

## Sustainable aquaculture of Asian arowana – a review

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### Abstract

Asian arowana, *Scleropages formosus* is a highly valued aquarium fish in the world, particularly in Asian countries, and has been listed as one of the most highly endangered species. This is a freshwater, carnivorous, fairly large mouth breeding fish belonging to the family Osteoglossidae. Arowana can be found in different colour varieties such as green, red, silver and golden. Among these varieties, Malaysian golden is the most valuable fish and is endemic to the Krian riverine system, Malaysia. However, overexploitation, habitat change and pollution have caused a serious decline of this arowana variety. Recently, arowana aquaculture industry is expanding rapidly in Southeast Asian countries. However, difficulties in an accurate differentiation of sex and strains, causing imbalanced stocking ratios for optimum spawning, remain major obstacles in maximizing arowana production. In addition, problems in sustainable water sources of suitable quality and prevention of diseases need to be addressed. Recirculating aquaculture system (RAS) and bioremediation are two possible technologies that could be used to minimize pollution and ensure adequate high-quality water for arowana culture. In addition, the application of appropriate molecular markers for sex and strain identification is also an important strategy required for the improvement of captive breeding. This review discusses several issues such as the importance of arowana as an aquarium fish, its market demand, current problems in the arowana aquaculture industry and the possible technologies to enhance reproductive capacity and increase culture production.

### Key words

Arowana aquaculture, Bioremediation, DNA markers, *Scleropages formosus*, Water quality

Arowana (*Scleropages formosus*) (Müller and Schlegel, 1844) is a freshwater bony tongue fish, also known as Asian arowana, barramundi, saratoga, kelisa and arwana. The name “bony tongue” is derived from the presence of big tooth plates on the tongue (basihyal) and basibranchial that bite against the roof of the buccal cavity (Hilton, 2001). It is a highly valuable aquarium fish and exclusively found in Asian countries and hence the name, Asian arowana (Bonde, 1979). However, overexploitation, habitat change and water quality deterioration have driven this species to near extinction.

Arowana is one of the few freshwater species classified under 'highly endangered' category by the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) and International Union for the Conservation of Nature (IUCN) (Joseph *et al.*, 1986; Tung, 1986; Moreau and Coomes, 2006). Arowana was quite an inexpensive species before the 1970s, but later became expensive due to sudden increase in market demand as a result of Chinese superstition that this fish brings good luck, strength, power and good fortune. This obsession is mainly

due to the close resemblance of arowana to the oriental dragon (Ng and Tan, 1997).

Arowana is a carnivorous fish, belonging to the family Osteoglossidae of the order Osteoglossiformes. The order Osteoglossiformes is divided into two sub-orders, *i.e.*, Notopteroidei and Osteoglossoidei (Nelson, 2006) (Fig. 1). The former comprises the old world knifefish (Notopteridae), elephantfish (Mormyridae), mooneyes (Hiodontidae) and the aba (Gymnarchidae), while the latter includes arowana (Osteoglossidae) and butterflyfish (Pantodontidae). Presently there are four species recognised in the genus '*Scleropages*' that include *S. formosus* (Asian arowana), *S. jardinii* (gulf arowana), *S. leichardti* (Australian arowana) and *S. inscriptus* (Myanmar arowana) (Roberts, 2012).

