THE "CATFISH ASIA" PROJECT: BACKGROUNDS, AIMS AND PROSPECTS

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Abstract

Catfishes, and in particular Clariidae and Pangasiidae, are important aquatic resources in Asia, where their culture represented an annual production of about 124,000 t in 1993. In the clariids, this production results mostly from the use of F1 hybrids between the introduced African catfish (*Clarias gariepinus*) and various local clariid species. In the pangasiids, various culturing techniques were empirically developed for some native species, whose juveniles are most often captured in the wild. Further development of this catfish culture industry faces serious problems related to the poorly known systematic of these fish groups, the scarce knowledge of the biology and aquaculture potential of autochthonous species, the limitation of seed supply and the declining performances reported in some cultivated stocks.

In this context, the "Catfish Asia" project which deals with the two catfish families, Clariidae and Pangasiidae, has the main following goals:

- To acquire a stronger knowledge of the biological diversity of SE Asian catfishes and to enhance its
 utilisation through a correct identification and characterisation of valuable species, populations and strains
 of aquaculture interest. This approach represents an important precondition to the sustainable
 management of cultivated and natural stocks and to guide conservation efforts of these economically
 important resources. It will also contribute to a better knowledge of their phylogeographic relationships.
- To acquire sound biological bases for the development of catfish culture in the SE Asian region. The evaluation of the aquaculture potential of the autochthonous species (diversification) and the optimisation of their rearing cycle (artificial propagation) through technologies adapted to the local conditions are essential elements for a better production in the future.

The research work associates six institutes and laboratories from Indonesia, Vietnam, France and Belgium. The specific objectives, general methodologies, first results and prospects of the project are presented.

INTRODUCTION

Among the freshwater fish, the Siluriformes (including both autochthonous and exotic species) represent an important group in Asia. Several species are actively exploited by fisheries and in a variety of aquaculture production systems. Although ranking beyond carps and tilapias, the total volume of cultured catfishes in Asia has shown fast increase during the last 20 years and was estimated around 124,000 t in 1993 (Csavas, 1994). In the Lower Mekong Basin, catfishes of the clariid and pangasiid families are of particular significance for aquaculture. In 1993, they represented an estimated annual production of about 21,000 t in Vietnam, 36,000 t in Thailand and 6,000 t (75 % of the total freshwater cultivated fish production) in Cambodia. In Indonesia, clariids are the main cultivated catfishes (4,000 t in 1992) but pangasiids present also a high potential for aquaculture, particularly in Sumatra and Kalimantan (Sudarto and Sumastri, 1994).

Indigenous culture techniques were developed for native species that are generally preferred by local consumers. However, in clariids, the actual trend is to cultivate F1 hybrids between the introduced African catfish (*Clarias gariepinus*) and various local species (*C. macrocephalus* or *C. fuscus* in Thailand or Vietnam, *C. meladerma* in Indonesia). These hybrids appear to combine the estimated flesh quality of local species and the faster growth rate and disease resistance of the introduced one. Because of the presence of a variety of pangasiid species and their omnivorous nature, culture techniques can be adapted to the local conditions. Pangasiids are used both in smallscale or industrial production systems and can be reared in high-density cage culture, low input polyculture systems, integrated livestock/fish farming or with human waste utilisation (Peignen, 1993; Cacot, 1994; Csavas, 1994). The ability of some of these catfishes to undergo aerial respiration allows their use for a valorisation of poorly oxygenated aquatic environments.

However, major constraints for further development and sustainable management of cultivated and natural catfish resources still remain. A part of the encountered problems is listed below, as they were identified in 1996.

