GENETIC MANIPULATION IN FARMED FISH

Enhancing Aquaculture Production

PROFESSOR DR. SITI SHAPOR SIRAJ

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PROFESSOR DR. SITI SHAPOR SIRAJ
BSc. (Hon) - UM
MSc. (Auburn University, USA)
PhD (Ehime University, Japan)

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ABSTRACT

Ever since the creation of human civilisation, breeding has been the pivotal struggles to increase and diversify agricultural production, enhance food security and incomes, and adapt farming to changing environmental conditions and social needs. This is achieved by exploiting variation of the plant and animal genetic resources' traits. These preferred genetic materials are improved through selection and reproduction and this practice remains the basis for producing new generations of domesticated and indigenous breeds.

Aquatic organisms particularly finfish and shellfish that are cultured today originated largely from the introduction of scientific breeding at the beginning of the twentieth century. Inclusion of crosses into breeding schemes prior to artificial selection and application of Mendel's laws of inheritance to improve both simple and quantitative traits follows. For effective breeding effort, selecting genetic materials with one or a combination of the required traits still relies mainly on physical features (phenotype) which are influenced by the environment thus could be misguided to the actual heritable genetic composition (genotype) of the material being considered.

The extent of aquatic diversity is both extremely large and relatively poorly understood. Thus, identification, selection and estimation of specific traits (such as growth rate, disease resistance) in wild and cultured fish and shellfish through genetic tools and breeding programmes are a must to secure future improvements in genetic resources for food. Ever since the discovering of DNA structure over 50 years ago, scientists have made tremendous strides in identifying genes and gene functions, making it increasingly possible to detect genetic differences (DNA polymorphisms) for traits among individuals in a much more direct way, thereby assisting in the selection of desired traits.

In fish, manipulation of the pre-embryonic stages rendering different ploidy levels is achievable and tolerable. Triploid fish is produced through various physical, chemical and biological stimulants. Triploids generally cannot reproduce, so the energy that is not channelled into reproduction would go instead to increasing growth rate. Induction of gynogenesis involves egg activation by irradiated homologous or heterologous sperm, and diploidization by retention of the second polar body (meiotic gynogenesis), or suppression of the first mitotic cleavage (mitotic gynogenesis). As a consequence, these gynogens are "instantly inbred" and can be screened for phenotypes quickly to avoid the generations of breeding necessary in a conventional manner.

Artificial breeding is a simple genetic technology for forced reproduction such as the use of pituitary gland extract and other hormones to initiate gamete development and induce spawning (the release of fish eggs) besides triggering factors of the environment.

INTRODUCTION

Freshwater ecosystems such as rivers, lakes and wetlands occupy less than 2% of the Earth's total land surface which provide a wide range of habitats for a significant proportion of the world's plant and animal species. Cosgrove and Rijsberman (2000) noted that the number of freshwater species worldwide is estimated at between 9,000 and 25,000, though yet remained to be discovered. This number is rapidly decreasing due to human interference. Physical alteration, habitat degradation, excessive water withdrawal and pollution have contributed directly or indirectly to the erosion of genetic variation and ultimately to the extinction of freshwater fish species. Other factors that reduce freshwater biodiversity include the invasion of non-native species and the mismanagement of inland fisheries as well as climate change.

Climate change is a compounding threat to the sustainability of capture fisheries and aquaculture development by modifying the distribution of marine and freshwater species. This in turn affects the seasonality of particular biological processes, altering marine and freshwater food webs, with unpredictable consequences for fish production and increased risk of species invasions and the spread of vector-borne diseases. An estimated 20% of the world's freshwater fish are vulnerable, endangered or extinct (Revenga and Mock, 2001).

According to the Food and Agriculture Organisation (FAO, 2005), the world fish harvest in 2005 consisted of 93.2 million tonnes captured by commercial fishing in wild fisheries, plus 48.1 million tonnes produced by fish farms. In addition, 14.8 million tonnes were produced by aquaculture (Table 1 shows harvest from selected South East Asian countries where tonnage exceeds 100,000). Fish production through aquaculture is expected to double in years to come (Table 2).

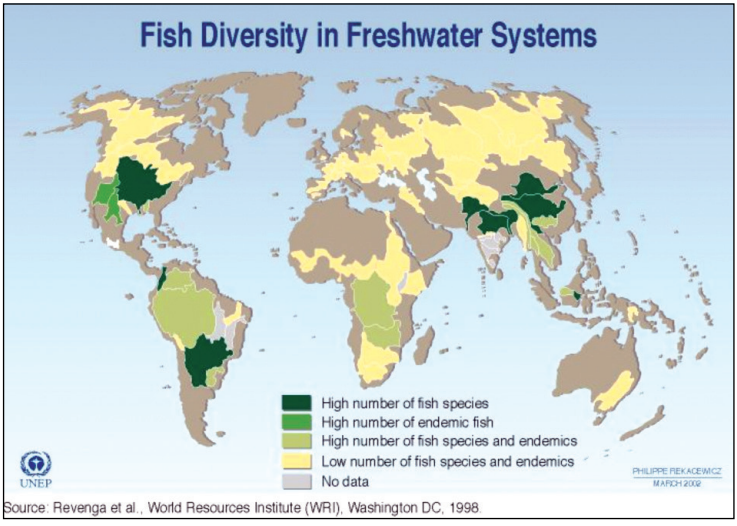


Figure 1 Areas of the world with high and low populations of fish species and of endemic species (Source: Revenga and Mock, 2001)

Table 1 Fisheries harvest from aquaculture (fish, crustaceans, molluscs) for 2005 in selected South East Asian countries **(**Adapted from FAO, 2005)

Country	Aquaculture (tonnes)
Indonesia	1,197,109
Malaysia	175,834
Myanmar	474,510
Thailand	1,144,011
Vietnam	1,437,300

Table 2 Fish production in 2004 and projections for 2010 and later simulation target years (in million tonnes) (Source: FAO, 2009)

	2004	2010	2015	2020	2020	2030
Information source	**FAO statistics**	**SOFIA 2002**	**FAO study**	**SOFIA 2002**	**IFPRI study**	**SOFIA 2002**
Marine capture	85.8	86		86		87
Inland capture	9.2	6		6		6
Total capture	95.0	93	105	93	116	93
Aquaculture	45.5	53	74	70	54	83
Total production	140.5	146	179	163	170	176
Food fish production	105.6	120		138	130	150
Percentage used for food fish	75%	82%		85%	77%	85%
Non-food use	34.8	26		26	40	26

The late 20th century has seen a revolution in the use of genetics and genomics in understanding distribution and abundance of fish populations for conservation and management. In the wild, fish genetic resources help determine the productivity of fish populations and their adaptability to environmental stresses such as climate change and human intervention. For aquaculture, the genetic resources affect the performance of farmed fish, help fish farmers satisfy consumer demands and even influence how farmed and wild fish interact in nature. However, information about aquatic genetic resources is still sporadic. Thus, application of the conventional and molecular genetics tools is a must for biological conservation and genetic resource preservation.

Various challenging genetic tools are developed to restore origin and potential of commercially important fish species. Some modern genetic technologies are already extensively applied by the diverse aquaculture industries, but not to the same extent for all important aquacultured species (FAO, 2009). Concentrated breeding efforts are given to major cultured species like common carp, Atlantic salmon, rainbow trout, channel catfish, Japanese ayu, Nile tilapia, and the Pacific oyster, while other major cultured species received, so far, relatively limited attention or not genetically improved at all. Most of the genetically improved strains reaching the aquaculture industry were developed through traditional selective breeding (selection, crossbreeding and hybridisation) (Hulata, 2001).