The market price for arowana depends mainly on the colour of the species. Asian arowana has three main varieties of red, golden and green. Further, red Asian arowana is divided into blood red and chilli red; and the golden variety is divided into Indonesian golden and Malaysian golden. Among these varieties, Malaysian golden is one of the world's most expensive and colorful strain. It is endemic to the Krian district, especially in Bukit Merah Lake, Perak, Malaysia. The price for the Malaysian golden variety is approximately 5-10 times higher than the other varieties (Ng and Tan, 1997). There are many sub-varieties of Malaysian golden arowanas and among these, the most popular in the market are the blue-based golden, high-backed golden and cross-backed golden. The value of golden arowana depends mainly on the metallic based colour and golden scale coverage. The fully golden variety or all round golden is known as 'cross backed' and the half golden scale is known as high backed or super high backed (Suleiman, 1999; Mohd-Shamsudin *et al.*, 2011). An adult specimen can cost more than US\$ 20,000 (Yue *et al.*, 2004) and US\$ 400-500 per juvenile fish. A platinum variety, originating from the golden variety, cost about US\$ 30,000 per piece (<http://www.qianhu.com/>). An adult arowana with Malaysian golden colour tone and other fine characteristics can fetch almost US\$50,000 to US\$ 100,000 per piece (<http://www.qianhu.com/>).

**Distribution, legislation and animal marking :** *Scleropages formosus* is widespread throughout the South-East Asia (Joseph *et al.*, 1986; Roberts, 1989). It is native to Malaysia, Indonesia, Brunei, Vietnam, Cambodia, Thailand, Darussalam, the Philippines, Laos People's Democratic Republic and Myanmar (Blanc and D'Aubenton, 1965; Kottelat *et al.*, 1993; Rainboth, 1996). Asian arowanas

spread to Asia from Australia about 140 million years ago, during the early cretaceous period (Kumazawa and Nishida, 2000) and the ancestors of Asian arowana passed through the Indian sub-continent and smaller islands to enter into Asia (Kumazawa and Nishida, 2000). Asian arowana was reclassified by CITES in 1980 which allowed commercial breeding. Classified as an endangered species, Asian arowana requires import and export permits for international trade. The permit will only be issued if the purpose is not detrimental to the survival of the species (CITES, 2007). Due to these restrictions, captive breeding facilities of arowana were first established in Singapore in 1981. After that, a number of captive breeding facilities were established in Malaysia, Singapore and Indonesia (Dawes *et al.*, 1999). The species produced by captive breeding would only be traded internationally with CITES permission and tagged with 'passive integrated transponders' (PIT), which are coded microchips used to mark and identify individual animals (Prentice *et al.*, 1990; Camper and Dixon, 2004). These PIT tags act as a lifetime barcode for an individual fish and, along with PIT tags, specimens can also be provided with a birth certificate that contains the microchip number and the name of CITES registered fish farm where the specimen was raised (Gibbons and Andrews, 2004).

**Biology, nutrition and breeding :** Arowana is a long streamlined fish and has a great value with unique characteristics. It is a large and aggressive species in its natural habitats. The head is bony with an elongated body that can reach up to 1 m in length and 7 kg in weight and are covered with large and heavy scales. Arowana lives in lakes, deep parts of swamps, flooded forests and stretches of deep rivers with slow currents and dense overhanging vegetation (Suleiman, 1999). Sexual differentiation among arowana is only possible after reaching maturity of about 3-4 years. In addition, sexual differentiation is also based on the body shape and size of the mouth cavity. Male arowanas generally contain a bigger mouth cavity and have more intense coloration but are slimmer with a shallower body depth than the females. Males are more aggressive than females (Scott and Fuller, 1976; Suleiman, 1999). Arowana are surface feeders and eat different types of foods such as crickets, grasshoppers, crayfish, worms, small fish, frogs, centipedes, animal organ and pellets, and prefer to eat live feed over artificial pellets (Suleiman, 2013).

