SEASONAL CHANGES IN SEXUAL MATURITY AND FECUNDITY, AND HCG-INDUCED BREEDING OF THE CATFISH, *HETEROBRANCHUS LONGIFILIS* VAL. (CLARIIDAE), REARED IN EBRIE LAGOON (IVORY COAST)

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ABSTRACT

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.

Oocyte maturation and ovulation were induced with human chorionic gonadotropin (HCG) in the African catfish, *Heterobranchus longifilis*. Females with a mean oocyte diameter of at least 1.1 mm were used. 100% ovulation was obtained after a single intramuscular HCG injection of between 1.0 and 2.5 I.U./g body weight; a lower dose led to high variability in individual response. Latency time depended more on temperature than on the hormone dose. Eggs could be stripped within 14 h of a dose of 1.5 I.U./g of HCG, at a temperature of 27 to 29°C. After stripping, most of the eggs were fertilized, and high percentages of normal larvae (76% ± 8) were obtained after hatching. Fish hatched in captivity and becoming sexually mature at 1 year of age (mean weight 1.5 kg) were, in turn, artificially reproduced.

Seasonal changes in oocyte diameter (determined by periodic intraovarian biopsy of brooders reared in lagoon enclosures) showed a clear decrease in sexual activity at the beginning of the dry season (December) which seemed to be related to higher water temperature. However, even at that time, good quality eggs were obtained after HCG injection. Thus, eggs and fry could be produced all year round, although the mean quantity of eggs collected per female kg proved to be much less at the beginning of the dry season (26 000 ± 14 000) than during the rainy season (68 000 ± 13 000).

INTRODUCTION

The general potential of Clariidae in aquaculture was initially demonstrated in the Central African region by Micha (1973). Since then, work dealing with the biology and culture of *Clarias*, especially *C. gariepinus*, has been carried out (see reviews by Richter, 1976; Bruton, 1979; Hogendoorn, 1983; Legendre and Jalabert, 1986), whereas no further work was undertaken with species of the genus *Heterobranchus*, despite initial indications of fast growth and a frequently mentioned potential interest for aquaculture

(Awachie, 1976; Bard et al., 1976; Maletoungou, 1976; Micha and Franck, 1976). It is only recently that grow-out trials, carried out in enclosure poly-culture with tilapias (Legendre, 1983) or in pond monoculture (Dia et al., 1986), have definitely confirmed the great potential value of *Heterobranchus longifilis* for fish culture. Although it is considered as a continental form only rarely found in mixohaline waters (Daget and Iltis, 1965), *H. longifilis* can be cultured in fresh water or brackish water. Its adaptation and growth appeared to be excellent in the lagoon environment, in waters up to $7 \text{ g} \cdot \text{l}^{-1}$ salinity (Legendre, 1983). However, to this day, the lack of fry supply has constituted a major obstacle to the culture of this species in Africa. To over-come this shortage, research has been initiated at the Abidjan Oceanographic Research Centre with a view to developing a reliable method of controlled reproduction allowing mass production of *H. longifilis* eggs and fry.

Successful induced breeding of fish using different substances with direct or indirect gonadotropic action has been reported for several species and has been recently reviewed by Fontaine (1976), Harvey and Hoar (1980) and Lam (1982). Even for fish species which can undergo natural spawning in captivity, induced ovulation followed by artificial fertilization is generally preferred in order to achieve a greater control over fry production. The prob-lem in a number of cases is that the procedures in use still suffer from a lack of standardization and thus may prove difficult to repeat in different con-texts because the various parameters affecting the results are not carefully defined.

The purpose of the current study was to determine the minimal effective dosage of human chorionic gonadotropin (HCG) needed to induce ovulation in *H. longifilis* and the corresponding latency response, and to establish techniques for the selection of brooders, injection, artificial fertilization and egg incubation.

In order to obtain information for broodstock management, parallel in-vestigations were conducted to determine seasonal changes in sexual matu-rity, the weight and age at first maturation and the fecundity of *H. longifilis* reared in the Ebrié Lagoon.

