EVALUATION OF HYBRIDISATION IN FIVE *CLARIAS* SPECIES (SILURIFORMES, CLARIIDAE) OF AFRICAN (*C. GARIEPINUS*) AND ASIAN ORIGIN (*C. BATRACHUS*, *C. MELADERMA*, *C. NIEUHOFII* AND *C. TEIJSMANNI*)

Sophie Lenormand [(1)], Jacques Slembrouck [(1)], Laurent Pouyaud [(1)], Jojo Subagja [(2)] and Marc Legendre [(1)]

(1) IRD (ex ORSTOM), Catfish Asia Project, Instalasi Penelitian Perikanan Air Tawar, Jalan. Ragunan-Pasar Minggu, P.O. Box 7220/jkspm, Jakarta 12540, Indonesia
and *GAMET, B.P. 5095, 34033 Montpellier Cedex 1, France*
(2) RIFF, Jalan Raya 2, Sukamandi Subang 41256, Java Barat, Indonesia

Abstract

Ten crosses between five *Clarias* species of African (*C. gariepinus*) and Asian origin (*C. batrachus*, *C. meladerma*, *C. nieuhofii* and *C. teijsmanni*) were evaluated on the basis of the fertilisation and hatching rates obtained. Zootechnical performances (growth, survival) of *C. batrachus* and *C. gariepinus* larvae were compared. Viable hybrids growth and survival were followed up until the age of 11 weeks. The genotype of the reciprocal crosses between *C. gariepinus* and *C. meladerma* was studied and the gonad development of two-years-old *C. meladerma* x *C. gariepinus* hybrids was examined (*inter-specific crosses are always given with the female parent in the first position*).

No hybrid between *C. gariepinus* and *C. batrachus* could be obtained. *Clarias gariepinus* x *C. nieuhofii* and *C. gariepinus* x *C. teijsmanni* hybrids did not survive more than a few hours after hatching and larvae obtained from *C. gariepinus* x *C. meladerma* cross were non viable beyond a few days after hatching. In the latter case, protein electrophoresis of progeny carried out at two loci diagnostic for *C. gariepinus* and *C. meladerma* (PGI and PGM) indicated that the larvae resulted from an haploid gynogenetic development. The crosses between female *C. meladerma* and male *C. gariepinus* or *C. nieuhofii* or *C. teijsmanni* produced viable hybrids. *Clarias gariepinus* and *C. batrachus* zootechnical performances comparison during larval rearing indicated that the growth potential of these two species is equivalent until the age of 12 days, though *C. gariepinus* is favoured by its larvae bigger initial size. The comparison of *C. gariepinus* and *C. meladerma* x *C. gariepinus* growth and survival until the age of 11 weeks showed good performances in the hybrid compared to the Asian species but slightly lower than the ones observed in *C. gariepinus*. The gonads observation made in *C. meladerma* x *C. gariepinus* pointed out the complete sterility of the hybrids that were sampled. On account of the low growth rates observed in *C. meladerma* and *C. meladerma* x *C. nieuhoffi* or *C. teijsmanni*, those fish seem to present only a limited interest for aquaculture.

INTRODUCTION

Species belonging to the *Clarias* genus are freshwater catfishes characterised by their ability to utilise atmospheric air and walk on land for several hundred meters using their pectoral spines (Teugels, 1996). *Clarias* species are present from Africa to south-east Asia, where they are frequently exploited by fishermen and produced in farms. Essential source of proteins from animal origin, they have gained a major economic importance (Legendre, 1992).

In Indonesia, five species from the *Clarias* genus are generally recognised (Kottelat *et al.*, 1993). The yellow and flabby flesh of *C. batrachus* is highly appreciated by consumers and this species was the first to be used for aquaculture on the archipelago. Its farming, based mainly on natural reproduction in captivity, is most often carried out in ponds or small tanks, as a side-line. At the beginning of the 90's, Indonesian researchers succeeded in reproducing a second local species, *C. meladerma*, in captivity (Sumastri *et al.*, 1994). That latter species potential for aquaculture has not been evaluated yet. However, generally speaking, Asian *Clarias* species seems to present a lower resistance to pathogens and a lower growth rate than the African *Clarias*,

C. gariepinus, whose potential for aquaculture is clearly established. The robust, omnivorous and fast growing African *Clarias* was introduced into Indonesia in 1985, via Taiwan (Sudarto & Sumastri, 1994). Its performances in aquaculture led to a rapid development of its farming but this species, whose flesh is tougher and whiter than *C. batrachus* one, remains less appreciated by the local population and is sold half-price on markets. Looking for a fish which could satisfy producers and consumers at the same time leads to think about the opportunities given by inter-specific hybridisation between the different *Clarias* species consumed in Indonesia.

Hybridisation can be attractive for aquaculture in many ways (Chevassus, 1983). First, it may produce sterile animals, avoiding growth loss or fragility related to sexual maturation. Hybrids sterility reduces potential interactions between domestic and wild fishes. Secondly, hybridisation may lead to one-sex population production which might be an advantage in case of a differential growth between males and females or in species whose proliferation has to be avoided (e.g. in tilapias). Finally, even if hybrids growth and survival are rarely higher than the ones of both of their parental species, they might present the growth rate of the faster growing parental species and one (or several) characteristic(s) sought-after in the other parent (robustness, salinity tolerance, morphology, flesh quality...).

It is in Thailand, at the end of the 70's that a first cross between *C. gariepinus* and an Asian *Clarias* species, *C. macrocephalus*, enabled farmers to associate the zootechnical performances of the African *Clarias* to the flesh quality of the species the most appreciated by the local population, *C. macrocephalus* (Csavas, 1994; Lazard, 1994). The hybrid *C. macrocephalus* x *C. gariepinus*[1], whose performances in culture and flesh quality are halfway between its parental species ones may have largely contributed to catfishes production expansion in that country, gone from 5,000 t in 1976 to 39,500 t in 1992, 26,500 t of which from *Clarias* spp. (Csavas, 1994). At the same period, *C. macrocephalus* was crossed with *C. batrachus* (Boonbrahm *et al.*, 1977) and in the 80's, the production of the hybrid *C. fuscus* x *C. gariepinus* developed in Northern Vietnam (Csavas, 1994). Three crosses between genera, involving *C. gariepinus* or *C. batrachus* have also been achieved: *C. gariepinus* x *Heterobranchus longifilis* (Hetch & Lublinkhof, 1985; Legendre *et al.*, 1992), *C. gariepinus* x *H. bidorsalis* (Salami *et al.*, 1993) and *C. batrachus* x *Heteropneustes fossilis* (Mukhopadathy & Dehadrai, 1987). Finally, Tarnchalanukit *et al.* (1986) crossed *C. batrachus* and *C. macrocephalus* with *Pangasius hypophthalmus*.

In such a context, a viable hybridisation between *C. batrachus* and *C. gariepinus* could have been expected and might have been fruitful for catfishes production in Indonesia. However, while crossing these two species was reported to be successful in Bangladesh (Ahmed & Sarder, 1994; Rahman *et al.*, 1995), hybrids between the African *Clarias* and an Indonesian stock of *C. batrachus* could not be obtained (Richter *et al.*, 1995). At the same period, Indonesian researchers studied hybridisation opportunities between *C. meladerma* and *C. gariepinus*. The first hybrids that were obtained between female *C. meladerma* and male *C. gariepinus* seem to present a growth potential higher than that of the local *Clarias* species. However, this latter hybridisation remains to be described accurately in terms of hatching results, zootechnical performances, biological characteristics and consumers appreciation of its products.

This study focused on an African *Clarias* species, *C. gariepinus*, and four Indonesian *Clarias* species, *C. batrachus*, *C. meladerma*, *C. nieuhofii* and *C. teijsmanni*. The feasibility of different crosses between these species was tested. Zootechnical performances (growth, survival) of *C. batrachus* and *C. gariepinus* larvae were compared. The growth and survival of the hybrids which could be obtained were followed up until the age of 11 weeks. The genotype of the reciprocal crosses between *C. gariepinus* and *C. meladerma* was studied and the gonad development of two-years-old *C. meladerma* x *C. gariepinus* hybrids was examined.

