CHAPTER 1

INTRODUCTION

The snakehead fish (*Channa striata*) is a freshwater fish species indigenous to Malaysia (Mat Jais 2007a). In Malaysia, *C. striata*, locally known as *Ikan Haruan* are traditionally found in paddy fields (Tan et al., 1973) where they are able to adapt well to the seasonal and temporary wetland cycles (Wee, 1982). This predaceous species is valued for its therapeutic properties as well as nutritive qualities. The flesh is believed to be rejuvenating due to its recuperative elements and, hence is given to elderly and those in convalescences (Kumar et al., 2008 and Mat Jais, 2007a). It is also rich in essential amino acids and fatty acids that accelerates wound amelioration (Baie and Sheikh, 2000) in addition to anti-fungal, anti-microbial and anti-inflammatory activities (Zuraini et al., 2006) among others. Besides that, the boneless, meaty and white flesh of snakehead fish is also a good and economical source of dietary protein (Gam et al., 2005).

The ever-growing comprehensive list of pharmacological properties discovered in *C. striata* has prompted many local entrepreneurs to build up a niche market commercialising *C. striata* products that are aimed towards enriching and supplementing the consumer's nutrition and health. They include edible extracts, canned foods, pills, creams, fish floss and many more. Due to its carnivorous nature, *C. striata* was regarded as a pest and was not considered a priority candidate fish species for aquaculture farming in Malaysia (Mat Jais, 2007a). However, the increasing popularity of *C. striata* as a reputable protein source, coupled with the extensive commercialisation of *C. striata* products meant that dependence of the wild capture of *C. striata* to meet the significant rise in demand for the species is no longer viable.

The wild population of *C. striata* in Malaysia are on a gradual decline due to several reasons. The great demand for *C. striata* in the nation has compelled farmers to collect juvenile wild fingerlings from ricefields and the continuous irrigation and drainage canals. Where once *C. striata* used to be in abundance, ricefields has been beleaguered with double cropping and concurrent increase of pesticides and herbicides which cause the dwindling of species (Ali, 1999). Other than over-fishing, anthropogenic activities such as dam constructions, industrial and domestic pollution, rapid deforestation for the development of plantation (palm oil and rubber) and public housing areas have destroyed the natural spawning areas, adversely affecting the survival of the species. Although Malaysia has never experienced drastic weathers, there are apparent unpredictable climate change such as haze phenomena, floods and drought that exert negative influences the population of *C. striata* in the wild (Mat Jais, 2007a).

Although *C. striata* are cultured commercially in Malaysia, production pales in comparison to established farms in Thailand, Indochina, Indonesia, Philippines, China and India (Li et al., 2006). In 2010, production of *C. striata* were grouped together in a "miscellaneous" category [which included snakeskin gouramy (*sepat siam*), climbing perch (*puyu*) among others] that accounted for 3.1% (2, 884.62 metric tonnes) of the total estimated fish production from freshwater ponds (92, 833.45 metric tonnes) in Malaysia (DOF, 2010). Currently, the small number of local mono- and poly-culture farms scattered throughout the country are able to cater to the rising demands for the species, although majority of them are small- to middle-scale based operational farms (usually due to insufficient start-up capitals).

Genetic diversity is a fundamental requirement for any successful long-term culture practices. These routines consists of domestication selection on the culture, species stock management and identification, selective breeding programmes to facilitate commercially superior breeds (hybrid vigour) as well as the sustainable use and restoration of wild resources (Ha et al., 2009; Liu and Cordes, 2004). In addition, sufficient genetic variation is a requisite in cultures in order to obtain genetically divergent lines for accommodating varying aquaculture conditions, which are frequently accompanied by disease outbreaks. Therefore, this necessitates appropriate documentation of genetic diversity characteristics that are present in wild stocks so that these stocks may also serve as an immediate resource for addressing genetic diversity in cultured lines. Nevertheless, in most cases, cultured species are susceptible to the loss of genetic variability compared to their wild progenitors. This is because cultured populations are more vulnerable to the intensified effects of inbreeding and the reductive pressures of genetic drift due to their closed environment. Hence, a comprehensive understanding on the genetic relationships among cultured and wild *C. striata* populations is vital for successful implementation and sustainable management of these culture programmes.

For that reason, molecular genetic markers such as microsatellites, can be aptly employed to assess genetic variation present in both cultured and wild populations. Microsatellites or simple sequence repeats (SSRs) are short DNA sequences containing tandem repeats of mono, di, tri, tetra and onwards (up to 6 base pair repeats) of nucleotide units ubiquitously distributed throughout an organism's genome (Riju et al., 2009). Microsatellites are highly valuable markers because of their multi-allelic nature, co-dominant mode of inheritance, even distribution in the genomes, wide coverage, small locus size and ease of genotyping by using polymerase chain reaction (PCR)

(Serapion et al., 2004). Besides that, microsatellites are highly polymorphic, which makes them a powerful evolutionary neutral marker system that is capable of detecting differences among closely related populations (Connell and Wright, 1997).

The objectives of this study were to compare the genetic diversity of cultured populations of *C. striata* to that of wild populations using microsatellite markers, and to examine the degree of genetic differentiation among them.

CHAPTER 2

LITERATURE REVIEW

2.0. Snakehead

Snakeheads (Perciformes: Channoidei: Channidae) are obligate, primary freshwater fishes distributed in African and Asian continents. Snakeheads belong to the family Channidae, whereby they are also known as murrel and serpent-headed fish (Courtenay and Williams, 2004; Paripatananont, 2002). There are only two genera in the family of Channidae. The Asian genus *Channa*, consists of 26 valid species whereas the African genus, *Parachanna* contains only three valid species. The former possess an expansive natural distribution, extending from Iran, across southern Asia (including Sri Lanka, Myanmar, Thailand, Laos, Cambodia, Vietnam, Malaysia, Indonesia, and Philippines) and to the Far East (China, Taiwan, Korea, and southern Russia) while the latter are confined to central West Africa (Li et al., 2006; Musikasinthorn, 2003).

Snakeheads are characterised by distinct morphological features, such as elongated cylindrical body; flattened head that superficially resemble that of a snake; long, entirely soft-rayed dorsal and anal fins; a large mouth with well-developed teeth on both upper and lower jaws; cycloid or ctenoid scales; and an accessory air-breathing apparatus known as the suprabranchial organ in the head region. A suprabranchial chamber, epibranchial respiratory fold and hydromandibular process constitute the suprabranchial organ (Musikasinthorn, 2003). The difference between the two genera lies in the morphology of the air-breathing (suprabranchial) apparatus, that of the genus *Parachanna* being less developed. *Channa* possess chambers that are bordered by two plates, from the epibranchial and the hyomandibular, unlike the simple cavity of the

Parachanna which involve neither plates and are lined with respiratory epithelium (Courtenay and Williams, 2004). As air-breathers, it is essential that snakeheads breach the water surface periodically to replace the atmospheric air in their suprabranchial chamber. It was reported that dead snakeheads were found trapped in underwater nets due to drowning as they are unable to surface to breathe (Musikasinthorn, 2003).