Arowana is a seasonal breeder, and normally their breeding season lies between July and November, but can breed anytime in ponds if sufficient nutritious food is available. While female arowana can only produce eggs 5-6

**Table 1 :** Types of DNA markers isolated from different varieties of arowana

No.	Marker type	Arowana varieties							Reference
		IG	R	G	S	MG	IR	MRTG MYTG OFO	
1	Sex		✓	✓					Yue <i>et al.</i> , 2002
	specific AFLP			✓			✓		Yue <i>et al.</i> , 2003 Yue <i>et al.</i> , 2004
2	Strain specific		✓	✓					Yue <i>et al.</i> , 2002
	RAPD	✓	✓	✓					Yue <i>et al.</i> , 2003
3	Strain specific		✓	✓					Yue <i>et al.</i> , 2002
	Microsatellites	✓		✓			✓	✓	Tang <i>et al.</i> , 2004
			✓	✓			✓		Yue <i>et al.</i> , 2004
			✓						Yue <i>et al.</i> , 2006
				✓		✓			Rahman <i>et al.</i> , 2008
				✓			✓		Rahman <i>et al.</i> , 2010
4	Strain specific		✓	✓					Manoharan <i>et al.</i> , 2011
	mtDNA markers	✓		✓			✓	✓	Mu <i>et al.</i> , 2011
	(CytB and COI)		✓	✓			✓	✓	Tang <i>et al.</i> , 2004
			✓	✓		✓			Hu <i>et al.</i> , 2009
			✓		✓	✓		✓	Rahman <i>et al.</i> , 2010
			✓	✓		✓			Mohd-Shamsudin <i>et al.</i> 2011
			✓	✓		✓			Mu <i>et al.</i> , 2012
								✓	Mu <i>et al.</i> , 2012

IG: Indonesia gold; R: Red; G: Gold; S: Silver; MG: Malaysian golden; IR: Indonesia red; MRTG: Malaysian red tailed golden; MYTG: Malaysian yellow tailed golden; OFO: Other species of the family Osteoglossidae

times a year, male arowanas can fertilize the egg at any time if the females are ready to spawn (Suleiman, 2013). Meanwhile, their breeding behaviour is unique since they spawn during night when the arowana swims closer to the water surface. Female arowana release orange-red eggs and the male fertilizes and then scoops the eggs in their mouth cavity for subsequent incubation. They incubate the eggs until the yolk sac is completely absorbed and the fingerlings become independent (Scott and Fuller, 1976; Dawes, 1999; Suleiman, 2013).

**Arowana industry in Malaysia :** The arowana industry in Malaysia has been growing rapidly since 2005 and currently, Malaysia is the second largest arowana producing country in Asia after Indonesia. The production value of arowana is almost half of the total ornamental fish production in Malaysia and is still increasing (DOF, 2011, 2012) (Fig. 2). Arowana breeding activity in Malaysia has expanded at an annual growth rate (AGR) of 21.8% from the year 2001 to 2008. In 2008, over 230 thousand Asian arowana pieces were produced with a total revenue of US\$ 31.1 million. In 2009, the arowana production increased to nearly 317 thousand pieces worth US\$ 47.45 million. From this total production,

almost 80% of the arowana was exported to China and the total overseas export value was approximately 95%. In 2009, the highest arowana producing states in Malaysia such as Perak and Johor generated about US\$ 16 million and US\$ 26 million revenue, respectively. In Malaysia, about 130 farmers are cultivating Malaysian golden arowana in about 800 ha of farm land. The farmers in Bukit Merah, Perak produce about 10,000 to 15,000 arowana fry every month. Arowana cultivation is expected to contribute more than US\$ 196 million to gross national income (GNI) by 2020. Furthermore, this industry is likely to provide 10,000 job opportunities. Arowana aquaculture industry in Malaysia has been identified as one of the high priority sectors under the National Key Economic Areas (NKEA) until 2020 (Tow and Nagaraj, 1989; Singh and Dey, 2006).