MATERIAL AND METHODS

Four hybridisation experiments were carried out between March and July 1997 at the Sukamandi RIFF research station, West Java, Indonesia. In that region at low altitude, climate is characterised by high temperatures, high atmospheric humidity and two main seasons: a dry season, from May to

[1] Inter-specific crosses are always given with the female parent in the first position.

September, and a wet season, from October to April.

Broodstock management

Clarias gariepinus broodstock used in this study had been bought from Sukamandi region farms. *Clarias batrachus* one also came from reproduction in captivity and descended from a wild Western Java stock. Part of the *C. meladerma* broodstock had been produced at Depok station of the Research Institute For Fisheries, from individuals initially caught in the wild near Jambi (Sumatra Island) on the Batang Hari River system. The rest of *C. meladerma* broodstock and *C. nieuhofii* and *C. teijsmanni* broodstocks were wild fish caught in the same area. Every stocks specimens were identified following Teugels (1986) and Kottelat *et al.* (1993). Finally, observations were made on hybrids between female *C. meladerma* and male *C. gariepinus* that had been produced at the Depok station in 1995 and whose parents came respectively from the Batang Hari River system and a RIFF stock. All specimens of brooders used in this study were deposited at the Musée Royal de L'Afrique Centrale (MRAC), Tervuren.

Every species or hybrids were stocked (males and females together), at a maximum density of 10 fish.m^{-3}, in 2 m^3 net cages set up in a 200 m^2 pond. During the experiments, the pond water temperature fluctuated between 26.5 and 32.0°C and pH between 7.5 and 8.5. Fish were fed a 35% crude protein pelleted feed, distributed twice per day and six days a week at a daily rate of 2% of fish biomass.

Artificial reproduction

Four hybridisation experiments were carried out (Table 1). For each experiment, spawners were selected on the day of reproduction. Genital papilla dimorphism between males and females, allowed to chose rapidly two to five males (depending on the species and the quantity of sperm required). For *C. gariepinus* and *C. batrachus*, females were selected according to the diameters measured on about thirty oocytes sampled by intra-ovarian biopsy. For a female to be chosen, observed diameters had to be homogeneous, with a mode higher than 1.5 mm and 1.2 mm for *C. gariepinus* and *C. batrachus*, respectively. Those diameters correspond to oocytes ending vitellogenesis and apt to respond to hormonal treatment for inducing maturation and ovulation. For *C. meladerma*, in which most of the broodstock came from natural environment, some atresia was observed, probably related to the stress induced by capture and captivity. The lack of individuals showing an advanced maturity stage led to the selection of small females, on which biopsies could not be made. Those females were chosen according to outward signs of maturity (rounded and supple abdomen, turgescent genital papilla).

However, oocytes could be sampled by intra-ovarian biopsy on the three biggest females, coming from Depok research station. Measured oocytes were grouped around a mode of 2 mm, close to the maximal diameter observed on prespawning females caught in natural environment (Catfish Asia Project, unpublished data).

Selected females were placed in individual aquariums or plastic tanks (from 40 to 300 L, depending on the fish size). Temperature was continuously taken by a data logger thermometer. Oocytes maturation and ovulation were induced by a single hCG (human chorionic gonadotropin) injection of 4.0 UI.g^{-1} body weight (Eding *et al.*, 1983; Zonneveld *et al.*, 1989). These authors' recommendations for *C. gariepinus* and *C. batrachus* being almost equivalent, a same latency time for all the species (depending on water temperature) was applied between injection

Experiment	Hybridisation attempts
1	*C. batrachus* x *C. gariepinus* and *C. gariepinus* x *C. batrachus* *C. gariepinus* x *C. meladerma* and *C. meladerma* x *C. gariepinus* *C. batrachus* x *C. meladerma* and *C. meladerma* x *C. batrachus*
2	*C. gariepinus* x *C. meladerma* and *C. meladerma* x *C. gariepinus* *C. gariepinus* x *C. nieuhofii*, *C. gariepinus* x *C. teijsmanni* *C. meladerma* x *C. nieuhofii*, *C. meladerma* x *C. teijsmanni*
3	*C. batrachus* x *C. gariepinus* and *C. gariepinus* x *C. batrachus*
4	*C. gariepinus* x *C. meladerma*

Table 1: Hybridisation attempts in the four experiments carried out between March and June 1997 (crosses are given with the female parent in the first position).

and eggs collection by abdominal stripping. Latency time ranged from 10 to 12 h for mean temperatures between 26 and 29°C.

Clariidae male genital apparatus is characterised by seminal vesicles, composed of many lobes and well individualised from the testis (Legendre & Jalabert, 1986). This can explain the troubles met when trying to collect sperm by abdominal stripping (Legendre, 1986). Thus, males had to be killed for testis dissection and sperm collecting. In *C. gariepinus*, testis multiple incisions let the sperm leak and the amounts collected from all the males (0.6 to 2 ml per male) were diluted 10 times in a NaCl 0.9% solution for a better preservation. In *C. batrachus*, *C. meladerma*, *C. nieuhofii*, *C. teijsmanni*, intra-testicular sperm quantity was too low to use this method. Thus, wide incisions were made on testis and the latest were rinsed successively in a known quantity of NaCl 0.9%. Sperm preparations were kept at 5°C during the few hours required for fertilisation trials, knowing that, in *C. gariepinus* and *Heterobranchus longifilis*, sperm can be kept this way at least 24 h without loss of its fertilising ability (Hogendoorn & Vismans, 1980; Legendre and Otémé, 1995). Spermatozoa concentrations in the sperm preparations were evaluated using a Thomas's hematimeter after a second dilution (dilution rate from 2.10^{-2} to 2.10^{-3}) in a NaCl 0.9% solution. Active spermatozoa proportion and motility duration were evaluated after mixing 2 µL of sperm with 50 µL of spring water on a microscope slide (x 200, black background).

In each experiment, hybrid and intra-specific crosses were made at the same time with gametes coming from the same parents. Batches of 100 to 300 eggs (two replications per cross), weighed beforehand (± 0,1 mg) were mixed with 200 µl of diluted sperm in a dry small plastic receptacle. Spermatozoa were then activated by adding 6 ml of spring water. After one minute of moderated shaking, eggs were rinsed to withdraw sperm excess and spread in another receptacle holding 300 ml of spring water for incubation.

The eggs of all the females (two to three per species) that were used were first tested individually in intra-specific fertilisation. In the first two experiments, the eggs of the different females coming from the same species were then mixed with a feather before intra and inter-specific fertilisations. However, a negative effect of this practice was observed on hatching rates. During the last two experiments, eggs coming from two females were fertilised individually in all the crosses that were made.

Incubation was carried out in stagnant water. The receptacles containing the oocytes were put to buoy on large aquariums in order to reduce temperature variations. Water temperature was recorded by a data logger thermometer during embryonic development.

During the third experiment, the embryonic development of *C. batrachus*, *C. gariepinus* and their crossed-fertilisation products was followed up on batches of about thirty eggs spread in Pétri dishes that held 100 ml of spring water at the laboratory temperature (29 to 29.5°C). Two batches per female and per cross were observed simultaneously by the authors, under two binocular microscopes, at 1h and 3h hours after fertilisation and then every 5 hours until hatching. Such a chronology, based on Legendre et Teugels (1991) and Legendre *et al.* (1992) observations on *Heterobranchus longifilis* and *C. gariepinus*, had been chosen in order to take into account all the main embryonic development stages. For estimating embryonic mortality, development was considered as aborted only when eggs were beginning to turn white. At the same time, hatching kinetics of the different products obtained in incubation containers (28.2°C to 29.5°C) was followed up on one batch of about 200 eggs per female and per cross, twice an hour from the beginning to the end of the hatching period. During the third and the fourth experiments, fertilisation rates of the different batches under observation were estimated by embryos percentages one hour after fertilisation.

After hatching, the proportions of normal and deformed larvae were determined for each batch of eggs (two replications per cross) by observations and counting over an illuminated table.

