PRELIMINARY DATA ON GENETIC VARIATION IN THE GENUS *CLARIAS* AND *PANGASIUS* ON THE BASIS OF DNA MICROSATELLITE LOCI

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Abstract

Fourteen dinucleotide DNA microsatellite loci were screened in the catfishes *Clarias gariepinus*, *C. batrachus* and *Pangasius hypophthalmus*. Six loci showed allelic variation in *Clarias batrachus*, 6 in *C. gariepinus* and 3 in *P. hypophthalmus*. They will be used for the screening of wild and cultured populations.

INTRODUCTION

Catfishes (Siluriformes) constitute a major taxon among the bony fishes. They are especially widespread in the neotropics and constitute a major component of the highly diverse fauna of south-east Asia, including the Indonesian archipelago and the Mekong rivershed. They are of growing interest in aquaculture; currently more than 100,000 tons of clariids and pangasiids are produced in SE Asia. Part of this aquaculture is supported by the exotic species *Clarias gariepinus* and its hybrids (Na-Nakorn *et al.*, 1993). Despite their significance in biological evolution and aquaculture, the genetics of indigenous species has been studied fragmentarily. The review by Volckaert and Agnèse (1996) includes few references on this topic apart from Daud *et al.* (1989), Ismail *et al.* (1989) and Na-Nakorn *et al.* (1997). The Catfish Asia Project aims at filling in some of the missing information.

In this report we detail on an interspecific comparison of in total 14 DNA microsatellite dinucleotide primersets in 3 catfish species (*Clarias gariepinus*, *C. batrachus* and *Pangasius hypophthalmus*).

MATERIALS AND METHODS

Two strategies have been envisaged to obtain microsatellite DNA primers specific to SE Asian catfishes of the family Clariidae and Pangasiidae: (1) testing of DNA microsatellite primers developed for *Clarias gariepinus*, in the related species *C. batrachus* and *Pangasius hypophthalmus* and (2) development of completely new DNA dinucleotide microsatellite primers for *C. batrachus* and *P. hypophthalmus*.

The testing of a selected number of primers developed for *C. gariepinus* has been performed on 2% agarose gels with the microsatellite DNA primers and according to the PCR directions of Galbusera *et al.* (1996).

The second strategy, namely development of microsatellite markers specific to *Pangasius hypophthalmus* and *Clarias batrachus*, has been done according to a modified version of the biotin capture method of Kandpal *et al.* (1994). It includes the following steps:

Selection of oligonucleotides:

The following dinucleotide oligonucleotides (Eurogentech, Belgium) were ordered:

- CA repeat: $(CA)_{15}$
- *MboI* adaptor:

5'-ATCGCAGAATTCGCACGAGTACTACC
GTCTTAAGCGTGCTCATGATGC-5'

Construction of a standard gDNA library:

Genomic DNA from the Asian Catfishes, *Clarias batrachus* and *Pangasius hypophthalmus*, was completely digested with *MboI*. The digested DNA (5µg) was ligated to the *MboI* adapter (15µg) in a 300µl reaction volume with 5µl T4-ligase (BRL). After overnight incubation at 22°C, the free linkers were separated from the DNA-fragments on a 1.2% agarose gel. The ligated DNA was purified from the gel with the Jetsorb extraction kit (Imtech). The purified fragments were amplified in a PCR reaction with one of the *MboI* adapter oligonucleotides as a primer. The fragments were cloned into the TA-cloning vector from the TA-cloning kit from Invitrogen and transformed into *E. coli* cells.

Construction of an enriched gDNA library:

Because of the low yield of positive clones with the traditional protocol, we used a modified version of the biotin capture method of Kandpal *et al.*, 1994. The protocol is identical up to the first PCR reaction with the *MboI* primer. The enrichment procedure is as follows. The amplified DNA was denatured in a boiling water bath for 10 min and hybridised to the biotinylated CA repeat. The hybridisation was carried out overnight in a 100µl volume containing 2 to 3 µg amplified DNA, 1 µg repeat, 0.5% SDS and 0.5M sodium phosphate (pH 7.4) at 50°C. The hybridisation mixture was incubated with 0.15ml streptavidin coated magnetic beads (Promega) for 30 min at ambient temperature. The beads were precoated with 100µg/ml sonicated salmon sperm DNA. The supernatant was removed by centrifugation after incubation. The beads were washed 3 times with 1ml 1xTris buffer (100mM Tris pH 7.5, 150mM NaCl) at ambient temperature, 50°C and 65°C, once with 0.1xTris at 65°C and once with distilled water at 65°C. The eluents were concentrated with centricontubes (Amicon) to a volume of 100µl. An aliquot of the concentrate was used to start the same PCR reaction as above. These fragments were cloned into the TA-cloning vector from the TA-cloning kit from Invitrogen and transformed into *E. coli* cells.

Screening of the library:

Two strategies were used. First we hybridised the colonies directly with the CA repeat probe after a colony lift, although occasionally problems were encountered to locate the positive clones. Alternatively we isolated individual colonies and performed a PCR on each colony. We spotted 5µl of this reaction on a dot blot, which was hybridised with the CA repeat probe. From each positive clone, we prepared a miniprep to sequence the insert (Amersham).

