FIRST RESULTS ON GROWTH AND ARTIFICIAL PROPAGATION OF *PANGASIUS DJAMBAL* (SILURIFORMES, PANGASIIDAE) IN INDONESIA

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Abstract

Observations on growth rate and occurrence of first sexual maturity were done on a population of 75 *Pangasius djambal* originally caught in the wild, individually tagged, and reared in 200 m^2 ponds at a stocking density of 0.4-0.5 fish.m^{-2} at the Sukamandi research centre (Java). These fish, weighing initially between 190 and 1100 g were fed with a 35-40% crude protein pelleted feed and followed up during a period of 16 months. Two trials of induced ovulation were also carried out on females from a small stock of a dozen adult fish reared in floating cages at the Danau Teluk fish culture station (Sumatra).

Between June 1997 and October 1998, the mean body weight of fish increased from 555 g to 4162 g, which corresponded to a daily weight gain of 7.4 g.d^{-1}. The highest individual growth rate observed between two successive sampling dates was of 16.5 g.d^{-1}. The mean growth rate of *P. djambal* females (8.9 ± 2.2 g.d^{-1}) was significantly higher than that of males (6.3 ± 1.9 g.d^{-1}) and first sexual maturity occurred several months earlier in males than in females.

Oocyte maturation and ovulation were induced with two successive injections of Ovaprim at 8 h interval, corresponding to a total dose of 0.9 mL.kg^{-1}. Among four females treated, three ovulated and could be stripped. Mean hatching rates obtained after artificial fertilisation of eggs varied between 8 and 31%. After 27 days of age, the survival rate of larvae fed live *Artemia* nauplii then dried feed was 61%. No cannibalism was observed during the larval rearing which did not appear as a critical phase of the breeding cycle.

Although preliminary, these results confirm the great potential interest of using *P. djambal* for aquaculture. The induced breeding and larval rearing carried out for the first time in this species represent a breakthrough in the control of its biological cycle in captivity.

INTRODUCTION

Catfishes of the family Pangasiidae are of great economic importance in Indonesia. Although 13 pangasiid species were reported to belong to the local ichthyofauna (see Roberts & Vidthayanon, 1991 and Pouyaud *et al.*, 1999), their biology and potential for aquaculture remain largely unknown. Nowadays, *Pangasius hypophthalmus* Sauvage, 1878, initially introduced to Indonesia from Thailand in 1972, remains the only pangasiid catfish produced in aquaculture in this country.

Among the local pangasiids, *Pangasius djambal* Bleeker, 1846 is one of the fish species most appreciated by consumers in Sumatra and other Indonesian areas. It reaches large size with individual body weight of more than 20 kg (unpublished data). However, up to now its culture has not been possible due to the lack of fry. In this context, the control of its reproduction in captivity represents a strategic goal. Contrarily to the statement of Roberts and Vidthayanon (1991, p. 98), *P. djambal* has never been utilised in aquaculture so far. This confusion may result from the fact that "jambal" is a common name given in Indonesian language to several *Pangasius*.

As a part of a programme of evaluation of the potential of autochthonous pangasiid species for aquaculture, this paper presents a preliminary assessment of growth performance and sexual maturation of *P. djambal* in culture conditions. The first success of hormonal induced ovulation, artificial fertilisation and larval rearing is also reported for this species.

MATERIAL AND METHODS

Origin of fish

Between March and May 1997, a captive stock of *P. djambal* has been constituted. The wild fish, captured by fishermen in the Indra Giri River (Riau province, Sumatra), were firstly stocked in floating cages in the river area of capture and were then transferred by car (about 8 h transportation) to the Sungai Gelam station (DGF-Loka) in Jambi (Sumatra) where they were adapted to pond environment during 2 to 4 weeks. In June 1997, a part of these fish remained at the Sungai Gelam station, while 75 individuals weighing between 190 g and 1100 g (mean body weight of 555 g) were transferred by plane and car to the Sukamandi research centre of RIFF (Java Island) (about 15 h transportation) to serve as future experimental broodstock. Fish transportation was carried out in plastic bags, under oxygen atmosphere, using a specifically adapted packing technique avoiding bags to be cut by the sharp pectoral and dorsal spines of fish (Pouyaud & Sudarto, in prep.). This technique was fully satisfactory and 100% of the fish remained alive after transportation. Based on growth rate observed subsequently in culture conditions they were estimated to be 0.5-1.5 years old.