Growth and survival

During the first experiment, only larvae obtained from *C. batrachus* and *C. gariepinus* intra-specific crosses were numerous enough to make growth and survival comparisons. The day after hatching (D1), normal larvae were sorted out and shared out in batches of 400 individuals (two replications per species) in 30 L tanks of a recirculating water system. They were observed during 12 days. From D2 (i.e. the beginning of exogenous feeding) larvae were fed 6 times a day with *Artemia* nauplii, on the basis of a daily food ration equal to 200% of estimated biomass. From

D9 to D13, *Artemia* was progressively replaced by a 35% crude protein pelleted feed (President n°1). During the whole of that period, 20 individuals per batch were weighed every 3 days to the nearest 0.1 mg. On the last day of larval rearing, all the fish remaining in each tanks were counted to determine survival rates.

After the second experiment, two batches of 50 *C. gariepinus* larvae and two batches of 50 hybrid larvae from *C. meladerma* x *C. gariepinus* cross were placed in the 30 L tanks of a recirculating water system. Those batches were fed 6 times a day with *Artemia* nauplii until D9, and then with President n°1 pellets, progressively introduced in food rations. Twenty individuals per replicates were individually weighed on D3, D5, D8, D9 (to the nearest 0.1 mg). After 15 days (D16), each batch was counted again and all larvae individually weighed. Respecting the mean weights obtained for each kind of larvae, fish were shared out again in two batches of 30 individuals per cross type and placed in 30 L aquariums, filled with stagnant oxygenated water renewed every two days. They were fed in excess, 4 times a day with 35% crude protein pellets (President n°1 and n°2,) until the age of 78 days. During that period, all the larvae in every batches were counted and individually weighed on D29, D48 and D78.

During the same experiment, the low quantities of normal larvae obtained after hatching in the other crosses, whether intra-specific (*C. meladerma*) or inter-specific (*C. meladerma* x *C. nieuhofii* and *C. meladerma* x *C. teijsmanni* hybrids) did not allow making two batches of 50 individuals, as in the case of *C. gariepinus* and *C. meladerma x C. gariepinus*. Nevertheless, those fish growth and survival were followed up to the same age, in the same conditions except for stocking densities and absence of replicate.

The main physicochemical characteristics of the water during larval rearing in the first two experiments were recorded every day and proved to be particularly stable (Table 2).

	Minimum	Maximum
Temperature (°C)	26	28
O_2 (mg.L^{-1})	7.5	7.7
pH	8.6	8.7
NH_4^+ (mg.L^{-1})	0.2	0.2
NO_2 (mg.L^{-1})	0.012	0.012
Water flow	8 L.h^{-1} (D2) to 40 L.h^{-1} (D15)	

Table 2: Main water characteristics during larval rearing in the first two experiments.

Genetic characterisation of* C. gariepinus, C. meladerma *and their reciprocal hybrid progenies

The genotypes of the progenies obtained from the reciprocal crosses between *C. gariepinus* and *C. meladerma* were compared between them and with their parents ones, studying phospho-gluco-isomerase (PGI) and phospho-gluco-mutase (PGM) expression polymorphism on starch gel. Both protein systems (PGI and PGM) are diagnostic for the two studied species, which means that each species is characterised by an original allelic form for each enzymatic system studied. This is revealed by a different electrophoretic migration of each allelic form. If allelic segregation is respected during gamete association, an heterozygous state of hybrid progenies must be expected.

One batch of larvae coming from the cross *C. gariepinus* x *C. meladerma* was ground in distilled water and centrifuged (6,000 *g*, 30 minutes, 4°C) in order to extract proteins. Eye samples coming from *C. gariepinus*, *C. meladerma* and hybrids of *C. meladerma* x *C. gariepinus* underwent the same treatment. Electrophoresis of the proteins extracts was made on starch gel (15%) using a Ridgway type migration buffer. The PGI and PGM systems revelation was carried out using the method of Pasteur *et al.* (1987).

Gonad development in* C. meladerma *x* C. gariepinus *hybrid

Eleven hybrids coming from a cross between female *C. meladerma* and male *C. gariepinus* (made by the RIFF in 1995) were killed in order to examine their gonads. Those were observed with the naked eye and under a binocular microscope. Besides, intra-testicular sperm samples were subject to a meticulous exam, using a microscope, in the same conditions as previously described. Ovaries and testis of the seven females and four males (531 to 900 g individual body weight) were weighed to the nearest 0.1 g, in order to calculate gonado-somatic index (GSI = 100 x ovaries weight or testis weight / body weight).

Statistical analysis

Experimental data were compared using Student t test or one way ANOVA. When analysis of variance concluded to a significant difference, homogeneous experimental groups were sought using Duncan multiple-range test ($p<0.05$). When necessary, analyses were performed after angular

Species	Oocyte diameter (mm)	Ova diameter (mm)	Weight of ova (mg)
C. batrachus	1.23	1.28	0.91
C. gariepinus	1.61	1.72	1.75
C. meladerma	2.00	2.08	3.11

Table 3: Mean size of oocytes (before induced ovulation) and mean size and weight of ova in three *Clarias* species used in the hybridisation study (2 to 10 females per observations).

(arc-sine) transformation of data in order to stabilise residual variance.

RESULTS

Gametes used in the different crosses

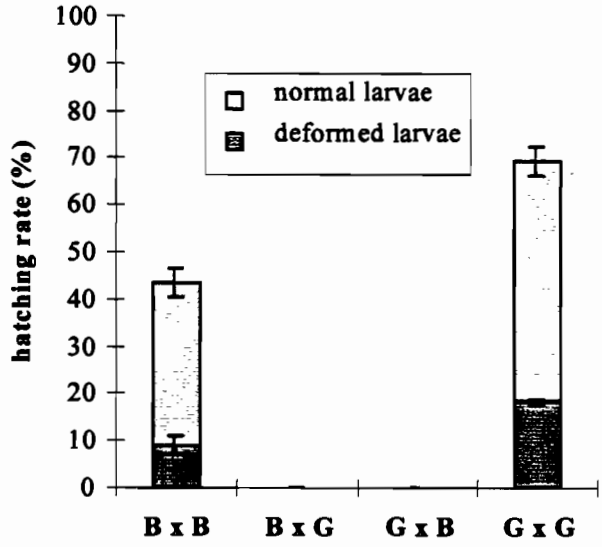
The three species that were used as female parent clearly differed by their egg size. Weight of *C. meladerma* ova was three times greater than that of *C. batrachus*, whereas *C. gariepinus* ova presented intermediate values (Table 3).

Among the five species used as male parent, *C. gariepinus* appeared clearly less oligospermic than the Indonesian species. As a matter of fact, the sperm quantities collected after rinsing testis of the Indonesian species were always very low in comparison with the sperm quantities obtained by simples incisions on the African *Clarias* testis. This has resulted in different numbers of spermatozoa per ovum during the fertilisation trials, from 1 to 5×10^6 spz per ovum with *C. gariepinus* sperm, 3×10^4 to 1×10^5 with *C. batrachus*, 1 to 2×10^4 with *C. nieuhofii* and 3 to 7×10^5 with *C. meladerma*. Spermatozoa concentration was not quantified when using *C. teijsmanni* sperm but it was also low. Moreover, the percentage of motile spermatozoa after dilution in water was usually lower than 50% in the local species, while it was generally greater than 90% in *C. gariepinus*. For those reasons, number of active spermatozoa per ovum in the artificial fertilisations carried out with sperm of the Asian species was always lower than in the ones carried out with sperm of *C. gariepinus*. However, spermatozoa motility duration (usually between 40 and 60 sec.) did not noticeably differ between the five species.

Cross-fertilisation success and embryonic development

Reciprocal crosses between C. batrachus *and* C. gariepinus

At 27°C (first experiment), hatching time ranged between 20h and 24h30 after fertilisation in *C. gariepinus* and between 25h15 and 29h30 after fertilisation in *C. batrachus*. In the third experiment, where incubation temperature was noticeably higher (28.2-29.5°C), hatching started earlier in the two species but did not end before 30h after fertilisation in *C. batrachus*. During those two experiments, no hybrid between *C. batrachus* and *C. gariepinus* was obtained (Fig. 1 and 2). With hatching rates rising up to 83.7% (of which 8.5% deformed larvae) in *C. batrachus* and to 73.6% (of which 10.3% deformed larvae) in *C. gariepinus*, gametes quality could not be responsible for the non-viability of the inter-specific crosses carried out.