**Challenges faced by the arowana industry :** The arowana population in lakes and rivers is facing a sharp decline due to habitat change and extensive harvesting of wild population for commercial purposes (Ng and Tan, 1997; Dawes *et al.*, 1999). In addition, there is no recent development in the conservation status of arowana. This is probably due to inadequate background knowledge on the population

structure and diversity of the wild arowana populations. Several countries have already established captive breeding programs for the development of arowana species. However, there are still some unresolved problems such as difficulties in the identification of sex and varieties, which are essential for the successful spawning and breeding of arowana (Yue *et al.*, 2003) as well as deterioration of water quality in ponds, lakes and rivers (Nur Atiqah *et al.*, 2013; Sharifhuddin *et al.*, 2013). In arowana farms, water quality management in nursery tanks and ponds is crucial to ensure good fish health, and to counteract any negative impacts on the environment (Marinho-Soriano *et al.*, 2011). Thus, sound water quality management, sex and variety differentiation, as well as inbreeding prevention are major issues that need to be addressed for the sustainable arowana production.

### Mitigating the arowana problems

**Water quality management :** Water quality is one of the main constraints in aquatic animals husbandry and plants. It is the total of the physical, biological and chemical parameters that influence growth and health of the cultured organisms. Rapid development of the arowana aquaculture industry in recent years has placed a great demand on good

water quality, without which, could ultimately affect arowana health and productivity (Nur Atiqah *et al.*, 2013; Sharifhuddin *et al.*, 2013). Successful breeding and production of arowana mainly depends on adequate supply of water with suitable attributes at minimal cost (Antony and Philip, 2006; Nur Atiqah *et al.*, 2013; Santos, 2013). Standard water quality parameters required for arowana aquaculture are: temperature, 29°C to 31°C; pH - 6.5 to 7.0; dissolved oxygen, >5 mg l<sup>-1</sup>; alkalinity, 60-100 mg l<sup>-1</sup> CaCO<sub>3</sub>; hardness, 100 mg l<sup>-1</sup>, Secchi disk transparency, 30-50 cm; and total ammonia-N, <1.0 mg l<sup>-1</sup> (Sabariah, 2011). Although arowana is a relatively hardy species, stressful environmental conditions can cause the fish to be more vulnerable to diseases. Therefore, to minimize these adverse conditions, adaptation and application of the current aquaculture technologies such as recirculating aquaculture system and bioremediation are essential. Recirculating aquaculture system ensures good water quality, and also guarantees a constant and adequate water supply during drought season when water from lakes and canals can be limited.

**Land-based recirculating aquaculture systems :** Recirculating aquaculture system (RAS) is a modern approach of fish farming and has the capacity to reduce water

