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Distribution, Environmental Physiology and Genetic Variability of Haruan *Channa striatus*

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Abstract. - A teleost, *Channa striatus* or locally known as haruan is a food fish and considered to be of biomedical value in traditional medicine. A distribution study to identify locality and environmental physiology of their habitat was done throughout Malaysia, including Sabah and Sarawak. Water parameters namely turbidity, conductivity, temperature, dissolved oxygen (DO), pH and salinity were determined using HORIBA water checker – U7 and the depth was measured using calibrated tape. Consequently, a HACH model DREL/1 was used to determine sulphate, nitrate, phosphate, chlorine and ammonia content, while atomic absorption spectrophotometer (Perkin-Elmer) model 2380 was used to measure the sodium, magnesium and calcium in both water and soil samples. Subsequently, haplotypic differences and genetic variability revealed by mitochondria DNA in haruan was determined as described by Kajima, *et al.*, 1994, where five 6 – base recognizing restriction endonucleases, *Hin* dIII, *Eco* RI, *Pst* I, *Kpn* I and *Bam* HI were used to establish the stock identity of haruan, *C. striatus*, in Malaysia. A total of 12 haplotypes was observed among the three populations and total size of the mtDNA was 20.3 ± 1.6 kbp. Haruan is widely distributed throughout Malaysia and the fish preferred slow running, otherwise calm or stagnant water, shallow with aquatic plants and hiding places, clean and good quality habitats where all the ionic composition in both water and soil are well below the toxic level. Haruan had also shown a physiological ability to adapt towards extreme pH from 4 – 8, temperature between 20 – 25°C and salinity of up to 10 ppt.

Keywords: Distribution, habitat, environmental physiology, stock identification, mitochondria DNA

Abstrak. - *Channa striatus,* sejenis teleost, yang dikenali sebagai haruan, diketahui adalah ikan makanan bernilai perubatan tradisional. Satu kajian sebaran untuk mengenalpasti lokaliti dan fisiologi persekitaran habitat telah dilakukan ke seluruh Malaysia, termasuk Sabah dan Sarawak. Parameter air umpamanya kekeruhan, pengaliran, suhu, oksigen terlarut (DO), pH dan kemasinan telah ditentukan menggunakan HORIBA water checker U-7 dan kedalaman telah diukur menggunakan tali bersengkat. Serentak dengan itu, HACH model DREL/1 telah digunakan bagi menentukan kandungan sulfat, nitrat, fosfat, klorin dan ammonia, sementara spektrofotometer serapan atom (Perkin Elmer) model 2380 telah digunakan bagi mengukur natrium, magnesium dan kalsium dalam kedua - dua sampel air dan tanah. Seterusnya, perbezaan haplotipik dan kepelbagaian genetik yang ditunjukkan oleh DNA mitokondria dalam haruan telah ditentukan berdasarkan kaedah yang dijelaskan oleh Kajima *et al.* (1994) di mana lima 6 – besendonukleas pengenalan terhad, *Hin* dIII, *Eco* RI, *Pst* I, *Kpn* I dan *Bam* HI telah digunakan bagi mengujudkan identiti stok haruan, *C. striatus*, dalam Malaysia. Kesemuanya 12 haplotip telah dikesan diantara tiga populasi dan jumlah saiz mtDNA adalah 20.3 ± 1.6 kbp. Haruan adalah tersebar luas keseluruh Malaysia dan ikan ini mengemari air mengalir perlahan, kalau tidak-pun tenang atau tidak bergerak, cetek dengan tumbuhan akuatik dan tempat menyorok, bersih dan habitat berkualiti baik di mana kesemua komposisi ioniknya di dalam kedua - dua air dan tanah adalah jauh di bawah paras toksik. Haruan juga menunjukkan keupayaan fisiologi beradaptasi kepada pH melampau dari 4 – 8, suhu diantara 20 – 25°C dan kemasinan sehingga 10 ppt.

