បំរែបំរួលភាពនៃពន្ធុរបស់ត្រីប្រាខ្មៅ នៅក្នុងអាងទន្លេមេគង្គកម្ពុជា

GENETIC DIVERSITY OF THE MIGRATORY CATFISH *PANGASIUS BOCOURTI* IN THE CAMBODIA'S MEKONG RIVER

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អត្ថបទសង្ខេប

ការប្រើប្រាស់បំរែបំរួលពន្ធុ នៅក្នុងគោលបំណងនៃការគ្រប់គ្រង និងអភិរក្សធនធានជលផលមានសារៈសំខាន់ជាខ្លាំង។ ការយកមីតូកុងទ្រី ឌីអិនអេ (Restriction Fragment Polymorphism of mitochodrial DNA) មកប្រើប្រាស់ដើម្បីធ្វើអង្កេតលើ បំរែបំរួលភាពនៃពន្ធុ (genetic diversity) និងរចនាសម្ព័ន្ធ (genetic structure) នៅក្នុងតំបន់ហ្សែន ដែលមានឈ្មោះថា សាយតូក្រូមប៊ី-ឌីលូប (Cytochrome b – D-loop region) ដែលមានប្រវែង ២.១គីឡូបេស (2.1 kb) របស់ត្រីប្រាខ្មៅ (*Pangasius bocourti*)។ ក្នុងត្រីប្រាខ្មៅចំនួន ៩០ក្បាល ដែលត្រូវបានចាប់ពីទន្លេមេគង្គកម្ពុជា នៅឆ្នាំ២០០១ ដើម្បីយកមកសិក្សាលក្ខណៈពន្ធុសាស្ត្រនោះ យើងបានស្រាវជ្រាវឃើញថាត្រីប្រាខ្មៅទាំង៩០ក្បាលនេះ មានមីតូកុងទ្រី ឯកប្រភេទ (mitDNA haplotype) ចំនួន ០៧។ ជាមធ្យមបំរែបំរួលភាពនៃពន្ធុរបស់ត្រីប្រាខ្មៅប្រែប្រួលពី ០.៤៤ ដល់ ០.៦៨។ ផ្អែកលើការវិភាគពន្ធុសាស្ត្រលើកដំបូងរបស់យើងទៅលើអត្រាសរុបនៃការចាប់យក (Total fixation Index) ផែរវ៉ាយ (Pair-wise F_{ST}) និងម្រ៉ឺស្ទ អំពីចម្ងាយពន្ធុរបស់ Slatkin (Slatkin's genetic distance) បានបង្ហាញអោយឃើញថា ត្រីប្រាខ្មៅមានចំនួនមួយពូជតែប៉ុណ្ណោះ នៅក្នុងទន្លេមេគង្គកម្ពុជា។

ការបំផ្លិចបំផ្លាញកន្លែងពងកូនរបស់ប្រភេទត្រីប្រាខ្មៅនេះ នឹងធ្វើអោយមានការជះឥទ្ធិពលអវិជ្ជមាន មិនត្រឹមតែដល់ទន្លេមេគង្គកម្ពុជាប៉ុណ្ណោះទេ ថែមទាំងដល់អាងទន្លេមេគង្គទាំងមូលទៀតផង។ ដោយសារតែត្រីប្រាខ្មៅមានតែចំនួនមួយពូជ ហើយពូជនេះត្រូវបានចែកចាយទៅក្នុងអាងទន្លេនៃប្រទេសជិតខាងទាំងបួន (កម្ពុជា ឡាវ ថៃ និងវៀតណាម) ដូច្នេះប្រទេសទាំងបួននេះត្រូវ មានយុទ្ធសាស្ត្ររួមមួយដើម្បីគ្រប់គ្រងធនធាននេះ ហើយត្រូវអនុវត្តយុទ្ធសាស្ត្រនេះរួមគ្នានាពេលអនាគត។

ABSTRACT

The application of genetic variation is of practical significance in both fisheries management and conservation objectives. Restriction fragment polymorphism of mitochondrial DNA (mtDNA-RFLP) was employed to investigate the genetic diversity and genetic structure in the mtDNA cytochrome b - D-loop region (2.1 kb) of the migratory catfish Pangasius bocourti collected in the Cambodia's Mekong River in 2001. We observed seven mtDNA haplotypes among the 90 individuals assayed. On average, intraspecific genetic diversity (i.e. genetic variation) ranged from 0.439 to 0.684. Based on our initial genetic analyses of the total fixation index (G_{ST}) among all samples, pair-wise F_{ST} values and Slatkin's genetic distances, P. bocourti seems to represent a single stock in the Cambodia's Mekong River.