MATERIALS AND METHODS

This study was carried out between February 1984 and July 1985 at the Layo Aquaculture Research Station, located 40 km west of Abidjan on the edge of the Ebrié Lagoon. In the station area, the water characteristics are strongly influenced by the vicinity of the Agneby River estuary in the lagoon (Albaret and Legendre, 1983; Guiral, 1983).

Origin and maintenance of brooders

Two groups of brooders were used; the first was composed of 20 wild-spawned individuals that had penetrated ponds on the station. These fish

were collected and placed in enclosures at a density of 1 fish per 5 m², and held there for 2 years prior to the beginning of the experiments. The second group was composed of individuals hatched and reared in captivity after the induced spawning of some of the females from the first group. These fish were reared in ponds for 10 months after hatching, then transferred to enclosures at a density of 1 fish per 2 m². The fish in this group became sexually mature at 1 year of age (mean weight 1.5 kg).

In both cases, the fish were fed with a 32% protein pelleted feed, distributed 6 days a week with a daily ration of 3% biomass.

Seasonal changes in sexual maturity

Seasonal changes in sexual maturity were followed within the first group of females on the criterion of their mean oocyte diameter. Oocyte diameters were measured with a micrometer using a binocular microscope ($\times 25$) on samples of 30 to 40 oocytes periodically obtained by in vivo intraovarian biopsy with a plastic catheter (external diameter 3 mm, internal diameter 1.5 mm). The induced ovulation trials, carried out at different times of the year, served to corroborate the observed changes. Salinity and surface temperature of the lagoon were measured daily. Water pH ranged between 6.5 and 7.5 during the investigation.

Selection of brooders

One day prior to reproduction, brood fish were taken out of the enclosures in order to evaluate their sexual state. Males, possessing an elongated and pointed genital papilla, were easily separated from females, which have a well-rounded genital papilla. For the reproduction trials, females were selected on the criterion of their mean oocyte diameter determined after intraovarian biopsy. Females with homogeneous-sized oocytes and mean oocyte diameter greater than 1.1 mm were used.

Large males were preferred because a greater quantity of sperm could be collected from them. In all cases, the selected males had a well-developed genital papilla.

Hormonal treatment and assessment of ovulation

HCG was chosen as the ovulating agent because its activity is well standardized; it is also easy to preserve in tropical climates and has the advantage of being more readily available in developing countries.

Six induced breeding experiments were conducted, in March, April, July and December 1984, and in June and July 1985 respectively. HCG efficiency to induce ovulation in *H. longifilis* was first tested with a dose of 2.5 I.U./g body weight. Then the doses were progressively reduced to 0.5 I.U./g body weight to determine the minimal effective dosage. Females,

after being weighed ($W \pm 1$ g), were injected in the dorsal musculature approximately under the separation between adipose and rayed dorsal fins. Injections were given in the evening. The volume injected never exceeded 1 ml in one place. If a larger hormone quantity was necessary, a second injection was given. The fish were manipulated without anaesthetic. After treatment, to avoid aggressive reactions between individuals, the fish were placed separately in aquaria (300 l) where water was permanently renewed by pumping from the lagoon. Water temperature was measured regularly during the latent period. Beginning 6 h post-injection, the fish were checked hourly for ovulation by gentle pressure applied in the antero-posterior direction on the abdomen. After stripping, females from the first brooder group were returned to the enclosures, while females from the second group were sacrificed and the stripping response evaluated by the ratio: weight of eggs stripped/(weight of eggs stripped + weight of the stripped ovary). In this study, the latency time is defined as the interval between injection and stripping.