Mitochondrial DNA (single locus) analyses conducted by Li and co-workers (2006) implied that the mutually monophyletic African and Asian snakeheads diverged in the early Cretaceous [117 $\pm$ 13 million years ago (Ma)] where divergence corresponded to geological evidence of continental breakup. On the contrary, based on a multi-locus approach, divergence between the lineages of the channid genera; *Parachanna* and *Channa* was estimated to have occurred between 40 – 50 Ma where Adamson and colleagues (2010) suggested that multiple broad scale dispersal events across India and Southeast Asia had exerted a profound influence on the evolution of the Asian taxa post-divergence. The species of snakeheads discovered in Malaysia include *Channa striata* (ikan haruan), *C. micropeltes* (ikan toman), *C. marulioides* (ikan toman bunga) and *C. lucius* (bujuk); three other species, *C. gachua*, *C. melasoma* and *C. bankanensis*, which have no common Malaysian names (Bolong et al., 2007; Lee and Ng, 1994; Rahim et al., 2009).

2.1. *Channa striata* (*C. striata*)

2.1.1. Geographical distribution, ecology and biology of C. striata

Among the Channidae family, *Channa striata* (*C. striata*) is the most widely naturally distributed snakehead species which spans from Pakistan through Southeast Asia (Myanmar, Laos, Vietnam, Thailand, Cambodia, Malaysia, Indonesia) to Yunnan, in Southern China (Figure 2.0). Additionally, *C. striata* is believed to be the most commonly introduced species of snakehead and has been established in tropical islands of Hawaii, Madagascar, the Philippines, Sulawesi (Indonesia), Papua New Guinea and Mauritius (Courtenay and Williams, 2004; Musikasinthorn, 2003).

C. striata has been acknowledged as a native Malaysian tropical freshwater fish species based on the genetic variability of the Haruan's mitochondrial DNA that dates backs to more than 600, 000 years ago (Kumar, 1995). In Malaysia, *C. striata* (Figure 2.1) is a common freshwater fish species that distribute extensively in tropical stagnant to slow-running lowland waters (≤ 119.5 metres above sea level) such as rivers, streams, lakes, ponds, reservoirs, ditches, irrigation canals, mining pools, borrow pits, swamps, marshes, earthen ponds and rice-fields (Ambak et al., 2006; Jamaluddin et al., 2011; Lee and Ng, 1994; Musikasinthorn, 2003).

By virtue of their accessory respiratory organs, *C. striata* have been known to aestivate under bottom mud crusts of lakes, swamps or canals that have dried up. These obligate air-breathing fish are highly prized as a food source due to the ease of transportation which in turn, has probably facilitated the wide distribution of species (Lee & Ng, 1994). On the Indian sub-continent, *C. striata* are transported to markets in

bamboo baskets since they can survive without water for prolonged periods, up to 8 hours (Chandra and Banerjee, 2004). Interestingly, in the wild, these amphibious fish species has a unique physiological adaptation that enables it to relocate between ponds by crossing on land using their bodies, pectoral fins and caudal fins in search of suitable and unpolluted water (Mat Jais, 2007a; Mat Jais, 1991; Musikasinthorn, 2003).

C. striata is a non-migratory species. Although they do embark on short lateral migrations between rivers and nearby floodplains, following the hydrological cycle of the moonsonal or floodplain river ecosystem (Poulsen et al., 2008), these migrations are mostly localised, and rarely exceeds an averaged dispersal distance of 500 m (Amilhart and Lorenzen, 2005). This behavioural pattern suggests that the movement is generally confined to individual lifetimes (Adamson, 2010).

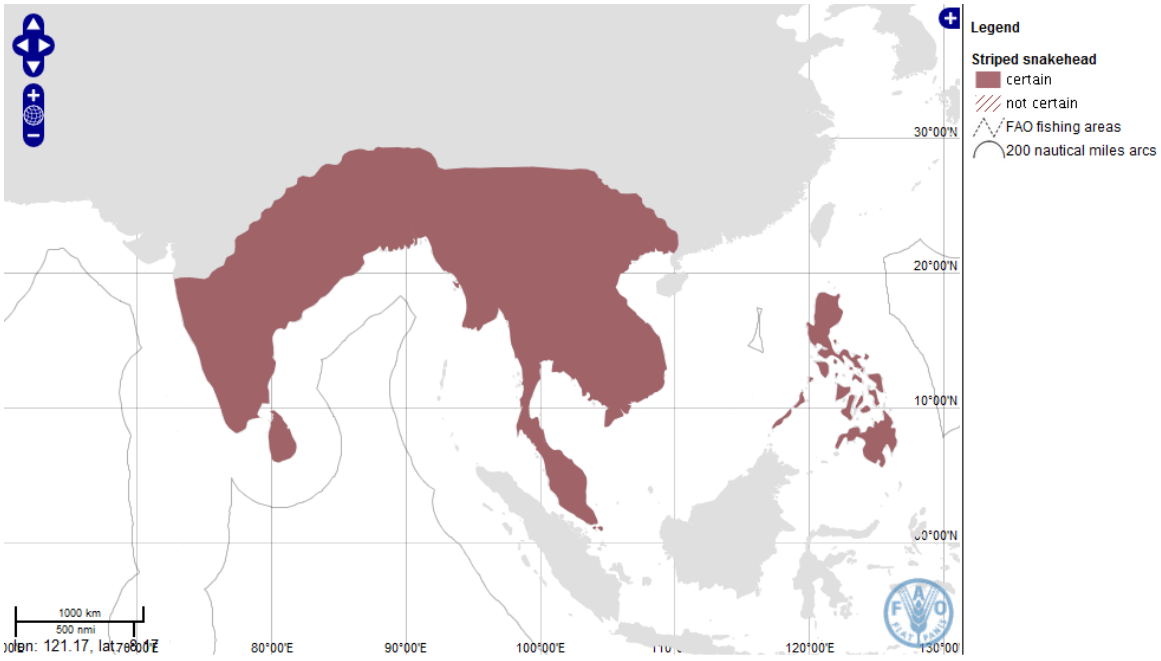


Figure 2.0. *C. striata* species distribution map (FAO, 2011a).