The destruction of the spawning ground of this species might have negative impacts on the Cambodia's Mekong River and probably the whole Mekong River basin. Since the stock is shared among the riparian countries of the Mekong, holistic and basin-wide resource management strategies have to be developed and implemented.

KEYWORDS

conservation, fisheries, management, mtDNA, Pangasiidae, restriction fragment length polymorphism (RFLP).

INTRODUCTION

Among the world largest and biologically richest rivers, including the Yangtze and Xijiang (East Asia), Amazon (South America), Mississippi (North America), Congo (Africa) and Danube (Europe), the Mekong River basin is no exception in its faunal characteristics (Banarescu 1992; Myers et al., 2000). With a mean annual discharge of 475×10^9 m^3 y^{-1} the Mekong has its source in High Asia, its middle course flows through the province of Yunnan (P.R. China), the lower course and its tributaries drain Laos, Cambodia, southern Vietnam and Thailand. Its aquatic fauna is very rich with at least 1,200 fish species (Rainboth, 1996). Furthermore, annual floods inundate larger areas, establishing temporary connections between various water-bodies that otherwise remain isolated during the dry season. These flooded areas provide abundant food, spawning areas and fry nurseries for fish. Many fish species migrate laterally between the floodplain and the deeper lake or tributaries, or carry out longitudinal migrations to and within the main Mekong stream. Typically many species spawn within the main Mekong River and its tributaries. Eggs and larvae are swept downstream by the river and into the floodplain where they grow. Other species may spawn directly on the floodplain and in the swollen lakes and reservoirs. The long-range migrations undertaken by some species appear to be spawning migrations, when the fish home to particular spawning areas (Singhanouvong et al., 1996). Environmental deterioration from human activities has disrupted migrations and decreased fish populations.

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Running title: Genetic diversity of Pangasius bocourti

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The Asian catfish family of the Pangasiidae includes riverine fishes generally occurring in freshwater, with exception of Pangasius pangasius, P. polyuranodon, P. krempfi, and P. kunyit, which may enter in saline waters. They occur from the Indian subcontinent and Burma to continental Southeast Asia (Indochina/Mekong) and Insular Southeast Asia (Indo-Malay Archipelago) (Roberts and Vidthayanon, 1991; Vidthayanon, 1993; Pouyaud et al., 1999). Pangasiidae are moderately to very large catfishes, with at least 21 living species (Vidthayanon, 1993; Rainboth, 1996). They are an important fisheries resource, some of them being cultured widely and some well known as aquarium fishes.

Today, unfortunately, human activities along the Mekong River watershed are increasing rapidly and threaten this heritage of mankind. Fish yield appears to be related to variations in the extent of the yearly flood regime (Ahmed et al., 1998). Since the 1950's nearly 4,000 large and small dams and associated reservoirs and irrigation schemes have been built in the Mekong watershed. This has led to large reductions in the coverage of aquatic habitats, the blocking of accesses of migratory fish species to spawning and fry nursery areas, the altering of the level and quality of water, and the ending of the seasonal ebb and flow that is vital to the cycle of mating and reproduction (Robert, 1993a; Moreau and Ernsberger, 2001). This has resulted in decreasing Mekong populations, including Pangasius bocourti (Roberts, 1993b). Hence assessment of population diversity and structure of Pangasius bocourti represents imperative scientific information.

Pangasius bocourti (Trey Pra Kchao in Cambodian) (Pangasiidae, Teleostei) plays a major role in the Mekong River ecosystem due to their migratory behavior, feeding ecology, development of aquaculture and commercial values as popular food fish. It is an omnivore feeding on fruit and plant material (Vidthayanon, 1993; Singhanouvong et al., 1996; FishBase, 2002) and molluscs, crustaceans and small fish in nature (Nam So, 2001: pers. obs.). The total estimated production of P. bocourti and Pangasianodon hypophthlmus was 20,000-40,000 tons for pond culture and 40,000-50,000 tons for cage culture in the An Giang and Dong Thap provinces of the Mekong delta in 2000 (Tung et al., 2001; Tong et al., 2002).