Artificial fertilization and egg incubation

Sperm could not be obtained from the males by stripping, probably because of the testicular anatomy (Fig. 1) common to clariid species (Legendre and Jalabert, 1986). Thus, it was necessary to sacrifice the males and to dissect the testes for collecting semen. The sperm quantity obtained ranged be-
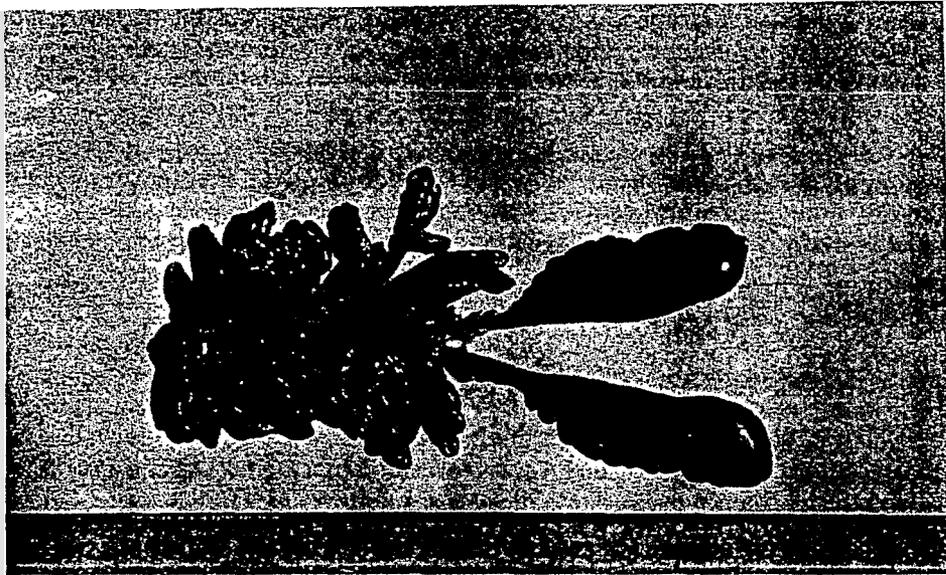

Fig. 1. Testicular anatomy of *H. longifilis*. The testes (on the right) are well individualized from the seminal vesicles (on the left), which are composed of numerous lobes connected to the posterior part of the vas deferens.

tween 0.5 and 5.8 ml depending on the size of the males (between 1200 and 4500 g).

As soon as it was collected, the sperm was diluted in a 0.9% NaCl solution (dilution rate 10^{-1}) because a positive effect of such dilution on fertilizing ability has been observed (unpublished data). Similar findings were also made with *Clarias gariepinus* (Hogendoorn and Vismans, 1980). The quality of the stripped oocytes was evaluated by percentages of normal and deformed larvae obtained after hatching. Aliquots of 200—300 oocytes (two replications per female), previously weighed ($W \pm 0.1$ mg) to estimate the total number of the stripped eggs, were mixed with 0.5 ml of diluted sperm in a dry receiver. Sperm activation was initiated by the addition of 5 ml fresh water. After 1 min of gentle stirring, eggs were rinsed to remove excess milt and transferred for incubation into a plastic box containing 300 ml fresh water. In a given experiment, the eggs collected from all the females were fertilized with the same sperm preparation. Egg incubation was done in stagnant water, at ambient temperature (27 to 29°C) and in darkness. Hatching took place 24 to 28 h after fertilization, and the hatching rates were evaluated 35 to 40 h after fertilization.

RESULTS

Seasonal changes in sexual maturity

The temporal variations in oocyte diameter, maximum and minimum surface water temperatures and salinity were recorded for the 17-months study period (Fig. 2).

There are two distinct periods in salinity fluctuations. The first, from January to May, coincides with the major dry season, with salinities rising above $6 \text{ g} \cdot \text{l}^{-1}$. The second begins in May—June with the first rains; then salinity rapidly decreases and fluctuates around $1 \text{ g} \cdot \text{l}^{-1}$ until December. The small increase in salinity in August and September coincides with the minor dry season.

Similarly, the highest water temperatures are found during the dry season, the lowest during the rainy season. The temperature decrease observed in December and January coincides with the cool north wind (Harmattan).

The seasonal variation in oocyte diameter mirrored that of temperature. The largest diameters (1.5 mm) are found when the water is coolest, from June to October, and a rapid decline in oocyte diameters is noted as water temperature increases during November and December. A larger proportion of oocytes in resorption, as well as more inter-female variability, was noted during the latter period. Both in 1984 and 1985, there was a progressive increase in oocyte diameters during the end of the major dry season (February to May), followed by a new increase with the onset of the rains and when lower water temperature was observed.