RESULTS

***Testing of DNA microsatellite primersets of* Clarias gariepinus**

Five primersets (Cga04, Cga06, Cga09, Cga11 and Cga14) developed specifically for *Clarias gariepinus* were tested on a wild population of the same species (Lake Mweru, Zambia), *C. batrachus* (collected at the fish market of Can Tho) and *Pangasius hypophthalmus* (collected at the fish market of Can Tho and from Can Tho University aquaculture station). The "Cga" primersets amplified highly repetitive dinucleotide loci in *C. batrachus* (3 cases) and *P. hypophthalmus* (2 cases) (Table 1). Since research is in progress, several combinations still have to be verified.

***Testing of DNA dinucleotide microsatellite primersets of* Clarias batrachus**

Five primersets (Cba02, Cba04, Cba06, Cba09 and Cba10) developed specifically for *Clarias batrachus* were tested on a wild population of the same species (collected at the fish market of Can Tho), *C. gariepinus* (Lake Mweru, Zambia) and *Pangasius hypophthalmus* (collected at the fish market of Can Tho and from Can Tho University aquaculture station). The "Cba" primersets amplified highly repetitive dinucleotide DNA sequences in *C. batrachus* (2 cases;1 primerset is polymorphic), *C. gariepinus* (1 case) and *P. hypophthalmus* (in progress) (Table 2). Since research is in progress, several combinations still have to be checked.

	Cga04-KUL	Cga06-KUL	Cga09-KUL	Cga11-KUL	Cga14-KUL
C. gariepinus	124/128/132/134/	141/143/145/	186/190/192/	181/183/185	179/195/197/199/
(n = 10)	136/140/150	147/151	195/202/203		201/205/207/217
Lake Mweru	(7)	(5)	(6)	(3)	(8)
C. batrachus	negative	negative	182/184/186/188/	197/199/211/	178/180/184
(n = 10)			194/196/198/200/	205/215/223/	
Can Tho			202/204/210	243	
			(11)	(7)	(3)
P. hypophthalmus	negative	negative	178/182/192	183	negative
(n = 10)					
Can Tho			(3) (n = 5)	(1)	

Table 1: Interspecific comparison of *C. gariepinus* DNA microsatellite primersets on *C. batrachus* and *P. hypophthalmus*. The alleles observed as well as the number of alleles observed is given in brackets. I.P.: in progress

	Cba02-KUL	Cba04-KUL	Cba06-KUL	Cba09-KUL	Cba10-KUL
C. batrachus	I.P.	184	221/247/251	208/210	I.P.
(n = 10)			(n = 8)		
Can Tho		(1)	(3)	(2)	
C. gariepinus	I.P.	I.P.	249/251	I.P.	negative
(n = 10)			(n = 7)		
Lake Mweru			(2)		
P. hypophthalmus	negative	I.P.	I.P.	I.P.	negative
(n = 10)					
Can Tho					

Table 2: Interspecific comparison of *C. batrachus* DNA microsatellite primersets on *C. gariepinus* and *P. hypophthalmus*. The alleles observed as well as the number of alleles observed is given in brackets.

	Phy05-KUL	Phy07-KUL	Phy09-KUL	Phy12-KUL
P. hypophthalmus	202/204/206/	214/215/216/	positive	I.P.
(n = 10)	208/210	260/270 (?)		
Can Tho	(5)	(5)	(8)	
C. gariepinus	negative	negative	negative	negative
(n = 10)				
Lake Mweru				
C. batrachus	negative	306/308/336/338	188	negative
(n = 10)				
Can Tho		(4)	(1)	

Table 3: Interspecific comparison of *P. hypophthalmus* DNA microsatellite primersets on *C. gariepinus* and *C. batrachus*. The alleles observed as well as the number of alleles observed is given in brackets.

***Testing of DNA dinucleotide microsatellite primersets of* Pangasius hypophthalmus**

Four primersets (Phy05, Phy07, Phy09 and Phy12) developed specifically for *Pangasius hypophthalmus* were tested on a population of the same species (collected at the fish market of Can Tho and from the CTU aquaculture station), *C. gariepinus* (Lake Mweru, Zambia) and *C. batrachus* (collected at the fish market of Can Tho, Vietnam). The "Phy" primersets amplified

highly repetitive dinucleotide DNA sequences in *P. hypophthalmus* (3 cases), *C. gariepinus* (1 case) and *C. batrachus* (1 case; 1 monomorphic locus) (Table 3). Since research is in progress, several combinations still have to be checked.

DISCUSSION

The African catfish loci have been successfully tested on 2 Asian species. The markers developed specifically for the Asian species are currently being tested on a siluriforme "zoo-pannel". So far, six loci showed allelic variation in *Clarias batrachus*, 6 in *C. gariepinus* and 3 in *P. hypophthalmus*. It is still too early to make conclusions about the genetic structure of wild populations (to be studied particularly in *Clarias nieuhofii*) and cultured populations (*C. batrachus*, *C. gariepinus* and *Pangasius hypophthalmus*). Such information should become available in the near future.

In general our data confirm that the more evolutionary distant a taxon, the lower the chance of successful amplification and the lower the genetic variability observed by heterologous primer sets. The loci Cga09 and Cga11 primers show major allelic variation in *C. batrachus*, a species which belongs to the Clariidae but is suspected from molecular data not to fit in the *Clarias* genus (Arndt, pers. comm.).