- Aquaculture has often been based on the utilisation of introduced species while the knowledge on the biology and the potential of autochthonous species remains scarce. As an example, the African catfish *Clarias gariepinus* has been spread all over SE Asia where it is cultured either as such or after hybridisation with local *Clarias* species. In Indonesia, although more than 10 pangasiid species were listed from the ichthyofauna, the only *Pangasius* cultured in this country remained *Pangasius hypophthalmus*, which was initially introduced from Thailand.
- Diversification of the cultivated species is required both for a better response of fish culturists to market demands, and for a better fit with the diversity of habitats and consumer preference. However, main limitations are the followings.
 - > The systematic of Siluriformes remains poorly known in this region and information genetic structure on their (species, populations) is very limited. In the pangasiid family, despite a systematic revision of the group (Roberts & Vidthayanon, 1991), numerous discrepancies were found in recent descriptions of the fish fauna. This was particularly the case for the Mekong delta where the available information relative to taxonomy and even the number of represented species was still inconsistent (Khoa & Huong, 1993; Lenormand, 1996). In Indonesia, the only local pangasiid tested for aquaculture was species misidentified as P. pangasius and remained to be correctly named. For SE Asian clariids, the situation was even more confusing as the most recent revision was made by David in

1935. In the absence of reliable identification keys, cultured species are often misidentified. This situation impairs a comprehensive view of the culture potential of these fishes and a correct interpretation of the information published on their biology and culture.

- In most cases seed supply is impaired by the absence of reproductive control in captivity and by fluctuating or limited natural wild juvenile resources (Csavas, 1994; Cacot, 1994).
- Declining performances in cultured fishes have been reported in several areas in SE Asia (Main and Reynolds, 1993).
- Introductions of exotic species for pure culture or hybridisation with native species could induce diseases due to parasites (Welcomme, 1988; Kottelat, 1990) and genetic impacts on native gene pools (Hindar *et al.*, 1991).

Therefore, the precise description and characterisation of species, populations and strains in these fish groups represent a condition *sine qua non* to the sustainable management of their cultivated and natural stocks and to guide conservation efforts of these economically important resources. They should also contribute to a better knowledge of their phylogeographic relationships.

The sound evaluation of the aquaculture potential of the autochthonous species (diversification) and the optimisation of their rearing cycle (particularly artificial propagation) through technologies adapted to the local conditions appears as essential elements for a better production in the future.

These topics were retained has the main goals of the "Catfish Asia" project, which focuses on the two main catfish families of economic importance, the Clariidae and Pangasiidae. The genesis, specific objectives, general methodologies, first results and expected outcomes of the project are presented in the present paper.

GENESIS OF THE PROJECT AND PARTNERSHIP

The first contact between the European and Asian partners today associated in the "Catfish Asia" project took place in 1992 during a prospective mission of two of us in the Southeast Asian region (Lazard & Legendre, 1993). This first contact allowed the identification of research fields related to fish biology and culture, and partner institutions to develop collaborative programmes. The cooperation was initially engaged by exchange of scientists and students between France, on one side, and Vietnam and Indonesia, on the other side.

In Vietnam, inquiries were made on catfish production systems (Peignen, 1993; Bazir, 1994; Cacot, 1994) and a preliminary study on the systematic, biology and aquaculture potential of pangasiid species from the Mekong Delta was carried out (Lenormand, 1996). Starting from 1994, the French Ministry of Foreign Affairs supported a collaborative programme on the control of reproduction of Pangasius bocourti, associating two French (CIRAD and IRD) and three Vietnamese institutions (the Can Tho University (CTU), the University of Agronomy and Forestry (UAF) and the AGIFISH Company). This programme led to the very first spawn of this species in captivity, obtained in May 1995. It represented an important success as, until this date, the millions of P. bocourti juveniles necessary to sustain the 15,000 tonnes of annual aquaculture production of this species in the Mekong delta were entirely dependent on captures from the wild.

The study of populations genetic of SE Asian *Clarias* species was also started in 1995 in a cooperation between IRD, the Central Research Institute for Fisheries (CRIFI-RIFF) based in Jakarta, Indonesia, and the University Montpellier II.