Pangasius bocourti might spawn in the Mekong River between the north end of Koh Rongiev (Kratie), through Koh Kha Nhae and Koh Ou Kandear next to the border at Stung Treng province and below the Khone Falls. Fish may concentrate in the Stung Treng province from Khoh Baychor (Siembok district) near the Kratie border to Koh Kei (Thalaboriwath district) during April-July (Touch, 2000: pers. comm.; Nam So, 2001: pers. obs.). Poulsen and Valbo-Jorgensen (2001) reported that spawning of P. bocourti may take place between Kratie and Stung Treng provinces. The upstream migration of adult fish towards the spawning grounds occurs from November to March and the downstream migration from April to October, followed by the fry and fingerling (Poulsen and Valbo-Jorgensen, 2001).

Within species diversity might be partitioned into variation within and among populations (Wright, 1968). It is necessary to maintain both types of variation to minimize the frequency of extirpation of local populations and to sustain species stability since genetic diversity is a requisite for evolutionary adaptation to a changing environment (Avise, 2000; Frankham, 2002). So far, genetic diversity and genetic differentiation at the population levels has proven to be the best method to manage the conservation of species (Templeton et al., 1995; Crandall et al., 2000), including fisheries (Ryman & Utter, 1987; Waples, 1991; Pullin et al., 1999). However, their application, particularly in tropical regions, is still in its infancy.

The specific objectives of this study are: (1) to optimize the mitochondrial DNA RFLP markers of P. bocourti; (2) to examine genetic diversity, levels of genetic differentiation and population structure; and (3) to assess the implications for fishery management of this species in the Cambodian Mekong River.

Materials and Methods

Biological material

Pangasius bocourti samples were collected from five major watersheds in the Cambodia's Mekong River (Fig. 1). Samples were typically taken at three life stages: adult, sub-adult and fingerling. There were difficulties to sample adult fish, therefore fingerlings include about 60% of the fish genotyped. A total of 90 specimens were collected from five sampling localities (Stung Treng, Kratie, Prey Veng, Bassac: Kandal, and Kampong Chhnang), with the sample sizes varying from 12 to 20 individuals per location (Table 1). Tissue samples consisted of fin clips, although muscle tissue was occasionally used. Most of the samples were collected from cage culture operations along the Tonle Sap and Mekong River; some samples were collected from fishers and retailers. Attention was paid to make sure that fish specimens sampled from each location originated from that locality in order to avoid mixing of populations. Fin clips were stored in a salt-saturated DMSO solution (20% DMSO; 250 mM Ethylenediaminetetracetic acid and 5 M NaCl) and kept at room temperature for transport to the laboratory for further DNA analysis. They were transferred to pure ethanol and stored at room temperate upon arrival.

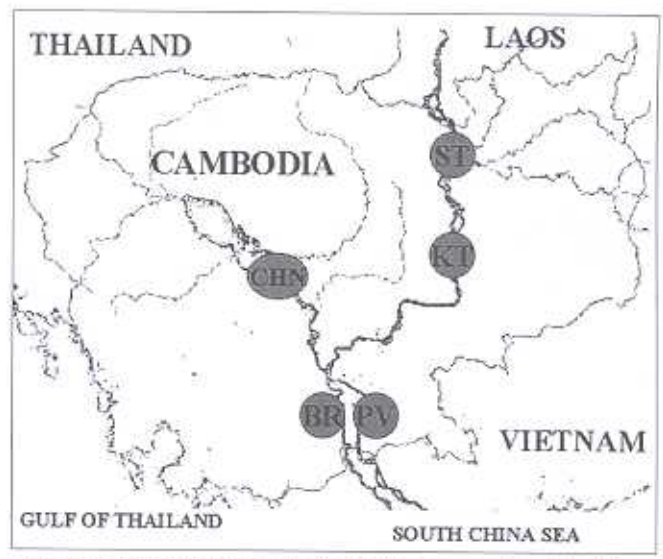

Figure 1. Map of Cambodia showing the geographical locations (named in Table 1) of Pangasius bocourti sampled from the Cambodia's Mekong River basin.

Each specimen was identified and classified according to Roberts and Vidthayanon (1991), Vidthayanon (1993) and Rainboth (1996).

Table 1 Sampling locations and sample sizes for Pangasius bocourti sampled in the Cambodia's Mekong River. Letters (BR, CHN, KT, PV and ST) correspond to sampling sites as illustrated in Fig. 1.