In addition, tetranucleotide microsatellite loci are being developed by our laboratory. It has become clear that the lower allelic variation of tetranucleotide DNA repeats suits better our goals of parental and population genetic characterisation (Jarne & Lagoda, 1996).

ACKNOWLEDGEMENTS

We thank G. Teugels, M. Legendre and the members of the Catfish Asia Project for providing us with biopsies of catfishes.

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PRELIMINARY DATA ON SPECIES COMPOSITION AND DISTRIBUTION OF PANGASIID CATFISHES (SILURIFORMES, PANGASIIDAE) IN THE LOWER MEKONG RIVER BASIN

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Abstract

The composition and distribution of Pangasiidae in the lower Mekong River basin, southern Vietnam, was studied through four periods, from April 1997 to April 1998. The fish were mainly collected by trawl net, but some were also collected by long-line, hand-line, gill net along Tien River and Hau River.

The investigation showed the presence of 9 species of *Pangasius* and 1 species of *Helicophagus*, in which *Pangasius bocourti* (ca Ba Sa), *Pangasius conchophilus* (ca Hu), *Pangasius hypophthalmus* (ca Tra), *Pangasius larnaudii* (ca Vo dem), *Pangasius krempfi* (ca Bong lao) and *Pangasius* sp1 present a high commercial value. Almost all pangasiid species are distributed in fresh water-bodies, but *Pangasius krempfi*, and *Pangasius* sp1 (ca Tra nghe or ca Tra ban respectively) were found mainly in brackish-water. Pangasiidae are distributed mainly from Vinh Xuong to Hong Ngu in Tien River, but at the Vam Nao confluence, in Hau River, they were particularly abundant, where water current is rapid and water depth important. The size and weight distributions of collected species changed in every investigated period. From July 1997 to December 1997, young fish were predominant. On the other hand, *Pangasius macronema* was always found in abundance during all sampling campaigns. Many kinds of fishing gears have been used to catch Pangasiidae in the Mekong delta, such as trawl net, long-line, hand line, gill net, seine net and stow net. Some of them represent a threat for the fish. Increased control and management from local government is needed for sustainable fishery resource development and protection.

INTRODUCTION

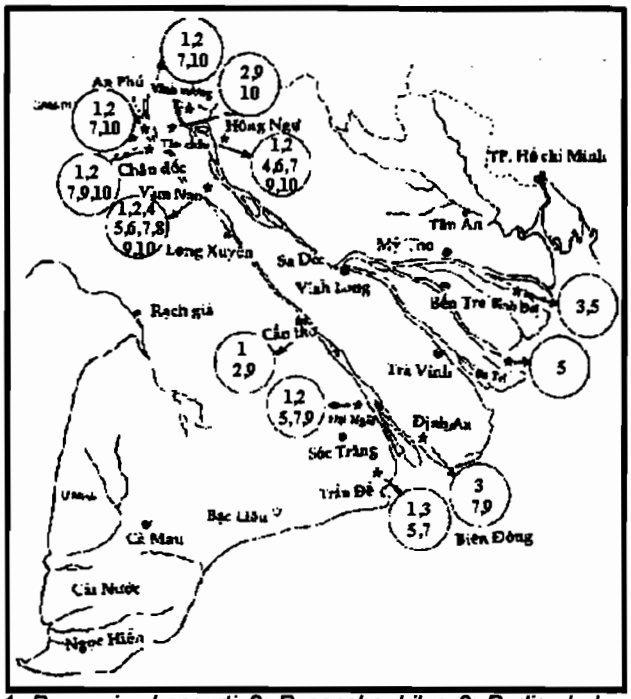
Pangasiidae is a family of fish, commonly known as catfish, belonging to Sub-Order Siluriformes, Order Siluriformes. Many species in this family are of high economic value and are important commodity for exportation. Most of the species distribute widely in the Mekong Basin. They are found in Thailand, Burma, Laos, Cambodia, Malaysia and Vietnam.

According to Tyson and Vidthayanon (1991), family *Pangasiidae* consists of two genera: *Pangasius* (Valenciennes, 1840) and *Helicophagus* (Bleeker, 1858). *Pangasius* consists of 19 species and *Helicophagus* has only two species. Most of the species in this family are freshwater fish, only two species (*Pangasius krempfi* and *Pangasius djambal*) are found in marine habitat and one species (*Pangasius polyuranodon)* is found in brackish-water habitat. Considering geographical distribution, eleven species have been found in Thailand, ten species in Indonesia and three in Malaysia. According to Lenormand (1996), eleven pangasiid species have been identified in the Lower Mekong Basin in Vietnam, among which ten belong to *Pangasius* and one to *Helicophagus*. Of the ten species in genus *Pangasius*, one species was found only in the floating fish-raising cages and could not be found in natural habitat.

This research, supported by the Catfish Asia project, is planned to be conducted from April 1997 to April 1999 to investigate the distribution patterns of catfish in the Lower Mekong Basin and to study the ecology of several species of high economic value. The results of this study will later contribute to the elaboration of plans for conservation of resource and promotion of sustainable catfish culture.

MATERIALS AND METHODS

The major sampling sites were chosen along the two main branches of the Mekong River (Tien and Hau Rivers) from the Cambodian border down to the river's mouths (Fig. 1).