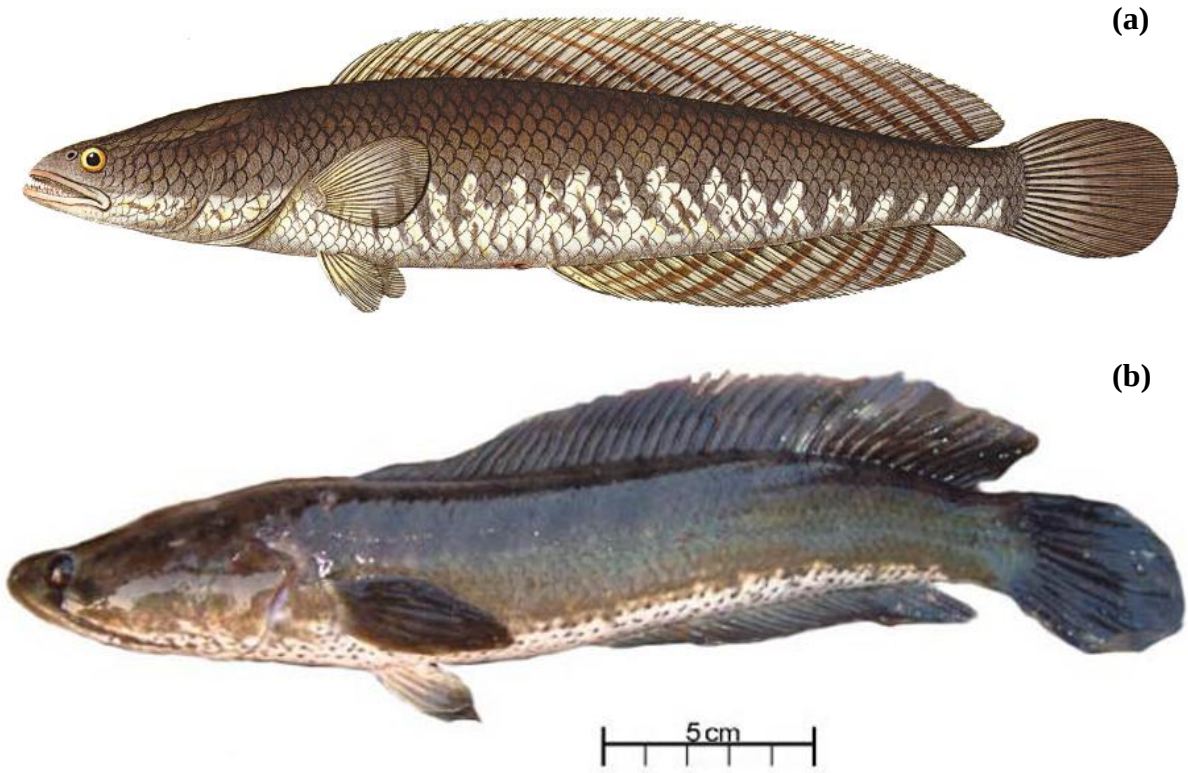


Figure 2.1. Images of *Channa striata* adapted from (a) Courtenay and Williams (2004), (b) DOF (2011).

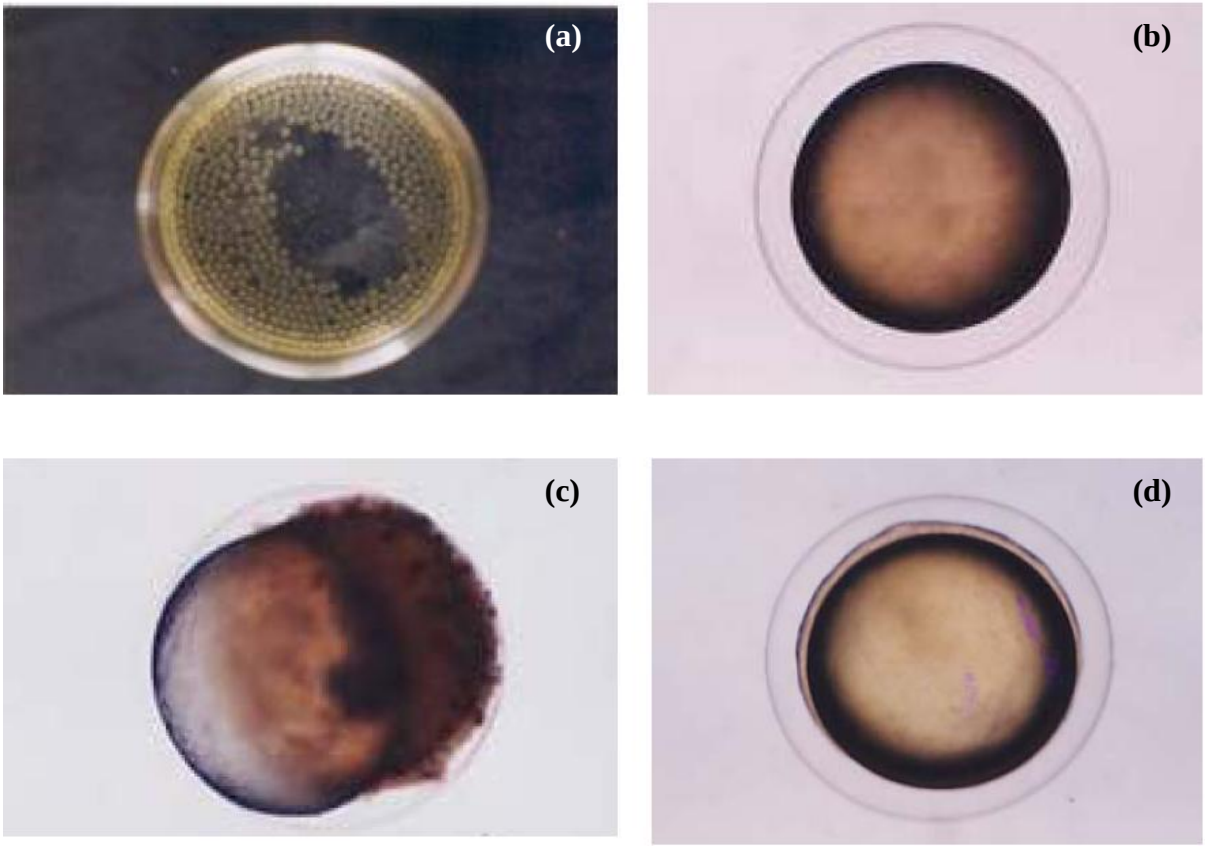
C. striata is a crepuscular, predaceous fish species that feeds on snakes, frogs, tadpoles, fishes, *Trichogaster* sp. (gourami), snails, insects, prawns (*Macrobrachium*) and worms (Lee and Ng, 1994). Although carnivorous in nature, *C. striata* catches its prey with a quick flip motion to compensate for its lack of robustness as a swimmer (Mat Jais, 2007a). For this reason, they prefer stagnant rather than flowing water although *C. striata* have been discovered in streams with flow rate of up to 0.47 ms^{-1} (Lee and Ng, 1994). *C. striata* can survive in water temperatures of 11 – 40 °C and tolerate pH values of 4.25 – 9.40 (Lee & Ng, 1994). They can also withstand the harshest of environments, such as waters that contain high ammonia concentrations. Qin and colleagues (1997) reported that *C. striata* can survive in conditions of up to 15.7 mg of unionized ammonia per litre, at pH 10.

According to a recent karyotype investigation by Supiwong and co-workers (2009), *C. striata* has a chromosome diploid number of *2n*=42 in both sexes. However, the diploid number of the species was identified as *2n*=44 in an earlier cytogenetic study conducted by Donsakul and Magtoon (1991). The variation in chromosome number of the fish indicates that *C. striata* correspond to a species complex, which is also reinforced by the biodiversity analysis on populations of *Hoplerythrinus unitaeniatus* (Diniz and Bertollo, 2003) as well as the current taxanomic status stated in the International Union for Conservation of Nature (IUCN) Red List of Threatened Species (2011).