Besides the fish stocks constituted at Sukamandi and Sungai Gelam, a dozen of older *P. djambal* caught from the wild 4 years ago were held at the Danau Teluk fish culture station (Dinas Perikanan, UPPPU) in Jambi. By courtesy of Dinas Perikanan Provinsi Jambi, these adult fish of 2-5 kg body weight and about 5 years of age, never reproduced so far, were used for induced spawning trials. The species identification of these fish was confirmed after genetic analysis (isoenzymes polymorphism) of their descendants obtained from the artificial propagation reported here (Pouyaud, pers. comm.).

Rearing conditions and sampling

At Sukamandi, between June 1997 and July 1998, the fishes were placed in two 200 m^2 ponds in mixed culture with *Pangasius nasutus*, at a total stocking density of 0.4-0.5 fish.m^{-2}. In July 1998, the 75 *P. djambal* were grouped in a same 200 m^2 pond and reared in monoculture. They were fed during the whole period with a 35-40% crude protein pelleted feed, distributed two time per day and six days a week at a daily ration decreased progressively from 2% to 1% of fish biomass.

In January 1998, each fish was implanted with a P.I.T. tag (Fish Eagle ©) in order to allow individual identification. From this moment and then every three months, all individuals were anaesthetised in a bath of 0.3 $mL.L^{-1}$ phenoxy-2-ethanol, measured for their standard length, weighed using an electronic balance (± 1 g) and examined for their sexual maturity.

No external characteristics allowed for distinction of sexes. Males were identified only when sexually mature by emission of sperm upon hand-pressure onto the abdomen and females when oocytes could be sampled by intra-ovarian biopsy. Measurements of oocyte diameter were carried out using binocular microscope (x 25) equipped with a micrometer.

At Danau Teluk, the broodfish were reared together with brooders of *P. hypophthalmus* in floating cages implanted in a lake connected to the Batang Hari River system. They were fed with various commercial pelleted feeds containing 25 to 40% crude protein. The site is characterised by important seasonal changes in water depth (± 8 m) and water quality, with periods of low oxygen concentration (Rusli Yulidar, pers. comm.).

Artificial propagation

Two trials of induced spawning were carried out at Danau Teluk in November 1997 and February 1998. Three females in the first case and one in the second, found with oocytes at an advanced stage of vitellogenesis after intra-ovarian biopsy, received hormonal treatment to induce ovulation. In November, the females were treated with two successive injections of Ovaprim done at 8 h interval and respective doses of 0.3 and 0.6 $mL.kg^{-1}$. In February, the female received two priming injections of hCG (500 $IU.kg^{-1}$) at 24 h interval and, 24 h after the last hCG injection, the same treatment with Ovaprim as in November was applied. At each injection time and then every 2-3 h after the second Ovaprim injection (in November fish were checked only 8 h after second injection), a sample of oocytes was taken by intra-ovarian biopsy to follow the evolution of oocyte diameter and maturation. The position and state of germinal vesicle was assessed after fixation of a sub-sample of oocytes in Serra's solution (60% ethanol, 30% formalin, 10% acetic acid). The males received a single Ovaprim injection (0.4 $mL.kg^{-1}$) given at the same time as the second Ovaprim injection of females. The sperm collected by stripping was diluted in a 9 $g.l^{-1}$ NaCl solution

in order to prevent its activation due to possible mix with urine. After ovulation, the stripped eggs were fertilised with sperm mixed from 3 males in November and sperm from one male in February. The procedures of artificial fertilisation, egg incubation and estimation of hatching percentages were the same as those previously described by Legendre (1986) for the African catfish, *Heterobranchus longifilis*.