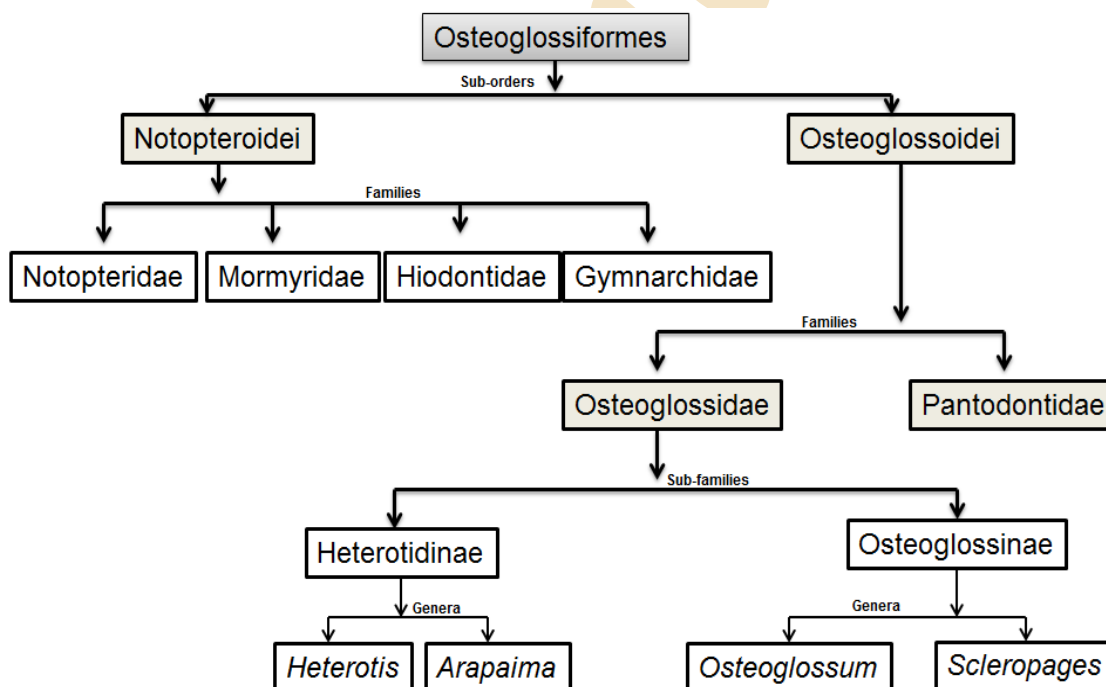


Fig. 1 : Classification of the order Osteoglossiformes (Greenwood and Wilson, 1998; Nelson, 2006)

usage. This system minimizes the occurrence of disease and dependence on lake water supplies which can be limited and/or polluted during certain times of the year (Martins *et al.*, 2010). The system is equipped with several components to continuously filter the water to remove impurities and therefore, water can be reused many times (Martins *et al.*, 2010b; Rijn, 2013). The main advantage of a RAS system is that requires less water and land area, enhances waste management, maintains good hygienic conditions and disease prevention, improves nutrient recycling, and involves biological pollution control (Zohar *et al.*, 2005; Verdegem *et al.*, 2006; Tal *et al.*, 2009). Different RAS types are currently utilized for the cultivation of a large variety of fresh and marine water species, including expensive ornamental fish koi carp (*Cyprinus carpio*) (Davis and Arnold, 1998; Lin *et al.*, 2003; Schulz *et al.*, 2003; Lymbery *et al.*, 2006; Halachmi *et al.*, 2006; Zachritz *et al.*, 2008). Arowana is an aggressive and insectivorous species, therefore integrated land-based RAS system is more suitable for sustainable arowana farming under natural conditions instead of using big expensive tanks. Land-based integrated RAS systems consist of constructed wetlands, biological ponds and an ecological ditch, which is coupled and connected to the recirculating land-based earthen ponds (Fig. 3). The constructed wetlands (CWs) serve as water treatment units and the advantage of constructed wetlands are that they are easy to build with low construction and maintenance costs (Kadlec and Wallace, 2009). Biological ponds act as pre-treatment units by treating the water before it flows into the

constructed wetland. Recently, Zhang *et al.* (2011) developed an integrated RAS system for land-based fish cultivation to prevent environmental pollution and to enhance the production rate in aquaculture ponds. This type of land-based RAS system can also be applicable for arowana cultivation during water scarcity conditions, to prevent environmental pollution and to enhance the healthy production. Even though land-based integrated RAS system is recommended for arowana farming, more technical advancements in the application of constructed wetlands (CWs) and improvement in the recirculation loop is still needed (Martins *et al.*, 2010a).

**Bioremediation :** Bioremediation is an other biotechnological method that can be used to maintain healthy environmental conditions in aquaculture ponds and hatchery tanks. It is a method of utilizing microbial metabolic potential to remediate polluted environments (Watanabe, 2001). Bioremediation of aquaculture ponds is generally carried out by bioremediating agents or bioremediators, which include microorganisms (e.g. bacteria) and enzymes.