2.1.2. Life cycle

C. striata are solitary by nature except during spawning seasons (Lee and Ng, 1991). The females in Malaysia become reproductively mature about 30 cm in length at about 2 years of age and produces an average of between 4, 326 – 9, 017 oocytes annually (Ali, 1999) although in nature, the maximum brood size does not exceed 5, 500 (Parameswaran and Murugesan, 1976). *C. striata* are opportunistic breeders that are capable of multiple extended spawning whenever environmental conditions are conducive. The multimodal oocyte distributions of the female *C. striata* allows year-round reproduction but spawning is normally reserved in the wild for the wet monsoon season (May to September) (Ali, 1999; Poulsen et al., 2008).

C. striata constructs nests in shallow (30 – 100 cm), swampy, stationary areas near the waters' border. The nest is wrought into a floating ring-shaped about 30 cm in diameter where the parents clear away the dense vegetation using their mouth and tail (Musikasinthorn, 2003). Spawning entails the male positioning its body close to the female, fertilising the eggs as they were released. The translucent, non-adhesive eggs (0.15 cm in diameter) floats to the surface as they are rich in lipids (Musikasinthorn, 2003; Yaakob and Ali, 1992). The male displays substantial parental care by guarding the nest and subsequently, the schooling larvae from predators. The strong recruitment into the adult population is most likely ascribed to the level of parental care displayed by *C. striata* (Ali, 1999; Musikasinthorn, 2003). A study conducted by Marimuthu and Haniffa (2006) provided a comprehensive information on the embryonic and larval development of *C. striata* (Figure 2.2).



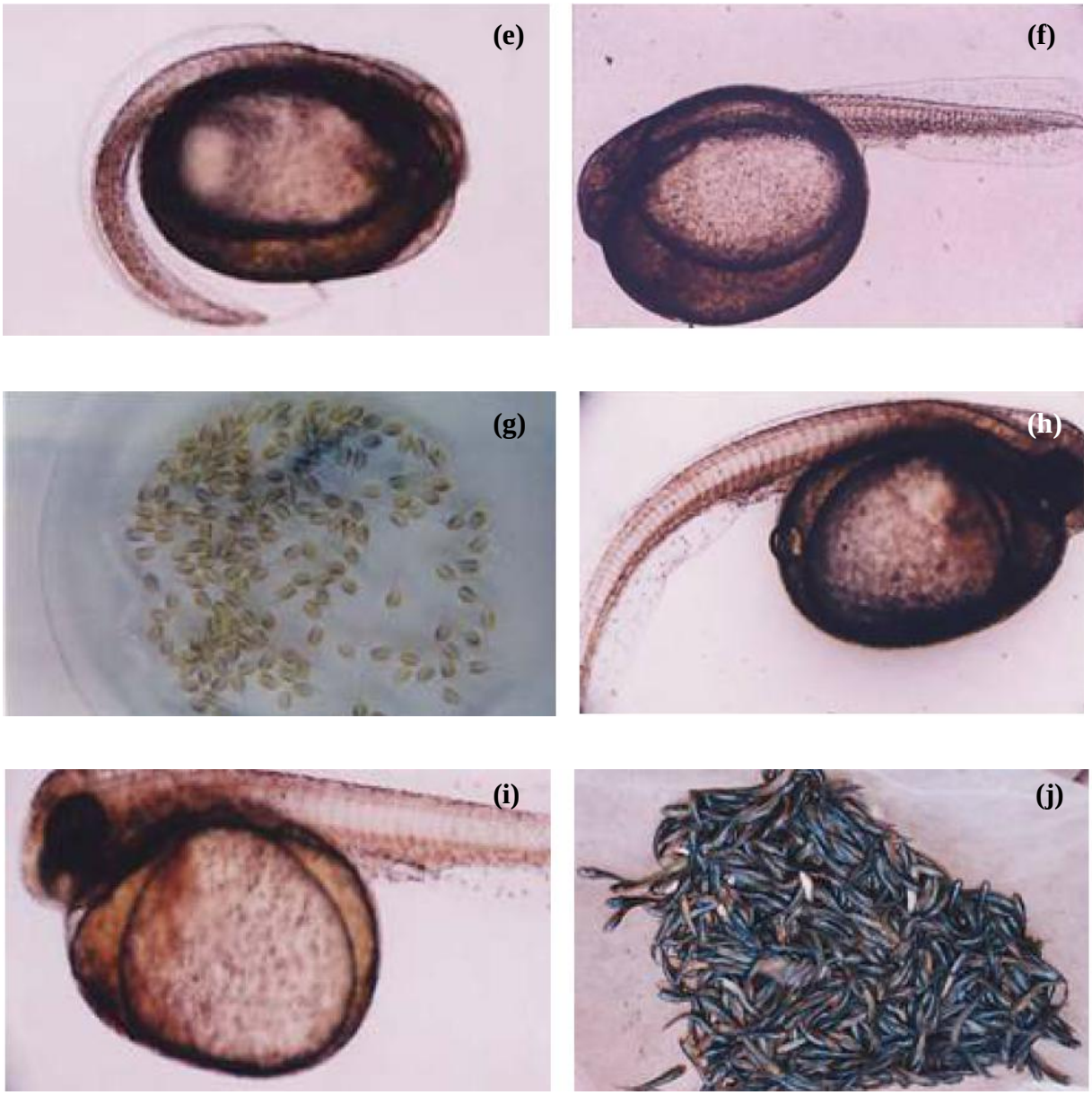


Figure 2.2. Embryonic and larval development of *C. striata*. (a) Fertilised eggs of *C. striata*, (b) Fertilised egg, (c) Morula stage, (d) Six hours old embryo, (e) At hatching, (f) Four hours old larva, (g) Eight hours old hatchlings, (h) Sixteen hours old larva, (i) Thirty-six hours old larva, (j) Twenty days old fry. Source: Marimuthu and Haniffa (2006).

2.1.3. Nutritional source

Fresh water and marine fishes are an integral part of the Malaysian diet, since the country is enveloped by sea and has an abundance of natural water resources in the form of river systems. Fish represents approximately 60 – 70% of the protein intake consumed by an average Malaysian (Rahman et al., 1995). The white flesh of the *C. striata* sans intra-muscular bones is a great source of economical dietary protein (78 ± 0.23%) with minimal lipid content (2.08 ± 0.08%). The fish is also rich in essential amino acids and fatty acids as well as dietary minerals such as magnesium, copper, calcium, manganese, iron and zinc (Mat Jais, 2007a; Zuraini et al., 2006).

It is noted that the majority of essential amino acids present in *C. striata* are higher compared to the Malaysian cat fish (local name: *Keli*), Rainbow trout (*Oncorhynchus mykiss*) or Atlantic Salmon (*Salmo salar*) (Gam et al., 2005). In addition, the roe of the species are edible and in many countries, considered a valuable delicacy (Mat Jais et al., 1998). *C. striata* is an important source of protein in many countries of the Asia–Pacific region, namely Indonesia, Singapore, Thailand, Indo–China, Hong Kong, Taiwan, South Korea and Philippines (Froese and Pauly, 2011; Mohsin and Ambak, 1983; Wee, 1982).