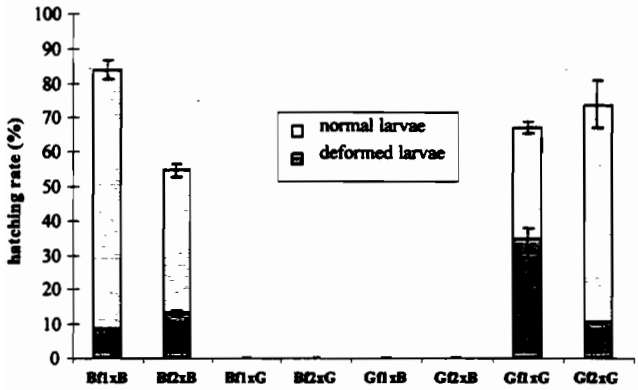


Vertical bars indicate range between replicates.

Figure 1: Mean hatching rates in *C. batrachus* (BxB), *C. gariepinus* (GxG) and their hybrid crosses (BxG and GxB) in the first experiment (pool of ova from 3 *C. batrachus* and 2 *C. gariepinus* females).

A more precise examination of embryonic development in the third experiment showed that the cross between female *C. batrachus* and male *C. gariepinus* was characterised by a very low fertilisation rate (9.3%), about 10 times lower than that obtained in the two parental species (Fig. 3.).

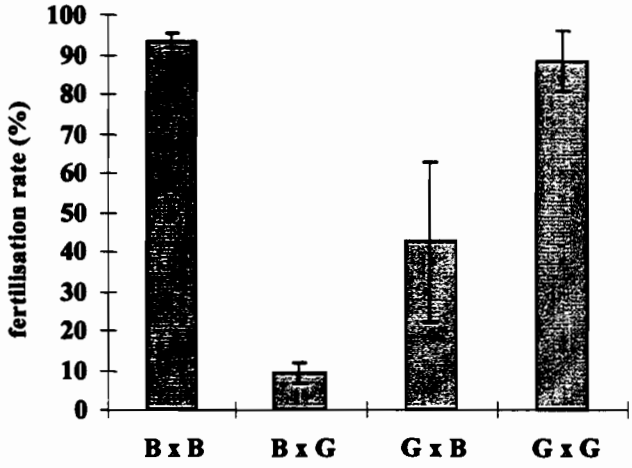
In the reciprocal cross, *C. gariepinus* x *C. batrachus*, fertilisation rate was noticeably higher (42.4%) but remained lower than the ones obtained in intra-specific crosses. In the two intra-specific crosses, most of the embryonic mortality occurred between beginning of gastrulation and the first somites apparition (Fig. 4). In *C. gariepinus* x



Vertical bars indicate ranges between replicates.

Figure 2: Mean hatching rates in *C. batrachus* (BxB), *C. gariepinus* (GxG) and their hybrid crosses (GxB and BxG) in the third experiment (crosses using individual females).

C. batrachus cross, the development of eggs containing embryos went on until gastrulation. However, all the observed gastrulations presented major anomalies (disorganised cells structures) and no embryo could develop beyond closure of the blastopore. In the reciprocal cross, *C. batrachus* x *C. gariepinus*, anomalies were observed as early as morula stage (cells dispersal in the perivitelline space) and only 9.1% of fertilised eggs entered gastrulation stage. In the two cases, all the embryos were dead (white eggs) 22h after fertilisation.



Vertical bars indicate range between replicates.

Figure 3: Mean fertilisation rates in *C. batrachus* (BxB), *C. gariepinus* (GxG) and their hybrid crosses (GxB and BxG) in the third experiment (two females per species, two replicates per female).

Reciprocal crosses between C. gariepinus *and* C. meladerma

In the first experiment, at an incubation temperature of 27°C, embryos of *C. gariepinus* were the first to hatch (20 to 24h30 after fertilisation). In *C. meladerma*, hatching occurred between 29h30 to 36h after fertilisation. In the hybrids, incubation times were in-between the parental species ones: 22h to 26h30 in *C. gariepinus* x *C meladerma* and 26 to 31h in *C. meladerma* x *C. gariepinus*. During that experiment, only one out of the three *C. meladerma* females presented an egg quality good enough to get hatching (21.7% hatching, of which 6.7% deformed larvae). Afterwards, eggs pools led to very low hatching rates, around 1% in intra-specific crosses and 5.4% after fertilisation with *C. gariepinus* sperm. In *C. gariepinus*, intra-specific control batches proved to be a lot better (69.2% hatching of which 26.5 deformed larvae; Fig. 1). After fertilisation with *C. meladerma* sperm, the same eggs pool gave only 28.0% hatching, with a particularly high proportion of deformed larvae (69.4%).

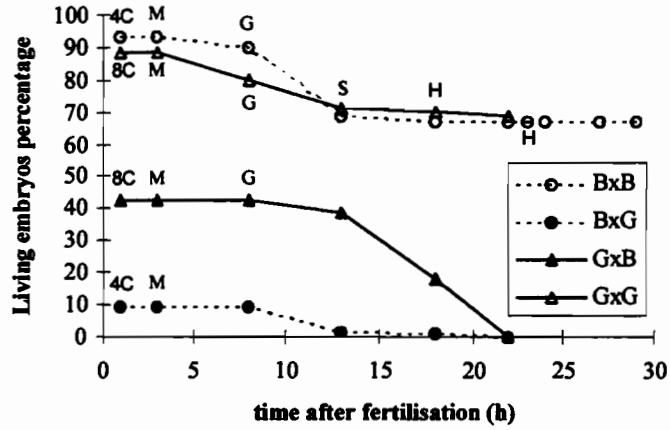
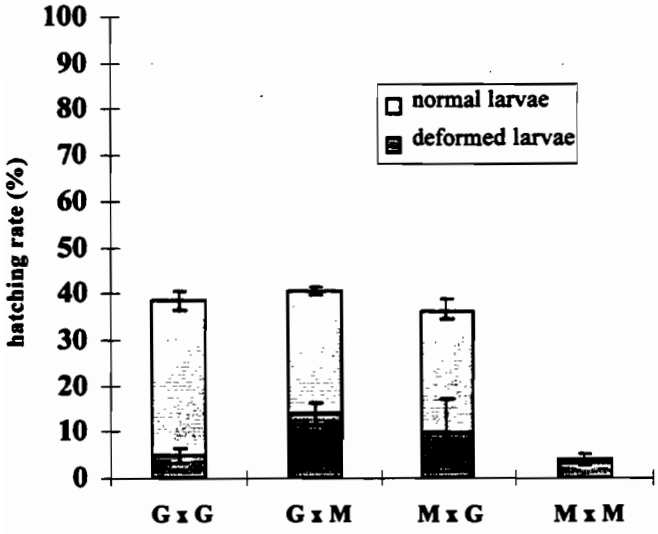


Figure 4: Kinetic of embryonic development and embryonic mortality at 29-29.5°C in *C. batrachus* (BxB), *C. gariepinus* (GxG) and their reciprocal hybrid crosses in the third experiment (4 or 8C: 4 or 8 cells stage; M: morula stage; G: gastrulation; S: somites stage; H: beginning of hatching).

During the second experiment, hatching rates obtained in *C. meladerma* intra-specific crosses were only slightly higher than those observed in the first experiment (Fig. 5). However, hatching rates obtained in inter-specific crosses with *C. gariepinus* rose up to 36.1% (of which 24.7% abnormal larvae). *Clarias meladerma* and *C. gariepinus* x *C. meladerma* hatching rates did not significantly differ but all the hybrid batches between male *C. gariepinus* and female *C. meladerma* presented more than one third strongly deformed larvae.

