ABSENCE OF MARKED SEASONAL VARIATIONS IN SEXUAL MATURITY OF *PANGASIUS HYPOPHTHALMUS* BROODERS HELD IN PONDS AT THE SUKAMANDI STATION (JAVA, INDONESIA).

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Abstract

An evaluation of the seasonal variations of sexual activity of *P. hypophthalmus* broodstock held in 50 m^2 ponds was carried out between April 1997 and April 1998 at the Sukamandi station (West Java, 6°S of latitude). These variations were assessed through the evolution of oocyte diameter of individual females, the proportion of fluent males and the success of hormonal-induced breeding trials.

High proportions of fluent males and mature females with post-vitellogenic oocytes of modal diameter superior to 1.00 mm were found at anytime. As a consequence, successful induced breeding of this species using hormonal treatments could be performed regardless of season and allowed an all year long production of fry. This continuous sexual maturity of *P. hypophthalmus* broodstock, during both the dry and rainy seasons, may have resulted to a large extent from rather stable conditions, with a monthly mean water temperature constantly high (28-31°C) and a low variation of day length during the year. Ovulation was successfully induced at 3, 2 and 4 months interval in a same *P. hypophthalmus* female. This indicated a quick recovery of oogenesis in females reared in the ponds.

INTRODUCTION

Pangasius hypophthalmus (Sauvage, 1878) (senior synonym of *P. sutchi*, Roberts and Vidthayanon, 1991) is an important economic freshwater species in South East Asia. The complete breeding cycle in captivity of this pangasiid catfish originating from the Mekong and Chao Phraya River Basins, was first obtained in Thailand about 30 years ago (Potaros & Sitasit, 1976). Since that time, the fish has been introduced to other areas where its use in aquaculture has progressively gained in importance. In Indonesia, the species was successfully induced to breed for the first time at the beginning of the eighties, after it was introduced in this country from Thailand in 1972 (Hardjamulia *et al.*, 1981).

Despite the economic interest of *P. hypophthalmus*, published data related to its biology and culture are still scarce. In particular, it was rather surprising that detailed study of the reproductive cycle of this catfish could not be found in the literature, either for wild or captive stocks. Nevertheless, Potaros and Sitasit (1976) mentioned that the spawning season of *P. hypophthalmus* was between June and September in the Thai climatic conditions, while Saidin and Othman (1986) reported broodstock to be mature from June to December-January in Malaysian ponds. In Indonesia, Hardjamulia *et al.* (1981) also reported that *P. hypophthalmus* females from a broodstock held in 18 m^2 tanks were sexually mature during a limited period of the year, corresponding mainly to the rainy season, from October to April. However, we observed recently the occurrence of mature *P. hypophthalmus* of both sexes even during the dry season in the ponds of the Sukamandi station located in West Java.

It was therefore decided to start an evaluation of the seasonal variations of sexual activity of *P. hypophthalmus* broodstock held in pond conditions. These variations were assessed through

the evolution of oocyte diameter of individual females, the proportion of fluent males and the success of hormonal-induced breeding trials. The demand for *Pangasius* seeds is high in Indonesia and it would be of major importance to be able to produce them all year round.

MATERIAL AND METHODS

The ponds experiments were carried out at the Sukamandi Station of the Research Institute for Freshwater Fisheries (Indonesia). Sukamandi is located in the Western part of the Java Island, at 6° S of latitude. In this area, two climatic seasons are generally distinguished: a rainy season from October to April and a dry season from May to September. The range of yearly variation of day length is about 30 minutes.

Fish origin and maintenance

The *P. hypophthalmus* brooders used in this study descended from the fish initially introduced from Thailand in 1972 and were 3-4-years-old and 2 to 5 kg individual body weight. Seasonal variations of sexual activity were followed up on broodstock of equilibrated sex ratio (1:1) reared at a stocking density of 0.3 fish.m^{-2} in two replicated 50 m^2 ponds. The fish were fed two times per day, 6 days a week, with a 35% crude protein pelleted feed distributed at a daily rate of 1-1.5% of total biomass. All fish were tagged using PIT tags (passive inductive transponder, Fish Eagle©) in order to allow their individual identification. Supplementary groups of *P. hypophthalmus* brooders were also maintained in other ponds of the station with equivalent rearing conditions, except for a higher stocking density (0.6 fish.m^{-2}). Fish from these latter groups were used for a part in the induced breeding trials.