1: *Pangasius bocourti*; 2: *P. conchophilus*, 3: *P. djambal*; 4: P. *hypophthalmus*, 5: *P. krempfi*; 6: *P. larnaudii*; 7: *P. macronema*; 8: *P. pleurotaenia*; 9: *P. polyuranodon*; 10: *Helicophagus waandersii*

Figure 1: Distribution map of Pangasiid catfishes in the Mekong basin (Vietnam).

Local fishing gears such as trawl net, gill net and some others were used for collecting fish samples. Samples were collected four times a year: two in the dry season and two in the wet season. Water temperature, pH, transparency, salinity, dissolved oxygen concentration (DO) and chemical oxygen demand (COD) were measured at each sampling area. Other environmental parameters - water depth, water current's velocity, bottom substrate and natural food source - were also noted. During the campaigns, local fishermen were interviewed on the distribution patterns of catfish, the harvest season and the harvest sizes of catfish. The fish samples were preserved in 10% formalin and transported to the laboratory of the Department of Environment and Natural Resources Management for examination and measurements.

RESULTS AND DISCUSSIONS

Environmental parameters

In the dry season: Table 1 shows that the water temperatures were high in the dry season, ranging between 29.8°C to 32.4°C. Salinity was low at the river's mouths (12-14 $g.L^{-1}$at Tien River's mouth; 4-9 $g.L^{-1}$ at Hau River's mouth). Values of pH were stable because they were less affected by water drained from inland fields. Water transparency was high due to low sediment content in the dry season. Water depths varied greatly between the sites, ranging from 5m to 30m. The sites with great water depths included Vinh Xuong, Tan Chau, Hong Ngu, Long Khanh, Vam Nao, Chau Doc. Many species of Pangasiidae were found at these sites, a large variety of fishing gears such as trawl net, gill net and fishing line, being used to catch them.

In the wet season: during the wet season the water temperature was lower than during the dry season, usually below 30°C (Table 2). Values of pH were low because they were affected by rainwater drained out from inland fields. Water transparency was low due to high sediment content in floodwater. Water salinity in the wet season was also low (1.0-2.1 $g.L^{-1}$ at Hau river's estuary; 1.9-2.3 $g.L^{-1}$ at Tien river's estuary) because the water was diluted by upstream water during rainy season.

Species composition of Pangasiidae at the sites (Table 3)

Among a total of 1400 specimens sampled, 10 species of *Pangasiidae* were found, nine of them belonging to the genus *Pangasius* and only one to the genus *Helicophagus*. At this stage, the specific identification of one of these species, *Pangasius* sp1, remains uncertain. This species, which is commonly found at the river's estuaries, was initially identified as *Pangasius djambal* by Lenormand (1996), but this identification could not be confirmed from both morphological observations and genetic analyses recently carried out (see Pouyaud *et al.*, 1999). In general, all the species found appeared to be common at the studied sites except *P. pleurotaenia* which was rarely observed in the natural habitat.

These results were in agreement with previous observations made by Lenormand (1996) in the same area. Three species (*P. gigas, P. micronema* and *P. sanitwongsei*), categorised as Indochina and Thailand species by Tyson and Vidthayanon (1991), were not found during our survey.

Distribution of Pangasiidae in the Lower Mekong River Basin, Vietnam

The results of the distribution of pangasiid catfishes in the main stream and estuarine areas of the Tien and Hau Rivers are summarised in Table 4. The Tables 5 and 6 indicate the pangasiid

Sites	Parameters						
	Temp. (°C)	pH	DO (ppm)	COD (ppm)	Salinity ($g.L^{-1}$)	Transparency (cm)	Depth ** (m)
Tien River							
Vinh Xuong	31.7	7.1	7.7	5.1	0	73	20-30
Tan Chau	31.1	6.8	8.4	5.3	0	97	25-30
Hong Ngu	29.8	6.8	-	-	0	-	10-20
Long Khanh	30.3	7.5	8.2	6.4	0	112	10-15
Ham Luong *	31.0	7.5	8.8	6.8	12	40	5-10
Cua Âai *	31.0	7.5	7.2	7.2	14	25	5-10
Hau River							
An Phu	32.1	6.8	7.5	4.6	0	96	< 10
Chau Doc	30.2	6.8	7.7	5.4	0	62	15-20
Vam Nao	32.5	7.1	8.8	4.8	0	32	20-30
Can Tho	31.2	7.4	7.8	6.5	0	78	10-15
Dai Ngai	32.3	6.9-7.2	7.4	4.7	0.1	12	10-15
Long Phu	30.8	6.8	7.0	4.5	2.5	-	10-15
Âinh An *	31.7	6.9	7.0	5.1	4.0	20	10-15
Tran De *	32.4	7.3-6.8	7.5	8.4	9.0	17	8-10

*: near river's mouths **: from interviews with local fishermen

Table 1: Measurements of aquatic environmental parameters in the dry season.