Sampling site	Sample size
ST Mekong Stung Treng	20
KT Mekong Kratie	12
PV Mekong Prey Veng	19
BR Bassac River Kandal	19
CHN Tonle Sap Kampong Chhnang	20

MtDNA extraction and RFLP genetic analysis

Tissue of 50-100 mg was digested with proteinase K in CTAB (Cetyltrimethyl-ammonium bromide) buffer and incubated at 55°C overnight. DNA was extracted using a standard phenol-chloroform extraction procedure (Sambrook et al., 1989).

The primers used to amplify the cytochrome b (cytb) and D-loop regions were the complimentary ND5/6 and HN20 (Bernatchez and Danzmann, 1993). Polymerase chain reaction (PCR) mixture contained approximately 10-100 ng of DNA, 0.8 µM of forward and reverse primer each, 200 µM of each dNTP, 0.5 U of Globalstar Taq polymerase (Eurogentec, Seraing, Belgium) and the corresponding 1x reaction buffer, 2 mM $MgCl_2$ and water in a final volume of 25 µl. Amplification was performed in a thermal cycler (Trioblock, Biometra, Goettingen, Germany) programmed as follows: 95°C for 3 min, 35 cycles at 95°C for 30 s, 46°C for 30 s, 72°C for 2 min 30, followed by a final extension at 72°C for 7 min. PCR amplification yielded a fragment of approximately 2100 base pairs, which were visualized by ethidium bromide in 1.2% agarose gel.

The amplified fragments were subsequently screened for polymorphism using five restriction endonucleases: HinfI, MboI, AluI, TasI, and FnuDII. All these enzymes recognize tetranucleotide palindromic sequences. Five or 7 µl of each PCR product were digested overnight with 10 U of the restriction enzyme in a final volume of 10-12 µl. The restriction fragments were visualized under UV light on 1.2 - 2% agarose gels stained with ethidium bromide. The molecular size of each restriction fragment was measured by densitometry (Gel documentation system, Amersham Biosciences, New Jersey, USA), based on comparison with a comigrating 100 bp molecular weight ladder.

The RFLP pattern produced by each endonuclease was assigned a letter so that each composite mtDNA haplotype was defined by a five-letter code (Table 2).

Data analysis

Endonuclease fragment patterns could be interpreted easily in terms of gain or loss of restriction fragments (Table 2), which were used as data to analyze genetic relationships within P. bocourti.

Data analysis, including mtDNA gene diversity (within population) (Nei, 1987), total fixation index (G_{ST}) among populations (Weir and Cockerham, 1984; Long1986), pairwise F_{ST} values between populations (Reynolds, Weir and Cokherham, 1983; Slatkin, 1995) and genetic distances (Slatkin 1995) was done with the software package ARLEQUIN version 2.0 (Schneider et al., 2000).

Results

Distribution of mtDNA haplotypes

Of the five restriction enzymes used, three (HinfI, MboI and AluI) were polymorphic in Pangasius bocourti (Table 2).

We observed seven mtDNA haplotypes among the 90 fish tested in the five samples (Table 2). The number of haplotypes within a single sampling locality varied from three to six: four were detected at Stung Treng, three at Kratie, five at Prey Veng, three at Bassac river and six at Kampong Chhnang. Haplotypes Pb1, Pb2 and Pb3 added up to 79 (87%) out of the 90 fish analyzed. The dominant haplotype (Pb1) was found in 55 (61%) P. bocourti. At Kratie, 75% of fish tested had the common haplotype (Pb1), followed by 70% at Stung Treng. Three singletons (unique haplotypes) were detected: two (Pb6 and Pb7) at Kampong Chhnang and one (Pb5) at Prey Veng.

Table 2. Absolute (in brackets) and relative frequencies and distribution of the composite haplotypes and presence/absence of restriction fragments resolved among Pangasius bocourti. Restriction enzymes are HinfI, MboI, AluI, TasI, and FnuDII. Capital letters identify fragment patterns. Abbreviation letters refer to sample locations illustrated in Fig. 1. HC denotes Haplotype Code.