During the fourth experiment, without any ovulation in *C. meladerma*, the hybrid cross *C. gariepinus* x *C. meladerma* only could be achieved. The ova coming from the two females of

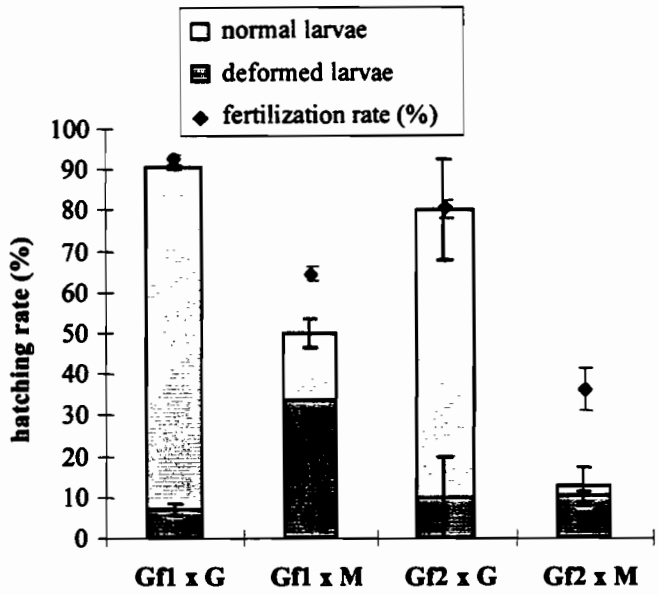


Vertical bars indicate range between replicates.

Figure 5: Mean hatching rates in *C. gariepinus* (GxG), *C. meladerma* (MxM) and their hybrid crosses (GxM and MxG) in the second experiment (pool of ova from 2 *C. gariepinus* and 3 *C. meladerma* females).

C. gariepinus were not mixed and the hatching rates obtained after intra-specific fertilisation underlined the quality of the gametes used (Fig. 6). The more meticulous examination of embryonic development showed that the hybrid cross was characterised by fertilisation rates significantly lower than those obtained in the female parent. Many hybrid embryos were abnormal during gastrulation and more than 40% died before hatching. After hatching, 67.8% of the hybrids coming from the first female and 85.7% of the hybrids coming from the second female presented major deformations.

In the three experiments, non deformed larvae obtained in crossing female *C. gariepinus* and male *C. meladerma* were characterised by a spherical yolk sac and a much more frail appearance than *C. gariepinus* larvae. As soon as D1, a very low percentage of that larvae were still active. Their eyes were relatively smaller and their barbels noticeably less developed than those of *C. gariepinus* larvae. On D2, many motionless larvae presented an abnormal, curvilinear vertebral column sketch. More than 60% of the hatched larvae did not survive after 72h. On D3, alive larvae which were still moving did not seem to have started yolk sac absorption and the scarce larvae still moving on D4 had not started eaten yet whereas, in *C. gariepinus*, the beginning of exogenous feeding had been observed on D2. On D5, no larvae were still alive.



Vertical bars indicate range between replicates.

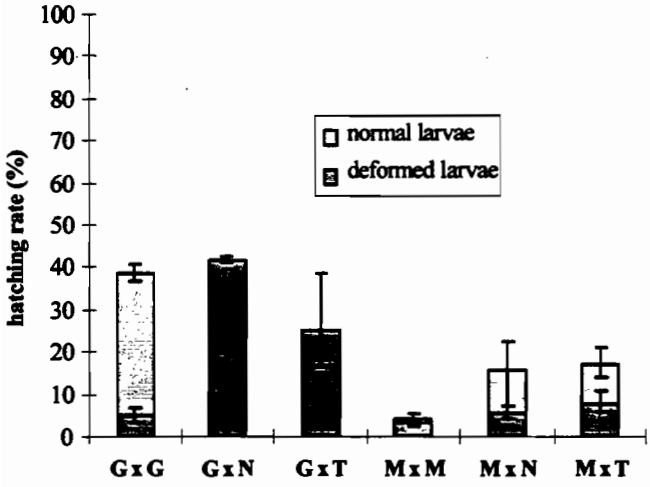
Figure 6: Mean fertilisation rates in eggs from two *C. gariepinus* females (Gf1 and Gf2) fertilised with *C. gariepinus* (G) or *C. meladerma* (M) sperm (fourth experiment).

Crosses between female C. gariepinus *or* C. meladerma *and male* C. nieuhofii *or* C. teijsmanni

Due to oocytes atresia observed on *C. nieuhofii* and *C. teijsmanni* wild stocks, the hybridisation experiments were restricted to the crosses between female *C. gariepinus* or *C. meladerma* and male *C. nieuhofii* or *C. teijsmanni* (experiment n°2). Larvae were obtained in the four crosses (Fig. 7). Hatching rates obtained in *C. gariepinus* x *C. gariepinus*, *C. gariepinus* x *C. nieuhofii*, and *C. gariepinus* x *C. teijsmanni* did not significantly differ. However, in the two inter-specific crosses, 100% of hatched larvae presented deformations and mortality began soon after hatching. In the best case, larvae did not survived more than a few hours. In that experiment, 3.9% of *C. meladerma* eggs only went to hatching in intra-specific cross. In inter-specific crosses with male of *C. nieuhofii* or *C. teijsmanni*, hatching rates passed over 15%, with a fraction of deformed larvae around 44% in the two crosses. These larvae proved to be viable.

Reciprocal crosses between C. batrachus *and* C. meladerma

In the first experiment, no hybrid between female *C. meladerma* and male *C. batrachus* was obtained. In the reciprocal cross, hatching rates were lower than 1% in the two replicates and the larvae did not survive. However, considering the



Vertical bars indicate range between replicates.

Figure 7: Mean hatching rates in *C. gariepinus* (GxG), *C. meladerma* (MxM) and their hybrid crosses with *C. nieuhofii* (N) or *C. teijsmanni* (T) males (pool of ova from 2 *C. gariepinus* and 3 *C. meladerma* females, second experiment).

poor results obtained in *C. meladerma* intra-specific cross during the same experiment, supplementary observations remain necessary before expressing any conclusion.

Growth and survival

C. batrachus *and* C. gariepinus *larval growth and survival*

Growth and survival of two batches of 400 *C. gariepinus* larvae and two batches of 400 *C. batrachus* larvae were compared from hatching up to 13 days of age. During that period, the mean body weight difference between the two species (already in favour of *C. gariepinus* at hatching) increased progressively. Mean weights of twelve-days-old fry were 81 ± 7 mg in *C. gariepinus* and 33 ± 4 mg in *C. batrachus*. However, specific growth rates (SGR) and relative weight gain (RWG) between D0 and D12, or between the beginning of exogenous feeding and D12, were very close in the two species and did not differ significantly (Table 4). Thus, final weight differences seemed to result essentially from the initial specific difference between larval weight of the two species. After 13 days of larval rearing, survival rate exceeded 93% in *C. gariepinus* while it was less than 27% in *C. batrachus*.

Growth and survival until 78 days in C. gariepinus *and hybrids between female* C. meladerma *and male of* C. gariepinus, C. nieuhofii, *or* C. teijsmanni

Growth and survival of two batches of 50 *C. gariepinus* larvae and two batches of 50 *C. meladerma* x *C. gariepinus* larvae were compared from hatching up to 16 days of age. Just after hatching, on account of the difference of egg size between the two species, *C. gariepinus* larvae mean weight was much lower than that of hybrid larvae (Table 5). However, this handicap was compensated as soon as D5, age at which *C. gariepinus* had reached a significantly higher weight than the hybrid. Mean weights of 16-days-old larvae were 494 ± 43 mg in *C. gariepinus* and 214 ± 17 mg in the hybrid. *Clarias gariepinus* growth was significantly higher than that of the hybrid during the whole period (Table 5). After 16 days of larval rearing, *C. gariepinus* and the hybrid survival rates were higher than 60% and did not differ significantly.