2.1.4. Beneficial properties

C. striata has been studied extensively for its therapeutic and recuperative qualities. In Malaysia, conventional mid-wives were strong advocates of *C. striata* for centuries as dietary treatment in ameliorating wound lesion and in post-partum involution (Mat Jais, 2007a ; Mat Jais et al., 1998), where the fish is consumed as either dry-fried, grilled and boiled as in a herbal soup or a rice porridge (Chen, personal commun., 2011; Mat Jais et al., 1994). Studies have discovered that *C. striata* contains the required biochemical components such as amino acids and fatty acids for promoting wound healing (Baie and Sheikh, 2000; Gam et al., 2005; Mat Jais et al., 1994; Rahman et al., 1995; Zuraini et al., 2006).

C. striata contains an unusually high level of arachidonic acid, but virtually no eicosapentaenoic acid (EPA). Arachidonic acid is a precursor for prostaglandins that may initiate blood clotting by inducing platlet aggregation and adhesion in endothelial tissue, and thus encourages tissue growth (Mat Jais et al., 1994). Furthermore, the presence of glycine, a key component of human skin collagen, accompanied with 13 other essential amino acids such as arginine, alanine, leucine, isoleucine, tyrosine, theonine, oxyproline, serine, methione, tryptophane, lycine, histidine, aspartic and glutamic acid establishes the foundation of wound amelioration (Gam et al., 2005; Mat Jais, 2007a; Mat Jais et al., 1998; Mat Jais et al., 1994). The relatively high content of Vitamin A also promotes the healing properties of the fish (Mat Jais et al., 1994). In addition, preliminary studies have shown *C. striata* extracts acting as positive but mild, anti-bacterial and anti-fungal agents that were believed to have facilitated the wound healing processes (Mat Jais, 2007a).

On top of that, *C. striata* also possesses potent anti-inflammatory and antinociceptive (analgesic) activities (Mat Jais et al., 1997; Somchit et al., 2004; Zakaria et al., 2007). Although the exact mechanisms have yet to be discovered, it was suggested that the presence of potential antinociceptive and anti-inflammatory cyclopentanone prostaglandins and lipo-amino acids contained in the fish probably contributed to these therapeutic properties (Zakaria et al., 2007). Moreover, a preliminary study conducted by Ng and co-workers (2004) revealed the efficacy of *C. striata* fish extract in supressing inflammation of arthritic joints. Hence, the authors expressed the possibility of a complementary treatment for osteoarthritis in the form of an orally administered extract. Other than the flesh of the fish, the mucus extracts of the fish have also shown potential wound healing and antinociceptive characteristics, implying the prospect of mucus, generally regarded as a valueless part of the fish, as an alternative candidate for commercial health products (Mat Jais et al., 1998; Mat Jais et al., 1997).

In addition, *C. striata* based cream exhibits effectiveness in treating exfoliation dermatitis such as psoriasis, eczema and ichthyosis (keratinisation or cornification skin disorder) (Mat Jais, 2007b). *C. striata* also facilitates the recovery of numerous skin-related problems, including acne, pimples, rashes as well as hormonal-imbalance and allergy induced skin afflictions. The clinical effects of the fish have been credited to its excellent nutraceutical values, in relieving the dermal conundrum, which is faced by a significant number of Malaysians living in the warm and humid tropical climate. This is due to docosahexaenoic acid (DHA), an essential fatty acid found in *C. striata* that exerts inhibitive actions on the skin complications (Mat Jais, 2007a).

2.1.5. Commercialisation of C. striata

As more and more of the nutritional and pharmacological properties of the species are being scientifically validated, with the resulting information being widely dispensed to the public, rising consumers' demands on the fish has nurtured a progressively developing domestic industry that capitalises on *C. striata*–based therapeutic products. These natural products are believed to boost and promote general well-being as well as to alleviate specific health conditions. Among the *C. striata*–based products (Figure 2.3) in the Malaysian market include *C. striata* essences, supplement pills, creams and processed foods such as floss and soups.

Figure 2.3. Commercialised *C. striata*–based products. (a) Pills, (b) Cream, (c) Instant soup, (d) Canned soup, (e) Essence, (f) Floss.

2.1.5. Cultural significance

In the Malaysian chinese culture, Chinese New Year celebrations are usually accompanied by *Yee Sang*, a raw fish salad where slices of *C. striata* are an essential element to this traditional dish (Mat Jais, 2007a). The act of tossing the salad during Chinese New Year symbolises abundance, prosperity and vigour and is believed to usher in an auspicious year. In Myanmar, *C. striata* plays an important part in a spiritual ceremony that aids the recovery of a sick individual (Musikathorn, 2003).

2.2. Aquaculture

Aquatic products can be harvested by two approaches, namely aquaculture and capture fisheries. Aquaculture comprises diverse systems of farming animals and plants in aquatic environments such as inland (brackishwater and freshwater), coastal and marine areas. Aquaculture commonly involves local species, but introduced (or alien) species have been employed to boost production, which brings about significant social and economic impact on nations (FAO, 2011b) however, often, with adverse ecological consequences (Na-Nakorn et al., 2004).

Aquaculture is the fastest growing food-producing sector set to surpass capture fisheries as a source of food fish (FAO, 2011c). This is because majority of capture fisheries worldwide are currently harvested at or above maximum sustainable levels and thus, are regressing due to over-harvesting and habitat degradation (Liu, 2007). Aquaculture can be easily discriminated from capture fisheries by two crucial criteria;

ownership of stock and intentional intervention or manipulation in the production cycle (husbandry) (Naylor et al., 2000).

The array of cultured species and diverse aquaculture production practices led to the development of two subsectors, one which consists of larger scale commercial farms that primarily engage in intensive (provision of all nutritional requirements) and semi-intensive practices (enrichment of food supply) to produce medium- to high- valued commodities for regional or global markets, for example salmon, oysters, prawns and so on. The second group comprises of family owned and co-operative produces that depend on extensive (elimination of natural predators, and control of competitors) and semi-intensive methods to supply low-value species for household subsistence or local markets such as tilapias, carps, catfish and others (Muir and Young, 1998).

Currently, global aquaculture production continues to be dominated by Asia and accounts for nearly 50 % of total food fish supply, with the average annual growth rate of nearly 7 %. This increasing trend is projected to outpace population growth and contribute significantly in the future to compensate declining capture fisheries and to relief the burden on marine resources as the population demand for aquatic food products rises (FAO, 2011c).

2.2.1. Aquaculture production in Malaysia

Malaysia is a tropical country located on the north of the Equator is blessed with luscious rainforest filled with exotic flora and fauna species. The abundance of a variety of water bodies such as rivers, lakes, marshes, streams and swamps further enhances the

diversity of aquatic organisms that flourish in them. In Malaysia, fresh water aquaculture is becoming increasingly popular as a means of intensifying local food production for food security as well as increasing export revenues. Besides that, aquaculture has assisted in the poverty alleviation programme involving the impoverished rural communities to improve their livelihoods by creating many job opportunities.

Compared to capture fisheries, the aquaculture market have yet been fully developed, as it contributes less than 0.2 percent to the nations' Gross Domestic Product (GDP) (FAO, 2010). In 2010, the Malaysian Department of Fisheries (DOF) estimated that RM2798.74 million was generated from 581, 048.41 tonnes of total aquaculture production (Figure 2.4) from which, 26.75% (155, 398.63 tonne) of the total consisted of freshwater fish production. With the declining catch of marine-based production due to over-fishing and sea pollution, aquaculture, a vital agricultural sector, is emerging as a viable alternative source of fish supply for the country.