GENETIC IMPROVEMENTS IN CATFISHES

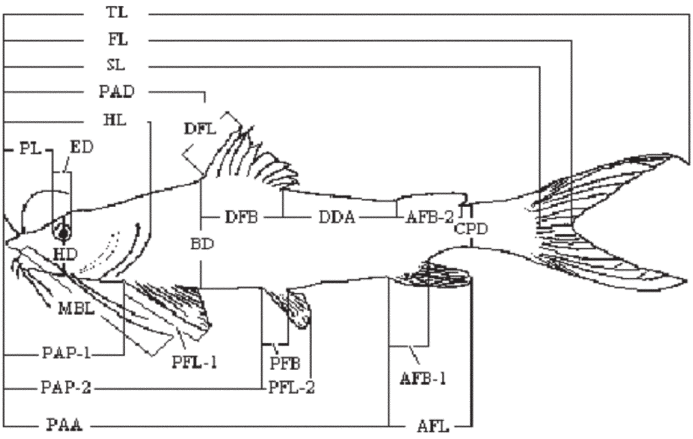
Order Siluriformes is composed of 38 families of catfishes that are widely distributed and highly diversified in freshwater (Sullivan et al., 2006). Catfishes can be found in major rivers, streams, ponds, canals, paddy fields or swamps throughout Malaysia and other parts of Asia. *Hemibagrus nemurus*, formally designated as *Mystus nemurus* (FishBase, 2008) commonly known as river catfish, yellow catfish or locally known as *ikan baung*, is one of the favourite economically important aquacultured species in Southeast Asia, especially in Malaysia and Thailand together with two other equally important species namely *Pangasius* (patin, dory) and *Clarias* (keli, walking catfish). Total catfish production in the country has doubled over the last five years at 497,556 metric tonnes (DoF, 2009). These catfishes are cultured in small cages, rice fields, ponds, cement tanks, canvas tanks and ex-mining pools for local consumption and export purposes. To date, the seed supply of majority of catfishes is seasonal and their inability to reproduce in captivity is a major hindrance to mass production.

A population's ability to adapt to environmental changes or stresses and thereby to survive is very much dependent on genetic variation. Estimation of genetic variation within and relationships among populations, species characterisation (Bardakci and Skibinski, 1994; Foo et al., 1995; Caccone et al., 1997; Ashley and Dow, 1994) are by using genetic tools such as morphological, karyological, biochemical and molecular markers.

Measuring Variation in Morphometric and Meristic Traits

Morphometric and meristic are characters used to determine and characterise two or more distinct morphs, each represented at a high enough frequency to be readily noticeable either inter or intra population besides determination by biochemical and molecular markers. Morphometric characters are body areas, such as head length and standard length, used for taxonomical characterization in fish (Figure 2). Usually, morphometric characters must be transformed to make them consistent in dimension with linear measurements (Strauss and Bond, 1990).

Among the morphometric characters, ratio of total length in relation to standard length (TL/SL), premaxilla to anterior end of dorsal fin to standard length (PAD/SL), dorsal fin length to standard length (DFL/SL), maxillary barbels length to standard length (MBL/SL), and premaxilla to anterior end of pectoral fin to standard length (PAP-1/SL) are conspicuous characters diagnostic in distinguishing *H. nemurus* populations (Lee, 2004).



Head length (HL), preorbital length (PL), eye diameter (ED), total length (TL), standard length (SL), fork length (FL), premaxilla to anterior end of pectoral fin (PAP-1), premaxilla to anterior end of dorsal fin (PAD), premaxilla to anterior of pelvic fin (PAP-2), premaxilla to anterior end of anal fin (PAA), the distance from posterior end of dorsal to anterior end of adipose fin (DAA), body depth (BD), pectoral fin length (PFL-1), dorsal fin length (DFL), pelvic fin length (PFL-2), dorsal fin base (DFB) and maxillary barbell length (MBL)

Figure 2a Morphometric characters of *Hemibagrus nemurus* (Cuv. & Val)

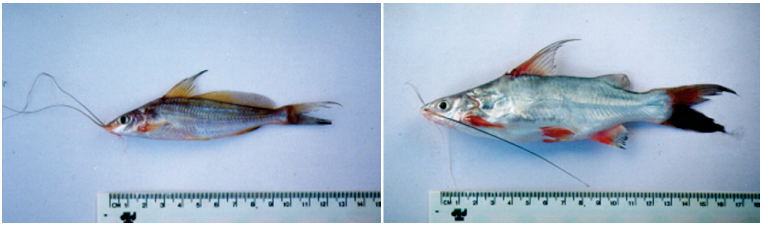
Figure 2b River catfish, baung, *Hemibagrus nemurus* (Cuv. & Val)

Meristic traits are countable characters in fish, such as fin rays, vertebrae and gill rakers. The caudal fin and the gill raker counts

are the meristic traits indicative of the *H. nemurus* population differentiation.

Genetic Monitoring using Allozyme Markers

Electrophoresis of protein variation is "taxonomically congruent" with morphological variation in interpreting phylogenetic and evolutionary relationships (Mickevich and Johnston, 1976). Allozyme markers using starch gel electrophoresis have proven to be very useful tools in determining genetic relatedness among species of catfishes (*Hemibagrus, Mystus* and *Tachysurus*) (Figures 3 and 4) at interspecific level (Leesanga et al., 2002; 2000) and population structure (Siraj et al., 1998). The biochemical differences between alleles of genes coding for metabolically important enzymes emphasise the relationship between genetic and functional diversity. The functional properties of different alleles often reflect a biochemical and genetic adaptation to life in a heterogeneous environment (Ward and Grewe, 1995). The concept is that if the different species or source populations have different frequencies of shared polymorphic genetic loci, then the genotypic arrays found in different species or source populations will be partially non-overlapping.



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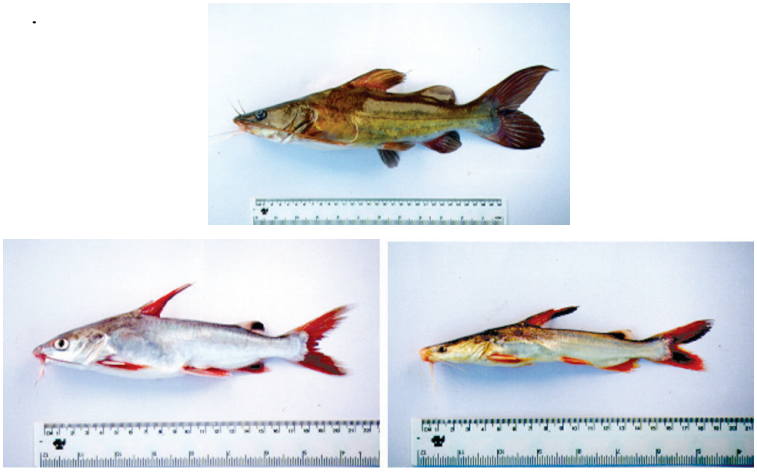


Figure 3 Selected river catfishes; (A) *Mystus cavasius* (Hamilton), (B) *M. gulio* (Hamilton), (C) *Hemibagrus nemurus*, (D) *Tachysurus caelatus* (Cuv & Val), (E) *T. truncates* (Cuv & Val)

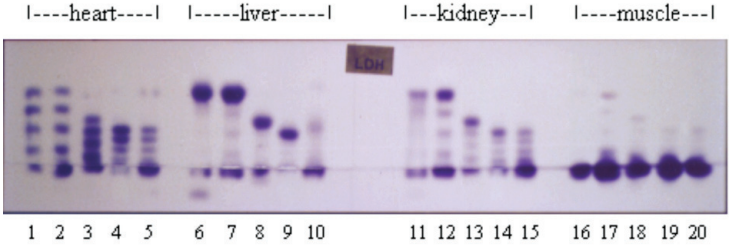


Figure 4 Electrophoresis pattern of lactase dehydrogenase (*LDH**) on *Mystus cavasius* (1,6,11,16); *M. gulio* (2,7,12,17); *Hemibagrus nemurus* (3,8,13,18); *Tachysurus caelatus* (4,9,14,19) and *T. truncates* (5,10,15,20)

Assessing Variability in Chromosome Structure

Chromosomal composition of any cells is a very useful tool for cytotaxonomy, ploidy determination, species identification and genotoxicity of pollutants (Padhi and Mandal, 2000).