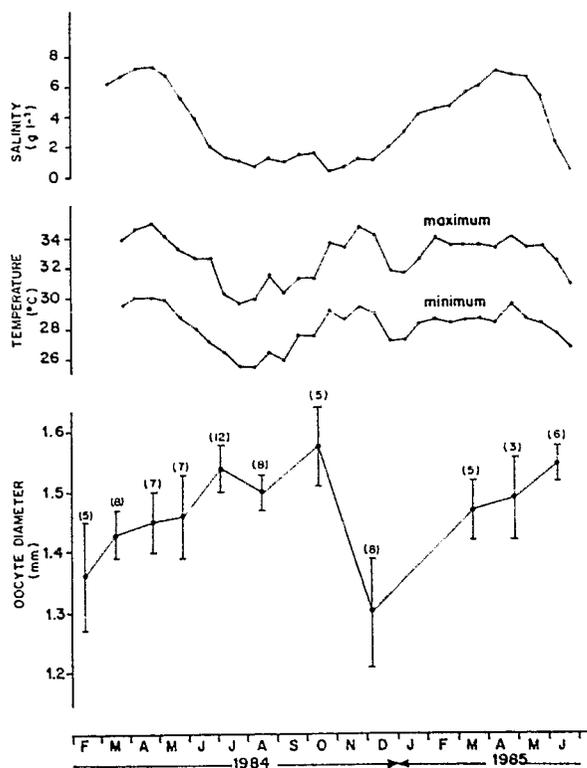The sexual maturity of *H. longifilis* seemed less dependent on salinity

Fig. 2. Seasonal development of mean oocyte diameters of *H. longifilis* females in the Ebrié Lagoon, salinity and surface temperature of the lagoon water. The vertical bars correspond to the confidence interval of the mean diameter at a 5% risk. (+): number of females sampled.

fluctuations: even though oocyte enlargement began with the onset of the rainy season and reduced salinities, the reduction of diameters occurred in December, when salinities were still at their lowest.

Induced oocyte maturation and ovulation: effects of HCG

In the series of experiments run between March 1984 and July 1985, oocyte maturation and ovulation were induced in all the females ($N = 26$) injected with HCG at doses of 1.0, 1.5, 2.0 and 2.5 I.U./g body wt. (Tables 1 to 4). On the contrary, a dose of 0.5 I.U./g body wt. did not induce ovulation in 3 out of 5 females (Tables 3 and 4). No ovulation occurred in placebo-treated females (Table 4).

Under equivalent temperature conditions, the latent period appeared to vary little as a function of the hormone doses given. However, latency time was slightly increased with lower hormone doses, as verified by comparison of the oocyte quantity collected from each female after the first and second

TABLE 1

Quantity of eggs stripped and hatching rate in *H. longifilis*, after injection with 2.5 I.U. HCG/g body wt. in March, April and July 1984. Females (group one) were stripped between 10 and 12 h after injection. During the latent period, water temperatures varied between 27 and 30°C in March and April, and between 27 and 28°C in July

Date	Body weight (g)	Initial oocyte diameter (mm)	No. of eggs stripped per female kg (× 1000)	Normal larvae (%)	Deformed larvae (%)
30 March 1984	1469	1.47	29	91.7	1.7
	1065	1.50	43	—	—
24 April 1984	4120	1.54	40	78.8	10.3
	2072	1.49	44	88.5	7.2
3 July 1984	2420	1.52	56	95.8	1.7
	3721	1.55	67	93.7	2.4

TABLE 2

Effects of different doses of HCG on induced ovulation, quantity of eggs stripped and hatching rate in *H. longifilis* (experiment of 6 December 1984). Females (group one) were stripped 10 h and 15 h after injection. During the latent period, water temperatures varied between 27 and 33°C

Dose (I.U./g body wt.)	Body weight (g)	Initial oocyte diameter (mm)	Weight of eggs stripped (g) 10 h	15 h	No. of eggs stripped per female kg (× 1000)	Normal* larvae (%)	Deformed* larvae (%)
2.5	2480	1.40	183	11	54	89.1	7.3
2.5	1968	1.15	4	1	2	86.1	3.3
2.0	2680	1.28	129	14	43	90.7	6.5
2.0	4668	1.13	58	7	11	86.7	9.2
1.5	3680	1.44	70	21	19	81.8	6.8
1.5	4232	1.25	102	33	20	95.7	3.3
1.0	3072	1.37	95	18	29	91.1	5.3
1.0	4400	1.35	129	52	31	93.7	4.9

*Percentages evaluated after fertilization and incubation of eggs collected 10 h after injection.

strippings (Tables 2 to 4). By contrast a more notable difference was observed between experiments in relation to the seasonal variations in temperature. Latent period was approximately 10 h at 27—33°C (Table 2), 10 to 14 h at 27—29°C (Table 3) and 14 to 18 h at 26—27°C (Table 4).