These different activities and their results provided a solid basis and motivated the preparation of a more ambitious collaborative research programme on the biodiversity and aquaculture of catfishes in SE Asia. Since November 1996, this programme, abbreviated as "Catfish Asia" ¹, is coordinated by IRD and supported by the European Commission. It associates 6 research institutions, from France (IRD and CIRAD), Belgium (Musée Royal de l'Afrique Centrale and Katholieke Universiteit Leuven), Indonesia (CRIFI-RIFF) and Vietnam (CTU) (Fig. 1).

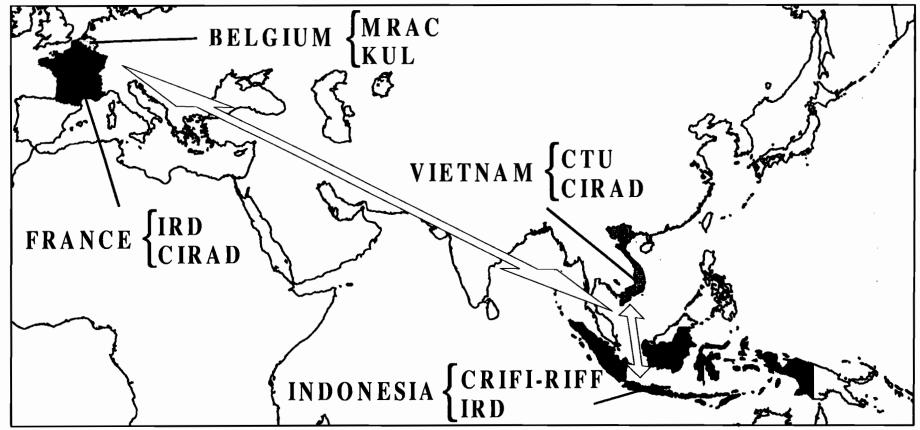
A part of the research is also conducted in close cooperation with the AGIFISH Company in Vietnam and the services of the Directorate General for Fisheries in Indonesia, allowing real possibilities for a rapid and efficient transfer of results from research to the production sector and fish farmers.

OBJECTIVES OF THE "CATFISH ASIA" PROJECT

In order to enhance the utilisation of the biological diversity of the local freshwater ichthyofauna, acquire sound biological bases for the development of aquaculture, provide an appraisal of the present situation in order to guide sustainable management of cultivated and natural fish resources, and strengthen North-South-South cooperation between the European Union, Indonesia and Vietnam by the transfer and exchange of technology, the project aims at the following specific objectives:

- To characterise species, populations and strains of autochthonous Clariidae and Pangasiidae catfishes for:
 - A thorough knowledge of their taxonomy and appraisal of their phylogeny and zoogeography.
 - A general inventory of available resources that could be used for culture.
- To contribute to the knowledge of their life history.
- To implement monitoring tools that could be used for the analysis of population microstructuration and monitoring of genetic diversity in cultivated fish stocks (i.e. development of DNA microsatellite loci).
- To assess and compare the aquaculture potential of species, populations and hybrids in the Pangasiidae and Clariidae.
- To develop artificial propagation and culture techniques adapted to local conditions for some target species for which captive broodstock can be available:
 - Identification of the environmental requirements to attain full sexual maturity under rearing conditions and optimisation of induced breeding and artificial fertilisation procedures.
 - Assessment of some nutritional, behavioural and environmental requirements of larval and juveniles stages and optimisation of larval rearing methods.

¹ Full title of the project : Characterisation, utilisation and maintenance of biological diversity for the diversification and sustainability of catfish culture in South-East Asia.



IRD : Institut de Recherche pour le Developpement (Coordinating Institute)

CIRAD : Centre de Coopération Internationale en Recherche Agronomique pour le Developpement

- **RIFF** : Research Institute for Freshwater Fisheries
- **CTU : Can Tho University**
- MRAC : Musée Royal de l'Afrique Centrale
- KUL : Katholieke Universiteit Leuven

The research work is divided into two major parts:

The first part aims at the identification and characterisation of species and populations of actual or potential interest for aquaculture. Three complementary approaches are used: 1) morphometric analysis, 2) estimation of genetic variation, and 3) characterisation of gill parasite communities.