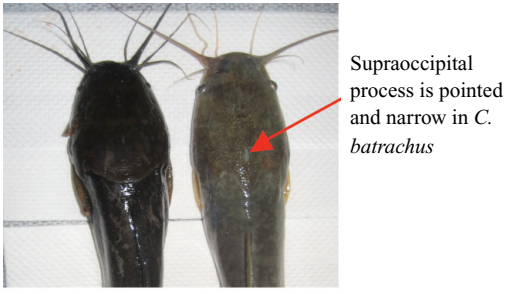


Figure 5a Walking catfish *Clarias macrocephalus* (left) and *C. batrachus* (right)

A wide karyological variation among selected catfishes (Figure 5a -e) was observed which play a key role in the karyotypic diversification (Siraj et al., 2009; Sahoo et al., 2007; Oliviera et al., 2007). Karyological analyses revealed that the diploid chromosome numbers in Malaysian catfishes ranged from 2n equal to 52 (*C. nieuhofii*); 54 in (*C. macrocephalus* and *C. batrachus*); 56 (*H. nemurus, C. gariepinus*) and 60 (*P. pangasius* and *P. sutchi*) (Siraj et al., 2009). Thus, the chromosomal taxonomy is a clear indication of its usefulness in determining the genetics and systematic of fishes (Gold et al., 1980).

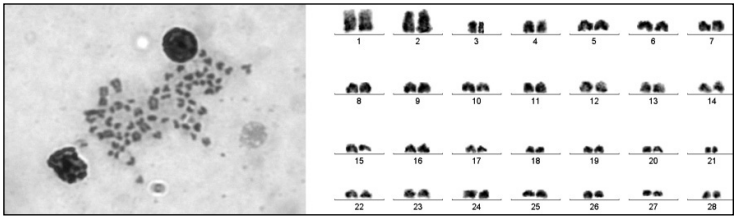


Figure 5b Metaphase spread and karyotype of *Clarias batrachus*

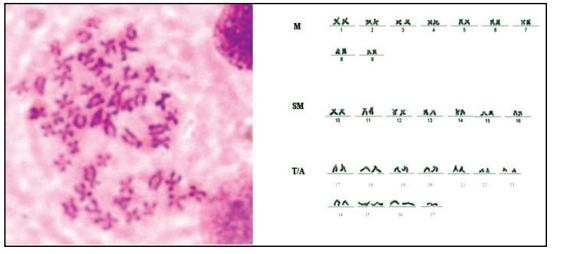


Figure 5c Metaphase spread and karyotype of *Clarias macrocephalus*

Figure 5d Patin, *Pangasius pangasius*

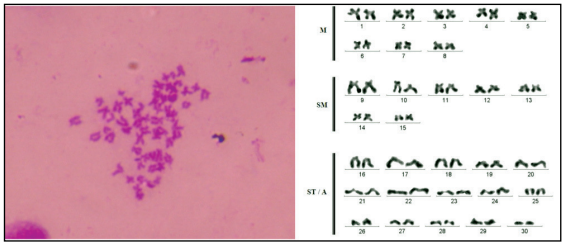


Figure 5e Metaphase spread and karyotype of *Pangasius pangasius*

Application of Molecular Markers

The application of molecular DNA based markers (Randomly Amplified Polymorphic DNA (RAPD), Restriction Fragment Length Polymorphisms (RFLP), Amplified Fragment Length Polymorphisms (AFLP) and microsatellites offer better and new insights into the taxonomy, population structure and conservation management of fish species (Smith and Wayne, 1996; Nguyen et al., 2006) than isozyme markers. These markers provide more reliable and consistent results for rapid species identification among the species (Ryan and Esa, 2006), levels of genetic variability, levels of gene flow and population subdivisions and for understanding factors contributing to fitness in freshwater fishes (Vrijenhoek, 1998). The RAPD markers have revealed high levels of genetic diversities of *H. nemurus* within the UPM and Sarawak populations and a low level of genetic variability in the Kedah population. Based on the position of each population in a dendrogram, the Sarawak population, which is located in East Malaysia is isolated from the rest of the populations in West Malaysia. However, clustering pattern differed from analyses of isozyme markers (Siraj et al., 1998).

Molecular markers also have an advantage over morphological marker since only a very small quantity of DNA is required from any tissues (scale, fin clip, muscle etc) of a particular organism for analysis using the Polymerase Chain Reaction (PCR) technology (Avise, 1994). This advantage is crucial particularly for endangered, protected or declined population or species where a non-destructive sampling is required for genetic analysis (Ward, 2000; Esa et al, 2008).

Comparatively, the AFLP is a more efficient marker system than RAPD (Figure 6) for identifying genotypes within populations of *H. nemurus* (Chong et al., 2000; Leesanga et al., 2004). The AFLP technique involves the use of a pair of selective primers to amplify

a subset of DNA fragments from a pool of restriction fragments generated by two restriction enzymes (Vos et al., 1995), while RAPD uses a single 10-mer primer to amplify randomly a few regions in the genome (Williams et al., 1990).

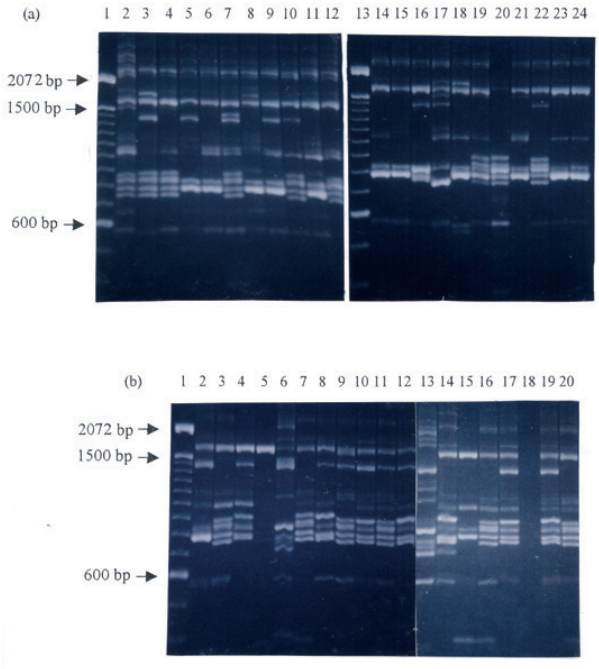


Figure 6 RAPD patterns generated by RAPD primer OPD18 on selected *H. nemurus* populations (A Perak; B Selangor)

DNA Microsatellite Markers Development

Successful attempts have been made in developing microsatellite markers based on Random Amplified Microsatellites (RAMs) in *H. nemurus* (Usmani et al., 2001; Chan et al., 2005; 2005b; Hoh et al., 2007) (Table 3). The developed technique is non-radioactive, rapid and cost effective in isolating the microsatellite DNA. The newly

designed microsatellites are polymorphic (Figure 7), amplified and characterised on various populations of *H. nemurus* across East and West Malaysia (Usmani et al., 2003).

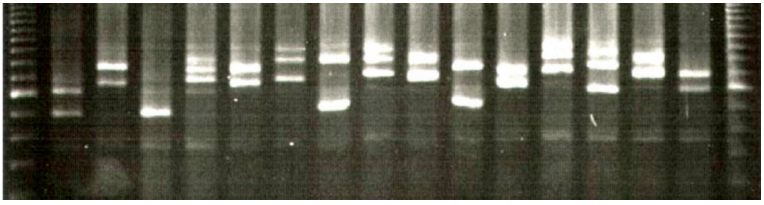


Figure 7 Sample of microsatellite profile of *H. nemurus*

The importance of microsatellites in genetic studies has been greatly acknowledged over the years (Chambers and MacAvoy, 2000). Being a co-dominant marker system, it is more informative and effective than RAPD, RFLP and AFLP for population, phylogenetic, linkage and quantitative traits loci (QTL) studies. It is useful in monitoring levels of heterozygosity in broodstocks.