HC	Haplotype	Restriction fragments	ST	KT	PV	BR	CHN	Total
			% (n=20)	% (n = 12)	% (n = 19)	% (n = 19)	% (n = 20)	% (n = 90)
Pb1	AAAAA	10111110100111011110101111111111111	70 (14)	75 (9)	53 (10)	58 (11)	55 (11)	62 (55)
Pb2	AABAA	10111110100111011110110011111111111	10 (2)	8 (1)	16 (3)	11 (2)	15 (3)	14 (13)
Pb3	ABAAA	10111110110101010100101111111111111	10 (2)	-	21 (4)	31 (5)	10 (2)	12 (11)
Pb4	ABBAA	10111110110101010100110011111111111	10 (2)	17 (2)	5 (1)	-	10 (2)	9 (8)
Pb5	ACAAA	10111110100111110101101111111111111	-	-	5 (1)	-	-	1 (1)
Pb6	ADBAA	10111110111001000110110011111111111	-	-	-	-	5 (1)	1 (1)
Pb7	BBBAA	01111111010101010100110011111111111	-	-	-	-	5 (1)	1 (1)

mtDNA haplotype diversity

The restriction fragments for each endonuclease in Pangasius bocourti (Table 2) were used to estimate genetic variation. Gene diversity was estimated to measure population fitness. We detected an average gene diversity of 0.505 at Stung Treng, 0.439 at Kratie, 0.684 at Prey Veng, 0.614 at Basac, 0.684 at Kampong Chhnang (Table 3).

Table 3. Sampling sites, number of individuals used for RCR-RFLP analysis, number of mtDNA haplotypes and gene diversity for Pangasius bocourti sampled in the Cambodia's Mekong River.

Sampling site	Number of individuals	Number of haplotypes	Gene diversity
ST	20	4	0.505 ± 0.125
KT	12	3	0.439 ± 0.158
PV	19	5	0.684 ± 0.092
BR	19	4	0.614 ± 0.095
CHN	20	6	0.684 ± 0.103

Table 4. Pairwise F_{ST} values (above diagonal) and corrected Slatkin' genetic distances (below diagonal) of Pangasius bocourti. No value is significantly different at $P< 0.05$.

	BR	CHN	KT	PV	ST
BR	-	0.0000	0.0000	0.0000	0.0000
CHN	-0.0108	-	0.0000	0.0000	0.0000
KT	-0.024	-0.0356	-	0.0000	0.0000
PV	-0.048	-0.0262	-0.0435	-	0.0000
ST	-0.029	-0.0123	-0.0657	-0.0374	-

Discussion

Based on the mtDNA PCR-RLFP analysis, it is obvious that: (1) The catfish Pangasius bocourti has a moderate level of genetic diversity in the Cambodia's Mekong River; (2) The populations of P. bocourti are not significantly differentiated; and (3) There is no obvious evidence for genetic structure in P. bocourti in the Cambodia's Mekong River.

P. bocourti samples had a similar distribution of mtDNA haplotypes in the Cambodia's Mekong River. Two haplotypes were ubiquitous and none were frequent and unique to a particular river. At Kampong Chhnang in Tonle Sap the highest number of haplotypes was found, which might suggest that we are either dealing with a mixed group or a more ancestral population. The location is known to be of recent geological origin and as a feeding ground, with an abundance of food and habitats for fish species adapted to periodic water-level change (Lim et al., 1999). Hence we opt for the mixed group hypothesis.

The overall genetic diversity in P. bocourti was higher than other fish such as whitefish Coregonus clupeaformis in North America, which was reported to range between 0.000-0.351 (Bernatchez and Dodson, 1991) and 0.067-0.700 (Lu et al., 2001); Atlantic salmon Salmo salar, 0.00-0.682 (King et al., 2000); brown trout Salmo trutta in a Danish river system, 0.000-0.860 (Hansen et al., 1995), in Spain, 0.000-0.712 (Machordom et al., 2000) and in tributaries of the Austrian Danube, 0.181-0.772 (Weiss et al., 2001). However, the values for brown trout (0.511-0.931) in Atlantic, Adriatic, Danubian, Mediterranean and marmoratus areas (Bernatchez, 2001) were higher than P. bocourti. Furthermore, the genetic diversity of P. bocourti in the Cambodia's Mekong River was higher than the Pimelodid catfishes Brachyplatystoma flavicans and Pseudoplatystoma fasciatum in the Bolivian Amazon basin (0.054-0.667) (Coronel et al., 2004).

The reduced genetic diversity at Kratie and Stung Treng might be attributed to four causes: (1) incomplete sampling (i.e. n = 12, Kratie), (2) stochastic consequences, including genetic drift and inbreeding (Hedrick 1992; Frankham, 2002), (3) historic bottlenecks (Wilson and Bernatchez, 1998), or (4) human-associated effects, including fishing pressure (Ryman and Utter, 1987; Frankham 2002). These genetic consequences might be caused by the small population size assumed to be present at Kratie and Stung Treng. The fisheries in the areas are poorly documented for various reasons and hence no population dynamic data are available.