On the day of hatching, a group of 350 larvae was transferred from Danau Teluk to the Sukamandi research center. During the first 3 weeks, they were reared in two 40 L aquarium in stagnant water changed every day by 50%, and fed in excess with live *Artemia* nauplii. They were then transferred to 80 L aquarium and progressively weaned to a 40% crude protein dried feed distributed ad libitum. Every week the fry were totally counted and twenty fish individually weighed (± 0.1 mg).

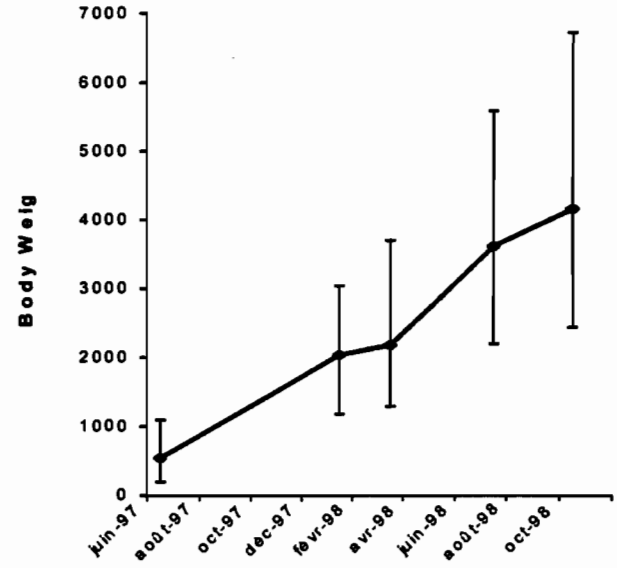
RESULTS

Survival and growth rates

The survival rate at the end of the whole period of observation was still of 100%.

Between each sampling dates, the observed growth rates were equivalent for the *P. djambal* held in the two ponds, therefore the data were pooled for presentation. The corresponding growth curve for the 16-months period of observation is given in Figure 1. During this period, from June 1997 to October 1998, the mean body weight increased from 555 g to 4162 g which corresponded to a daily weight gain of 7.4 $g.d^{-1}$. A mean growth rate of 6.3 $g.d^{-1}$ was also observed over a 5 months period for *P. djambal* in the ponds of the DGF-Loka station in Jambi (Maskur, pers. comm.). It should be noticed for comparison that the mean growth rate of *Pangasius hypophthalmus* observed in similar culture conditions at the Sukamandi station is generally around 5 $g.d^{-1}$ (unpublished data).

Electronic tagging of all fishes allowed to estimate growth variations between individuals. The minimal and maximal individual growth rates observed during the 9 months period from January to October 1998 were of 2.6 $g.d^{-1}$ and 12.8 $g.d^{-1}$, respectively. The highest individual growth rates found between two successive sampling dates were 14.9 $g.d^{-1}$ in male and 16.5 $g.d^{-1}$ in female fish.



Vertical bars indicate range between the smallest and biggest individuals within the population of 75 fish.

Figure 1: Growth of *Pangasius djambal* cultivated in pond at a stocking density of 0.4 $fish.m^{-2}$ at the Sukamandi station..

These results illustrate the remarkable growth potential of this species.

Individual identification of all fishes also permitted to test for a possible growth difference related to sex. Individual daily weight gain calculated between January and October 1998 indicated a significantly faster growth in females (8.9 ± 2.2 $g.d^{-1}$) than in males (6.3 ± 1.9 $g.d^{-1}$) (Table 1).

First sexual maturity

In October 1998, a total of 38 males and 19 females could be identified in the population of 75 fish, while sex of 18 individuals remained undetermined.

