Transboundary Coservation of the Mekong Giant Catfish

in Chiang Rai (Thailand) and Bokeo (Lao PDR) Provinces ,

Mekong River

Final report of
Analysis of fish larvae to determine spawning sites of the Mekong Giant Catfish at Chiang Rai Province, Thailand
year 2009

By
Siriwan Suksri, Apiradee Hanpongkittikul, Nakorn Pila, Khathawut Panboon, Sophit Chaiyao and Jutamas Jivaluk

Inland Fisheries Resources Research and Development Institute,
Department of Fisheries Thailand

Facilitate and support by WWF Thailand

Analysis of fish larvae to determine spawning sites of the Mekong Giant Catfish at Chiang Rai Province, Thailand

Siriwan Suksri, Apiradee Hanpongkittikul, Nakorn Pila, Khathawut Panboon, Sophit Chaiyao and Jutamas Jivaluk

Inland Fisheries Resources Research and Development Institute,
Department of Fisheries Thailand

Background

The Mekong Giant Catfish, *Pangasianodon gigas* which is one of the largest freshwater fish species in the world belong to the family Pangasiidae of order Siluriformes. It is endemic to the Mekong River Basin. The current population of wild stocks of Mekong Giant Catfish has greatly declined in number due to unsustainable levels of fishing pressure since the early 1980's. The traditional fishery targeting the Mekong giant catfish in the stretch of Mekong River between Chiang Khong district of Chiang Rai Province, Thailand, and Houayxai district of Bokeo province, Lao PDR, were mostly catching mature giant catfish in reproductive condition. Therefore, it has been assumed that this area of the Mekong River includes important spawning habitat for the species which begins to spawn during the period of low water level from mid April to early June. There were reports on small size of giant catfish caught in Mun River in Ubon Rachathani, with the weight of about 30 kg and there was no report on finding the small size of giant catfish in Mekong River (Pholprasit and Tevaratmaneekul, 1997). While fishermen and scientists recognize that this area of the Mekong between Chiang Rai and Bokeo provinces is important for spawning, there are no positive identifications of spawning habitat, and very little research has been done to investigate the spawning behaviors and location of the wild stocks of Mekong Giant Catfish in the Mekong River. Most of the reports on Mekong giant catfish ecology to date have focused on aquaculture. Knowledge on the ecology and migration behavior in the Mekong is very limited. To protect the species and improve the management of the wild population it is essential to improve the understanding of the spawning habitats in Mekong River. This report is a summary of collaboration between WWF and the Department of Fisheries (DOF), Thailand, to conduct research into the spawning habits and location in the upper Mekong River between Lao PDR and Thailand.

Objectives

The objectives of this research plan were twofold;

1. To determine the location of the Mekong giant catfish spawning habitats in the stretch of Mekong River between Chiang Rai province of Thailand and Bokeo province, Lao PDR during 1-19 May 2009

2. To provide additional samples of Mekong Giant Catfish DNA for long-term storage and future reference by researchers from the Fish DNA Bank and Genomic Laboratory of the Inland Fisheries Resources Research and Development Institute, Department of Fisheries, Thailand.

Methodology

A. Research Planning

It planned to study the Mekong giant catfish's spawning habitats to collect its eggs and larvae samples by using the plankton net in 3 surveying areas in the Mekong River on Chiang Kong district of Chiang Rai province during 1-19 May 2009. (see Figure 1).

- 1st sampling station was at Meuang Kran village, Rim Kong sub-district, Chiang Kong district.
- 2nd sampling station was at Pak Ing village, Sri Don Chai sub-district, Chiang Kong district.
- 3rd sampling station was at Had Khai village, Vieng sub-district, Chiang Kong district.