Table 3 Sequence of selected microsatellite primers designed for *H. nemurus*, primer sequences and the expected size of amplicon (Hoh, 2006)

No Locus	Repeat motif	Primer sequences (5' – 3')	Product size (bp)
Mnc340	(T)8(GT)4	F: GTCACTAGCACTGCACTTCA R: TGATAAAATAAACCCGTGCT	186
Mnc441	(AAAT)4AAT (TGG)3	F: CAGGTGGAACATTTTGGAT R: TTTAGAGCTATTCCCTTGGA	172
Mnc65B	(GT)10N4(ATA)3	F: CCTGGTTTTTCAGCAGTATT R: GGATCAGCATGCAACTAAA	180

Mnc434A	(TAT)3N5 (CA)10	F: ATCAGCATGCGACTAAAACA R: TGGTTTTTCAGCAGTATTGG	176
Mnc434B	(AC)5	F: CCCAATACTGCTGAAAAACC R: TCTTTGGGCAATTAGTGGAC	132
MnV2-261	(CTC)5CTT (CTT)2N2(CT)5 (CTT)5	F: GCTGAAGGCTCCTCCTCCT R: TTCAGCACAGAGCTCTAACA	197
MnBp5-1-28	(CA)12; (GAA)3 (GT)7	F: GCGCGCCTACCACACACAC R: TGCTCGTCTCGTACACAC	156

a) Mendelian Segregation on the Microsatellite Markers

The co-dominant nature of the newly developed microsatellite markers enables the identification of both homozygous and heterozygous individuals at a particular locus (Table 4) (Hoh, 2006). The genotypes and phenotypes of the parents and their offspring could be determined directly. Consequently, this feature makes them to be very attractive markers.

Table 4 Selected microsatellite loci genotype numbers among the F1 progeny and $\chi 2$ values for the expected Mendelian segregation ratio (Hoh, 2006)

Locus	FA / FB (parental population)	Parent genotype (♂ X ♀)	Expected F1 genotype ratio	Observed F1 genotype ratio	$\chi 2$	P value
Mnc434a	FA	BB x AB	50:50	45:55	1.000	0.317
MnBp5-2-06b	FB	AA x AB	25:25	31:19	2.88	0.090
MnRm30-1	FB	AB x AB	12.5:25:12.5	10:28:12	0.880	0.644
MnBp8-1-63a	FA	AA x AB	50:50	58:42	0.256	0.110
MnBp5-1-20b	FA	AC x BC	25:25:25:25	16:19:36:29	10.16	0.017
MnBp5-2-02b	FA	AB x AB	25:50:25	30:50:20	2.000	0.368

In contrast, the genotypes of the parents could only be inferred through the phenotypic ratios observed in the progeny for Mendelian inheritance testing of dominant markers as it was done for the RAPD and AFLP markers developed for this catfish species by Chong et al. (1999).

b) Genetic Linkage Mapping

The tools and methods used in genetic linkage mapping are polymorphic DNA markers, statistically analysed and advanced molecular biology techniques. The rationale is that DNA can be described by gene maps and thus the genes affecting traits can be located on the genetic map too. In aquaculture, a map of channel catfish was constructed using isozymes in 1994 (Morizot et al., 1994). Gibley et al. (2004) generated a significant microsatellites based genetic linkage map of Atlantic salmon with only 50 loci. For walking catfish, a preliminary genetic map was generated based on the AFLP markers and a total of 31 linkage groups were generated from 134 loci (Poompuang and Na-Nakorn, 2004).

Consequently, a preliminary linkage analysis was performed on Malaysian *H. nemurus* based on three mapping populations involving a total of 75 newly designed polymorphic microsatellite markers (Hoh, 2006). The linkage maps generated (Figure 8) were from segregating populations derived from a strategy described by Grattpaglia and Sederoff (1994) called "pseudo-testcross strategy".

Molecular breeding and genetic mapping based on the use of DNA based markers can expedite the progress of breeding programmes to improve important economic traits. A high density genetic linkage map is essential to improve the efficiency of breeding by marker-assisted selection and for the identification, isolation and eventual cloning of commercially important genes. In the effort to construct such a map, many molecular markers are

needed including highly polymorphic co-dominant ones such as microsatellite loci.

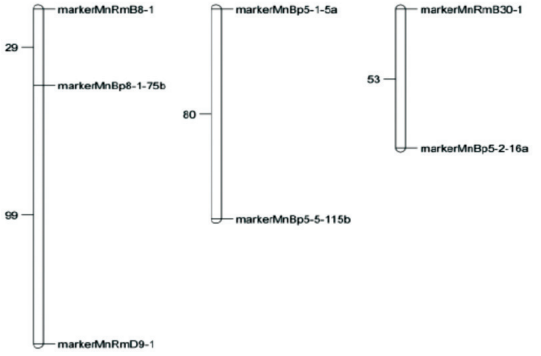


Figure 8 Linkage map generated in *H. nemurus*

One of the challenges in genetics is the identification of genes that contribute to quantitative traits, and genetic linkage maps are an important tool for dissecting this variation into underlying genetic factors. These are mostly traits of evolutionary and economic importance, such as fertility, fecundity, disease resistance, and growth performance. The tools to dissect continuous variation have been developed a long time ago, yet these methods are only becoming routine over the last decade. However, the black box of quantitative traits still remains unknown.

c) Cross-Species Amplification

The flanking sequences of microsatellites were shown by several studies which may be conserved well enough through evolution to serve as primer-annealing sites for closely related species (Primmer et al., 1996; Tong et al., 2002). Hence these markers may be a valuable resource for identifying polymorphic loci in other species

via cross-species amplification. In majority of catfishes no genetic markers are available as developing microsatellites are laborious, tedious, time consuming and costly. Chan et al. (2005) tested the ability of the developed microsatellite loci transfer from *H. nemurus* to other catfish species. The *H. nemurus* microsatellite loci were successfully cross-amplified in *Clarias* sp and *Pangasius* sp (Figure 9). This serves as an alternative approach to study other organisms without having to go through the technically demanding process of microsatellite isolation.

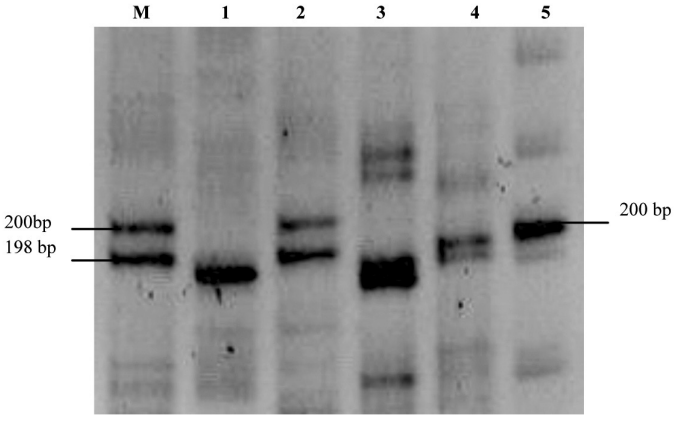


Figure 9 Microsatellite banding profile of cross-species amplification on *P. micronemus* using primer pair of *H. nemurus*

Triploid Production

The triploidization technique was introduced to aquaculture and fisheries management to increase the probability of producing sterile fishes. The interest is especially focused on triploidy, as triploid fish have been assumed to be sterile and potentially can avoid the growth depression, poorer feed conversion and survival losses which are associated with sexual maturation in normal fish (Purdom, 1983;

Thorgaard and Gall, 1979). Triploidy is a condition in which cells possess three haploid chromosomes (Figure 10a, b) and the fish is normally sterile (Siraj et al., 1993) and may be of great practical value in fish cultivation.

Triploidy in *H. nemurus* and *C. batrachus* was induced by applying cold shock treatment to the fertilised eggs (Figure 10c) (Lee, 2004; Siraj et al*.,* 1997). Lee (2004) found the nomenclature of a normal diploid (2n) *H. nemurus* to be equalled to 56 (XY male) and 2n, 56 for XX female. On the other hand the triploid male was 3n, 84 (XXY) and 3n, 84 (XXX) for female. In *C. batrachus* the female is heterogamety with ZZ-ZW mechanism, having 2n equal to 50 and triploid possessing chromosome number of 75 (Siraj et al., 1992; Pandey and Lakra, 1997).