Seasonal changes in sexual maturity

The sexual state of broodfish was checked monthly between April 1997 and April 1998. In order to follow the vitellogenesis stage without killing the females, fish were anaesthetised in a 0.3 mL.L^{-1} phenoxy-2-ethanol solution and a sample of oocytes was taken by intraovarian biopsy using a polypropylene tube of 3.5 mm external diameter (Pipelle de Cornier™). The diameters of 30 to 50 oocytes per sampled fish were measured with a micrometer using a binocular microscope (x25). The modal oocyte diameter was used as the main criterion of female sexual maturity (Legendre, 1986). The sexual stage of males was determined by the possibility of obtaining semen after gentle stripping.

All along the study, the daily fluctuations of pond water temperature were registered continuously with electronic thermometers (Stowaway Optic Prosensor©). Rainfalls were measured daily with a pluviometer implemented nearby the ponds.

Induced oocyte maturation and ovulation

Between December 1996 and April 1998, 54 *P. hypophthalmus* females were treated at different period of the year with Ovaprim© (n=50) or human chorionic gonadotropin (hCG; n=4) for inducement of oocyte maturation and ovulation. The treated females were chosen after intraovarian biopsy on the basis of a diameter of their oocytes greater than 1.0 mm. They received two successive hormone injections at 8 h interval with corresponding doses of 0.3 ml.kg^{-1} then 0.6 ml.kg^{-1} female BW with Ovaprim, and 500 IU.kg^{-1} then 2,000 IU.kg^{-1} with hCG (Legendre *et al.*, 1999; Cacot, 1999). Selected males were producing milt at stripping, they received a single Ovaprim injection of 0.3-0.4 ml.kg^{-1} applied at the moment of first injection of females.

When ovulation was detected, ova were collected by stripping, weighed and immediately fertilised. A sample of ova was also weighed to the nearest mg and fixed in 5% formalin for subsequent counting and total fecundity estimates. The sperm was collected by stripping directly in a syringe containing a 0.9% NaCl solution (dilution rate of 1/5), then preserved at 5°C for a maximum period of 3 hours before being used for fertilisation.

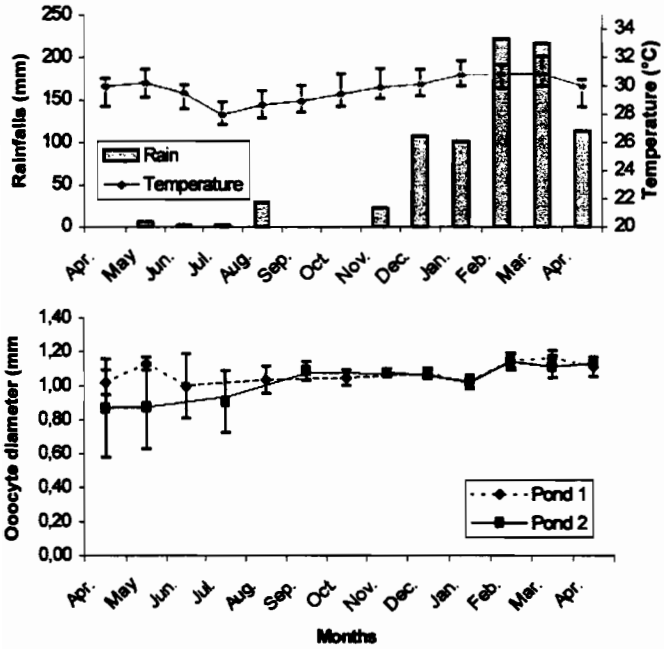
For each female the quality of ova was assessed by hatching rates obtained in replicated batches of 200-300 eggs fertilised with 0.2 ml of diluted sperm. Spermatozoa activation was obtained by addition of 10 ml freshwater. After 1min of gentle stirring, eggs were rinsed to remove excess milt and transferred for incubation in a plastic recipient containing 300 ml of standing water at ambient temperature (27-30°C). Hatching ended after 26-29 h of incubation, and the hatching rates were evaluated 35-40 h after fertilisation.

RESULTS

Seasonal changes in sexual maturity

The follow up carried out between April 1997 and April 1998 clearly showed that sexually mature *P. hypophthalmus* could be found all year round in the ponds of the Sukamandi station.