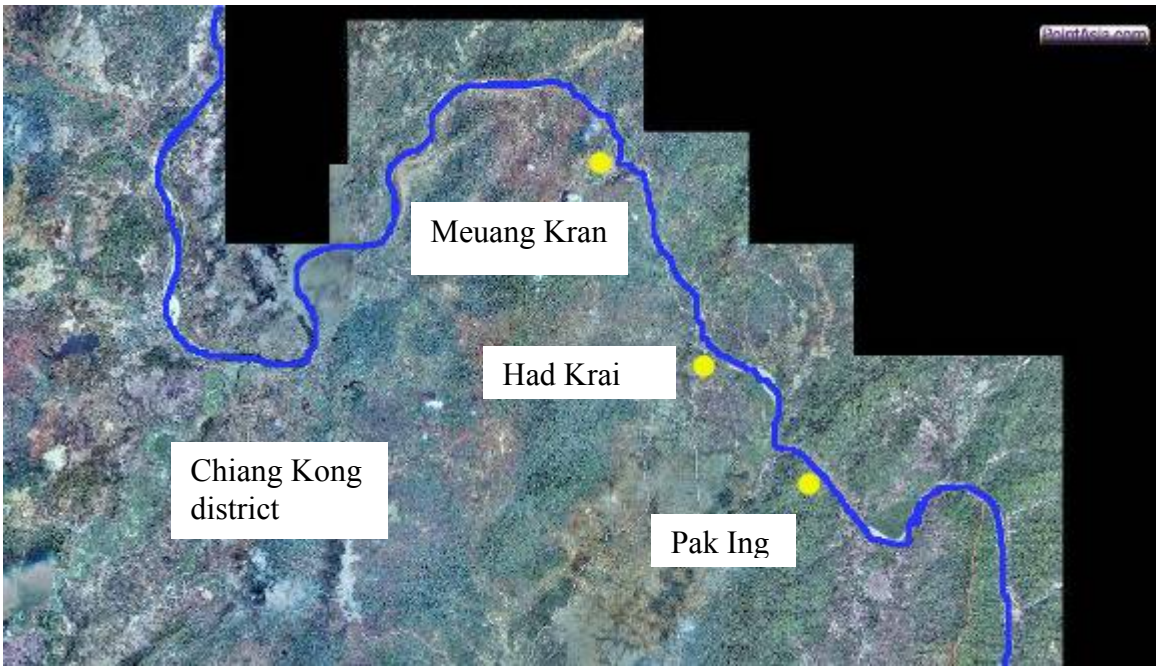

Figure 1: Map shown the sample collecting stations of Mekong giant catfish's eggs and larvae in Chiang Kong district, Chiang Rai province. Meuang Kran (2253755N: 47641657E), Had Krai (2239182N: 47649520E), Pak Ing (2234461N: 651700E)

B. Sample Collection

The plankton net, the triangular shape opening of the size 1.2 m x 1.2 m x 1.2 m with a 6-mm mesh size and 4.8 m length, was used to randomize for the eggs and larvae in those three stations at two different water depths (water surface and mid water). Two haul were operated at each stations with sampling time was about 20 minutes. Sampling was made 3 times per day i.e. 8.00 am at Meuang Kran village, 5 pm at Pak Ing village and 9 pm at Had Krai village. Then, the samples were sorted out and kept in 99.5% alcohol. Later on, those eggs and larvae samples were sent to the laboratory for species identification and DNA analysis at Inland Fisheries Resources Research and Development Institute, Department of Fisheries, Thailand.

C. Identification of Fish Larvae

1. The samples were identified by using their morphological characteristics based on fish larvae key of Aphichat (2003, 2005), Neira (1998) and Pholprasit and Tevaratmaneegul (1997); and fish key of Rainboth (1996) and Kottelet (2001) and Order classification by using Nelson's system (2006).

2. Bring those data obtained from fish larvae species and numbers counted to analyze for frequency of occurrence in percentage.

3. Bring those 19 larvae samples, as classified into Family Pangasiidae by using their morphological characteristics, to classify into species by using Micro Satellite DNA genetic marker analysis technique by performing DNA extraction from its fins and tissues by using the "eyeball extraction" technique (Jones and Hutchings, 2001). The extracted DNA was diluted until obtaining 25 ng/μL before analyzing for Micro Satellite DNA variation in different positions. The primers used were PSP G509, PSP G427, and PG9, which were primers of fish in Family Pangasidae. DNA was multiplied by using Polymerase Chain Reaction (PCR) and DNA size classification was done by using electrophoresis through polyacrylamide gel at the electrical current at 50 volts for 3 hours and read the results by comparing to the DNA standard marker (10bp DNA ladder; Invitrogen). After gel staining with fluorescent reflection light (Cyber Gold®), read the sizes of allyl through gel reader (FluorChem 8000, Alpha Innotech Corp.), then compare these alleles with those Mekong giant catfish's alleles stocked at the DNA bank of aquatic animals in the Fishery Department.

Results

1. Fish Larvae Identification using morphological characteristics

There were 180 eggs and 137 individuals of fish larvae belong to 6 orders obtained from the surface and mid-water haul. The most abundant order was Siluriformes which found 83 individuals (59.7%). The most abundant fish larvae were Family Pangasiidae (51 individuals, 36.7%), Family Sundasalangidae (22 individuals, 15.83%) and Family Siluriidae (17 individuals, 12.23%) (Table 1). In this study some fish larvae could identified into order or family only due to the unclear morphological characteriestis.