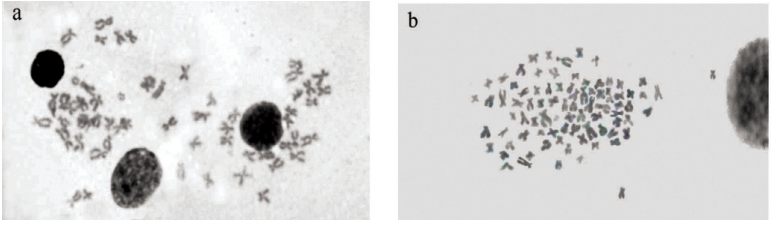


Figure 10a (a) Diploid male (b) triploid female chromosomes spread of *H. nemurus*

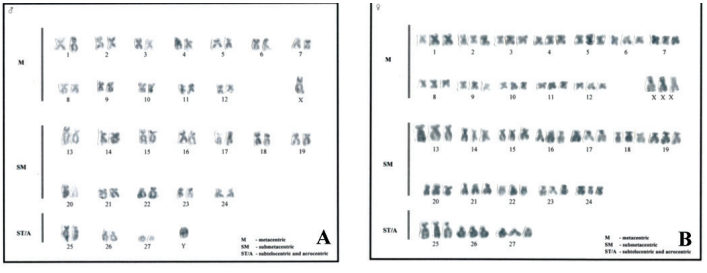


Figure 10b Karyotype organisation for diploid male (A); and triploid female (B) *H. nemurus*

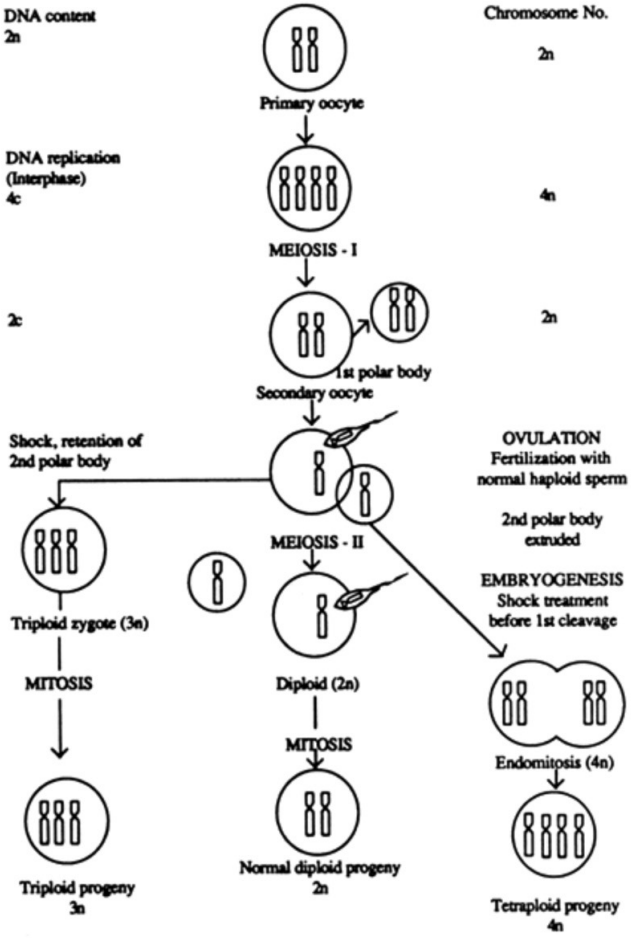


Figure 10c Illustrations of events for inducing triploidy and tetraploidy in fish (Source: Reddy et al., 1997)

Induced Spawning

The basic requirement of the controlled fish culture industry is the fish seed. The major constraints in fish seed supply are spontaneous captive breeding, short supply of quality seed and dependency on wild seeds, which is unreliable, time consuming and uneconomical. Generally, the reproduction of fish is controlled by both the external and internal environmental factors such as temperature fluctuations, rainfall, water and food qualities, and photoperiod as well as food availability and a series of hormone production in both the male and female fish (Figure 11a and b). To overcome such problems, induced spawning is thought to be the only alternative method for quality seed supply (Cheah et al., 1990; Sharma et al., 2010).

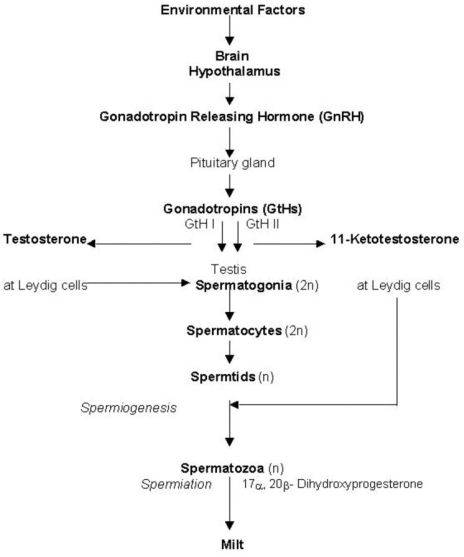


Figure 11a Events in the reproductive endocrine control of maturation and spermiation, amongst male teleosts (Source: Harvey and Carolsfeld (1993))

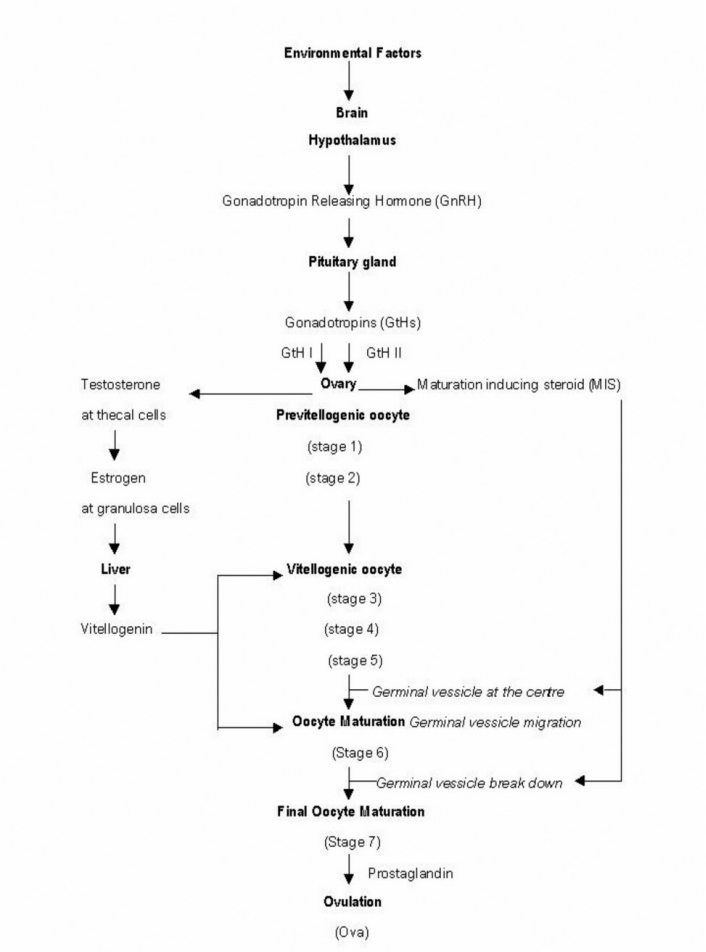


Figure 11b Events in the reproductive endocrine control of maturation and ovulation, amongst female teleosts (Source: Harvey and Carolsfeld (1993))

Among several inducing agents used in fish breeding, salmon gonadotropin releasing hormone (sGnRH) or luteinising hormone releasing hormone (LHRH) analogues in combination with dopamine antagonists was found to be effective in fish breeding

(Lin and Peter, 1996). Gonadotropin releasing hormone analogue (GnRHa) has been successfully used for induced spawning of some fish species which include winter flounder (*Pseudopleuronectes americanus*), Asian catfish (*Pangasius hypophthalmus*) (Harmin and Crim, 1992; Legendre et al., 2000). The use of synthetic inducing agents (Ovaprim, Wova) for successful ovulation followed by stripping is a common practice and found to be efficient in successful spawning of fish (Peter et al., 1988;, Nandeesha et al., 1990; Brzuska and Adamek, 1999; Cheah and Lee, 2000; Lee, 2004; Siraj et al., 2006) (Figure 11c).

Figure 11c Administration of hormone at the dorsal musculature of *H. nemurus*

Natural hormones such as carp pituitary extract (CPE), contains the hormone gonadotropin (GtH) and human chorionic gonadotropin (HCG) that act on the gonads. Cheah et al. (1990) were among the pioneers in using CPE to successfully induced spawn *C. batrachus* in the country.