Introduction

Malaysia is endowed with rich flora and fauna species. The freshwater animals, fishes in particular, are long associated with daily life and some has become legendary in various ways. Haruan, *Channa striatus*, a local freshwater species, belonging to family Channidae, is known and widely consumed throughout the nation not only as food, but also as a remedy for wound healing. Thanks to mid-wives, who constantly and aggressively promote haruan for wound healing but without knowing the scientific basis of their claims. People in China, Indochina, Thailand, Singapore, Indonesia, Philippines and India consumed and believed in the biomedical properties of the fish. Obviously, Channidae are well distributed within this region (Mat Jais, 1991; Mohsin and Ambak, 1983; Wee, 1982).

There are thirty species identified and eight were reportedly found in Malaysia (Inger and Kong, 1962; Mohsin and Ambak, 1983; Wee, 1982). Among them is haruan *C. striatus*, commonly found in rice fields and the surrounding areas, as well as various natural and man made water bodies. Being carnivorous, haruan is considered a pest and never been listed as priority in the farming activity in Malaysia. However, many other countries in the region such as Thailand, Indochina, Indonesia, The Philippines, China and India, have extensively cultivated this fish and it is among the most popular table fish (Mat Jais, 1997). This is also one of the sought after freshwater fish for Ee Sang during the Chinese New Year, and many believe that haruan contain all the essential elements to bring about good health and helps to recover the lost energy after long illness (Mat Jais, 1997; Mat Jais *et al.*, 1994; 1998).

In this study, we are trying to establish the population variation of haruan within Malaysia, the environmental physiology of their habitat and the nutritional value of the fish including vitamin A and minerals. These information will help to understand their natural habitat for future intensive culture and breeding. The nutritional value of the fish from various places will provide us with the information on the association of food and water quality of each locality. This will encourage commercial farming, and promote systematic marketing of this fish.

Materials and Methods

Kelantan, Johor and Penang in the west and Sabah and Sarawak in the east were selected to represent geographical distribution throughout Malaysia. Three localities within these states were chosen where all the environmental physiological parameters measurement were performed. The selection of the stations were based on the nature of the water body, suggestion by local farmers and on the advise of local Department of Fisheries. The captured haruan were released back to the environment after morphological observation to confirm the species. Both water and soil samples were collected for further laboratory testing.

Water quality for each station were check using HORIBA Water Checker U-7, to measure pH, temperature, turbidity, conductivity and dissolved oxygen and HACH Model DREL/1 was used to detect sulphate, nitrate, phosphate, chlorine and ammonia. These two equipments provide almost spontaneous *in situ* reading. Three readings were made at every locality, one in the morning at 8.00 am, one in midday 12.00 noon and the last one was at 6.00 pm for three consecutive days. Sodium, magnesium and calcium in benthos soil were determined using an atomic absorption spectrophotometer Perkin-Elmer Model 2380. This oven-dried soil was then meshed into finer particles using mortar before adding 50 ml of 1 M HCl and left to stand for 24 hrs before being filtered and analyzed using the spectrophotometer. Water samples were pretreated with concentrated nitric acid and kept at 4°C before being analysed for ionic composition using similar calorimetric analysis.