**Microorganisms :** Beneficial bacterial species can also be used widely as bioremediators to improve water quality in aquaculture ponds (Moriarty, 1997). Bioremediation bacteria added to the aquaculture ponds have many advantages such as increasing the decomposition of organic matter; reducing nitrogen and phosphorus concentrations; improving beneficial algae growth; increasing dissolved oxygen

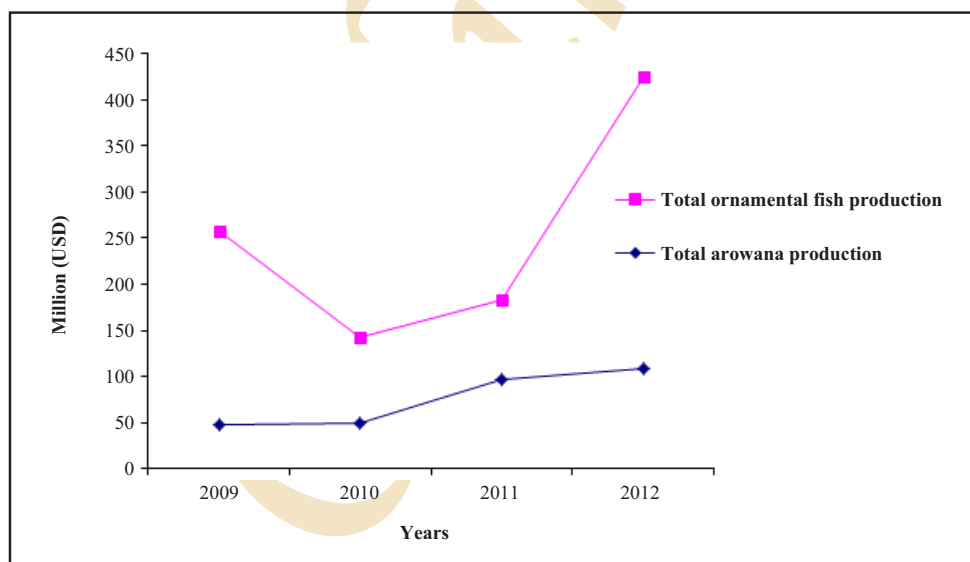
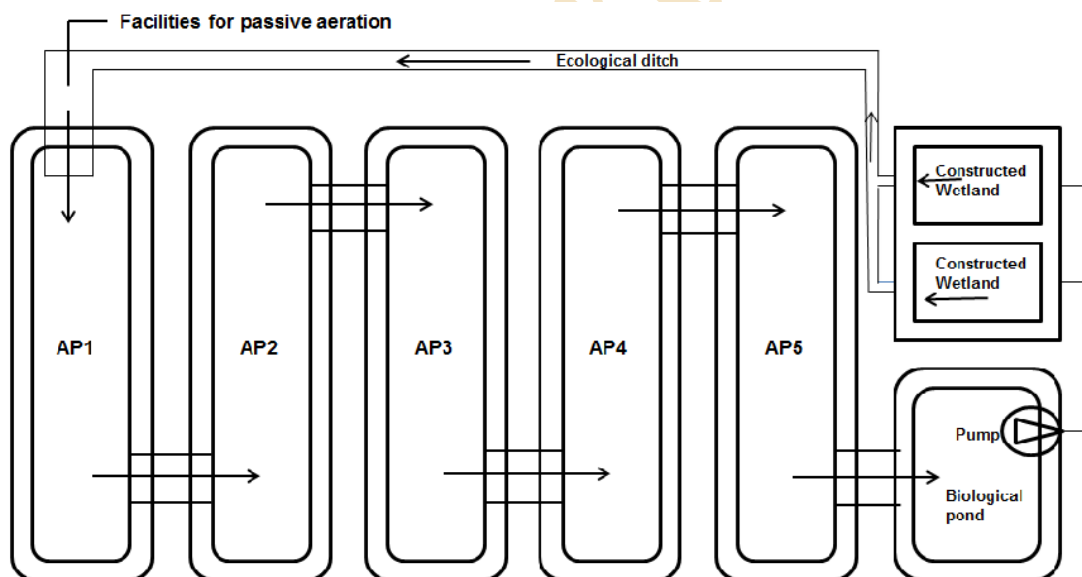