Figure 2.4. Estimated Value and Aquaculture Production from all Aquaculture System (2000-2010) in Malaysia. Source (DOF Malaysia, 2010).

In Malaysia, freshwater aquaculture has been mainly practised in ponds, followed by ex-mining pools, floating net cages, pen culture, cement tanks and canvas tanks. Freshwater fish aquaculture production is dominated by the catfish (freshwater catfish and river catfish), followed by tilapias (red tilapia and black tilapia), and carps (Javanese carp, common carp, grass carp, bighead carp and river carp) (DOF Malaysia, 2010). The freshwater catfish that is extensively cultured in Malaysia is the hybrid between the native walking catfish, *Clarius batrachus* and *Clarias gariepinus*, an exotic North African catfish with enhanced performance traits, viz hatching rate, viability of larvae, growth and survival of individuals than their pure progenitors (Rahman et al., 1995).

2.2.2. Aquaculture of C. striata

C. striata is one of the most common freshwater species that is harvested as a staple food source across its native range (Figure 2.5 a). The unique features of this species enables it to be cultured in ponds at densities of 40 – 80 fish/m^2 with annual yields that ranged from 7 – 156 tonnes per hectare (Wee, 1982). The species is generally marketed alive and is commonly encountered at wet markers throughout the lower Mekong basin (Poulsen et al., 2008). The therapeutic properties of wild *C. striata* is not lost on the cultured fishes (Mat Jais, 2007a) and hence, is considered of equal importance as the wild. In Malaysia, *C. striata* commands a lucrative market with the price ranging from USD$ 5 – 7 kg^{-1} (personal observation, 2011).

In recent years, over-fishing in addition to other anthropogenic elements such as habitat loss, environmental pollution, industrial activities and urbanisation have resulted in perpetual depression in the harvests of wild *C. striata*. These pressing factors along with the species' air-breathing ability, hardiness, fast growth rate, high tolerance to adverse environmental condition as well as scientific acknowledgement of its therapeutic properties have led to a progressive rise in the culture of the fish (Figure 2.5b).

C. striata juveniles are associated with high levels of cannibalism. Cannibalism in snakeheads are mouth size limited where the adolescent snakehead can devour siblings less than two-thirds of their body length (Qin and Fast, 1998). This posed a serious threat in the culture of snakeheads with factors underlying cannibalism such as diverse size variation, inadequate food supply, dense populations, limited refuge areas and light conditions (Hetch and Pienaar, 1993).

Gam and colleagues (2006) reasoned that the unavoidable cannibalism in *C. striata* most likely contribute to the robustness of muscle protein synthesis activity in smaller fishes as opposed to larger sizes. Results from their findings showed elevated levels of myofibrillar protein and collagen secretion in the smaller fishes to facilitate their motion for survival against larger predators. In snakehead culture, losses due cannibalism can be minimised by grading juvenile fishes based on their size into approximately similar groups and to supply *ad libitum* feeds for the cultures (Qin and Fast, 1996).

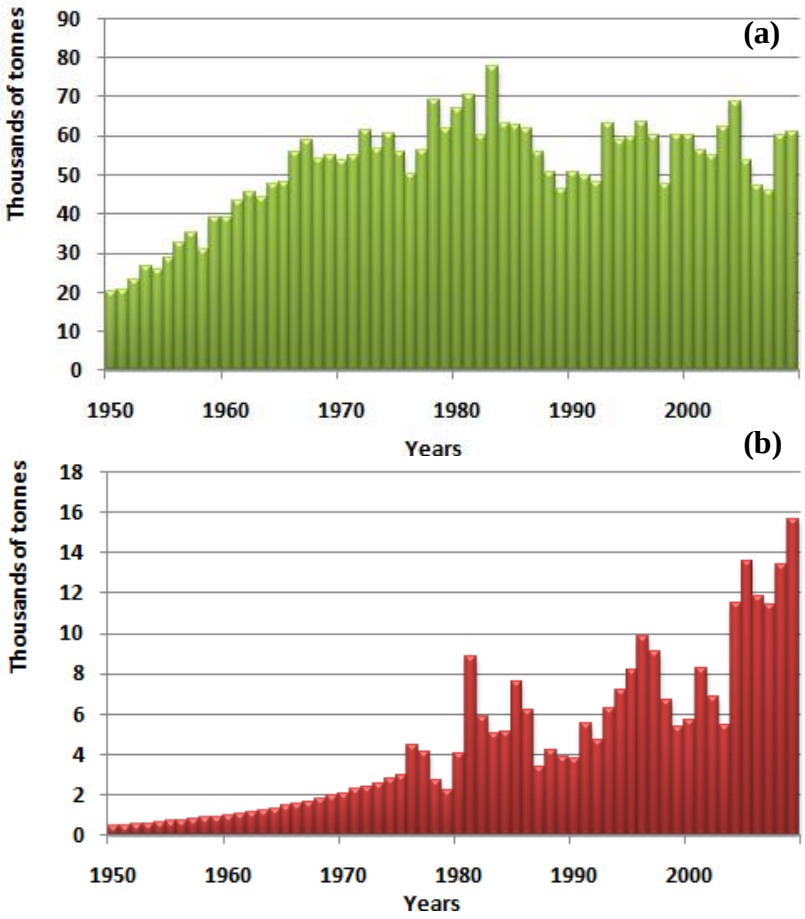


Figure 2.5. Global fisheries production for *C. striata* 1950-2010. (a) Global capture production for *C. striata* from 1950-2010, (b) Global aquaculture production for *C. striata* from 1950-2010. Source: Fisheries Statistics Data (FAO, 2011a).

C. striata have a strong resistance to disease and parasites although Vitamin B_6 deficiency disease was detected in fishes harvested in Thailand (Boonyaratpalin et al., 1985). In 1986, an outbreak of ulcerative skin disease which resulted in inferior growth of the diseased fishes were reported in Malaysia (Ali, 1990). Among the pathogens that have been found in *C. striata* are bacteria (*Aeromonas puctata*, *Flavobacterium* spp., *Pseudomonas* spp.), internal parasites (cestode, nematode, *Spinitectus* spp.) and external parasites (protozoa, trichodina, epistylis, costia) (Boonyaratpalin et al., 1985).

2.2.2.1. Aquaculture of C. striata in South East Asia (SEA).

C. striata is a popular fish for freshwater farming although other *Channa* species, such as *Channa micropeltes* and *Channa marulius* are also being farmed (Paripatananont, 2002). *C. striata* are commonly cultured in Malaysia, Thailand, Indochina, Indonesia, Philippines, China and India, with varying degrees of commercialisation ranging from subsistence to commercial ventures.

There is a lack of literature reporting the current status of aquaculture management and farming practices of *C. striata* in Malaysia. The Malaysian *C. striata* aquaculture industry is less established compared to the neighbouring South East Asian countries. This is because in Malaysia, *C. striata* is regarded as a menace due to its carnivorous nature and hence, does not occupy the top precedence in the local fish breeding industry (Mat Jais, 2007a). In addition, while *C. striata* is widely accepted by the locals, the species faces stiff competitions from its' other, more commercial counterparts such as tilapias and catfishes, which are widely available throughout the country and are a much cheaper source of protein.