Based on our analyses, the overall G_{ST} value among all samples suggests very weak or no genetic structure and insignificant differentiation in P. bocourti. Additional analyses, including pair-wise F_{ST} values and Saltkin's genetic distances also reflect no genetic structure and differentiation at all along the stretches of the Cambodia's Mekong River.

The Mekong River has, so far, been poorly studied, which complicates a comparative approach. However, one possible comparison could be made with several carp species, which showed minimal phylogeographical differentiation along a 500-km stretch of the Yangtze River in China (Lu et al., 1997). Also, the migratory pirarucu (Arapaima gigas) shows a lack of genetic structuring, a high level of gene flow and low effective population sizes in the Amazon basin (Hrbek et al., 2001).

There are no obvious natural or artificial barriers among all sampling sites (Stung Treng, Kratie, Prey Veng, Bassac River and Kampong Chhnang) covering the four major branches of the Cambodia's Mekong River. Hence, it is possible that P. bocourti might constitute a single stock, probably due to strong migration or high gene flow among all sampling locations in the Cambodia's Mekong River. This scenario parallels a hypothesis based on ecological surveys along the Mekong River using indigenous fishers' knowledge that there are two distinct populations of P. bocourti in the Mekong River. One population occurs from the Mekong delta through Cambodia to the Mukdahan-Sovannaket area

in southern Laos; another population occurs from around Boulikhamxay-Nong Kai provinces to around Chiang Rai-Bokeo provinces (Lao-Thai border) in the north (Poulsen and Valbo-Jorgensen, 2001).

In conclusion, the migratory catfish P. bocourti should initially be considered as a single stock in the Cambodia's Mekong River basin. The destruction of the spawning ground of this species might have negative impacts on the Cambodia's Mekong River and probably the whole Mekong River basin. Since the stock is shared among the riparian countries of the Mekong, holistic and basin-wide resource management strategies have to be developed and implemented.

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REFERENCES

Ahmed, M. Hap, N., Ly, V. and Tiengco, M. (1998). Socio-economic assessment of freshwater capture fisheries of Cambodia. A report on a Household Survey. MRC/DoF/DANIDA- Project for the Management of the Freshwater Capture Fisheries of Cambodia. Mekong River Commission, Phnom Penh, Cambodia.

Avise, J. C., and Hamrick,J.L. (1996). Conservation genetics: case histories from nature. *Chapman and Hall New York (N.Y.)*, XVII, 512 p.: ill. (ISBN: 0-412-05581-3).

Avise, J.C. 2000. Phylogeography: The history and formation of species (2nd edn.). *Harvard University Press*, Cambridge, Massachusetts (ISBN: 0-674-66638-0).

Banarescu, P. (1992). Distribution and dispersal of freshwater animals in North America and Eurasia. *Zoogeography of freshwater* **2,** *520-1091* (ISBN: 3-89104-482-8).

Bernatchez, L. & Danzmann, R. G. (1993). Congruence in control-region sequence and restriction site variation in mitochondrial-DNA of brook charr (*Salvelinus Fontinalis*, Mitchill). *Molecular Biology and Evolution* **10,** *1002-1014*.

Bernatchez, L. & Dodson, J. J. (1991). Phylogeographic structure in mitochondrial DNA of the lake whitefish (*Coregonus*, Clupeaformis) and its relation to Pleistocene Glaciations. *Evolution* **45,** *1016-1035*.

Bernatchez, L. (2001). The Evolutionary history of brown trout (*Salmo Trutta* L.) inferred from phylogeographic, nested clade, and mismatch analyses of mitochondrial DNA Variation. *Evolution* **55,** *351-379*.

Coronel, J. S., Maes, G. E., Clause, S., Van Damme, P. A.andVolckaert F. A. M (2004). Differential population history in the migratory catfishes *Brachyplatystoma flavicans* and *Pseudoplatystoma fasciatum* (Pimelodidae) from the Bolivian Amazon assessed with nuclear and mitochondrial DNA markers. *Journal of Fish Biology* **65,** *859–868*.

Crandall, K. A., Bininda-Emonds, O. R. P., Mace, G. M., & Wayne, R. K. (2000). Considering evolutionary processes in conservation biology. *Trends in Ecology & Evolution* **15,** *290-295*.

FishBase Website 2002. *http://www.fishbase.org*. Published by ICLARM and FAO.