Fig.2 : Comparison of the arowana aquaculture production with ornamental fish production in Malaysia (DOF, 2011; DOF, 2012)

availability; suppression of cyanobacteria blooms; controlling ammonia, nitrite, and hydrogen sulphide concentrations; lowering incidences of disease; enhancing the survival of the farmed organism and improving production (Boyd and Cross, 1998; Balcazar *et al.*, 2006). Generally, *Bacillus* spp. are used as bioremediators in aquaculture ponds as compared to Gram negative *Bacillus* spp. This is because Gram positive *Bacillus* spp. are more efficient in the conversion of organic matter to CO<sub>2</sub> (Verschuere *et al.*, 2000; Bandopadhyay and Mohapatra, 2009; Ngan and Phu, 2011). Several studies conducted on ornamental and edible fish, as well as in shrimp ponds showed improved water quality conditions and higher growth rate when bacteria were used as bioremediators (Queiroz and Boyd, 1998; Wang *et al.*, 2005; Taoka *et al.*, 2006; Laloo *et al.*, 2007). Some of the other beneficial bacterial species which could be used as bioremediators are *Nitrobacter*, *Enterobacter*, *Cellulomonas*, *Rhodopseudomonas*, *Aeromonas media*, *Aeromonas* CA<sub>2</sub>, *Photorhodobacterium* sp., *Pseudomonas fluorescense*, *Pseudomonas* sp. and *Roseobacter* sp. BS 107. Other than microorganisms, plant sources such as yucca extract, potassium ricinoleate, tannic acid and citrus seed extract were also reported as efficient bioremediators that could improve water quality in aquaculture ponds (Boyd and Cross, 1998; Verschuere *et al.*, 2000; Antony and Philip, 2006).

**Enzymes :** Enzymes are biocatalysts which are produced by living organisms to accelerate a particular biochemical processes. One way of improving water quality in aquaculture ponds is direct application of enzymes to pond water or soil (Mayer, 2012). However, this approach can be expensive and impractical due to large volume of water in ponds. Thus, enzymes could be used in tanks or other small culture units. Enzymes have the capability to transform all organic pollutants present in the aquaculture water and bottom soil. A variety of enzymes produced by different organisms such as bacteria, fungi and plants have been described to be involved in the bioremediation of toxic contaminants (Karigar and Rao, 2011). Compared to microorganisms, enzymes are more efficient bioremediators because they remain active in a wide range of environmental conditions (such as pH, temperature and dissolved oxygen concentration) (Ahuja *et al.*, 2004; Gianfreda and Rao, 2004). Furthermore, immobilized enzymes are active even during drastic environmental changes and their enzyme activity can be preserved and reused (Whiteley *et al.*, 2002; Gianfreda and Rao, 2004). One particular enzyme cannot act effectively in all cases and thus, a combination of different types of enzymes may work well in the removal of toxic contaminants in aquaculture ponds (Ruggaber and Talley, 2006). The major disadvantage associated with these enzyme preparations as bioremediators is the high cost of isolation,



**Fig. 3 :** Schematic diagram of integrated land-based recirculating aquaculture system (adapted from Zhang *et al.*, 2011): Consisting of purification units (biological pond, two parallel constructed wetlands [CWs], and a lengthy ecological ditch) and five recirculating arowana ponds (AP1-AP5)

purification and production. In spite of their high cost, there is a lot of interest in the preparation of these enzymes and at present there are already some enzyme-based products used as bioremediators in aquaculture industry (Mayer, 2012). The success of enzymes as bioremediators depends on the kind of reaction and the type of pollutant that needs to be degraded. Enzymes which are involved in bioremediation include microbial oxidoreductases, microbial laccases, microbial peroxidases and microbial hydrolytic enzymes (e.g. lipases, cellulases, proteases, amylases, pectinases and xylanases) (Karigar and Rao, 2011; Mayer, 2012). Therefore, friendly bacteria and enzymes could be used as potential bioremediators in water quality management of arowana aquaculture ponds for their sustainable production (Devaraja *et al.*, 2002)