During the same experiment, growth and survival in small batches (without replicates) of *C. meladerma* x *C. meladerma*, *C. meladerma* x *C. nieuhofii* and C. *meladerma* x *C. teijsmanni* (respectively 13, 31 and 27 fish) was followed up in the same conditions except for stocking densities. The low quantities of fry available for these groups were due to the very low hatching rates obtained in those crosses. After 16 days, the mean body weight obtained for each kind of cross was below 90 mg (Table 5). Their growth rates were thus lower than that of the hybrid *C. meladerma* x *C. gariepinus*. On the other hand,

Species	Initial fish number	Initial body weight (mg)	Final body weight (D12) (mg)	SGR (%day^{-1})	RWG (%day^{-1})	Survival rate at 13 days (%)
C. batrachus	(2x) 400	D0 : 0.7 D2*: 1.6	33.0 ± 3.9	from D0 : 32.1 from D2 : 30.3	from D0 : 461.4 from D2 : 196.3	94.3
C. gariepinus	(2x) 400	D0 : 1.8 D2*: 3.4	81.1 ± 7.1	from D0 : 31.7 from D2 : 31.7	from D0 : 440.3 from D2 : 228.4	26.0

(*): beginning of exogenous feeding

Table 4: Mean growth from 0 to 12 days and mean survival rates after 13 days for *C. batrachus* and *C. gariepinus* larvae in the first experiment (SGR = specific growth rate, RWG = relative weight gain).

Species or hybrids	Initial fish number	Initial body weight (D0) (mg)	Final body weight (D16) (mg)	SGR (%day^{-1})	RWG (%day^{-1})	Survival rate at 16 days (%)
C. gariepinus	(2x) 50	1.8	497 ± 43	35.1	1708	69.0
C. meladerma x *C. gariepinus*	(2x) 50	3.3	214 ± 17	26.1	399	61.0
C. meladerma	13	3.3	65 ± 17	18.6	116	61.5
C. meladerma x *C. nieuhofii*	31	3.3	89 ± 7	20.6	162	48.1
C. meladerma x *C. teijsmanni*	27	3.3	85 ± 13	20.3	155	61.3

Table 5: Mean growth and survival rates from 0 to 16 days in *C. gariepinus*, *C. meladerma* and *C. meladerma* x *C. gariepinus* or x *C. nieuhofii* or x *C. teijsmanni* in the second experiment (SGR = specific growth rate, RWG = relative weight gain).

the survival rates of the hybrids between female *C. meladerma* and male of *C. gariepinus*, *C. nieuhofii* or *C. teijsmanni* did not differ significantly from that observed in *C. meladerma*.

After the 16 days of larval rearing, two batches of 30 *C. gariepinus* and two batches of 30 *C. meladerma* x *C. gariepinus* were reared in aquarium during two months. At the end of this period, the mean body weight of *C. gariepinus* fingerlings (8.4 ± 1.9 g) was still higher than that of hybrid fingerlings (6.2 ± 0.8 g). However, during that period, a bacterial infection developed in some aquariums. Mortality began on D25 in *C. gariepinus* and spread (at a lower level) to the hybrids batches a few days latter. This could explain a growth decrease observed between D29 and D48, particularly obvious in *C. gariepinus*. Although SGR on the whole period was higher in the hybrid than in *C. gariepinus* (Table 6), the last weighing period revealed a higher growth in *C. gariepinus* from D48 to D78. Survival rates, accounting for the mortality observed during the bacterial infection, were higher in the hybrid (93.3%) than in C. *gariepinus* (63.3%).

From 16 to 78 days, *C. meladerma* fingerlings and hybrids between female *C. meladerma* and male of *C. nieuhofii* or *C. teijsmanni* were also transferred to aquariums and presented SGR similar to that of *C. meladerma* x *C. gariepinus* (Table 6). However, on account of their growth lateness on D16, their mean body weights remained very low in comparison with *C. meladerma* x *C. gariepinus* one until D78. Within those three crosses, *C. meladerma* x *C. teijsmanni* only presented a high mortality during bacterial infection.

Genetic characterisation of* C. gariepinus, C. meladerma *and their reciprocal hybrid progenies

The PGI and PGM locus revelations led to the same results (see Fig. 8 for PGI locus). As expected hybrids genotypes resulting from the cross *C. meladerma* x *C. gariepinus* were heterozygous. They displayed allelic forms from both parental species. However, in the reciprocal cross, *C. gariepinus* x *C. meladerma*, progeny genotype was identical to the one observed on the female parent (*C. gariepinus*) and revealed no trace of *C. meladerma* genes.

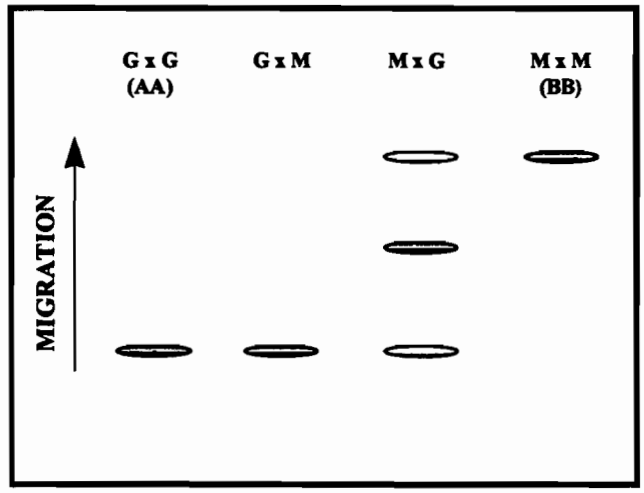


Figure 8: Electrophoretic profile (starch gel) at the PGI diagnostic locus in *C. gariepinus* (G x G), *C. meladerma* (M x M), *C. gariepinus* x *C. meladerma* (G x M) and *C. meladerma* x *C. gariepinus* (M x G).

***Gonad development in the hybrids between female* C. meladerma *and male* C. gariepinus**

The *C. meladerma* x *C. gariepinus* hybrids that were observed for their gonad development were about two-years-old. Females weighed between 647g and 900g and males between 531 and 879g. The sexual dimorphism of the genital papilla was much less obvious in these individuals than in their parental species at the same age. The maximum GSI observed on females was 0.68%. This value is much lower than GSI reported in *C. gariepinus* at the same age (generally around 15%) and much lower than GSI observed on wild *C. meladerma* females as well (4-11%). Gonads of four out of the

Species or hybrids	Initial fish number	Initial body weight (D16) (g)	Final body weight (D78) (g)	SGR (%day^{-1})	RWG (%day^{-1})	Survival rate From D16 (%)
C. gariepinus	(2x) 30	0.493	8.4 ± 1.9	4.6	25.9	63.3
C. meladerma x *C. gariepinus*	(2x) 30	0.214	6.2 ± 0.8	5.4	45.1	93.3
C. meladerma	8	0.064	1.6	5.2	38.7	100
C. meladerma x *C. nieuhofii*	13	0.089	1.9	4.9	32.8	100
C. meladerma x *C. teijsmanni*	19	0.085	2.3	5.3	42.0	36.8

Table 6: Mean growth and survival rates from 16 to 78 days in *C. gariepinus*, *C. meladerma* and *C. meladerma* x *C. gariepinus* or x *C. nieuhofii* or x *C. teijsmanni* in the second experiment (SGR = specific growth rate, RWG = relative weight gain).

seven female hybrids examined were reduced to two white long filaments. In the three other females, gonads looked like small pinkish sacs surrounded with blood vessels. Within the most developed gonads, some translucent and vacuolated areas of the ovarian tissue presented an organisation similar to that of perivisceral adipose tissue. In any case, no developing oocytes could be observed, even in the biggest ovaries,. The GSI of the 4 male hybrids were low (0.02-0.05%) in comparison to those of the *C. gariepinus* (0.32-0.89%) and *C. meladerma* (0.06-0.16%) males used during this study. By contrast, three hybrids presented particularly well developed seminal vesicles. The pinkish and almost translucent testis observed in the hybrids contained no spermatozoa and backcross fertilisation attempts using *C. gariepinus* ova did not lead to any fertilisation. At the same age, *C. meladerma* broodstock coming from the Depok station had reached full sexual maturity. In farm reared *C. gariepinus*, the first sexual maturation occurs when fish are 5 to 7 months old (Legendre *et al.*, 1992).