The hatching rates, as determined after artificial fertilization, were not significantly different for the different amounts of HCG given (Tables 2 to 4). The percentages of normal larvae were generally high (76% ± 8), while

TABLE 3

Effects of different dose of HCG on induced ovulation, quantity of eggs stripped and hatching rate in *H. longifilis* (experiment of 7 June 1985). Females were stripped 10 h and 14 h after injection. During the latent period, water temperatures varied between 27 and 29°C

Dose (I.U./g body wt.)	Body weight (g)	Initial oocyte diameter (mm)	Weight of eggs stripped (g)		No. of eggs stripped per female kg (× 1000)	Stripping response (%)	Normal* larvae (%)	Deformed* larvae (%)
			10 h	14 h				
2.5	1556[a]	1.23	14	61	45	62.5	41.7	22.1
2.5	1689[a]	1.25	46	130	88	77.2	76.1	6.5
2.5	3532[b]	1.54	193	251	97	—	—	—
2.5	2924[b]	1.52	317	66	90	—	74.1	18.0
1.5	4026[b]	1.54	270	224	87	—	78.8	14.7
1.5	3125[b]	1.55	161	250	93	—	73.9	18.0
0.5	5100[b]	1.60	0	42	5	—	79.1	2.5
0.5	4900[b]	1.55	91	429	75	—	56.4	17.3

[a]Females from group two. [b]Females from group one.
*Percentages evaluated after fertilization and incubation of eggs collected 14 h after injection.

TABLE 4

Effects of different dose of HCG on induced ovulation, quantity of eggs stripped, stripping response and hatching rate in *H. longifilis* (experiment of 9 July 1985). Females (group two) were stripped 14 h and 18 h after injection. During the latent period, water temperatures varied between 26 and 27°C

Dose (I.U./g body wt.)	Body weight (g)	Initial oocyte diameter (mm)	Weight of eggs stripped (g)		No. of eggs stripped per female kg (× 1000)	Stripping response (%)	Normal* larvae (%)	Deformed* larvae (%)
			14 h	18 h				
1.5	1257	1.50	11	67	48	69.8	32.7[b]	30.3[b]
1.5	2299	1.53	175	36	73	73.5	41.2[a]	37.6[a]
1.5	1939	1.41	49	15	31	60.9	87.8[a]	6.0[a]
1.0	1766	1.39	77	42	61	75.3	81.1[b]	5.3[b]
1.0	1696	1.39	10	148	85	69.9	52.2[b]	25.9[b]
1.0	2079	1.44	1	90	36	40.3	42.5[b]	29.3[b]
0.5	1872	1.43	No ovulation					
0.5	1774	1.45	No ovulation					
0.5	1708	1.43	No ovulation					
Placebo	1994	1.44	No ovulation					
Placebo	1604	1.39	No ovulation					
Placebo	2426	1.33	No ovulation					

*Percentages evaluated after fertilization and incubation of eggs collected 14 h[a] or 18 h[b] after injection.

the percentages of deformed larvae stayed within an acceptable range (12% ± 4). The mediocre quality of the eggs obtained in July 1985 (Table 4) may be a consequence of the stressed condition of the brooders which were not fed for a week prior to injection because of an oxygen depletion in the lagoon.

The stripping responses (Tables 3 and 4) were high and indicated that the majority of the oocytes had reached the post-vitellogenic stage.

At ovulation, the mean oocyte diameter was significantly ($P < 0.01$) larger than that observed before hormonal treatment. As determined from samples taken from 22 females, the size increase was 10% (from 1.40 ± 0.06 mm before injection to 1.54 ± 0.04 mm at ovulation). The minimal egg diameter observed after stripping these 22 females was 1.20 mm. Considering the 10% increase in diameter during the course of oocyte maturation, the minimum diameter of oocytes apt to respond to hormonal treatment was estimated to be approximately 1.0 mm.