- The morphometric analysis consists in a number of measurements, meristic counts and special morphological observations taken on representative samples of species or populations. This part of the work should result in correct and detailed descriptions of the different species, populations or hybrids.
- The estimation of genetic variation includes different techniques adapted to the research goals: protein electrophoresis, mitochondrial DNA analysis and microsatellite DNA analysis.
 - Protein electrophoresis is the most suited to examine evolutionary relationships within a great number of species belonging to the same or different genera. It gives rapidly accurate data in systematic investigations. However, in closely related taxa, differences in variation in allelic frequencies are often indicative but not sufficient to assign a single fish to a particular stock.
 - The restriction endonuclease analysis of mitochondrial DNA (mt-DNA) is a more accurate approach for population analysis at the intraspecific level. As the differences in mt-DNA base sequences (generated by mutation) are transmitted maternally without recombination, the mt-DNA analysis provides a strong support for stock identification and zoogeography.
 - The microsatellites DNA analysis allows much greater stock discrimination and is used as a complementary approach to screen wild populations for overall genetic variation.

The investigation of intraspecific differentiation may allow the identification of differentiated populations of potential interest for future use in aquaculture. In addition, the development of DNA microsatellite loci provides highly discriminating tools which could be used subsequently for the characterisation and monitoring of genetic diversity in cultivated fish stocks.

• The characterisation of gill parasite communities (Monogenea) completes the

genetic and morphometric analyses and provide complementary elements for the study of host's phylogeography. Monogenea have, toward their hosts, a specificity which is generally oioxenous (one species of parasite is present on only one host species). When the specificity is larger (stenoxenous) it is either due to lateral transfer or to genetic relatedness of the hosts. Parasites are identified by morphological studies (optic microscopy) of their sclerotized parts (genital and haptoral apparatus).

For these studies, representative fish samples from wild or culture origin are collected at the regional scale. The morphology, genetics and parasitology of this material are studied concurrently. During the sampling campaigns, observations are also made on the biological traits (reproductive strategies, feeding habits...) of the collected species.

The second part of the research is oriented toward a diversification of the cultivated species, the identification of the best performing ones (including hybrids) and an optimisation of the culture practices (particularly artificial propagation).

- The global and comparative evaluation of the aquaculture potential of pangasiid species is carried out following a two-stage procedure (Legendre, 1992; Lenormand, 1996):
 - On the most objective biological and economical criteria, a preliminary screening, the preselection stage, aim to retain those species that, among the entire group, present the greatest potential for a given type of aquaculture.
 - Then, in the <u>selection</u> stage, an evaluation of the aquaculture performances of the preselected species is made in culture trials.

The choice of an aquaculture candidate depends directly on biological characteristics of the species and on the environment, as well as on the economic and cultural context of the areas or countries concerned (e.g. pangasiids are more appreciated in Sumatra and Kalimantan than in Java). The preselection is based on information obtained from field sampling, market studies and literature analysis. In this view, the maximal size of species represents a useful biological criterion, both in regards to specific market demand for fish size and as a rapid estimator of growth rate (Legendre & Albaret, 1991). The culture trials are carried out in experimental aquaculture stations from juveniles caught in the wild. Three main characteristics are studied: survival, growth and sexual maturation. The fish are generally tagged for individual identification.

In the clariids, the research work is mostly oriented toward a comparative evaluation of zootechnical performances of different hybrids and of their parental species. The following criteria are generally considered: survival, growth, age/size at first sexual maturity, gonadal development, viability of the gametes and possibilities of obtaining viable F2 or back cross fry. Despite an increasing use of Clarias hybrids in SE Asian aquaculture, there is a lack of reliable and detailed data on their biological traits and zootechnical performances in comparison to parental species. preliminary investigations Similarly, on hybridisation have been started in the pangasiids.