Frankham, R., Ballou, J.D. and Briscoe, D.A. (2002). Introduction to conservation genetics. *Cambridge University Press*, Cambridge (ISBN: 0-521-63985-9).

Hansen, M. M., Hynes, R. A., Loeschcke, V., & Rasmussen, G. (1995). Assessment of the stocked or wild origin of anadromous brown trout (*Salmotrutta* L.) in a Danish River system, using mitochondrial DNA RFLP Analysis. *Molecular Ecology* **4,** *189-198*.

Hedrick, P. (1994). The Natural history of inbreeding and outbreeding. Theoretical and empirical-perspectives. Thornhill,Nw. *Science* **263:** *107-108*.

Hedrick, P. W. & Miller, P. S. (1992). Conservation genetics. Techniques and fundamentals. *Ecological Applications* **2,** *30-46*.

Hindar, K., Ryman, N., & Utter, F. (1991). Genetic effects of aquaculture on natural fish populations. *Aquaculture* **98,** *259-261*.

Hrbek T., Farias I., Crossa M., Meyer A., Cunha J. & Sampaio, I. (2001). Population genetic structure of *Arapaima gigas*: implications for conservation. Meeting of the European Society of Evolutionary Biology, Aarhus, Denmark . Book of abstracts p. 240.

King, T. L., Spidle, A. P., Eackles, M. S., Lubinski, B. A., & Schill, W. B. (2000). Mitochondrial DNA diversity in North American and European Atlantic salmon with emphasis on the Downeast Rivers of Maine. *Journal of Fish Biology* **57,** *614-630*.

Lim, P., Lek, S., Touch, S. T., Mao, S. O., & Chhouk, B. (1999). Diversity and spatial distribution of freshwater fish in Great Lake and Tonle Sap River (Cambodia, Southeast Asia). *Aquatic Living Resources* **12,** *379-386*.

Long, J.C. 1986. The allelic correlation structure of Gainj and Kalam speaking people. I. The estimation and interpretation of Wright' F-statistics. *Genetics* **112,** *629-647*.

Lu G., Basley D.J., and Bernatchez L. (2001). Contrasting patterns of mitochondrial DNA and microsatellite introgressive hybridization between lineages of lake whitefish (*Coregonus clupeaformis*); rele-

vance for speciation. *Molecular Ecology* **10**, 965-985.

Lu, G. Q., Li, S. F., & Bernatchez, L. (1997). Mitochondrial DNA diversity, population structure, and conservation genetics of four native Carps within the Yangtze River, China. *Canadian Journal of Fisheries and Aquatic Sciences* **54**, 47-58.

Machordom, A., Suarez, J., Almodovar, A., & Bautista, J. M. (2000). Mitochondrial haplotype variation and phylogeography of Iberian brown trout populations. *Molecular Ecology* **9**, 1325-1338.

Moreau, R. and Ernsberger R.J.R. (2001). Strangling the Mekong: A spate of dam building has stopped up Southeast Asia's mighty river and may threaten the livelihood of millions who live along its banks. In *Newsweek newsletter* March 19, 2001.

Myers, N., Mittermeier, R. A., Mittermeier, C. G., Da Fonseca, G. A. B., & Kent, J. (2000). Biodiversity hotspots for conservation priorities. *Nature* **403**, 853-858.

Nei, M. 1987. Molecular evolutionary genetics. Columbia University Press, New York, NY, USA (ISBN: 0231063202).

Panulu V.R. (1992) The Mekong River system. *In* 'Distribution and dispersal of freshwater animals in North-America and Eurasia' (Ed. P. Banarescu). *Zoogeography of freshwater* **2**, 520-1091 (ISBN: 3-89104-482-8).

Poulsen, A.T. and Valbojorhensen, J. 2001. Editors. Fish migration and spawning habits in the Mekong mainstream: a survey using local knowledge (basin-wide). *Fish migration and spawning. Version* **1**. *CD ROM. Mekong River Commission, Phnom Penh.*

Pouyaud, L., Teugels, G. G., & Legendre, M. (1999). Description of a new Pangasiid Catfish from Southeast Asia (Siluriformes). *Cybium* **23**, 247-258.

Pullin R.S.V., Bartley, D.M. and Kooiman, J., Editor (1999). Towards policies for conservation and sustainable use of aquatic genetic resources. *ICLARM/FAO Proceeding of an international conference held on 14-18 April 1998 in Bellagio, Como, North Italy*. ICLARM, Makati City, Philippines, and FAO, Rome, Italy (ISSN: 0115-5545/ISBN: 971-802-003-9). 277p.