Seasonal changes in fecundity

The mean oocyte diameters measured from females raised in enclosures were higher than the minimal oocyte diameter for response to HCG all year round (Fig. 2). In fact, ovulation was induced in all females treated with HCG (doses above 0.5 I.U./g body wt.), regardless of season. The high hatching percentages obtained in December (Table 2) showed that good quality oocytes and ripe males can be found even during the dry season. However, the quantity of oocytes collected per female kg after HCG injection in De-
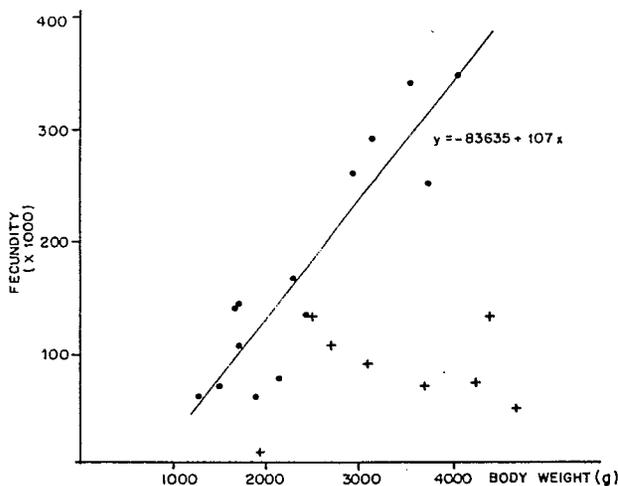

Fig. 3. Relationship between body weight and fecundity of *H. longifilis* females treated with HCG. Only females having received a dose of HCG higher than 0.5 I.U./g body wt. were considered. The regression ($r = 0.91$) was calculated using values obtained in June—July (●); values obtained in December (+) are presented only for comparison.

cember (26 000 ± 14 000; Table 2) was much lower than the quantity collected during the cool season (June—July), with both the first and second group of brooders (68 000 ± 13 000; Tables 1, 3 and 4). In addition, a significant correlation ($r = 0.91$) between the number of oocytes stripped after hormonal treatment (doses larger than 0.5 I.U./g body wt.) and female body weight was observed only in June and July (Fig. 3). The absence of correlation in December (Fig. 3) and the comparatively low number of oocytes collected confirm the existence of a greater disparity in the physiological state of the ovaries of different females during this season.

DISCUSSION

HCG efficiency to induce oocyte maturation and ovulation in *H. longifilis* is clearly demonstrated in this study. A single HCG injection induced ovulation in 100% of treated females when applied at between 1.0 and 2.5 I.U./g body wt. A lower dose leads to high variability in individual response.

Clemens and Sneed (1962) recommended that the hormone dosage used in practice to induce ovulation in a given species should be in excess of the minimal dose determined experimentally. This precaution can be useful to avoid failure due to a possible difference of sensitivity between brooders of different origins or ages. In this investigation, only females of the first group ovulated (Table 3) while no response was observed within the second group (Table 4), after HCG injection at a dose of 0.5 I.U./g body wt. As a low stripping response was observed for one of the treated fish with a dose of 1.0 I.U./g body wt. (Table 4), the use of 1.5 I.U. HCG/g body wt. is therefore recommended for routine application in induced breeding of *H. longifilis*. Higher doses, which give similar responses in terms of quantity and quality of the stripped eggs, are unnecessary. It should be noted that the minimal effective dose of HCG determined for *H. longifilis* is lower than those determined for other clariids; 2.0 I.U./g body wt. in *C. macrocephalus* (Mollah and Tan, 1983) and 2.5 I.U./g body wt. in *C. gariepinus* (Eding et al., 1982).

As has been observed in other fish species, the latent period is dependent both on temperature and hormone dosage (Clemens and Sneed, 1962; Harvey and Hoar, 1980). However, in *H. longifilis*, latency time appeared to be influenced much more by temperature than by the injected dose of HCG. Mollah and Tan (1983) also observed very little variation in the latency response of *C. macrocephalus* despite the different dosage of HCG (between 1.0 and 5.0 I.U./g) used in their study. In *H. longifilis*, at a temperature of 27 to 29°C, the latency period is approximately 14 h. At that time, most of the eggs could be stripped and displayed an overall good quality. On the basis of the generally high percentages of normal larvae (76% ± 8) obtained after hatching, the procedures for artificial fertilization and egg incubation described above can be considered satisfactory.