DISCUSSION - CONCLUSION

In this study, ten crosses between four Asian *Clarias* species and one African *Clarias*, *C. gariepinus*, were evaluated according to the fertilisation and hatching rates that were obtained. Zootechnical performances (growth, survival) of *C. batrachus* and *C. gariepinus* were compared in larval rearing. Viable hybrids were followed up until 11 months old. The genotypes of the reciprocal crosses between *C. gariepinus* and *C. meladerma* were studied and sexual maturation state of two years-old hybrids between female *C. meladerma* and male *C. gariepinus* was examined.

On a methodological point of view, analysis of fertilisation trials underlined a decrease of egg quality after mixing the eggs coming from several females in *C. batrachus* and *C. gariepinus*. Hatching rates of mixed eggs were 15 to 40% lower than the mean hatching rates observed in individual intra-specific fertilisations. This phenomenon may be explained by the low fluidity of the ova masses that were collected by abdominal stripping (almost complete absence of ovarian fluid), in favour of mechanical shocks during gametes homogenisation. The low quantities of sperm that could be obtained in the oligospermic Asian species raises the issue of the minimum number of spermatozoa required to achieve fertilisation in optimal conditions in the different female parental species that were used. The few data available on Clariidae point out that, in *C. macrocephalus*, required number of spermatozoa per ovum is about 40,000 to 80,000 (Tambasen-Cheong *et al.*, 1995). In *Heterobranchus longifilis*, a decrease in hatching rate is observed under 50,000 spermatozoa per ovum (Legendre, 1992). In some of the fertilisations made with *C. batrachus* or *C. meladerma* sperm, mean number of spermatozoa per ovum were close to those critical values, or even lower. Moreover, in some fish families (such as Salmonidae), large egg size, lengthening spermatozoa trajectory to the micropyle, may result in an increase of the required number of spermatozoa per ovum (Billard, 1990). The low spermatozoa concentration observed when fertilising with *C. meladerma* sperm, associated with the relatively large egg size of this clariid, may partly explain the low hatching rates obtained in this species after intra-specific fertilisation in comparison to those observed after fertilisation with *C. gariepinus* sperm.

Table 7 summarises the results obtained in terms of hatching achievement and offspring viability for the different crosses tested. The two hybridisation experiments between *C. batrachus*

Cross tested (female x male)	Hatching	Offspring viability
C. gariepinus x *C. batrachus*	NO	/
C. gariepinus x *C. meladerma*	YES	NO
C. gariepinus x *C. nieuhofii*	YES	NO
C. gariepinus x *C. teijsmanni*	YES	NO
C. batrachus x *C. gariepinus*	NO	/
C. batrachus x *C. meladerma*	NO	/
C. meladerma x *C. gariepinus*	YES	YES
C. meladerma x *C. batrachus*	less than 1%	NO
C. meladerma x *C. nieuhofii*	YES	YES
C. meladerma x *C. teijsmanni*	YES	YES

Table 7: Hatching achievement and offspring viability in the different crosses tested.

and *C. gariepinus* did not lead to any hatching. In *C. batrachus* x *C. gariepinus*, this result was expressed by a significant decrease of fertilisation rates compared to the intra-specific control cross and by the mortality of the scarce embryos that were obtained. In the reciprocal cross, fertilisation rate decrease was not significant but none of the embryos was viable. Theses observations match with the ones made by Richter *et al.* (1995) on an Eastern Java *C. batrachus* stock. These authors assigned the fertilisation rate decrease in the cross between female *C. batrachus* and male *C. gariepinus* to the relatively small size of the first species micropyle compared to the latter species spermatozoa size. The success reported in the hybridisation of these two species in Bangladesh (Ahmed & Sarder, 1994; Rahman *et al.*, 1995) could then only be explained by a genetic differentiation between Indian and Indonesian populations of *C. batrachus* from Indian Peninsula and Indonesia, or by the fact that fish called "*C. batrachus*" in these two areas may not correspond to the same species. The latter hypothesis is actually supported by recent caryotype and morphometric comparison analyses, suggesting that "*C. batrachus*" from the Indian Peninsula may in fact be misidentified and could correspond to *C. fuscus* (Garcia-Franco, 1993).

Larvae obtained in the crosses between female *C. gariepinus* and male of *C. nieuhofii* or *C. teijsmanni*, all strongly deformed, did not survive more than a few hours. Mortality that was observed in *C. gariepinus* x *C. meladerma* within the first five days of life seems to come from a different cause. Those larvae, of which many were not deformed, presented no trace of *C. meladerma* genes at the PGI and PGM loci, whereas in the reciprocal cross *C. meladerma* x *C. gariepinus*, the expected hybrid genotype was actually observed. This kind of situation (no paternal genome contribution and early mortality) recalls the one observed in artificial gynogenesis by sperm irradiation without diploidism restoration (Chourrout, 1980). In Salmonidae, haploid larvae that are obtained are characterised by a short body, few blood vessels around vitellus and small eyes. Those individuals die before yolk sac resorption. Larvae obtained after crossing female *C. gariepinus* and male *C. meladerma* presented similar characteristics and could therefore have resulted from haploid gynogenetic development of *C. gariepinus* ova. The fact that no embryo could be obtained from non fertilised *C. gariepinus* ova suggests that the developments obtained in that cross were activated by *C. meladerma* spermatozoa. Establishing those larvae caryotype would allow to fully confirm the haploid state of their genome.

By contrast, *Clarias meladerma* ova were successfully fertilised by spermatozoa coming from three different species: *C. gariepinus*, *C. nieuhofii* and *C. teijsmanni*. In all cases, obtained larvae proved to be viable.

Clarias batrachus and *C. gariepinus* larval rearing showed that those two species possessed an equivalent growth potential until the age of 12 days. Thus, mean body weight differences observed at the end of the experiment should have resulted from the initial differences in eggs and larvae size (bigger in *C. gariepinus*). A similar conclusion is given by Verreth and Eding (1993), who specify that *C. batrachus* growth potential could be under-exploited in Indonesian farms. Growth and survival of these two species should therefore be studied, under optimal farming conditions and until getting the size required on local market, in order to specify their relative interest for fish culture in that area.

Growth and survival comparisons between the hybrid *C meladerma* x *C. gariepinus* and its male

parental species until the age of 11 weeks showed a higher global growth potential in *C. gariepinus*. However, the hybrid reached a noticeably higher weight than its female parental species and than the two hybrids between Asian *Clarias* species that were obtained (*C. meladerma* x *C. nieuhofii* and *C. meladerma* x *C. teijsmanni*). Gonads examination of two years-old *C. meladerma* x *C. gariepinus* hybrids showed an abnormal development of the ovaries and testis that were observed: very low GSI, presence of adipose-like tissue in ovaries and total absence of spermatozoa in testis. These hybrids seem therefore completely sterile but this result has to be specified by (ongoing) histological analysis of samples taken on the gonads that were observed. It would be also interesting to extend the growth comparison that was made between this hybrid and the African *Clarias* until the latter species reaches sexual maturity, in order to test for an eventual effect of gonads development in *C. gariepinus* on the relative growth of these two fishes.

However, it should be noted that three Indonesian populations of *C. meladerma* were found to present a sufficient genetic distance to be considered as three different species inside the Clariidae family (Catfish Asia Project, 1997). The present study used one of these stocks, originating from Jambi (Sumatra Island). New hybridisation experiments with the two other "*C. meladerma*" groups that were identified could lead to different results.

Generally speaking, *C. meladerma* and its hybrids with *C. nieuhofii* and *C. teijsmanni* do not seem to present a high fish farming potential on account of the very low growth rates that were observed. On the opposite, relative zootechnical performances of *C. batrachus*, *C gariepinus* and *C. meladerma* x *C. gariepinus* are worth being specified. They have to be analysed taking into account the flesh and organoleptic characteristics of these fishes, compared to local market requirements. The sterility of the hybrid *C. meladerma* x *C. gariepinus* would be an important advantage as it could be used in aquaculture without possible impacts on gene pools of wild species.

REFERENCES

Amhed G.U. & Sarder M.R.I. (1994) Growth of hybrid catfishes under different supplemental diets. *In*: Chou L.M., Munro A.D., Lam T.J., Chen T.W., Cheong L.K.K., Ding J.K., Hooi K.K., Khoo H.W., Phang V.P.E., Shim K.F., Tan C.H. (eds), *The third Asian Fisheries Forum*. Asian Fisheries Society, Manila, Philippines.