Some problems are found associated with artificial propagation in fish broodstock which include simultaneous maturation of both

male and female fish, egg and sperm quality and survival of larvae. Thus there is a need to understand their reproductive biology so as to be able to use such information for aquaculture production and management. Khan et al. (1990) provided baseline information for the induced breeding of *H. nemurus* such as fecundity, gonadosomatic index (GSI) and seasonal variations in oocyte diameters. Whilst Christianus et al. (1998; 1999) tabled information on the reproductive biology of male *H. nemurus* with respect to gonadal development prior to spawning. Besides, fluctuation of the weather conditions was found to also affect the spawning ability of the fish broodstocks.

GENETIC IMPROVEMENTS IN CYPRINIDS

The cyprinids belong to the family Cyprinidae, consists of the carps, the true minnows, and their relatives (e.g. the barbs and barbels; attractive smaller cyprinids from both tropical and subtropical waters). It is the largest family of freshwater fish, with over 2,400 species (Fishbase, 2004). Cyprinids are highly important food fish; they are fished and farmed across Eurasia.

Kelah, Malaysian Mahseer

Cyprinids of the genus *Tor* (Gray), commonly known as mahseer *Tor tambroides* (Valenciennes) (Figure 12a), locally referred to as "kelah" in Peninsular Malaysia (Mohsin and Ambak, 1983) and "empurau" in Sarawak (Litis et al., 1997) is one of the important cyprinids for food, the aquarium industry and game fishing in the country (Ng, 2004). *T. tambroides* inhabits the upper streams of clean and unpolluted river systems with rocky beds and hilly terrains (Singh and Menon, 1994).

Figure 12a *Tor tambroides*

T. tambroides is morphologically identified based on the presence of a long median lobe character that is shorter in the other two mahseer described in Malaysia (Figure 12b) (*Tor douronensis* Valenciennes and *Tor tambra* Valenciennes) (Kottelat et al., 1993; Kottelat and Whitten, 1996; Rainboth, 1996). *T. tambroides* collected from Peninsular Malaysia tends to exhibit two colour-types (silver-bronze and reddish) based on its colouration (Ng, 2004; Siraj et al., 2009). Thus, species identification strictly on the basis of morphological characters (Figure 12c) alone is quite unreliable, because of considerable geographical and ecological variability (Tsigenopoulos and Berrebi, 2000).

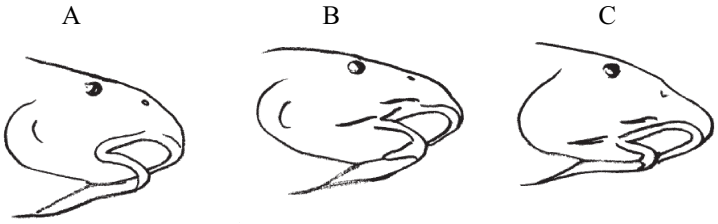


Figure 12b Comparisons of kelah based on presence of median lobe. (A) Lower lip without median lobe, *Acrossocheilus hexagonolepis* (Tengas); (B) Lower lip with long median lobe, *Tor tambroides;* (C) Lower lip with short median lobe, *Tor duoronensis* or *Tor tambra*

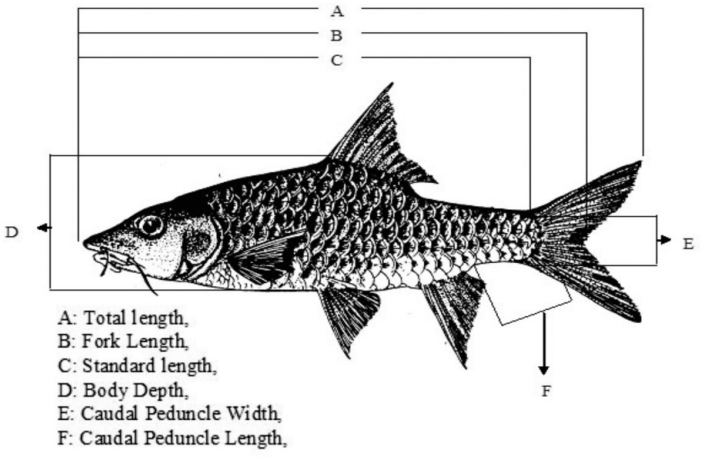


Figure 12c Morphometric characters of *Tor tambroides* (Valenciannes)

In addition, very little taxonomic work has been done to systematically sort out Malaysian mahseer for proper reference. The most cited work is by Mohsin and Ambak (1983) who described *Tor tambroides* and *Tor soro* as two valid mahseer found in Peninsular Malaysia, while a more recent opinion by Ng (2004) suggested the occurrence of three species; *T. tambroides*, *T. tambra* and *T. douronensis*. Thus, the identification of mahseer samples collected

from Peninsular Malaysia mostly rely on reference specimens examined from other Southeast Asia regions, the closest being specimens from North Borneo (Inger and Chin, 1962) and Western Kalimantan (including the Sarawak province (Roberts, 1989)) where *T. tambroides* and *T. douronensis* are two valid mahseer described from the region. Mitochondrial DNA analyses carried out by Esa et al. (2008) confirmed the reciprocally monophyletic status between *T. tambroides* and *T. douronensis* (Figure 12d) reinforcing their taxonomic status as distinct species.

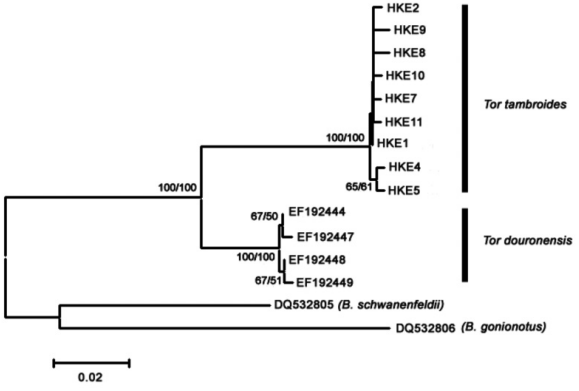


Figure 12d Neighbour-joining (NJ) phylogram showing the relationships among COI haplotypes of *T. tambroides* and *T. douronensis*

Environmental degradation (i.e. river pollution, deforestation, watershed erosion etc) of water heads and upper streams has led to the rapid destruction of *T. tambroides* natural habitat. Furthermore, excessive demand on the highly priced *T. tambroides* flesh (can reach up to 450 Malaysian Ringgit per kg in Sarawak) has led to uncontrolled fish harvest or destructive fishing practices by locals and illegal fish poachers, sometimes using highly damaging methods such as poison and bomb, resulted in rapid reduction

in its population size (Ng, 2004). *T. tambroides* distribution, particularly in Peninsular Malaysia are currently limited to the less or undisturbed upper streams and protected areas (such as natural parks).

Although currently not listed under the IUCN list as a protected or an endangered species, the drastic decline of the natural populations of *T. tambroides* has increased awareness of many parties including the relevant authorities (e.g. Fisheries Department and policy makers) on the importance of the conservation and proper management of the species.

Captive Breeding in Kelah

One of the problems of captivating broodstock in artificial environment is spontaneous spawning. There is no reported document where mahseer spawn naturally in cultured ponds. In culture environment, factors such as water quality, current, dissolved oxygen, tides and temperatures are dissimilar from the natural environments, and this could interrupt natural spawning cycle of the cultured mahseer. The environmental differences in the wild and captivity (ponds or tanks) may induce specific physiological responses or fail to entrain the culmination of the normal reproductive cycle. This may lead to a prolong stress to the fish which subsequently will affect the endocrine control process of reproduction (Milla et al., 2009).

Gonad maturation is one of the most important developmental stages during the life cycle of an animal, which undergo reproduction. Fish usually spawn when maturation is completed, producing mature eggs and sperms. In freshwater tropical fish, such as giant catfish (Manosroi et al., 2003), mahseer (Ismail et al., 2011) and tilapia (Campos-Mendoza et al., 2004) gonadal maturity (Figure 13a) is found to be highly correlated with daylight and temperature

changes throughout the year and classified as non-seasonal and multiple spawners. Spawning, ovulation and final maturation are known to be triggered by changes in water quality caused by rainfall or drought season (Cornish, 1998).