Table 1 Kinds and abundance of fish eggs and larvae using bongo net in Mekong River during 1-19 May 2009

Order	Family	Species	individual
Osteoglossiformes	Notopteridae	*Notopterus notopterus*	2
Cypriniformes	Cyprinidae	Unknown Cypriniid	13
		Esomus metallicus	2

		Rasbora sp.	1
	Cobitidae	*Pangio oblonga*	2
Siluriformes	-	Unknown Siluriform	2
	Bagridae	*Mystus* sp.	2
	Pangasiidae	Unknown Pangasiid	51
	Schilbeidae	Unknown Schilbeid	11
	Siluridae	Unknown Siluriid	15
		Micronema sp.	1
		Wallago attu	1
Osmeriformes	Sundasalangidae	*Sundasalanx mekongensis*	22
		Unknown	
Synbranchiformes	Mastacembelidae	Mastacembeliid	1
Perciformes	-	Unknown Perciform	2
	Ambassidae	*Parambassis wolffi*	1
	Gobiidae	Unknown Gobiid	8
		eggs	180

The result of the surface and mid-water haul showed that most of the fish larvae was found at the mid-water haul which the most abundant of fish larvae were Family Pangasiidae (51 ind.), Family Sundasalangidae (22 ind.) and Family Siluriidae (17 ind.). While at the surface haul found only Family Gobiiidae (2 ind.) (Table 2 and 3).

Table 2 Kinds and abundance (individuals) of fish larvae at 3 stations and 2 depths using bongo net in Mekong River during 1-19 May 2009

Kinds		Mid-water			Surface		
		Meuang Kran	Pak Ing	Had Krai	Meuang Kran	Pak Ing	Had Krai
Osteoglossiformes							
Notopteridae	*Notopterus notopterus*	-	1	1	-	-	-
Cypriniformes							
Cyprinidae	Unknown Cypriniid	5	-	8	-	-	-
	Esomus metallicus	1	-	1	-	-	-
	Rasbora sp.	1	-	-	-	-	-
Cobitidae	*Pangio oblonga*	1	-	1	-	-	-
Siluriformes	Unknown Siluriform	3	-	-	-	-	-
Bagridae	*Mystus* sp.	-	2	-	-	-	-
Pangasiidae	Unknown Pangasiid	10	35	6	-	-	-
Schilbeidae	Unknown Schilbeid	-	11	-	-	-	-
Siluridae	Unknown Siluriid	-	2	13	-	-	-

	Micronema sp.	-	1	-	-	-	-
	Wallago attu	-	1	-	-	-	-
Osmeriformes							
Sundasalangidae	*Sundasalanx mekongensis*	3	16	3	-	-	-
Synbranchiformes							
Mastacembelidae	Unknown Mastacembeliid	-	-	1	-	-	-
Perciformes							
	Unknown Perciform	-	-	-	-	-	1
Ambassidae	*Parambassis wolffi*	-	1	-	-	-	-
Gobiidae	Unknown Gobiid	-	4	2	-	2	-
	Total	24	74	36	-	2	1
	Fish eggs	33	28	112	4	-	3

Table 3 Kinds and abundance (persentage) of fish larvae at 3 stations and 2 depths using bongo net in Mekong River during 1-19 May 2009

Kinds		Mid-water			Surface		
		Meuang Kran	Pak Ing	Had Krai	Meuang Kran	Pak Ing	Had Krai
Osteoglossiformes							
Notopteridae	*Notopterus notopterus*	-	0.73	0.73	-	-	-
Cypriniformes							
Cyprinidae	Unknown Cypriniid	3.65	-	5.84	-	-	-
	Esomus metallicus	0.73	-	0.73	-	-	-
	Rasbora sp.	0.73	-	-	-	-	-
Cobitidae	*Pangio oblonga*	0.73	-	0.73	-	-	-
Siluriformes	Unknown Siluriformes	2.19	-	-	-	-	-
Bagridae	*Mystus* sp.	-	1.46	-	-	-	-
Pangasiidae	Unknown Pangasiid	7.30	25.55	4.38	-	-	-
Schilbeidae	Unknown Schilbeid	-	8.03	-	-	-	-
Siluridae	Unknown Siluriid	-	1.46	9.49	-	-	-
	Micronema sp.	-	0.73	-	-	-	-
	Wallago attu	-	0.73	-	-	-	-
Osmeriformes							
Sundasalangidae	*Sundasalanx mekongensis*	2.19	11.68	2.19	-	-	-