Up to now, HCG has been successfully used to induce spawning in several fish species (Lam, 1982). However, this mammalian, high molecular weight

substance could provoke an immune reaction in some species, making it inefficient after repeated use. Such a reaction has been observed in *Sparus aurata*, which, for this reason, can be used only during a single reproductive season (Gordin, personnal communication, 1983), and in two Chinese carp species which developed a resistance to HCG when spawned for several consecutive years (Anon., 1977a,b). Although the long-term effects of repeated HCG injection cannot be predicted in *H. longifilis*, the fact that ovulation has been successfully induced at 6-, 5- and 2-month intervals in the same *H. longifilis* female with 2.5 I.U. HCG/g body wt. (unpublished data), shows that in this species the same individual can be spawned with HCG four consecutive times at least without any inconvenience.

These results also indicate that after hormonal treatment there is a quick recovery of oogenesis in *Heterobranchus* females reared in enclosures.

Oocyte maturation and ovulation were successfully induced with HCG at the most extreme season of the lagoon hydroclimate at the level of our station. Oocytes of good quality and fertilizing sperm were collected in the rainy season (June and July) as well as in the dry season (December, March and April). This indicates that a continuous egg and fry supply can be obtained from *H. longifilis* brooders reared in the lagoon. However, it is important to consider that, although able to mature sexually in slightly brackish water, *H. longifilis* cannot propagate in this environment because when salinity reaches $6 \text{ g} \cdot \text{l}^{-1}$ all the fertilized eggs abort before hatching (Legendre, unpubl.). For this reason, it is advisable to practice artificial fertilization and incubation in fresh water, particularly in the dry season.

Mean oocyte diameters all year round are greater than the minimal oocyte diameter for response to HCG. Nevertheless, seasonal variations in the sexual state of females are observed and seem to be correlated with temperature. The mean oocyte diameters show a marked decrease at the beginning of the dry season. At that time, the egg quantity collected from HCG-treated females is much lower than during the rainy season and a greater proportion of degenerating oocytes is also observed in the samples obtained by intraovarian biopsy. Thus, although post-vitellogenic oocytes can be found all year round in the ovaries, these observations clearly indicate that the rainy season corresponds to the primary reproductive season of *H. longifilis*. In the Ubangui River (Central Africa), sexually mature individuals were captured only in August and September during the major rainy season when there was a rise in water level (Micha, 1973).

In fish the process of atresia is temperature dependent (Yamazaki, 1965; Richter et al., 1982; Rowland, 1983). The temperature increase registered in this study at the beginning of the dry season may be responsible for oocyte resorption observed at this period. The decrease in oocyte diameter and fecundity (in December) may thus be a consequence of the resorption of the bigger oocytes in the ovaries. A histological study should provide a final answer to this question and a better understanding of the dynamics of the ovary in *Heterobranchus*.

Reproduction and the complete biological cycle of *H. longifilis* have been observed in captivity. Fish hatched at the station reached first sexual maturity at 1 year of age and 1.5 kg mean weight. This result contrasts with observations of Micha (1973), who indicated that, in ponds, *H. longifilis* matures only at 3 or 4 years of age when fish weight reaches several kg. In a natural environment (the Niger basin), *H. longifilis* was reported to mature after 2 years (Motwani, 1970).

In conclusion, several qualities confer on *H. longifilis* a promising future as a culture fish. It is omnivorous (Micha, 1973), and its growth, which can exceed 10 g · day^{-1} (Legendre, 1983), is one of the fastest yet observed among African fish species tested in aquaculture. It is a highly resistant species and its performances in monoculture are very encouraging (Dia et al., 1986). The possibility of obtaining several spawns per female per year, added to a high fecundity (almost 4 million eggs were collected in the course of this investigation), give a further advantage to the species, as a mass and non-stop egg and fry production can be envisaged from a brood stock of relatively limited size.

The control of reproduction of *H. longifilis* in captivity now makes the culture of this species possible.

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