The establishment of reliable artificial propagation techniques is sought in pangasiids actually identified for their interest in aquaculture (particularly *P. djambal* in Indonesia, *P. bocourti* in Vietnam and *P. hypophthalmus* in both countries). This task includes three complementary research actions.

- The *identification of the environmental requirements to attain full sexual maturation* under rearing conditions is done through investigations on the gonadal development of tagged brooders kept in earthen ponds and/or floating cages. The sexual maturity of the females is regularly followed using especially the oocyte diameter and the position of their germinal vesicles determined after intraovarian biopsy. In males, spermiation, volume of collected semen and motility of spermatozoa are used as the main maturity criteria.
- The optimisation of the induced breeding procedures involves the following steps: a) defining selection criteria for receptive brooders, b) establishing efficient hormonal treatments to induce oocyte maturation and ovulation, c) determining the optimal latency period between injection and stripping of ova and, d) proposing appropriate standardised techniques of artificial fertilisation and egg incubation.
- The optimisation of larval rearing methods requires precise knowledge on larval biology and the development of specifically adapted larval rearing systems. Adequate preys, feeding

requirements, behavioural particularities (e.g. occurrence or not of cannibalism) and appropriate weaning time are characteristics particularly considered.

Finally for some *Pangasius* species already used in aquaculture, two supplementary topics were added to the initial objectives of the project: the evaluation of the nutritional requirements of juveniles and a survey of the main pathological problems encountered in various culture systems.

FIRST RESULTS AND PROSPECTS

In 1998, at its mid-term, the Catfish Asia project has already led to many significant results, in terms of both basic and applied research. These results are presented into details all along the contributions compiled in this volume. Some of the main aspects and their implications in terms of further research and development are commented hereafter.

- Representative fish samples from wild or culture origin have been collected from the following areas: Java, Sumatra, Kalimantan, the Lower Mekong Basin (particularly Mekong Delta) and the Chao Phraya River Basin. These campaigns helped to precise the zoogeography of several taxa. Supplementary sampling campaigns remain to be done from other areas in order to complete the collection of species and improve the assessment of their phylogeography.
- In the pangasiids, 18 of the 21 nominal species described by Roberts and Vidthayanon (1991) were already collected and genetically analysed. Considering the observed genetic distances, the results suggest that several groups previously recognised as possible subgenus of Pangasius should be elevated at the genus level. Two possible new species, Pangasius spl and sp2 were also identified. As no specimens of P. pangasius could be collected yet, the exact status of Pangasius sp1 remains unclear. Up to now, it was however misidentified as P. diambal. A detailed morphometric analysis of all specimens collected remains to be done in this family. The fact that Pangasius sp1 was collected mostly in the estuarine part of the rivers (in the Mekong Delta, Sumatra and Kalimantan) confer to this fish a special interest, as it might represent a candidate for brackish water aquaculture both in Vietnam and

Indonesia.

In the clariids, the morphometric analyses and genetic investigations carried out (not all presented in this volume) confirm the needs for a systematic revision of this group, as far as the Asian species are concerned.

The establishment of reliable identification keys in these two families should be a major objective of the second half of the project.

- The parasitic analysis of pangasiid species showed the occurrence of more than fifty species of gill monogenea, of which 3 or 4 only were already described. An important work of description of the newly discovered monogenean species is currently carried out. As about 80% of these monogenea are host specific, the analysis of monogenean parasite communities at the host species or populations levels will represent a useful complement to the morphometric and genetic studies for the inference of hosts phylogeny.
- Several functional new microsatellite markers have been developed in both *C. batrachus* and *P. hypophthalmus.* Some markers previously developed for *C. gariepinus* were also operational in these two species. In a short term, these markers will be used for the molecular identification of wild caught *Clarias* species in Sumatra and the study of their smallscale genetic structure. Samples of cultured *C. gariepinus* from various Asian localities will be also analysed to identify their level of inbreeding.
- Among the 21 recognised species of Pangasiids (now probably 23), only 2 of them (P. bocourti and P. hypophthalmus) are actually cultured on a large scale in SE Asia. Investigations were also performed on P. gigas in Thailand, initially for a conservation purpose. Assuming the validity of the two new species discovered during the project, 13 pangasiids are present in Indochina (Mekong Basin) and 13 in the Indo-Malay Archipelago (5 endemic to the Borneo Island); 5 species are common to both geographical areas. Due to their relatively small size, 5 species do not seem to present much interest for culture in comparison to the others pangasiids: P. macronema, P. micronema, P. pleurotaenia, polyuranodon **P**. and Helicophagus waandersii. Among the species reaching larger sizes, captive stocks of 5 of them have been constituted during the first half