Restriction fragment length polymorphisms (RFLPs) of mitochondria DNA (mtDNA) were performed to estimate genetic differences and variability. Wild haruan, *Channa striatus*, 10 from Penang, 15 from Kelantan and 16 from Johor with a mean size of 22.4 cm total length and mean weight of 121.4 g were sampled randomly, and the heart, liver and eggs were used for the RFLPs analysis of mtDNA. Five 6 – base recognizing restriction endonucleases, *Hin* dIII, *Eco* RI, *Pst* I, *Kpn* I and *Bam* HI were used based on the method described by Kajima *et al.* (1994). The tissue was first rinsed in 0.25 M Sucrose – TEK and homogenized before being centrifuged at 3,000 rpm for 10 min and at 4°C, in Nalgene tube using Centrikon Model H – 401B (Kontron Instruments) centrifuge. The supernatant was removed and centrifuged again at a higher speed of 15,000 rpm for 60 min and at 4°C using Beckman J2 – 21 M/E centrifuge, and 4,050 µl of TEK (1x) solution was added to the pellet. Subsequently, the mitochondria membrane was dissolved in 450 µl of 10% non - idet P40 (TEK) and vortexed. A 900 µl, fresh 0.4 N NaOH was added to the solution and finally, a 660 µl, 3 M Na - acetate was added and vortexed, before being centrifuged at 5,000 rpm for 5 min at 4°C. An equal amount of TE – phenol, about 7 ml, was added and thoroughly mixed by shaking for 10 min, and centrifuged at 5,000 rpm for 10 min at room temperature. The aqueous top solution was collected and another equal amount of TE - phenol was added and shaken for 5 min, and centrifuged at 5,000 rpm for 5 min, first in phenol:chloroform (1:1) and thrice in choloform:isoamylalcohol (24:1). The aqueous phase from the last centrifugation, about 450 µl, was added with 50 µl (about 1/10 of the volume), of 3 M Na - acetate and 1,000 µl (about 2:2.5 of the volume), of cold 100 % ethanol, and was mixed thoroughly and kept at – 20°C for an overnight in Appendorf tube. The following day, the mixture was centrifuged at 12,000 rpm for 15 min at 4°C and the pellet was rinsed with 0.5 ml 70% cold ethanol and 0.5 ml 100% cold ethanol, respectively. The pellet was briefly dried at room temperature, and 50 µl of TE solution was added before keeping at 4°C. The sample was electrophorized at 55 Volts for 2 hrs to check for the presence of mtDNA.

The details on the extraction and digestion of mtDNA by restriction endonucleases *Hin* dIII, *Eco* RI, *Pst* I, *Kpn* I and *Bam* HI to detect RFLPs in mtDNA, and calculation of the haplotypic frequency and diversity, nucleotide diversity and the number of substitutions were based on the methods prescribed by Kajima *et al.* (1994). Similarly, all the data analysis and detection of RFLPs in mtDNA were based on Kajima *et al.* (1994). Subsequently, the calculation of the haplotypic frequency and diversity has been described in Kumar, 1995.

Results and Discussion

As a carnivorous fish, *C. striatus* is not a good swimmer but with a fast flip action to catch prey, and as an air breather the fish need to surface for air. Therefore, *C. striatus* prefer slow running or stagnant waters, not more than 2 meters in depth, with aquatic plants and some dead log to hide or to hunt. Their natural enemies are snakes, eagles, monkeys, boomerangs and human beings. Although most of the habitats were remote but some are within close proximity to human settlements. Haruan were also found in waters up to 12 m deep, and the width between 4 - 80 m. Ponds, small lakes, agriculture canals, small rivers, rice fields and water catchments areas of 10 - 12 m wide seems the most ideal, but haruan were also found in various unexpected locality such as river mouth with salinity about 10 ppt and higher ground with water temperature around 20°C. The pH of the habitat was 4.3 - 7.9, temperature was between 20.7 and 26.4°C, conductivity between 0.1 and 1.3 $mScm^{-1}$, turbidity between 2 and 268 ppm and dissolved oxygen between 1.2 and 6.1 ppm (Table 1). As for Malaysia, a tropical country, the weather is constantly hot and humid, throughout the year (Ling, 1977; Minear and Keith, 1982; Mohsin and Ambak, 1983). Similarly, the water parameter seems to have minimal changes, within a day, weeks and months. There were hardly any changes even after a heavy storm, and any torrential effects will be buffered by in and out flow of the water. Although Malaysia had never experienced such a drastic weather change in the last 20 years, the recent La Nina and El Nino, and the haze problem in August to October 1998 impeded aquatic photosynthesis (Mat Jais, 2000).