Ali (1999) in his study, briefly described the culture of *C. striata* in the irrigated rice agrosystem of North Kerian, Perak (Malaysia) whereby, the rice farming landscapes are able to support various aquaculture systems such as capture-based culture which harvests wild *C. striata* fingerlings from ricefields as well as production in the form of rice-fish culture of wild populations. Both of which contribute significantly to the livelihood of local farmers by supplementing their income (Tan et al., 1973). The wild fingerlings from irrigation canals and sump ponds were entrapped during the wet season and allowed to mature into marketable size in the ricefields before

being harvested at the end of the rice growing season. The fishes subsisted on naturally occurring food in the ricefield ecosystem with the nutrients essentially supplied by chemical (NPK and Urea) fertilisers. The sump-ponds served as a refugia for fishes during periods of fluctuating water temperature and also as a fish harvesting basin (Ali, 1990).

C. striata is one of the most common staple food fishes among the Thai where it is generally cultured in monoculture systems. In Thailand, a high capital investment is required for the intensive farming operations of this species due to high feed costs and a longer raising period upon harvest (7–9 months). The fishes are fed an assortment of feed such as trash fish, rice bran, broken rice, vegetable and kitchen wastes depending on the scale of production and growth phase of the cultures (Wee, 1981). Other than pond culture, the conventional culturing via ricefield-trap pond system was also practised, whereby, Amilhat and colleagues (2009) categorised *C. striata* as a self-recruiting species that occurs naturally and do not require stocking of hatchery-reared juveniles. The ricefields serve as a major wet season habitat for the fish to feed and spawn while the trap ponds are constructed to provide dry season habitat for wild fishes for the purpose of harvesting during the arid spell or as spawning stock (Amilhat and Lorenzen, 2005).

Experienced fishers in the lower Mekong basin takes advantage of the conspicuous spawning behaviour of the wild *C. striata* that guards its' young by visually identifying its parents in order to collect these fry for stocking in grow-out cages. This specialised "juvenile" fisheries capture large bulk of seasonal surplus *C. striata* at various juvenile stages (low-value yields) during the monsoon season, subsequently confining them in ponds and cages till maturity prior to harvest as high-

value products for the market or consumption at other times of the year. In Cambodia, snakehead fingerlings composed of the species *C. striata* and *C. micropeltes* are frequently exploited in juvenile fisheries, which is a type of wild-seed based cage and pen culture system. In Thailand and Lao People's Democratic Republic, *C. striata* seeds are available from hatcheries while in Vietnam, hatchery-produced seeds are employed in addition to collection of wild seeds due to the rising demand for stocking in grow-out systems. By and large, wild seed capture-based aquaculture mainly dominates snakehead culture industry throughout the lower Mekong delta, with Thailand being the only exception. With the massive recruitment of snakeheads occurring within the natural ecosystem accompanied by their relative ease of capture, the prospect of large-scale hatchery production appear financially unattractive to the local farmers (Poulsen et al., 2008).

2.3. Overview of molecular markers

Application of molecular genetic approaches in fisheries was pioneered in the 1950s. Initial studies performed on tunas, salmonids and cod were constrained to serological based methods, namely blood group type variant which progressed to allozymes electrophoresis (Ward and Grewe, 1994). The advent of restriction endonucleases and DNA amplification via Polymerase Chain Reaction (PCR) technology gave rise to DNA marker technologies that have revolutionised the research of aquaculture genetics. At present, the genetic markers that have been developed and utilised in the field of aquaculture genetics include allozymes, mitochondrial DNA (mtDNA), restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP),

microsatellite, single nucleotide polymorphism (SNP) and expressed sequence tags (EST) markers (Liu and Cordes, 2004; Okumus and Ciftci, 2003).

With the myriad array of molecular techniques available in the field, it is crucial to choose a system that best addresses the nature of the research. The objectives, species/questions of interest and evolutionary time frame of the study have to be aptly matched with the applications, rate and mode of evolution in addition to mode of inheritance (maternal, biparental) and expression (dominant, co-dominant) of the marker. Factors that contribute to the selection of molecular marker are availability of samples from species of interest, sensitivity and availability of marker, rapid development and screening of putative markers, multi- (RAPDs, AFLPs) or single-locus (microsatellites) technique, relative cost of marker development and, organelle or nuclear DNA based genotypes (Okumus and Ciftci, 2003) .

2.3.1. Microsatellite DNA marker

Microsatellite are unique short tandem arrays of DNA sequences that range in size from 1 – 6 basepairs (bp) and are evenly distributed throughout the genome. Alternative terms that represent microsatellites include simple sequence repeats (SSRs), simple tandem repeats (STRs), simple sequence length polymorphisms (SSLPs) and variable number tandem repeats (VNTR). They have been discovered in gene coding regions, introns as well as non-gene sequences (Liu and Cordes, 2004). Microsatellites have a low recurrence in the protein-coding regions due to a functional selection pressure in the translated sequences unlike non-gene sequences which mutate without inhibition, exhibiting higher levels of polymorphism (Chistiakov et al., 2006).

Ubiquitous in nature, microsatellites are found in prokaryotes and eukaryotes as well as in bacterial genomes (Gur-Arie et al., 2000). A large portion of the microsatellites (30 – 67%) ascertained in the genome are composed of dinucleotide repeats (Chistiakov et al., 2006).

Although microsatellites are regarded as selectively neutral markers, they are of functional relevance in DNA structure, chromatin organisation, regulation of DNA recombination, transcription and translation processes, gene expression and cell cycle dynamics (Chistiakov et al., 2006). Microsatellites are co-dominantly inherited in a Mendelian manner. SSRs have been estimated to occur approximately once every 10 kilobases (kb) in fishes (O' Connell and Wright, 1997), with a mutation rate evolving at approximately $10^{-2} – 10^{-6}$ per locus per generation, making them hyper variable and highly polymorphic. Since the evolution rate of microsatellites are $10^{-2} – 10^{-3}$ faster than single-copy nuclear DNA, the markers are highly informative for analysing recent and contemporary events (Ellegren, 2000).

Polymorphism in microsatellite has been attributed to differences in sizes of the varying number of repeat units contained by alleles at a given locus (Liu and Cordes, 2004). The generation and evolution demonstrated by microsatellite have been suggested to be influenced by two models, namely DNA replication slippage and unequal recombination. The first model, which appears to be the predominant mechanism in microsatellites involves transient dissociation of replicating DNA strands with subsequent re-association, during which, the nascent and template strand realigns out of register. Continued replication of these strands will lead to differences in length of the succeeding strands, due to the alteration of the microsatellite repeat number (Schlotterer, 2000). A loop introduced by misallignment in the template strand would

initiate a decrease in repeat length while formation of a loop in the nascent strand leads to an increase in repeat length (Ellegren, 2004).