Rainboth, W.J. 1996a. Fishes of the Cambodian Mekong. *FAO identification sheets for fishery purposes*. Food and Agriculture Organization, Rome. M-40, (ISBN 92-5-103743-4). 265 p.

Reynolds, J, Weir B.S., and Cockerham, C.C. (1983). Estimate for the coancestry coefficient: basis for a short-term genetic distance. *Genetics* **105**, 767-779.

Roberts T.R. (1993a). Mekong mainstream hydropower dams: run-of-the-river or ruin-of-the-river? *Natural History Bulletin of the Siam Society* **43**, 9-19.

Roberts, T.R. (1993b). Artisanal fisheries and fish ecology below the great waterfalls of the Mekong River in Southern Laos. *Natural History Bulletin of the Siam Society* **41**, 31-62.

Roberts, T. R. & Vidthayanon, C. (1991). Systematic revision of the Asian Catfish family Pangasiidae, with biological observations and descriptions of 3 new species. *Proceedings of the Academy of Natural Sciences of Philadelphia* **143**, 97-144.

Ryman, N., and Utter, F. (1987). Population genetics and fishery management. *University of Washington press Seattle (Wash.)*, XVII 420 p. (ISBN: 0-295-96435-9).

Sambrook J., Fritsch, E.F. and Maniatis, T. (1989). Molecular cloning: A laboratory manual. 2 nd edn. Cold Spring Harbor Laboratory Press, New York.

Schneider, S, Kueffer, J.M., Roesseli, D. and Excoffier, L. (2000). Arlequin: A software for population genetic data analysis, Version **2.0**. Genetic and Biometry Lab., Dept. of Anthropology, University of Geneva, Switzerland.

Singhanouvong, D., Soulignavong, C., Vonghachak, K., Saadsy, B. and Warren T. 1996. The main wet-season migration through Hoo Som Yai, a steep gradient channel at the Great Fault line on the Mekong River, Champassaek province, Southern Lao PDR, Ministry of Agriculture-Forestry, Department of Livestock-Fisheries, Technical Section.

Slatkin, M. (1995). As measure of population subdivision based on microsatellite allele frequencies. *Genetic* **139**, 457-462.

Templeton A.R., Routman, E. and Philips, A. (1995). Separating population structure from population history: a cladistic analysis of the geographic distribution of mitochodrial DNA haplotypes in the tiger salamander, Ambystoma tigrinum. *Genetics* **140**, 767-782.

Trong, Trinh Quoc, Nguyen Van Hao and Don Griffiths (2002). Status of Pangasiid aquaculture in Vietnam. *MRC' Technical Paper No.* **2**, Mekong River Commission, Phnom Penh. 16 pp. (ISSN: 1683-1489).

Tung N.T., Tuan, N., Tuan,T.T., and Hao, N.D. (2001). Development situation of two fish species of Pangasiidae cultured in the Mekong Delta of Vietnam. Assessment of Mekong Fisheries: Fish migration and spawning and the impact of water management project (AMFC), Mekong River Commission. Ho Chi Minh City, Vietnam.

Vidthayanon, C. 1993. Taxonomic revision of Catfish family Pangasiidae. *Thesis submitted to Tokyo University of Fisheries*, Laboratory of Aquatic Biology, Department of Aquatic Biosciences, Tokyo University of Fisheries, Tokyo.

Waples, R. (1991). Pacific salmon, *Oncorhinuchus spp.*, and the definition of 'species' under the endangered species act. *Marine Fisheries Review* **53**, 11-22.

Weir, B.S. and Cockerham, C.C. 1984. Estimating F-statistics for the analysis of population structure. *Evolution* **38**, 1358-1370.

Weiss, S., Schlotterer, C., Waidbacher, H., & Jungwirth, M. (2001). Haplotype (mtDNA) diversity of brown trout *Salmo trutta* in tributaries of the Austrian Danube: massive introgression of Atlantic basin fish - by man or nature? *Molecular Ecology* ***10,*** *1241-1246*.

Wilson, C. C. & Bernatchez, L. (1998). The ghost of hybrids past: Fixation of Arctic charr (*Salvelinus Alpinus)* mitochondrial DNA in an introgressed population of lake trout (S-Namaycush). *Molecular Ecology* **7,** 127-132.

Wright, S. (1968). Evolution and the Genetics of Populations. Vol. 1: Genetic and Biometric Foundations. University of Chicago Press, Chicago.