Sites	Parameters					
	Temperature (°C)	pH	DO (ppm)	COD (ppm)	Salinity ($g.L^{-1}$)	Transparency (cm)
Tien River						
Vinh Xuong	28.5	6.0	7.8	4.4	0	12
Tan Chau	28.5	6.5	8.9	5.1	0	14
Long Khanh	28.0	6.5	8.1	6.4	0	16
Ham Luong *	30.1	6.8	7.7	8.8	2.3	25
Cua Dai *	29.3	6.7	9.2	5.2	1.9	8
Hau River						
An Phu	28.5	6.5	7.7	3.8	0	14
Cháu Doc	28.0	6.5	8.3	4.2	0	14
Vam Nao	28.5	6.5	7.6	6.0	0	14
Can Tho	28.5	6.7	8.2	7.2	0	21
Âai Ngai	27.5	-	7.2	8.1	0	18
Âinh An *	27.5	6.8	8.0	7.6	1.0	16
Tran De *	28.0	6.8	9.4	8.0	2.1	8

*: near river's mouths

Table 2: Measurements of aquatic environmental parameters in the wet season.

	Species composition	Local name
1	*Pangasius bocourti* Sauvage, 1880	Ca Ba sa
2	*Pangasius conchophilus* Roberts and Vidthayanon, 1991	Ca Hu
3	*Pangasius hypophthalmus* Sauvage, 1878	Ca Tra nuoi
4	*Pangasius krempfi* Fang and Chaux, 1949	Ca Bong lao
5	*Pangasius larnaudii* Bocourt , 1866	Ca Vo dem
6	*Pangasius macronema* Bleeker,1851	Ca Xac soc
7	*Pangasius pleurotaenia* Sauvage, 1878	Ca Xac bau
8	*Pangasius polyuranodon* Bleeker, 1852	Ca Dua
9	*Pangasius* sp1 *(?)*	Ca Tra Nghe, Tra Ban
10	*Helicophagus waandersii* Bleeker, 1858	Ca Tra chuot

Table 3: Species composition of Pangasiidae samples collected on Tien and Hau River (from 04/97 to 05/98).

specific richness at the different localities studied and for different sampling periods on the Tien River and Hau River, respectively.

Results from Tien River's sites

On the Tien River, the sampling sites at which the highest of pangasiid species were observed were Long Khanh, Hong Ngu and Vam Nao (Table 5). The most common species observed were: *H. waandersii, P. conchophilus, P. macronema* and *P. polyuranodon*. The two species *Pangasius spl* and *P. krempfi* were found only in the estuarine brackish-water areas. However, local fishermen, during the interviews, stated that these species could be also found in the freshwater area of Vam Nao far upstream from the estuaries.

The number of species found at Vinh Xuong and Tan Chau was low. These areas had great water depths and large current's velocities. The types of local fishing gears used in the investigation may not have been appropriate for deep-water areas with high current's velocities. Particularly, the trawling speed may have been too slow to catch this kind of fish. Further investigation with improved methods and equipment need to be done in these deep-water areas to obtain more reliable data.

Results from Hau River's sites

The results from the investigation at the sites on the Hau River showed that the numbers of species were the highest at the following sites: An Phu, Chau Doc, Can Tho and Dai Ngai (Table 6). The number of species found in the vicinity of Chau Doc appeared to be the more stable over time. This may be due to the facts that there are many floating fish-raising cages in this area and that the water current was not very strong which may be suitable for many species. The species which are common in freshwater habitat, such as *Pangasius bocourti, Pangasius conchophilus, Pangasius macronema, Pangasius polyuranodon*, were found at similar frequencies through the sites from An Phu to Dai Ngai (near the river's estuary). The two species *Pangasius krempfi* and *Pangasius* spl also appeared with equal frequencies through the estuarine sites such as Dai Ngai, Bai Gia, Tran De and Dinh An.

No.	Species composition	Tien River		Hau River	
		River	Estuary	River	Estuary
1	*Pangasius bocourti*	+	-	+	+
2	*Pangasius conchophilus*	+	-	+	-
3	*Pangasius hypophthalmus*	+	-	-	-
4	*Pangasius krempfi*	-	+	+	+
5	*Pangasius larnaudii*	+	-	-	-
6	*Pangasius macronema*	+	-	+	+
7	*Pangasius pleurotaenia*	+	-	-	-
8	*Pangasius polyuranodon*	+	-	+	+
9	*Pangasius* spl	-	+	-	+
10	*Helicophagus waandersii*	+	-	-	-

Table 4: Distribution patterns of Pangasiidae species.

No	Sampling location	Surveying time			
		I (Avril.97)	II (July.97)	III (Dec.97)	IV (Avril.98)
1	Vinh Xuong	0	4	0	2
2	So Thuong	0	3	0	0
3	Tan Chau	0	3	0	3
4	Long Khanh	3	4	7	7
5	Hong Ngu	6	0	6	2
6	Vam Nao	3	4	7	4
7	Binh Dai (estuaries)	1	2	1	1
8	Ba Tri (estuaries)	0	0	1	0

Table 5: Variation of the number of pangasiid species at the different sampling sites over time on the Tien River (from April 1997 to April 1998).

Several authors have conducted surveys and published data on species composition and distribution patterns of pangasiid catfishes in the Mekong River. The compiled results are presented in Table 7.