The yearly range of variation of mean oocyte diameters were between 1.00 - 1.16 mm, and 0.89 - 1.14 mm, in females of the two fish populations reared in 50 m^2 ponds (Fig. 1). A greater variability of oocyte diameter was observed between individuals in fish from the two ponds in June-July. The monthly proportion of females with post-vitellogenic oocytes of diameter greater than 1.00 mm was comprised between 57 and 100%, and 75 and 100%, in the two ponds respectively. The lowest values (57 and 75%) were observed in June-July. This period corresponded to a slight lowering of water temperature (reaching a minimal mean value of 27.9°C in July) and very low rainfalls (Fig. 1). It is to be noted that despite a total absence of rain, the proportion of mature females (oocyte diameter greater than 1.00 mm) was more than 85% in September-October 1997.



(*): no data.
Monthly mean temperature ± mean of daily minimum and maximum.

Figure 1: Seasonal evolution of rainfalls and mean water temperature, and mean (± sd) oocyte diameters of two groups of *P. hypophthalmus* females reared in ponds at the Sukamandi station between April 1997 and April 1998.

Similarly to females, fluent males were observed at anytime. The proportion of mature males (giving milt at stripping) was greater than 78% at all sampling dates, except in June-July. During these latter months, the proportion of fluent males dropped down to minimal values of 38 and 50% in the two ponds, respectively. However, several individuals were constantly observed at a fluent stage at all sampling performed.

Induced oocyte maturation and ovulation

In total, 54 *P. hypophthalmus* females were used for induced breeding trials between December 1996 and April 1998. The ovulation rate, quantity of egg collected by stripping and hatching percentages obtained for these fishes are presented in Table 1 as a function of the month at which trials were performed.

Successful induced ovulation could be obtained all year long and, among the 54 fish treated, only 7 did not respond to the hormonal treatment. The monthly means of fecundity and hatching rates varied between 63,000 and 226,000 eggs per kg body weight and between 47 and 97%, respectively. Despite the low number of fish examined in some months, it appeared clearly that ova and sperm of good quality could be obtained at all period of the year, as indicated by observation of individual hatching rates greater than 70% at anytime (Table 1).

Moreover, several females of the *P. hypophthalmus* broodstock were successfully induced to ovulate 2 to 4 times successively at a few months interval. As an example, ovulation was induced 4 times successively in the same female at 2-4 months interval between July 1997 and March 1998. No detrimental effect was found of such successive induced breeding treatments on the quantity and quality of eggs produced (Table 2).

DISCUSSION

The present study demonstrated that high proportions of fluent males and mature females with post-vitellogenic oocytes of modal diameter superior to 1.00 mm can be found at anytime in the *P. hypophthalmus* broodstock held in ponds at Sukamandi. As a consequence, successful induced breeding of this species using hormonal treatments could be performed regardless of season and

	January	February	March	April	May	June	July	August	September	October	November	December
Nb of female treated	6	2	8	8	2	2	2	0	2	3	2	17
Female BW (kg)	3.6 [3.5-3.7]	3.2 [3.1-3.3]	2.7 [2.3-4.0]	3.4 [2.3-4.2]	3.2 [3.0-3.4]	3.4 [3.2-3.5]	3.1 [2.6-3.6]	-	3.3 [3.1-3.5]	3.6 [3.4-3.8]	4.5 [3.3-5.8]	3.1 [2.2-4.5]
Oocyte diameter (mm)	1.15 [1.12-1.16]	1.14 [1.12-1.16]	1.19 [1.16-1.20]	1.15 [1.12-1.20]	1.17 [1.16-1.18]	1.18 [1.16-1.20]	1.16 [1.16-1.16]	-	1.14 [1.12-1.16]	1.12 [1.12-1.12]	1.08 [1.08-1.08]	1.08 [1.00-1.16]
Ovulation (%)	50	100	87.5	62.5	100	100	100	-	100	100	100 (*)	100 (*)
Relative fecundity (egg.kg-1) x 1000	201 [126-295] (3)	ND	180 [62-269] (6)	225 [125-322] (5)	95 (1)	ND	130 [108-153] (2)	-	226 [180-272] (2)	184 [148-231] (3)	63 (1)	87 [28-204] (9)
Hatching (%)	56.8 [30.2-83.3] (2)	ND	51.5 [21.3-81.6] (2)	67.2 [25.4-90.3] (3)	46.9 [21.6-72.2] (2)	95.5 (1)	96.9 [95.5-98.2] (2)	-	87.0 [83.0-91.0] (2)	79.7 (1)	74.0 (1)	88.0 [0.0-95.0] (8)

[ ] : Extreme values
( ) : Number of observations
(*) : Ovulation was partial in 1 of 2 females in November and in 5 of 17 females in December; data obtained for these 6 females are not included in analyses of fecundity and hatching rate.