**The role of molecular markers in the conservation of Asian arowana :** The main problems involved in the conservation and commercial production of Asian arowana is identification of sex and strains. Sexing of arowana is an important factor in determining suitable male-female ratio in broodstock ponds, which is necessary for successful fish spawning and breeding. However, maintaining the right sex ratio is often difficult under captive breeding conditions because the sex of arowana can only be recognized after sexual maturity at 3-4 years. Although experienced farmers can identify the sex based on morphology, accuracy is still below 100%. Molecular markers are the best suitable tools for the management of aquaculture animals (Carvalho and Pitcher, 1994; Ferguson and Danzmann, 1998), and are recognized as possible technologies to provide accurate information for sexing and varietal forms of arowana population at any developmental stage (Teletchea, 2009).

To date, very little information is available about the genetic diversity of arowana population. In recent years, few studies have assessed the genetic variability and sexing among different arowana species (Table 1), which include microsatellites (Rahman *et al.*, 2008; Mu *et al.*, 2011), random amplified polymorphic DNA (RAPD) (Yue *et al.*, 2002), and amplified fragment length polymorphism (AFLP) (Yue *et al.*, 2004). Furthermore, mitochondrial genes are extensively used in the taxonomic identification of many organisms due to high abundance of cell mitochondria. In addition, mitochondrial DNA has the advantages of rapid evolutionary rates, limited exposure to recombination, lack of introns and high copy numbers (Brown, 1985; Wilson *et al.*, 1985; Luo *et al.*, 2011). Cytochrome B (Cyt B) is commonly used mitochondrial DNA marker in phylogenetic

studies (Briolay *et al.*, 1998). Besides, DNA barcoding method has been extensively used in phylogenetic studies and species identification by using mitochondrial cytochrome C oxidase (COI) gene as a possible barcode region (Mohd-Shamsudin *et al.*, 2011).

Microsatellite and mtDNA markers are also used for short-term genetic differentiation amongst arowana species. In these experiments, microsatellite analysis was observed to be more efficient in estimating short-term genetic distance within population as compared to mtDNA. However, mtDNA analysis is more suitable for resolving long-term divergence (Tang *et al.*, 2004). Shotgun sequencing and phylogenetic analyses of mtDNA of Asian arowana confirmed its relationship with its ancestral teleost lineage (Yue *et al.*, 2006). Hu *et al.* (2009) studied the differences between the red and green arowana by comparing mtDNA cytochrome-B (cytB) gene sequences. These studies provided adequate evidence that molecular markers could be used as efficient tools for sex, varietal and species identification of Asian arowana. However, some advanced genetic research is still necessary to eliminate the challenges in arowana sex and species identification for their successful conservation.

Sex-specific markers help farmers to establish sex ratios based on their requirements. For example, a sex ratio of one male and one female is more suitable for conservation purposes, as this ratio will reduce the process of genetic drift. In the case of commercial arowana production, a different sex ratio can be maintained for efficient production. The sex-specific markers can also be useful for genotypic sex identification of individual strains (Yue *et al.*, 2003). Strain-specific molecular markers could be beneficial to the farmers who are involved in trading of different arowana strains, because the colour of the strains did not fully develop until they reached market size (10-12 cm) of eight months, and there is also a drastic color change depending on different arowana strains. Strain-associated molecular markers are helpful to commercial arowana farmers to check the identity of a particular shipment. Strain-specific markers can also be used to quickly test the ratios of colour varieties of young arowana caught from the wild, and for the identification of individuals for maintaining genetic diversity among the farmed stocks (Yue *et al.*, 2003).

Therefore, for sustainable arowana production and conservation, accurate sex differentiation protocols, maintenance of genetic vigour and water quality management practices are essential.

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