Table 7 indicates that most of the species in *Pangasius* family distribute widely in the Indochina region. These species include: *bocourti, conchophilus, hypophthalmus, krempfi, macronema, polyuranodon* and *pleurotaenia.* From the present study, ten species have been found in the Mekong Delta so far, among which nine species belong to genus *Pangasius* and one species belongs to genus *Helicophagus*, while Lenormand (1996) found only 8 species in natural habitat (including *H. waandersii*) and two species in floating fish-raising cages (*P. micronema, P. sanitwongsei*). Species composition in Cambodia is richer than other areas in the region. However, It should be noted that the effective presence of some Pangasiid species in the Mekong River is still controversial (see below). The two species *P. gigas* and *P. sanitwongsei* are found only upstream from the Vietnamese part of the Mekong (Rainboth, 1996; Roberts & Vidthayanon, 1991). However, according to the interviews with local fishermen, these two species were previously present in Vietnam, at Long Khanh and Hong Ngu (on the Tien River). The *Pangasius gigas* fish that were caught by Vietnamese fishermen before could reach individual weight up to 250 Kg.

Rainboth (1996) stated that a specimen of *Pangasius pangasius* was collected at My Tho market in 1974. According to this author, this

No.	Sampling location	Surveying time			
		I (Avril 97)	II (July 97)	III (Dec. 97)	IV (April 98)
1	An Phu	0	4	0	1
2	Kinh Xang	2	3	0	0
3	Chau Doc	2	3	3	3
4	Can Tho	1	3	0	5
5	Cai Con	1	2	0	5
6	Dai Ngai	3	0	5	1
7	Bai Gia	3	1	0	1
8	Tran De (estuary)	0	1	1	0
9	Dinh An (estuary)	0	3	0	0

Table 6: Variation of the number of pangasiid species at the different sampling sites over time on the Hau River (from April 1997 to April 1998).

Species composition	Mekong Delta	Laos	Cambodia	Thailand
Pangasius bocourti	6 ; 8	3	1 ; 7	2
Pangasius conchophilus	6 ; 8	3 ; 4 ; 5	7	2
Pangasius djambal	-	-	1 ; 7	2
Pangasius gigas	-	3	1	-
Pangasius hypophthalmus	6 ; 8	2 ; 3	1	2
Pangasius krempfi	6 ; 8	3 ; 5	1 ; 7	2
Pangasius larnaudii	6 ; 8	-	1 ; 7	2
Pangasius macronema	6 ; 8	3 ; 5	1 ; 7	2
Pangasius micronema	6	-	1 ; 2 ; 7	2
Pangasius pangasius	-	-	1(?) ; 7(?)	-
Pangasius pleurotaenia	8	4 ; 5	7	2
Pangasius polyuranodon	2 ; 6 ; 8	3 ; 5	1 ; 7	2
Pangasius sanitwongsei	2 ; 6	3 ; 5	1 ; 7	2
Pangasius sp1	8	-	-	-
Helicophagus waandersii	6 ; 8	4 ; 5	1 ; 7	2

Sources: (1): Kottelat, 1985; (2): Roberts & Vidthayanon, 1991; (3): Roberts, 1993; (4): Warren, April 1994; (5): Roberts & Baird, 1995; (6): Lenormand, 1996; (7): Rainboth, 1996; (8): Thuong *et al.*,1997.

Table 7: Pangasiid species listed from the Mekong Delta and other neighbouring areas in the region.

species distribute from India to Vietnam in estuarine brackish-water habitat, while Roberts and Vidthayanon (1991) considered that this species is absent from the Mekong.

Pangasius micronema is not a common species in the Lower Mekong Basin. It distributes mainly from Indonesia to Thailand (Rainboth, 1996). In Vietnam, Lenormand (1996) found this species in a floating fish-raising cage but the fish were brought from Cambodia originally. There has been no clear information indicating the presence of this species in the Mekong Delta either.

The species *Pangasius spl* appears mostly limited to the estuarine habitat. This species, which was tentatively identified as *P. djambal* by Lenormand (1996), is reported for the first time in Vietnam from the Tien and Hau rivers' estuaries.

The species *Helicophagus waandersii* which distributes widely in Indochina region is also found in Vietnam.

Size variation of Pangasiidae fish collected in natural habitat in the Lower Mekong Basin:

Indication of the size structure of the pangasiids sampled in this study is summarised in Table 8.