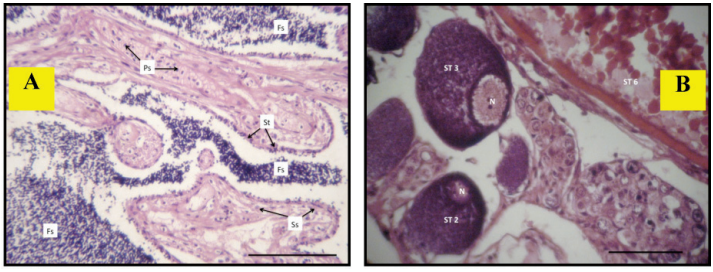


Figure 13a Testicular (A) and oocytes (B) developmental stages in mahseer

The recent success in the captive breeding of *T. tambroides* (Ingram et al., 2005) has opened an opportunity for the mass production of this highly valued mahseer both as an aquacultured fish for commercial production and for fish restocking for conservation purposes (Nguyen et al., 2006). Ismail et al. (2011) provided the first analyses of male and female mahseer hormones that might have been related to gonad development. This fundamental information on the annual gonad hormonal profiles and gonad maturation of Malaysian mahseer is pertinent for the conservation and breeding programme of this fish.

Several rise of 11KT (11-keto-testosterone) production indicate active spermatogenesis throughout the year, while E2 production indicates continuing vitellogenesis (Figures 13b, c).

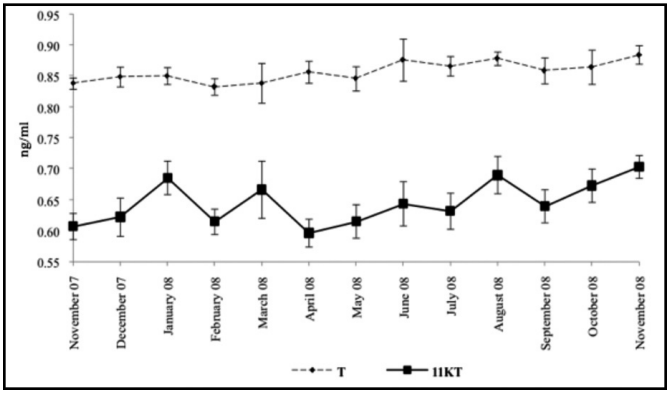


Figure 13b Changes of testosterone and 11-keto-testosterone in male mahseer throughout the year

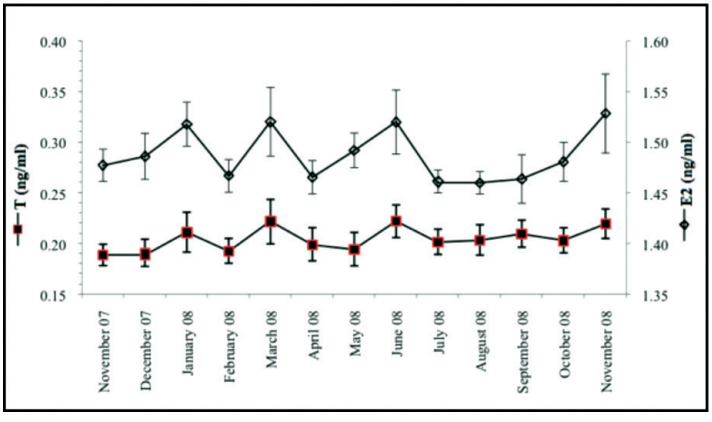


Figure 13c Changes of T (Testosterone) and E2 (17β-estradiol) in female mahseer throughout the year

Lampam, *Puntius (Barbodes)* spp

Puntius spp are the most abundant species among the cyprinids in Malaysia. They contribute substantially to the fisheries industry and are economically important as aquarium as well as food fish. The puntioid fish exhibit a wide range of sizes from 20mm to 250mm (Mohsin and Ambak, 1983). *Puntius gonionotus* (lampam jawa) being introduced from Indonesia in the early 50's (Soong, 1963) is the most widely cultured followed by *P. schwanenfeldii* (lampam sungai) (Figure 14a). The ornamental groups (*P. fasciatus, P. binotatus, P. lasteristriga* and *P. pentazona*) (Figure 14b) are normally cultured for the local aquarists and also exported.

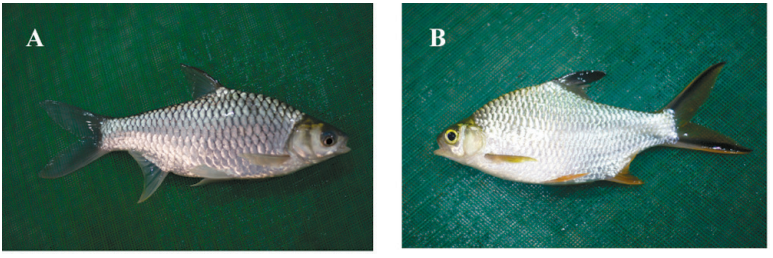
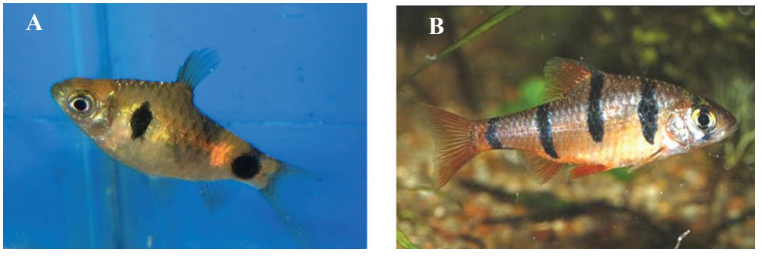


Figure 14a Lampam jawa, *Puntius gonionotus* (Bleeker) (A) and lampam sungai, *P. schwanenfeldii* (Bleeker) (B)



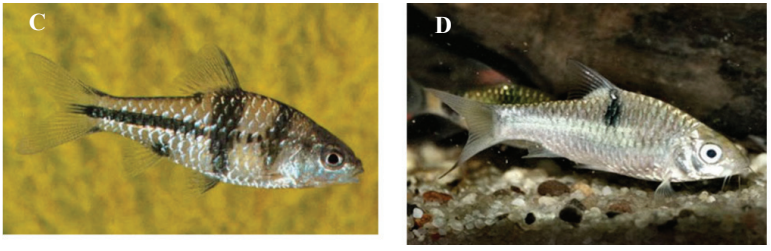


Figure 14b The ornamental puntioids; *Puntius fasciatus* (A), *P. binotatus* (B), *P. lasteristriga* (C) and *P. pentazona* (D) (Source: www.MyFishForum.com)

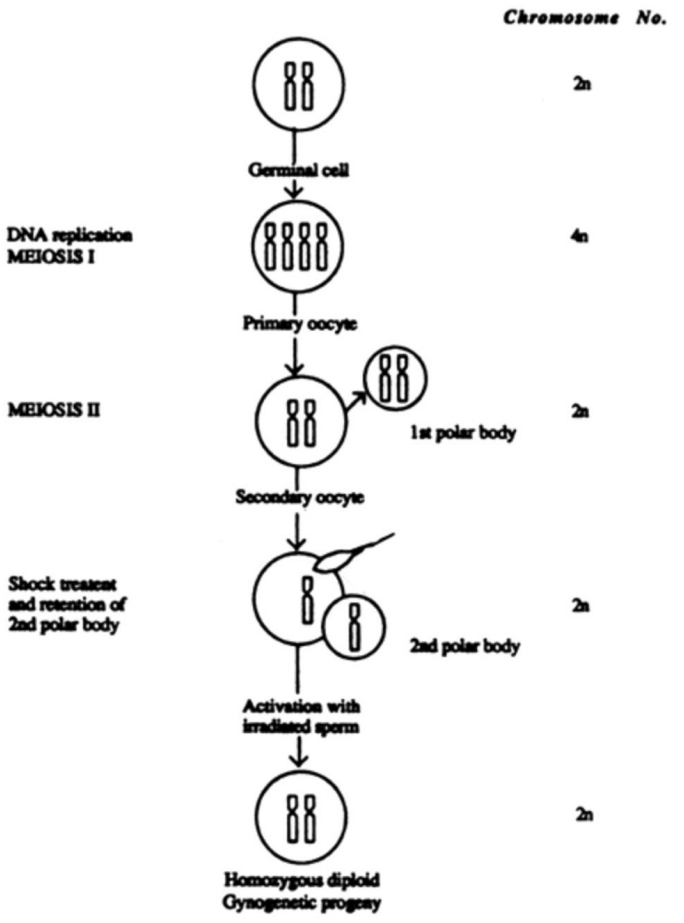
Siraj et al. (1993) categorised the Malaysian *Puntius* spp into four groups based on allozyme markers; the big group (making up food fish), and three small groups, one consists of the indigenous puntioid; and the other two small groups each represents origin from Indonesia and China, respectively.