Synbranchiformes							
Mastacembelidae	Unknown Mastacembeliid	-	-	0.73	-	-	-
Perciformes							
	Unknown Perciform	-	-	0.73	-	-	0.73
Ambassidae	*Parambassis wolffi*	-	0.73	-	-	-	-
Gobiidae	-	-	2.92	1.46	-	1.46	-

There was difference between the species compositions of fish larvae in the sampling time and stations. At 5 pm at Pak Ing village could collected highest individuals of fish larvae (78 ind., 56.93%) followed by 9 pm at Had Krai village (37 ind., 27.11%) and 8 am at Meuang Kran village. Anyway, we found family Pangasiidae was the most abundant at these 3 periods (Table 4).

Table 4 Kinds and abundance (ind. and persentage) of fish larvae at 3 stations using bongo net in Mekong River during 1-19 May 2009

	8-9 am		**5-6 pm**		**9-10 pm**		**Total**	
	Meuang Kran		**Pak Ing**		**Had Krai**			
Order	**ind.**	**percentage**	**ind.**	**percentage**	**ind.**	**percentage**	**ind.**	**percentage**
Osteoglossiformes	-	-	1	0.73	1	0.73	2	1.46
Cypriniformes	8	5.84	-	-	10	7.30	18	13.14
Siluriformes	13	9.49	52	37.96	19	13.87	84	61.31
Osmeriformes	3	2.19	16	11.68	3	2.19	22	16.06
Synbranchiformes	-	-	-	-	1	0.73	1	0.73
Perciformes	-	-	8	5.84	2	1.46	10	7.30
total	**24**	**17.52**	**77**	**56.20**	**36**	**26.28**	**137**	**100.00**
eggs	37		28		115		180	

If consider only family Pangasiidae, the result showed that at Pak Ing village found the most abundant (35 ind.) followed by Meuang Kran village (10 ind.) and Had Krai (6 ind.) (Table 5).

Table 5 Abundance and frequency of occurrence fish larvae of Family Pangasiidae using bongo net in Mekong River during 1-19 May 2009

Stations	Time	Depth	Individual	Percentage	Percentage of frequency of Occurence
Meuang Kran	8-9 am	Mid-water	10	19.61	9.80
Pak Ing	5-6 pm	Mid-water	35	68.63	21.57
Had Krai	9-10 pm	Mid-water	6	11.76	7.84

For the analysis of 180 fish eggs, only 57 eggs could observe their development, the rest could not due to the turbidity of egg shell. The results showed 8 Bastula, 15 Tail-free and 20 Late Embryo.

In this study, we could identify fish larvae belong to family Pangasiidae but could not go to genus or species due to unclear pattern and site of chromatophore which important to identify species of family Pangasiidae. Anyway, 17 Prolarvae stage with age 6-12 hours and 34 Larval stage with age 1-3 days were found.

2. DNA Analysis

Analysis of 19 fingerling samples were conducted by only one microsatellite marker at PSP-G 509 position due to amount of low quality larval DNA. The results shown that allele sizes of unknown fingerings were 190-200 bp in range with non specific allele but their allele forms were differ from *Pangasianodon gigas* and *P. hypophthalmus* which had not non specific allele. These unknown fingerings are expected to be *Pangasius macronema* due to there allele sizes and allele forms are similar. (Figure 1)