Sixteen fluent males were found as early as January 1998 in the population at an estimated age of about 1-2 years. Supplementary males that could be identified by sperm emission were found at each sampling examination: 3 in March, 12 in July and 7 in October. On this latter month, 36 males (95%) showed abundant sperm production while only 2 were not fluent, indicating an increased sexual activity at this period. By contrast the number of females that could be identified for the first time by intra-ovarian biopsy showed a slower evolution: 4 in January, 4 in March, 4 in July and 7 in October. It is only during this latter sampling that some females (21%) were found with developing oocytes of 0.68-1.28 mm maximum diameter. In

	MALE (n = 38)	FEMALE (n = 19)
Body weight at tagging (g) (08/01/1998)	2148 ± 428 [a] [1420 – 2972]	2159 ± 465 [a] [1535 – 3040]
Final body weight (g) (23/10/1998)	3929 ± 760 [a] [2436 – 5758]	4622 ± 983 [b] [2862 – 6720]
Daily weight gain ($g.d^{-1}$) (between January and October 1998)	6.3 ± 1.9 [a] [2.6 – 10.4]	8.9 ± 2.2 [b] [3.2 – 12.8]

Figures with same superscripts in the same line are not significantly different ($p<0.05$).
Mean ± sd ; [-] : extreme values

Table 1: Growth of males and females of *Pangasius djambal* in pond.

all other situations, oocyte diameter never exceeded 0.2-0.3 mm.

These results clearly indicate that sexual maturity occurs several months earlier in males than in females. If the development of gonads is at the expense of somatic tissue, then the early maturity of male could be responsible, at least in part, for their lower growth rate in comparison to female. An earlier sexual maturity of males was also observed in cultured stocks of *Pangasius hypophthalmus*. In this species, males were already fully mature (presence of intra-testicular sperm) at the age of 10 months and a mean body weight of 472 ± 78 g, while the first females with ovaries containing post-vitellogenic oocytes were observed at an age of 19 months and a mean body weight of 2249 ± 279 g (unpublished data).

Artificial propagation

From the broodfish stocked in floating cages at Danau Teluk, Three females in November 1997 and one in February 1998 were found with oocytes at an advanced stage of vitellogenesis after intra-ovarian biopsy. The mean oocyte diameter before hormonal treatment is given for each female in Table 2. After Ovaprim injections, three of these females ovulated and could be stripped. Mean ova diameter was of 1.8, 1.9 and 1.8 mm for the three females, respectively. Examination of oocytes sampled by intra-ovarian biopsy and fixed in Serra's solution after the second Ovaprim injection, indicated that only oocytes of size equal or superior to 1.55-1.60 mm reached the stage of germinal vesicle breakdown and ovulated. As also reported for *Pangasius bocourti* (Cacot, 1999), oocytes smaller than this threshold did not respond to hormonal treatment in *P. djambal*. As a matter of fact, the smallest diameter of ova found within the population of ova collected by stripping was of 1.64, 1.68 and 1.64 mm for the three females, respectively. Oocytes of the female that did not ovulate were the smallest compared to other females used (Table 2) and probably not fully achieved their vitellogenesis at the moment of experiment.

The weight of eggs collected could not be determined due to absence of appropriate balance, it was estimated to approximately 20 g, 200 g and 6 g for the three females, respectively. Mean hatching rates obtained after artificial fertilisation varied between 8 and 31% (Table 2). Depending on females and experiments, this rather low egg quality and quantity could be attributed either to inappropriate latency time between injection and egg collection, incomplete gonad development or unsuitable rearing conditions of broodstocks. Obviously, further investigations have to be done to define seasonal variations of sexual activity, and optimal conditions for broodstock management and induced breeding in this species.

Fry produced in November 1997 were reared at the Danau Teluk station but all died after 3-6 weeks of age as a result of disease outbreak due to *Ichthyophthirius* infection (Rusli Yulidar, pers. comm.). The larvae obtained in February 1998 were reared in aquarium after transfer at the Sukamandi station where first observations on their development (Table 3) and behaviour could be performed. After 27 days of age, the survival rate of fry was of 61%. It was still of 60% after two months of rearing. In contrast to the situation prevailing in *P. hypophthalmus* (Subagja *et al.*, 1999) and similarly to what is known from *P. bocourti* (Hung *et al.* 1999), no cannibalism was observed in *P. djambal* during the larval rearing which did not appear as a critical phase of the breeding cycle.