Table 1: Monthly repartition of number of *P. hypophthalmus* female induced to ovulate by hormonal treatment (between Decembre 1996 and April 1998) at the Sukamandi station; with corresponding mean female body weight, mean oocyte diameter before treatment, percentage of ovulation, mean relative number of egg collected and mean hatching rate obtained.

Female n°	Date	Hormonal treatment	Body weight (g)	Initial oocyte diameter (mm)	Relative fecundity ($egg.kg^{-1}$) x 1000	Hatching rate (%)	fraction of deformed larvae (%)
1	01/07/97	Ovaprim	3600	1.16	153	98.2	3.7
1	04/10/97	Ovaprim	3750	1.12	175	3.0	-
1	02/12/97	hCG	3500	1.16	37	72.0	15.3
1	27/03/98	Ovaprim	4024	1.20	269	81.6	6.4
2	02/12/97	hCG	2600	1.08	204	84.0	9.5
2	14/04/98	Ovaprim	3160	1.18	322	90.3	3.2

Table 2: Number of egg collected, hatching rate and fraction of deformed larvae (deformed larvae/total hatched) after repeated induced ovulation in two *Pangasius hypophthalmus* females reared in pond at the Sukamandi station and individually identified by their PIT tag.

allowed an all year long production of fry.

It is the first time that a continuous sexual activity is reported in this species, either in wild or captive conditions. All previous reports on the breeding cycle of *P. hypophthalmus* in Thailand, Malaysia or Indonesia, concluded to a reproductive season limited to 4-8 months in the year (Potaros & Sitasit, 1976; Saidin & Othman, 1986; Hardjamulia *et al.*, 1981). Unfortunately the range of variation of environmental factors, such as temperature, was not given in these papers. Recently, a study carried out on broodstock cultivated in ponds or floating cages in Vietnam also showed a clearly marked spawning period, restricted to March-September for both males and females (see Cacot, 1999). In Vietnam (Mekong Delta), this period corresponded to the end of the dry season and the beginning of the rainy season, and was associated to the highest water temperature (28-31°C) and longest day length in the year.

In the present study, the continuous sexual maturity of *P. hypophthalmus* broodstock, during both the dry and rainy seasons, may have resulted to a large extent from rather stable conditions with a monthly mean water temperature constantly high (28-31°C) and a low variation of day length during the year. The range of variation of day length is narrower in Java Island (half an hour) than in the Mekong Delta (1h15).

The fact that ovulation could be successfully induced at 3, 2 and 4 months interval in the same *P. hypophthalmus* female, also shown a quick recovery of oogenesis of fish reared in the ponds. The absence of negative effects of such repeated treatments on fecundity or hatching rates indicated the completeness of oogenesis during these short periods. However, the minimal period of time between two successive induced ovulations remains to be assessed in this species. In the African catfish *Clarias gariepinus*, a same female can be stripped every 6-8 weeks when maintained at 25°C (Hogendoorn & Vismans, 1980). In *Heterobranchus longifilis*, an other African clariid, Nunez-Rodriguez *et al.* (1995) reported that a new reproductive cycle can be achieved in less than one month after an hCG-induced ovulation.

Although mature *P. hypophthalmus* males and females were found all year long at the Sukamandi station, the sexual maturity tended to be reduced in June-July. This was seen at the population level by a decrease in the proportion of fluent males and mature females, as well as a wider variation of oocyte diameters between individuals. It should be noted that the months of June-July corresponded to a slight drop down of water temperature and to the period of shortest day length in the year. However, some individuals were systematically at an advanced stage of sexual maturity (fluent males and females with post-vitellogenic oocytes) at each time they were observed during the monthly checking. No clear trend of cyclic changes in fish fecundity or hatching rates were observed in relation to season. Nevertheless, due to the low number of fish induced to spawn in certain months, supplementary observations remain necessary before a conclusion could be drawn for these traits. A continuous reproduction together with seasonal variations of fecundity were already reported for *H. longifilis* cultivated in lagoon enclosures (Legendre, 1986).

Complementary investigations are currently carried out at the Sukamandi station in order to assess more precisely the seasonal variations in the intensity of the sexual activity of *P. hypophthalmus* brooders.

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

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