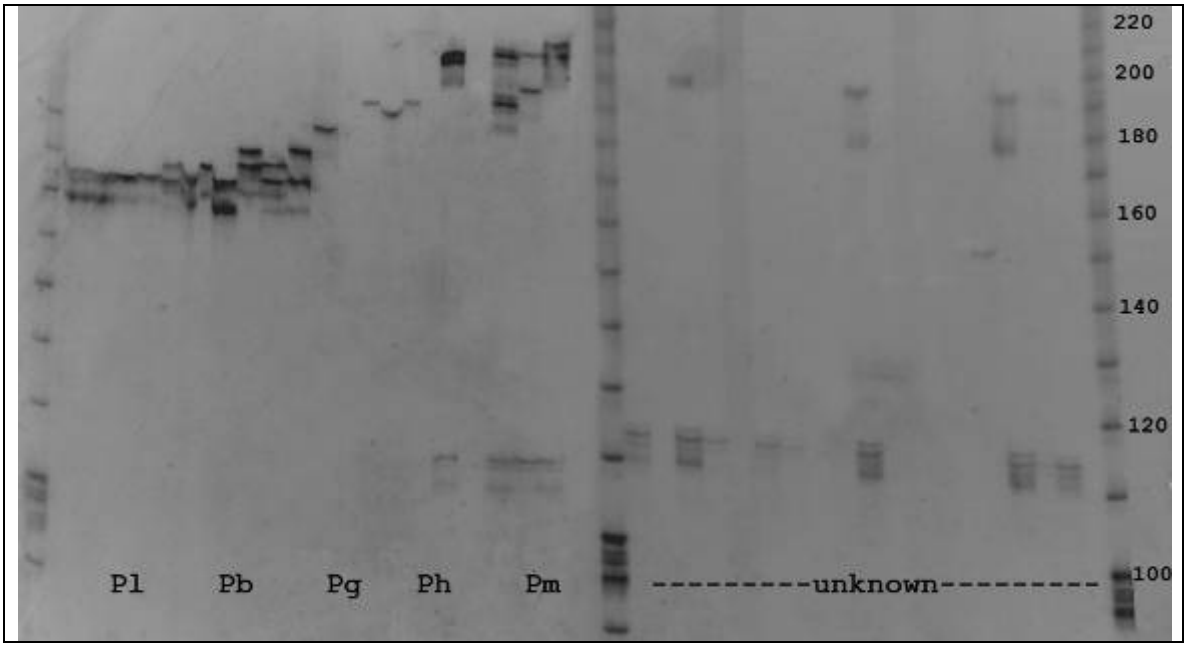

Figure 1 Forms and sizes of alleles in Microsatellite at PSP G509 position from 19 fingerlings samples analysis (Pb: *Pangasius bocourti*, Pl: *Pangasius larnardii*, Pg: *Pangasianodon gigas*, Ph: *Pangasianodon hypophthalmus*, Pm: *Pangasius macronema*)

Conclusion

1. This study found 180 eggs and 137 fish larvae from 6 Orders. The most abundant order was Siluriformes which found 59.7%.
2. This study, by using morphological characteristics, did not find any Mekong giant catfish larvae – which was the main objective of this study – but 51 larvae of Family Pangasiidae were found.
3. From sample analysis by using Micro Satellite DNA genetic marker analysis technique, those 19 samples were not Mekong giant catfish's larvae.

Recommendations

1. The results showed that most of the larvae of family Pangasiidae were found at Pak Ing village therefore the next year study should consider this station.
2. The most abundant fish larvae were collected at 5 - 6 pm similarly of the result in 2008. Thus more replicates at this period should be considered.
3. Trawl should be add for next year sampling because we could found fish larvae of family Pangasiidae at Larval stage in year 2008 and 2009. If we choose appropriate site for trawling, may be we can collect more larval stage of Pangasiidae.

References

Aphichat Teumvitchakorn. (2003). Freshwater fingerlings. Freshwater Fishery Resource Development and Research Institute, Fishery Department. 130 pp.

Aphichat Teumvitchakorn. (2005). Freshwater fingerlings of Thailand II. Freshwater Fishery Resource Development and Research Institute, Fishery Department. 125 pp

Hogan, Z. S. and B. P. May. 2002. Twenty-seven new microsatellites for the migratory Asian catfish family Pangasiidae. *Molecular Ecology Notes* 2: 38-41.

Jones, M.W., and J.A. Hutchings. 2001. The influence of male parr body size and mate competition on fertilization success and effective population size in Atlantic salmon. *Heredity* 86: 675-684.

Kottelat, M. 2001. Fishes of Laos. WHT Publ. (Pte) Ltd., Colombo, Sri Lanka. 198 pp.

Neira, F.J., A. G. Miskiewicz and T. Trnski. 1998. Laboratory Guide foe Larvae Fish Identification. University of Western Australian Press, Australia. 474 pp.

Nelson, J. S. 2006. Fishes of the World. 4 th ed., John Wiley & Sons, Inc., New Jersey. 601 pp.

Pholprasit, S. and Tevaratmaneegul, P. 1997. Biology and Culture of the Mekong Giant Catfish *Pangasianodon gigas* (Chevey, 1931). Thai Fisheries Gazette. 50 (6): 441-457.

Rainboth, W. J. 1996. Fishes of the Cambodian Mekong. FA0 Species IdentificationField Guide for Fishery Purposes. Rome. 265 pp.