Several other biological characteristics of *P. djambal*, such as size of ova and larvae (Table 3), appear to be very similar to those of *P. bocourti* (see Cacot, 1999; Hung *et al.*, 1999). These two species are genetically closely related (Pouyaud *et al.*, 1999) and similar morphologically, differing mostly by the number of rakers on first gill arch

Date	Water temperature (°C)	Female body weight (g)	Initial oocyte diameter (mm)	Latency time after 2nd Ovaprim injection	Number of egg collected	Hatching rate (%)
November 97	30-31	4300	1.58	No ovulation		
November 97	30-31	4200	1.68	12	-	22.0
November 97	30-31	4100	1.84	8	-	7.8
February 98	30-32	1900	1.66	6	5200	31.0

Table 2: Ovulation success, number of egg collected and hatching rate obtained during the first trials of induced-ovulation of *Pangasius djambal*.

Mean ova diameter before fertilisation	1.8 ± 0.1 mm (a)
Mean weight of ova	2.95 mg (b)
Range of incubation duration at 27-30°C	29-36 hours
Mean total length of larvae at hatching	4.7 ± 0.2 mm (a)
Duration of yolk sac absorption at 28-29°C	3 days
Mean total length of larvae at first feeding	8.6 ± 0.3 mm (a)
Mean body weight of larvae at first feeding	4.1 mg (b)
Mean body weight of fry at 27 days of age	607 ± 304 mg (a)
Survival rate after 27 days from hatching	61%

(a) Mean ± sd ; (b) Mean from global weight of 100 eggs and 10 larvae

Table 3: Size of ova, duration of egg incubation and growth and survival of *Pangasius djambal* fry obtained from induced-breeding trial of February 1998.

(Roberts & Vidthayanon, 1991). Both of them possess the biggest eggs and larvae reported so far among pangasiid species. Induced breeding of a local pangasiid, called "*Pangasius pangasius*", was previously reported in Sumatra (Indonesia) by Meenakarn, 1986 and Arifin, 1987. From the results of the Catfish Asia project (Pouyaud *et al.*, 1999, and unpublished data), it is now confirmed that this fish was misidentified and could not be *P. pangasius*. However, as the specimens used by these authors or descendants of these fishes could not be found for identification, it is impossible to precise the correct name of the species they used. It seems clear, however, that it could not be *P. djambal* when looking at the biological data given in these two papers.

Meenakarn (1986) reported fecundity of 100,000-130,000 egg per female kg and an incubation period of 40-44 h at 27-30°C. Such a fecundity with the eggs of *P. djambal* (mean egg weight of about 3 mg) would represent an unrealistic gonado-somatic index of 30-40%. Also, the duration of egg incubation observed in *P. djambal* was shorter at a same temperature (Table 3). Arifin (1987) reported that the size of ova of "*P. pangasius*" was 1.4-1.6 mm for a weight of 2.0-2.2 mg and that cannibalism displayed by the larvae could explain their low survival rate. However, from our data, *Pangasius djambal* presents bigger ova (by 30% in weight) and the larvae do not show cannibalistic behaviour.

CONCLUSION

The good adaptation of *Pangasius djambal* to pond environment, as well as its resistance to handling, high growth rate and ability to become sexually mature in captivity, confirm the great potential interest of this species for aquaculture. So far, the culture of this catfish which is among the *Pangasius* most appreciated by consumers in Indonesia, has not been possible due to lack of fry.

The feasibility of fry production from captive broodstock has been demonstrated in this study. Although preliminary, the present results represent an important breakthrough: it is the first time that induced ovulation, artificial fertilisation and larval rearing of *P. djambal* are performed successfully. The limited number of mature broodfish available

on fish farms is currently the main constraint for the start of its production in aquaculture.

ACKNOWLEDGEMENT

The authors are grateful to Laurent Pouyaud (ORSTOM) and Sudarto (RIFF) for providing the wild living specimens of *Pangasius djambal* followed up for growth and sexual maturity at the Sukamandi station. Special thanks are also addressed to Ir. Maskur, Chief of Loka BAT station, for its precious and constant collaboration ; Ir. H. Ali Supardan, Chief of Dinas Perikanan Provinci Jambi, for authorising reproduction experiments with fish held at the Danau Teluk UPPPU station and Mr. Rusli Yulidar for his assistance during these experiments.

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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
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