Billard R. (1990) Discussion de quelques données sur la spermatogenèse des poissons et sur l'adaptation des spermatozoïdes aux conditions de fécondation dans divers milieux. *La pisciculture française*, **101**, 24-40.

Boonbrahm M., Tarnchalanukit W. & Suraniranat P. (1977) Experiments on hybridisation of fresh-water catfish, *Clarias macrocephalus* Günther and *Clarias batrachus*. *Research Report of the Kasetsart University*, 143 p.

Catfish Asia Project (1997) First progress report to the European Commission, contract ICT 18-CT 96-0043, 34 p.

Chevassus B. (1983) Hybridisation in fish. *Aquaculture*, **33**, 245-262.

Chourrout D., Chevassus B. & Herioux F. (1980) Analysis of an Hertwig effect in the rainbow trout (*Salmo gairdneri* Richardson) after fertilization with γ-irradiated sperm. *Reprod. Nutr. Dévelop.*, **20** (3A), 719-726.

Csavas I. (1994) Status and perspectives of culturing catfishes in East and South-East Asia. *FAO Aquaculture Newsletter*, **8**, 2-10.

Eding E.H., Janssen J.A.L., Kleine Staarman G.H.J. & Richter C.J.J. (1982) Effects of human chorionic gonadotropin (HCG) on maturation and ovulation of oocytes in the catfish *Clarias lazera* (C.&V.). *In*: Richter C.J.J. & Goos H.J.Th.(eds), *Proceedings of the International Symposium on Reproductive Physiology of Fish*, Wagenigen: PUDOC, 195.

Garcia-Franco M. (1993) *Intra- and interspecific relationships of the clariid catfish* Clarias batrachus. Thesis, Tokyo University of Fisheries, 81 p.

Hetch T. & Lublinkhof W. (1985). *Clarias gariepinus* x *Heterobranchus longifilis* (Clariidae: Pisces): a new hybrid for aquaculture ? *South African Journal of Science* **81**, 620-621.

Hoogendorn H. & Vismans M.M. (1980) Controlled propagation of the African catfish, *Clarias lazera* (C & V). II. Artificial reproduction. *Aquaculture*, **21**, 39-53.

Kottelat M., Whitten A.J., Kartikasari S.N. & Wirjoatmodjo S. (1993) *Freshwater Fishes of Western Indonesia and Sulawesi.* Periplus Editions, 107-108.

Lazard J. (1994) Introduction et transferts d'espèces en pisciculture. Nécessité ou opportunisme? *Revue Elev. Méd. Vét. Pays trop.*, **47** (4), 435-438.

Legendre M. (1986) Seasonal changes in sexual maturity and fecundity, and HCG-induced breeding of the catfish *Heterobranchus longifilis* Val. (Clariidae), reared in Ebrié lagoon (Ivory Coast). *Aquaculture*, **55**, 201-213.

Legendre M. (1992) *Potentialités aquacoles des Cichlidae (*Sarotherodon melanotheron, Tilapia guineensis*) et Clariidae (*Heterobranchus longifilis*) autochtones des lagunes ivoiriennes.* ORSTOM, Paris, Collection Travaux et Documents Microédités, **89**, 83 p + annexes.

Legendre M. & Jalabert B. (1988) Physiologie de la reproduction. *In*: Lévêque C., Bruton, M.N., Ssentego G.W. (eds), *Biologie et écologie des poissons africains d'eau douce.* ORSTOM, Travaux et Documents, **216**, 153-187.

Legendre M. & Teugels G.G. (1991) Développement et tolérance à la température des oeufs de *Heterobranchus longifilis* et de *Clarias gariepinus* (Teleostei, Clariidae). *Aquat. Living Resour.*, **4**, 227-240.

Legendre M. & Otémé Z.J. (1995) Effect of varying latency period on the quantity and quality of ova after hCG-induced ovulation in the African catfish, *Heterobranchus longifilis* (Teleostei, Clariidae). *Aquat. Living Resour.*, **8**, 309-316.

Legendre M., Linhart O. & Billard R. (1996) Spawning and management of gametes, fertilized eggs and embryos in Siluroidei. *Aquat. Living Resour.*, **9**, Hors série, 59-80.

Legendre M., Teugels G.G., Cauty C. & Jalabert B. (1992) A comparative study on morphology, growth rate and reproduction of *Clarias gariepinus, Heterobranchus longifilis* and their reciprocal hybrids (Pisces, Clariidae). *J. Fish. Biol.*, **40**, 59-79.

Mukhopadathy S.M. & Dehadrai P.V. (1987) Survival of hybrids between air-breathing catfishes *Heteropneustes fossilis* (Bloch) and *Clarias batrachus* (Linn.). *Matsya* **12-13**, 162-164.

Pasteur N., Pasteur G., Bonhomme F., Catalan J. & Britton-Davidian J. (1987) *Manuel de génétique de l'électrophorèse des protéines.* Collection Technique et Documentation, Lavoisier, Paris, 217 p.

Rahman M.A., Bharda A., Begum N., Islam M.S. & Hussain M.G. (1995) Production of hybrid vigor through cross breeding between *Clarias batrachus* Lin. and *Clarias gariepinus* Bur. *Aquaculture*, **138**, 125-130.

Richter C.J.J., Eding J.A.J., Verreth J.A.J & Fleuren W.L.G. (1995) African Catfish (*Clarias gariepinus*). *In*: Bromage N.R., Roberts R.J. (eds), *Broodstock management and egg and larval quality.* Blackwell Sciences, 242- 276.

Salami A.A., Fagbenro O.A. & Sydenham D.H.J. (1993) The production and growth of Clariids catfish hybrids in concrete tanks. *Isr. J. Aquacult. Bamidgeh*, **45**, 18-25.

Sudarto S. & Sumastri S. (1994) Prospects of using catfish hybrids for aquaculture in Indonesia. *In*: Basil '94 - RESUMES - *Atelier international sur les bases biologiques de l'aquaculture des Siluriformes* - Montpellier, France, 24-27 mai 1994. Editions du CEMAGREF, 60.

Sumastri S., Priyadi A., & Rusmaedi (1994) Breeding Technique of *Clarias melanoderma. Penelitian Perikanan Darat*, Sukamandi, Indonésie, **12** (2), 17-20.

Tambasen-Cheong Ma V.P., Tan-Fermin J.D., Garcia L.M.B. & Baldevarona R.B. (1995) Milt-egg ratio in artificial fertilisation of the Asian freshwater catfish, *Clarias macrocephalus*, injected salmon gonadotropin-releasing hormon analogue and domperidone. *Aquat. Living. Resour.*, **8** (4), 303-307.

Tarnchalanukit W. (1986) Experimental hybridisation between catfishes of the family Clariidae and Pangasiidae in Thailand. *Enviromental Biology of Fishes*, **16**, 317-320

Teugels G.G. (1986) A systematic revision of the African species of the genus *Clarias* (Pisces, Clariidae). *Annales du Musée Royal de l'Afrique Centrale*, **247**, 1-99.

Teugels G.G. (1996) Taxinomy, phylogeny and biogeography of catfishes (Ostariophysi, Siluroïdei): an overview. *Aquat. Living Resour.*, **9**, Hors série, 9-34.

Verreth J. & Eding E.H. (1993) A Review of Feeding Practises, Growth and Nutritional Physiology in Larvae of the Catfishes *Clarias*

gariepinus and *Clarias batrachus*. *Journal of the World Aquaculture Society*, **24** (2), 135-144.

Zooneveld N., Wilbrink A.C., Soeprijanto A., Viveen W.J.A.R. & Nursalam Y. (1989) Induced spawning of the Asian Catfish (*Clarias batrachus*) by means of HCG. *In*: Hirano R., Hanyu I. (eds), *Proceedings of The Second Asian Fisheries Forum*, 17-22 avril 1989, Tokyo, Japan, 587-590.

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

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

Edited by :
Marc LEGENDRE
Antoine PARISELLE

CONTENTS

PATHOLOGY