The second model, non-reciprocal recombination (gene conversion) affects the genetic stability of some microsatellites, particularly the triplet motifs (Jakupciak and Wells, 2000). Gene conversion mechanism was observed from comparative sequence analysis of microsatellite locus in the differentiation and evolution of paralogous sequences (duplicated loci within the species) (Angers et al., 2002). The disparity in evolutionary dynamics of microsatellites could be attributable to several factors such as repeat number, sequence of repeat motif, length of repeat unit, flanking sequence, interruptions in the microsatellite along with the recombination rate and transcription rate (Schlotterer, 2000). Regardless of mechanisms, variations in numbers of repeat units bring about a great number of alleles at each microsatellite locus in a population (Liu and Cordes, 2004).

The initial stage of developing efficient microsatellite markers requires construction of microsatellite-enriched partial genomic DNA libraries (Liu and Cordes, 2004). The enrichment technique usually entails selective hybridisation of fragmented genomic DNA with a repeat-unit oligonucleotide probe. Subsequently, DNA fragments that screened positive were isolated and sequenced. Following identification of microsatellite loci, flanking PCR primers were designed to amplify the tandem repeats (O' Connell and Wright, 1997). The allelic state at a given locus can be observed by differences in lengths of the PCR products due to the variable number of repeats following size segregation by gel electrophoresis (O' Connell and Wright, 1997; Sekar et al., 2009).

Microsatellites can be described and differentiated based on the specific composition of their core sequence. The term perfect or uninterrupted microsatellite refers to only a single type repeat unit found within the array at a given microsatellite locus, while imperfect (or interrupted) microsatellite are recognised from the disruption of the core repetitive unit by base substitutions. Composite microsatellites are composed of many different types or lengths of tandem repeated sequences internal to the array whereas cryptic simple sequence consists of many interruptions including the addition of a few different motifs (Schlotterer and Zangerl, 1999).

2.3.2. Theoretical Models of Microsatellite Mutation

Various mutation models have been proposed to describe variations at microsatellite loci, which includes the infinite alleles mutation model (IAM) and stepwise mutation model (SMM). The former assumes that mutation will create only new allelic states and is not limited to a number of repeat units. Subsequently, this model does not permit homoplasy, which is an analogy of traits or genes for reasons other than co-ancestry. In contrast, the latter mechanism predicts that all mutational events involves gain or loss of a single repeat unit. Hence, mutation will result in allelic states that are already present in the population (O' Connell and Wright, 1997). The SMM model allows examination of genetic divergence based on the differences in allele sizes as well as frequency. As a result, alleles of very different sizes will be more distantly related than alleles of similar sizes. Therefore, SMM has a memory of allele size (Balloux and Lugon-Moulin, 2002). R_{ST} is one of the distance estimates that have adopted this model (O' Connell and Wright, 1997).

Other models include *K*-allele model (KAM) and two-phase model (TPM) (Balloux and Lugon-Moulin, 2002). Assessment of these models are of paramount importance so that the microsatellite data would be represented by a suitable mutation model that best describes the accurate estimate of population size and structuring events.

2.3.3. Advantages and Disadvantages of Microsatellite Markers

The large interest in microsatellite markers is mainly due to their abundance in the genome, even genomic distribution, single locus nature, simplicity of assay, high levels of polymorphism as a result of allelic diversity, Mendelian inheritance, co-dominance and selective neutrality (Liu and Cordes, 2004; Sekar et al., 2009). In addition, microsatellites' small locus size complements PCR-facilitated genotyping which presents a significant leverage over non-PCR based methods as it is a non-invasive procedure and allows amplification and analysis of DNA from minimal amounts of tissue, including that from preserved otoliths, scales, eggs, larvae and small fry (Okumus and Ciftci, 2003; Ward and Grewe, 1994). With the availability of high-throughput capillary fluorescent sequencers, a substantial volume of samples can be analysed at a particular time compared to conventional approaches (O' Connell and Wright, 1997).

Despite the advantages of microsatellite markers, their drawbacks include high cost and labour-intensiveness associated with the initial phase of primer development (Magoulas, 1998). A major obstacle faced by researches working with microsatellite markers is the occurrence of null alleles, which are defined as any allele at a locus that

consistently fails amplification to detectable levels via PCR (Dakin and Avise, 2004). Null alleles may be generated from point mutations within the primer annealing sites, large insertion/deletion (indel) events between the array and primer binding regions, low or inconsistent DNA template quality, or mutations within the array (O' Connell and Wright, 1997).

Null alleles, together with stuttering patterns and large-allele dropout constitute the common sources of scoring errors in microsatellite data (DeWoody et al., 2006). Stutter bands are initiated by polymerase slippage during PCR amplification, which generates secondary products containing one or more repeats units less than the primary allelic band (Liu and Cordes, 2004). Large-allele dropout refers to the preferential amplification of the smaller sized allele in a heterozygous genotype due to the competitive nature of PCR. Committance of these errors tend to create consistent allelic and genotypic scoring bias that may lead to bias data interpretation (DeWoody et al., 2006). Differing views on statistical parameters such as appropriate sample sizes and number of loci required in a study, as well as the various mutation model assumptions on which the statistical estimate is based are among the issues that need to be taken into consideration when employing microsatellite markers (O' Connell and Wright, 1997).

2.3.4. Application of Microsatellite in Fisheries and Aquacultures

Microsatellites are powerful markers with a great differentiating power and are utilised extensively to provide a great wealth of information on various aspects of aquaculture research such as genetic identification and discrimination of aquaculture stocks, evaluation of genetic variability, population structure and inbreeding,

comparisons between cultured and wild stocks, species and strain identification, parentage assignments, assessment of the escaped or released cultured fish (stocking) into natural populations, molecular epidemiology and pathology investigations on diseases, hybridisation and marker-facilitated identification of quantitative trait loci (QTL) via the construction of genetic linkage maps with the resulting markers involved in breeding programmes (marker assisted selection, MAS) (Chistiakov et al., 2006; Liu and Cordes, 2004; Magoulas, 1998).

Table 2.0. Methods available for genetically characterising individuals and populations and their applicability to each issue. Techniques with + can be used for the purpose specified, with several + indicating the technique has high utility, Δ are cases where the technique is useful in certain cases, while – indicates that the technique is not useful in this context. * can detect only female contributions (Adapted from Frankham et al., 2002).

Issue	Chromo-somes	Allo-zymes	mtDNA	RAPD	Micro-satellites	DNA Fingerprint
Non-intrusive sampling	-	-	+++	++	+++	-
Population size	-	-	+++	+	+	**Δ**
Estimating N_e	-	++	++*	-	+++	**Δ**
Demographic history	-	-	++	-	+	**Δ**
Detecting bottlenecks	-	++	++*	++	+++	**Δ**
Detecting selection	+	+	+++	+	+++	++
Migration and gene flow	-	++	+*	++	+++	++
Individual identification and tracking	-	-	++	+	+++	-
Population structure	-	++	+ **Δ**	++	+++	++
Phylogeography	-	-	+++	-	+++	-
Introgression	+	++	+*	++	+++	++
Paternity	-	+	-	+	+++	+++
Founder relationships	-	**Δ**	-	+++	++	+++
Detecting diseases	-	-	++ **Δ**	++	+	++