of *P. larnaudii* in Thailand. This study recommends that stocks must not be transferred among hatcheries outside the region of origin of a particular population in order to maintain its genetic integrity.

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

**PERBEZAAN GENETIK INDUK IKAN PATIN (*PANGGASIVS LARNAUDII*
BOCOURT)**

Oleh

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Ikan Patin, *Pangasius larnaudii* Bocourt, 1866 adalah salah satu ikan makanan yang dominan dan spesies pangasiiid komersil yang penting di Thailand. Jabatan Perikanan Thailand terlibat dalam program pembiakan untuk akuakultur dan juga untuk stok semula perairan semulajadi bagi meningkatkan pengeluaran ikan. Pada masa ini, anak ikan *P. larnaudii* dihasilkan oleh sepuluh hatcheri DoF dengan induk yang diperolehi samada dari liar (P_0), progeni F_1 atau F_2 dan sebahagiannya yang telah dipindahkan di kalangan mereka. Sedikit maklumat mengenai latarbelakang stok diperolehi akibat daripada penyimpanan rekod yang tidak lengkap. Oleh itu, kajian ini dijalankan untuk menentukan variasi genetik, perhubungan dan perbezaan di kalangan sepuluh populasi hatcheri Jabatan Perikanan Thailand dan tiga populasi sungai semulajadi dengan menggunakan penanda mikrosatelit. Empat pasangan primer mikrosatelit, iaitu PSP-G507, PSP-G513, PSP-G576 dan PSP-G579 digunakan di dalam kajian ini. DNA diekstrak daripada sirip individu menggunakan

prosedur ekstrak satu tiub untuk darah ikan dengan beberapa modifikasi. Prosedur ini telah digunakan untuk mengekstrak DNA dengan kualiti DNA yang baik untuk amplifikasi PCR.

Sejumlah 27 pasangan primer digunakan untuk menyaring dan mengoptimumkan lokus mikrosatelit bagi kajian variasi genetik dalam *P. larnaudii*, hanya empat pasangan primer mikrosatelit dipilih untuk analisis selanjutnya. Keputusan menunjukkan terdapat satu julat yang besar dalam kepelbagaian genetik di kalangan populasi. Purata alel per lokus menjulat daripada 2.8 hingga 15.8 dan heterozigositi tercerap menjulat daripada 0.319 hingga 0.864. Satu julat frekuensi alel yang besar didapati pada semua lokus, dengan nilai terbesar pada populasi Uthaitani diikuti oleh populasi Mekong, manakala yang terkecil terdapat pada populasi Chainat. Purata jarak genetik (D_T) dan perbezaan genetik (F_{ST}) di antara populasi masing-masing adalah 0.120 hingga 0.539 dan 0.013 hingga 0.349.

Dendrogram UPGMA menunjukkan bahawa populasi *P. larnaudii* dikelompokkan ke dalam dua kumpulan utama: kumpulan hatcheri Jabatan Perikanan Thailand dan sungai semulajadi. Kebanyakan populasi dari hatcheri Jabatan Perikanan Thailand adalah jelas terpisah dari populasi semulajadi. Oleh yang demikian, populasi dari sepuluh hatcheri Jabatan Perikanan Thailand boleh dibahagikan kepada empat subkumpulan: Subkumpulan I merangkumi Lopburi dan Uthaitani, Subkumpulan II merangkumi Ubonratchatani, Srisaket dan Nakhonpanom, Subkumpulan III merangkumi Yasothon, Surin, Buriram dan Chainat, manakala Roi-ed adalah satu-satunya yang berada dalam Subkumpulan IV. Selain itu, populasi hatcheri Jabatan Perikanan Thailand juga boleh dikategorikan kepada dua bahagian, tengah dan timur laut. Populasi Lopburi and Uthaitani mewakili kumpulan

tengah dan yang lainnya mewakili kumpulan timur laut. Populasi Chainat dimasukkan ke dalam kumpulan timur laut walaupun lokasi sebenarnya menunjukkan bahawa stok telah dipindahkan dari hatcheri Yasothon. Perbezaan genetik yang kecil di kalangan populasi kumpulan timur laut menunjukkan bahawa terdapat pertalian rapat di kalangan induk-induk tersebut. Daripada kajian ini, kesan pembiakbakaan dalaman adalah lebih jelas pada populasi hatcheri Jabatan Perikanan Thailand berbanding dengan populasi sungai semulajadi. Perbezaan genetik yang kecil di kalangan semua populasi menunjukkan bahawa wujudnya migrasi semulajadi di antara populasi – populasi *P. larnaudii* di Thailand. Hasil kajian ini adalah relevan dan berguna untuk pengurus hatcheri Jabatan Perikanan Thailand untuk mempertingkatkan kecekapan program pembiakbakaan dan mengekalkan integriti genetik *P. larnaudii* di Thailand. Hasil kajian ini mengesyorkan stok tidak sepatutnya dipindahkan di kalangan hatcheri yang berada di luar kawasan asal sesuatu populasi tertentu untuk mengekalkan integriti genetiknya.

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I certify that an Examination Committee met on 29th March 2006 to conduct the final examination of Thipsuda Tangprakhon on her degree of Master of Science thesis entitled “Genetic Differentiation of the Broodstocks of Black Ear Catfish, (*Pangasius larnaudii* Bocourt, 1866)” in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulation 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

THIPSUDA TANGPRAKHON

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