- *Pangasius bocourti* only appeared at the inland rivers. Sizes varied greatly between sampling times. Small-size fish appeared mostly in the samples of sampling 2 and 3 from July to December 1997. This is the breeding season of most of the fish species. *P. bocourti* was more particularly abundant at Vam Nao and Tan Chau on the Tien River.
- *Pangasius conchophilus* only appeared in the freshwater part of the rivers. Small-size fish appeared mostly in fieldtrip 2 in July 1997. Fish size ranged from 3 cm to 4 cm at Can Tho. The largest fish were 21 cm collected in fieldtrip 4 in December 1997 at Vam Nao on the Tien River. The sites in which this species was found in abundance were Tan Chau, Long Khanh, Hong Ngu, Vam Nao on Tien River; and Chau Doc, Can Tho, Cai Con on Hau River.
- *Pangasius hypophthalmus* only appeared on Tien River on the third field trip (Dec/1997). The fish size ranges from 16.0-30.8cm. This species was not found to be abundant. One the reason may be that the trawling net was not an appropriate way to catch this species. The better way to collect sample of this species would be using fishing lines and gillnet.
- *Pangasius larnaudii* appeared only on Tien River in samples collected at Tan Chau, Hong Ngu and Vam Nao, ranging in size from 10 cm to 25 cm.
- *Pangasius macronema* was mostly represented in the samples at Long Khanh, Hong Ngu, and Vam Nao on the Tien River and also at the river's estuary in the wet season. The smallest fish sizes observed were 3 cm at Dai Ngai on the Hau River and 4 cm at Long Khanh in July. This species is a widely distributing one with small-sized individuals that can be caught easily by trawl net..
- *Pangasius pleurotaenia* seems to be rare in the Mekong Delta. During the investigation, only one individual, 19-cm in size, was caught at Vam Nao on Tien River in December 1997.
- *Pangasius polyuranodon* appeared mainly in freshwater areas. In the wet season, they were also found in the river's estuaries. The sites where this fish was the most abundant in the samples were Tan Chau, Long Khanh, Hong Ngu, Vam Nao on the Tien River; and Chau Doc, Cai Con and Dai Ngai on the Hau River. Most of the fish collected were small (5 cm at Tan Chau in July and at Dinh An (Hau River's estuary) in December 1997). Like *P. macronema*, this species represented an important part of fish collected in the samples.
- *Pangasius krempfi* distributed mostly at the rivers' estuaries. It was found only once at Vam Nao on Tien River in December 1997. The average size was 64 cm raging from 32 cm to 77 cm on Hau River and from 42 cm to 58 cm on the Tien River.
- *Pangasius* spl was collected at the river's estuaries using gill net and trawl net. One fish of 72 cm in size was also collected at Vam Nao using trawl net. The size of the specimens collected at the river's estuaries varied greatly ranging from 5 cm in July to 34 cm in October and to 12 cm in December (caught by trawl net at Hau River's estuary). The specimens collected at Tien River's estuaries were larger in size, ranging from 31 to 72 cm.
- *Helicophagus waandersii* was generally small in size. Fish size remained stable over the sites; from 10 cm to 23 cm on the Hau River and 11 cm to 14 cm on the Tien River. Most of the specimens were collected at Vinh Xuong, Tan Chau, Long Khanh and Hong Ngu on the Tien River, and Chau Doc on the Hau River. None was found at the river's estuaries.

Species	Field trip	Tien River		Hau River	
		River	Estuaries	River	Estuaries
Pangasius bocourti (*Ca Ba Sa*)	1	15.3-32.5	-	-	-
	2	7.8-13.3	-	6.6 -14.5	-
	3	7.4-19.9	-	6.0-16.2	8.7-16.0
	4	27.2-28.5	-	-	-
Pangasius conchophilus (*Ca Hu*)	1	9.8-29.6	-	-	-
	2	2.7-12.8	-	3.3-13.5	-
	3	2.6-21.8	-	2.5-23.7	-
	4	16.8-25.3	-	4.5-7.6	-
Pangasius hypophthalmus (*Ca Tra nuoi*)	1	-	-	-	-
	2	-	-	-	-
	3	16.0-30.8	-	-	-
	4	-	-	-	-
Pangasius larnaudii (*Ca Vo dem*)	1	22.1-26.0	-	-	-
	2	-	-	-	-
	3	17.9-27.3	-	-	-
	4	-	-	-	-
Pangasius macronema (*Ca Xac soc*)	1	1.8-14.5	-	3.1-7.3	-
	2	3.0-21.4	-	3.0-4.6	-
	3	7.4-18.9	-	2.0-11.5	-
	4	6.7-18.3	-	5.4-9.4	-
Pangasius pleurotaenia (*Ca Xac bau*)	1	-	-	-	-
	2	-	-	-	-
	3	19.0	-	-	-
	4	-	-	-	-
Pangasius polyuranodon (*Ca Dua*)	1	6.0-19.0	-	3.2-14.3	-
	2	3.9-13.8	-	4.2-8.2	5.5
	3	8.2-21.0	-	5.1-15.9	8.6
	4	8.8-24.8	-	6.7-11.2	-
Pangasius krempfi (*Ca Bong lao*)	1	-	-	25.2-37.0	-
	2	-	30.5-56.0	-	-
	3	64.0	17.5-72.0	49.5	28.2-49.5
	4	-	55.5-61.0	76.5	-
Pangasius sp1 (*Ca Tra ban, Tra nghe*)	1	-	27.6-33.2	-	-
	2	-	50.0	-	4.4-5.6
	3	72.0	-	-	7.2-20.5
	4	-	-	-	-
Helicophagus waandersii (*Ca Tra Chuot*)	1	6.7-15.4	-	-	
	2	7.2-25.7	-	8.0-16.2	
	3	13.8-26.7	-	-	
	4	10.0-11.7	-	12.9-16.0	

Table 8: Size range of *Pangasiidae* fish collected in natural water bodies in the Lower Mekong basin, Vietnam (Total length: cm).

Current situation of exploitation and proposed solution for sustainable fishery of Pangasiidae in the lower Mekong river of Vietnam.

Fishery of *Pangasiidae* in the lower Mekong River basin had been established for a long time. Based on characteristics of fishing ground and habitat and biology of Pangasiidae several styles of fishing gear have been developed such as encircling net, seine net, trawl net, gill net, hand line, long line, stow net and scrub traps. These are the main fishing gears contributing to the total fishery production of pangasiids. However, resource reservation could be hampered by the improper use of these fishing gears.