Chromosome Manipulation in Lampam, *Puntius (Barbodes) gonionotus*

Lampam jawa easily breeds in the ponds during the onset of the rainy season, and its propagation through induced spawning by pituitary extract has been successful as an alternative to ensure a constant supply of seed (Tajuddin et al., 1977).

Gynogenesis is a mode of reproduction involving all maternal inheritance (Purdom, 1983) (Figure 14c). It has become increasingly important and popular as a rapid method of producing inbred lines in fish. The gynogens are generated by fertilising eggs with genetically inactive sperms giving rise to haploid individual (Figure 14d). Diploidisation is achieved through shock treatments (temperature or pressure) to obtain normal and viable fish. Siraj et al. (1993) produced 66.6% viable diploid gynogenesis in *P. gonionotus* by

using UV-irradiated sperms of *P. schwanenfeldii* followed by cold temperature shock treatment.



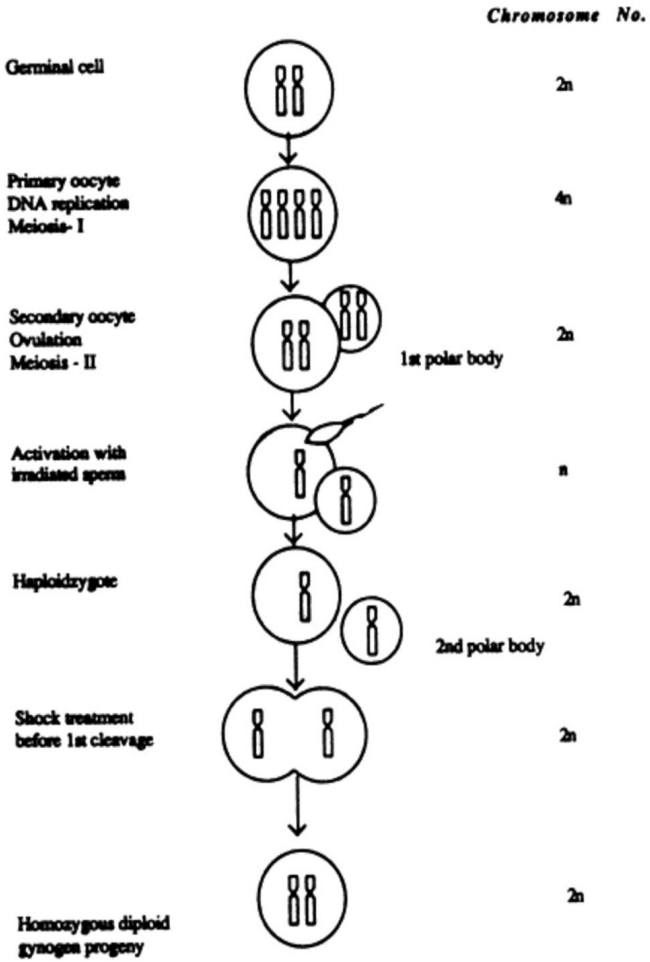


Figure 14c Illustration of events for inducing meiotic (A) mitotic (B) gynogenesis (Source: Reddy et al., 1997)

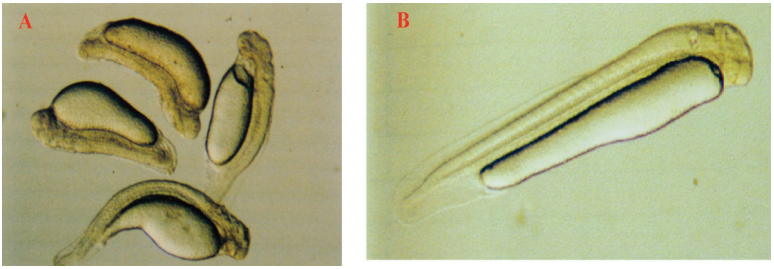


Figure 14d Haploid (A) and normal diploid (B) day-old larvae of *P. gonionotus*

Administration of early shock treatment to the activated egg with UV-irradiated milt and retaining the second polar body gives rise to meiotic gynogens. The meiotic gynogens may be heterozygous or homozygous. On the other hand, mitotic gynogen is induced when activated eggs are given later shock treatment. The mitotic gynogens are completely homozygous in one generation due to the combination of two identical chromosome sets which arise from duplication of the maternal genome in the first mitosis (Siraj et al., 1994; Nagy et al., 1983; Han et al., 1991) (Figure 14e).

Figure 14e Meiotic (A) and mitotic (B) gynogens of *P. gonionotus*

CONCLUSION

Understanding the distribution of genetic diversity in wild stocks is essential for long-term wild stock sustainability as genetic diversity levels may be declining. Before any breeding and conservation programme is initiated, it is essential that the populations and stocks are managed, cultured and used in selection work to be characterised by genetic (morphological, karyological, biochemical and molecular) markers. These markers have been widely used in species characterisation, estimation of genetic variations within and relationships among populations and as tools in marker assisted

breeding programmes. The identification of highly polymorphic genetic markers that provide high reliability is a crucial step to generating population genetic data.

The main goals of fish and shellfish breeding programmes are to increase the profitability and sustainability of production enterprises, while maintaining genetic variability in the cultured stock. Farmers' knowledge on the internal and external environmental cues that influence reproduction and the natural reproductive isolating mechanisms help them to domesticate aquatic species and thus assist a steady and consistent supply of fish to the markets.

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BIOGRAPHY

Siti Shapor Siraj was born in 1954 in Port Dickson, Negeri Sembilan. She received her primary and secondary education in Sekolah Kebangsaan SiRusa and Sekolah Menengah Tinggi, Port Dickson, respectively before continuing at King George V, Seremban. She graduated in Genetics at the University of Malaya in 1979 and soon after was employed as a tutor in the Faculty of Fisheries and Marine Science, UPM. She obtained her Master of Science in Fish Breeding at Auburn University, USA (1983) and continued her service as a lecturer at the same Faculty in 1983. She gained her doctorate in Fish Genetics and Breeding (1993) at Ehime University, Japan.

To her credit she has supervised and co supervised more than 50 postgraduate and 100 undergraduate students. She heads research projects which focus on genetics and reproductive biology of freshwater fish, the culture of finfish and also culture and genetics of freshwater and marine prawn from local and foreign grants. Out of which more than 200 papers were published in international and local journals, proceedings, abstracts, bulletins and reports. Her expertise and interest has been generously shared with the public particularly to fish farmers. Her work has also gained recognition and presented to the public via radio (Era Jaya, RTM) and television ("Malaysia Hari Ini" and "Majalah 3", TV3).

Leadership quality she possesses reckoned her the appointment as chairperson and member of organising committees of conferences at national and international levels and also as manager of MAHEVA (European Union students and staff mobility programme – from 2009 to 2012). She was a Secretary for the Malaysian Fisheries Society (MFS) in 1999 - 2000 and as the Vice President of the Society from 2008 – present and life member for Malaysian Society

of Applied Biology (MSAB) and Genetics Society of Malaysia (GSM).

She moved to Faculty of Science (then Faculty of Science and Environmental Studies) in 1996 (as Faculty of Fisheries and Marine Science moved to Kuala Terengganu) and was entrusted to lead the Department of Biology, Faculty of Science from 2003 till June 2007; her continuous and unfailing contribution reckoned her the position as the Deputy Dean (Academics) of the Faculty (2007-2008). In June 2008 she was transferred to Department of Aquaculture, Faculty of Agriculture, UPM and was appointed as Deputy Dean (Graduate Studies, Research and Internationals) (2009-2010). Currently she heads the Marine Science Station in Port Dickson, UPM. She was appointed associate professor in 1998 and full professor in 2008.

She is alhamdulillah blissfully married to Rosli Mohammad and blessed with four wonderful children (two girls and two boys).

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To All - Thank You and May Allah Bless You

LIST OF INAUGURAL LECTURES

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