Table 1 : The range of water quality parameters of water bodies where haruan, *Channa striatus,* were fished

Physical parameters	Range value
Depth (meter)	4 - 80 m
Width (meter)	10 - 12 m
Temperature (°C)	20.70 - 26.40 °C
pH	4.30 - 7.90
Dissolve Oxygen	1.20 - 6.10 ppm
Turbidity	2 - 268 ppm
Conductivity	0.10 - 1.30 $mScm^{-1}$
Salanity	0 - 10 ppt

Chlorine in the water was between 0.2 and 0.4 mg l^{-1}, ammonia 0.6 and 3.0 gm l^{-1}, nitrate between 0.5 and 6.0 mg l^{-1}, phosphate 0.5 and 0.8 mg l^{-1}, sulphate 2.0 and 13.0 mg l^{-1}, calcium 3.4 and 19.6 ppm, magnesium 1.7 and 25.1 ppm and sodium 2.37 and 9.81 ppm (Table 2). This is definitely a good quality and non-polluted environment. Although many believe that haruan is a hardy fish and certainly could tolerate to some extend the deterioration of the water quality, but the actual physiological adaptation unique to the species is the ability to move across land to more suitable and cleaner waters (Evans, 1993; Mat Jais, 1991). Similarly, haruan is also known to bury in the mud during dry spells and only come out when the situation improved. Subsequently, the calcium, magnesium and sodium content in the soil were 1.7 to 16.8 ppm, 0.28 to 29.32 ppm and 2.67 to 34.60 ppm, respectively. The environmental condition and physiology of all the stations in this study could be categorized as relatively clean, and coincidently these localities were away from the 25 rivers identified as being polluted in Malaysia.

Table 2 : The range value of chemical compositions in water and soil samples from the five states in Malaysia where haruan, *C. striatus,* were fished

Samples (ionic compositions)	Water	Soil
Chlorine	0.20 - 0.40 mg l^{-1}	-
Ammonia	0.60 - 3.00 mg l^{-1}	-
Nitrate	0.50 - 6.00 mg l^{-1}	-
Phosphate	0.50 - 0.80 mg l^{-1}	-
Suphate	2.00 - 13.00 mg l^{-1}	-
Calcium	3.40 - 19.60 ppm	2.67 - 34.60 ppm
Magnesium	1.70 - 25.10 ppm	0.28 - 29.32 ppm
Sodium	2.37 - 9.81 ppm	1.70 - 16.80 ppm

As food, haruan was proven to contain high protein, 78.32 ± 0.23% and low lipid, 2.08 ± 0.08%. This composition make haruan one of the better choice for protein, with 14 amino acids being detected namely leucine, isoleucine, methione, tryptophane, lycine, histidine, alanine, oxyproline, tyrosine, theonine, glycine, serine, aspartic and glutamic acid. All these amino acids are essential and are the basic elements for wound healing (Mat Jais *et al.*, 1994; 1998). The lipid was further categorized into phospholipid, partial glyceride, cholesterol, fatty alcohol, triglyceride and cholesterol ester. Subsequently, haruan contained 0.265 ± 0.013 mg per total lipid Vitamin A. As expected, a carnivorous *C. striatus* should contain Vitamin A, and the amount is relatively high and is essential for wound healing (Mat Jais *et al.*, 1994; Westaby, 1985). Furthermore, although magnesium seems to be relatively high (29.20 ± 0.06 ppm), but all these minerals in haruan tissue namely, calcium (3.99 ± 0.19 ppm), magnesium (29.20 ± 0.06 ppm), cuprum (0.74 ± 0.26 ppm), ferric (1.65 ± 0.15 ppm), manganese (0.31 ± 0.10 ppm), nickel (0.23 ± 0.03 ppm), lead (4.05 ± 0.25 ppm) and zinc (3.98 ± 0.05 ppm), were much below the toxic level to humans and this make haruan one of the safest live food and protein source (Heimann, 1982; Jaafar, 1985; Lands, 1986; Mat Jais, 1997).

As a conclusion, haruan *C. striatus,* is widely distributed within east and west Malaysia, and in general the habitats had similar environmentals factors. Furthermore, wild and cultured species proved to have almost similar biochemistry (Mat Jais *et al.*, 1994; 1998) and of the same stock (Kumar, 1995). Based on the present genetic variability study of the mitochondrial DNA, the haruan now present in Malaysia is of two stocks, as reported by Kumar, 1995.

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