Hand line and long line are popular gears. Hand

lines are used seasonally (mainly from June to November) to exploit small sized fish in species including *P. bocourti, P. conchophilus* and *P. macronema*. It is suitable for water depth of 10-20 m, with an average daily production of 10-20 fish. Long lines are used widely along the Tien and Hau Rivers, particularly at Vam Nao (from January to April) and in the estuaries. The long line gears have a total length of 500-1000 m (or 400-500 hooks). At Vam Nao, they was used mainly for exploiting *P. polyuranodon, P. bocourti* and *P. macronema* weighing from 50-200 g/fish, or up to 1-4 kg/fish in some cases. In Vam Nao site, the highest catch were observed in October-November for *P. conchophilus* and *P. polyuranodon* and February-March for *P. krempfi*, which could be up to 15-20 kg/day. In the estuaries, Long lines are mainly used to catch *Pangasius* spl at the confluence or along the riversides where there are important quantities of "Ban trees" (*Sonneriatia casceolaria*) living.

Gill nets are used all along the Tien and Hau Rivers. They are made of nylon with a mesh size of 2a=140 mm, length of 500-700 m and width of 8-10 m. They are set either floating with water current or fixing across the river at about 1-3 m above the river bottom. Gill net mainly caught large size fish of 3-5 kg such as *P. krempfi, P. bocourti* and *P. larnaudii*. Average catch of gill net varied from 6 to 9 kg/day, the main catching season for Pangasiids being from January to April at Vam nao. However, at the estuaries, it could be operated all year-round for catching *P. krempfi* mainly. Fishing-ground for *P. krempfi* is from seashore up to 10 km far offshore, where the water depth is about 3-10 m. During the main fishing season, from February to July, catches of one net unit reach about 50-600kg/day.

Stow net for *P. hypophthalmus* larvae collection were seasonally operated. They were used during the period from June to August (or May to July lunar year), and operated mainly in the area from Vinh Xuong, Thuong thoi Hau to Phu Thaun in the rivers. However, in the estuarine areas, stow net (Day hang khoi) mainly caught big size *P.* sp 1 in March-May with a yield of about 200kg/day and small *P.* sp 1 in October-November with 200-300 kg/day.

Trawl net consisted of two kinds: beam trawl net and bottom otter trawl net. The design of beam trawl net is 5-7 m wide; 0.4-0.7 m high; 10-20 m long and 2a=8-16 mm mesh size and is operated by a boat equipped with a 10-20 HP engine. The bottom otter trawl net are of 10-12 m wide; 1-1.5 m high; 15-20 m long, mesh size at cod-end of 2a=8-20 mm and boat engine of 20-30 HP. The trawl net operated all year round in estuaries, while some trawl net could operate in the main rivers (e.g. in Long Xuyen, Chau Doc and Dong Thap) excepting during the flood season due to strong water current. The best fishing season of trawl net was from April-July mostly for small size *P. conchophilus* and *P. polyuranodon* of 4-6 cm body length and from September-January for larger sized fish of 0.2-1 kg/fish. However, *P. macronema* could be caught all year long with the highest production reaching 30-100 kg/day.

Pull cash net were used in any location along Tien and Hau Rivers. Diameter of pull cash net mouth is 20-30 m. This was operated all around the year and could caught all kind of pangasiids.

There are also many other fishing gears (encircling net, seine net, scrub trap ...) operated in the surveyed areas to catch pangasiid catfishes along main and secondary river systems.

Some recommendations for sustainable fishery and protection of Pangasiidae

For sustainable fishery of Pangasiidae, efforts should be made to (i) limit over-fishing; (ii) forbid and control strictly the use of electrical fishing gears (such as electrical beam trawl net, electrical shock fishing gear); (iii) prevent the use of fishing gears with very small mesh size of barrier net and of cod-end of trawl net ("mesh size" should be >20 mm); and (iv) prohibit the use of stow net along the rivers, except for fry collection of *P. hypophthalmus* at Thuong Phuoc and Thuong Thoi Hau from June to August

CONCLUSIONS

The results obtained through the 4 surveys of the Pangasiidae resources realised on the Tien and Hau Rivers, indicate that :

- 10 species of Pangasiidae were found, they include 9 species belonging to *Pangasius* genus and one species belonging to *Helicophagus* genus. Almost all of them are large sized species which are suitable for culture and fishing in the waterbeds of Mekong delta.
- Three species, *P. gigas, P. sanitwongsei* and *P. micronema*, previously reported in this area, were not found in the Mekong basin during our surveys and sampling.

- Most of catfish species which belong to Pangasiidae live in freshwater and are widely distributed. Two species, *Pangasius krempfi* (Ca bong lao) and *Pangasius* spl (Ca tra nghe), were mainly found in estuaries. However, these two latter species were also widely distributed in freshwater in the area of Vam Nao (Tien River).
- Most Pangasiidae were found at Long Khanh, Hong Ngu, and Vam Nao areas. Further studies should be done on the influence of hydrographic conditions on the distribution of pangasiids.
- Due to recent over-fishing of Pangasiidae and the threat that it represents for some of these species, there is a need that the local government and fishery resource protection agencies increase their control and management on fishing gears along to rivers effectively.

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Proceedings of the mid-term workshop of the
"Catfish Asia Project"
Cantho, Vietnam, 11-15 May 1998

Edited by :
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