of the project in order to evaluate their

aquaculture potential: *P. conchophilus, Pangasius* sp1 and *P. larnaudii* in Vietnam, and *P. nasutus* and *P. djambal* in Indonesia. The latter species already showed a much faster growth rate than *P. hypophthalmus*. Besides *Pangasius* sp1, another species, *P. krempfi*, found in the estuaries of the Mekong Delta, may present a particular interest for brackish water culture and should be investigated.

- An important part of the work has been oriented towards an optimisation of induced breeding and larval rearing procedures of species already reproduced in captivity (P. bocourti and P. hypophthalmus). Significant progresses were obtained for both species and reliable methodologies were established, taking into account the seasonal variations of reproduction, the hormonal treatments applied and the viability of gametes and embryos. In P. hypophthalmus, the possibility of an all year round production of fry was demonstrated, and the survival and growth of larvae in controlled environment were strongly improved by using appropriate prophylactic treatments and feeding strategies. The transfer of these optimised artificial propagation methods to fish farmers already started in Vietnam and in Indonesia.
- Besides these two species, the reproduction in captivity of *P. conchophilus* and *Pangasius* sp1 in Vietnam and of *P. djambal* in Indonesia was also obtained for the first time from wild specimens acclimatised to culture conditions. Although artificial propagation techniques still needs to be specifically adapted for those fishes, these first successes now open strong opportunities for the development of their use in aquaculture.
- The availability of juveniles in some species also permitted to start investigations on their feeding strategies and nutritional requirements. The clear tendency of *P. bocourti* juveniles to start fat deposition even when fed at a low feeding rate and the apparent inverse relationship between fat deposition and gonad maturation in adult females of this species are aspects requesting further studies.
- Hybridisation is a manipulation permitted by the control of reproduction in captivity. Several crosses were tested in the clariids and the reciprocal hybrids between *P. bocourti* and *P. hypophthalmus* also proved to be viable in Vietnam. However, although hybrids may present several valuable qualities for

aquaculture and are always fascinating to produce, evaluation of their performances should be reserved to research station with closed facilities. Uncontrolled hvbrid production trials on fish farms have been made both in Vietnam and Indonesia between various pangasiid species. The risk that individuals could escape from fish farms is high and may have serious impacts on the native gene pools. Therefore the production of hybrids in aquaculture should be considered only after a full evaluation of their performances in comparison to those of their parental species and an assessment of their possible fertility.

The possibility or not of making hybrids between two species is also a good indication of their genome compatibility. The fact that the hybridisation between C. gariepinus and C. batrachus was successful in Bangladesh and neither in Indonesia nor in Vietnam, suggests that the nominal species C. batrachus corresponds to a species complex. It should be noticed that high genetic divergences were also observed between the low and highlands C. batrachus populations of Sumatra. Therefore the actual status of this important species for aquaculture clearly requests further investigations.

The final workshop of the "Catfish Asia" project is planned in May 2000 and will be organised in Bogor, Indonesia. A full synthesis of the results obtained during the 4 years of this collaborative programme will be presented at this occasion.

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THE BIOLOGICAL DIVERSITY AND AQUACULTURE OF CLARIID AND PANGASIID CATFISHES IN SOUTH-EAST ASIA



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