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Comparative study of vitellogenesis of two African catfish species *Chrysichthys nigrodigitatus* (Claroteidae) and *Heterobranchus longifilis* (Clariidae)

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Abstract

Chrysichthys nigrodigitatus and *Heterobranchus longifilis* are two commercially important African catfish species that have been studied for their reproduction and breeding possibilities in the lagoon waters of Ivory Coast. *C. nigrodigitatus* is characterized by an annual reproductive cycle with a vitellogenesis period from March to June. The reproductive season coincides with the short rainy season which is characterized by lower water temperature and salinity at the beginning (September) and by a progressive rise of these parameters at the end of the season (November). The previtellogenic oocytes enter vitellogenesis at an average diameter of 320 μm. This initial oocyte stock is reconstituted progressively as soon as the spawning is achieved and lasts for a period of approximately 3 months. Fully vitellogenic oocytes reach an average diameter of 2.4-2.8 mm by the end of June. The maturation is possible only if a male and a female are confined in artificial spawning receptacles. Maturation and spawning generally occur within the following 3 to 5 weeks for 30 to 80% of the females. When the non-spawning females are kept in the nests they maintain fully vitellogenic oocytes for 3 months with a continuous atresia of the larger oocytes, while the ovaries of the non- spawning females. placed out of the receptacles, exhibit a complete atresia of all the vitellogenic oocytes.

After an HCG-induced ovulation, a new reproductive cycle of *H. longifilis* can be achieved in less than a month. The previtellogenic oocytes enter vitellogenesis at an oocyte diameter of about 380 μm and become fully vitellogenic after 15 days (1.2-1.6 mm in diameter). If maturation is not artificially stimulated the females remain at the same stage of vitellogenesis while larger oocytes undergo atresia and are continuously replaced by growing vitellogenic oocytes.

Keywords: *Chrysichthys nigrodigitatus, Heterobranchus longifilis,* African catfish, reproductive cycle, oogenesis, vitellogenesis, egg.

Étude comparative de la vitellogenèse chez deux espèces de poissons-chats africains Chrysichthys nigrodigitatus *(Claroteidae) et* Heterobranchus longifilis *(Clariidae).*

Résumé

Chrysichthys nigrodigitatus et *Heterobranchus longifilis* sont deux espèces présentant un grand intérêt pour l'aquaculture en Afrique. Elles ont fait l'objet de travaux sur leur biologie de reproduction et sur les possibilités d'élevage dans les lagunes saumâtres du sud de la Côte d'Ivoire. *C. nigrodigitatus* est caractérisé par un cycle reproducteur annuel avec une période de vitellogenèse entre mars et juin. La période de reproduction coïncide avec la petite saison des pluies au début de laquelle la température et la salinité diminuent sensiblement (septembre) puis augmentent à la fin de celle-ci (novembre). Les ovocytes prévitellogéniques commencent à entrer en vitellogenèse à un diamètre moyen de 320 μm. Ce stock initial d'ovocytes est reconstitué progressivement dès la fin de la ponte pendant une période de 3 mois. Les ovocytes atteignent une taille de 2,4-2,8 mm en fin de vitellogenèse vers la fin juin. La maturation ovocytaire n'intervient que si un mâle et une femelle sont confinés dans des réceptacles de ponte artificiels. Dans ce cas la maturation et la ponte interviennent généralement pour 30 à 80 % des femelles dans les 3 à

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5 semaines qui suivent. Lorsque les femelles qui n'ont pas pondu sont maintenues dans les réceptacles leurs ovaires présentent un aspect de fin de vitellogenèse avec seulement une atrésie des plus grands ovocytes. Au contraire, les ovaires des femelles n'ayant pas pondu et maintenues hors des réceptacles de ponte subissent une atrésie totale de tous les ovocytes vitellogéniques. Chez *H. longifilis* la maturation ovocytaire et l'ovulation peuvent être induites par HCG et un cycle reproducteur peut s'effectuer en moins d'un mois. Les ovocytes prévitellogéniques entrent en vitellogenèse à un diamètre moyen de 380 μm et atteignent (1,2-1,6) mm en fin de vitellogenèse. Si la maturation n'est pas provoquée artificiellement les plus gros ovocytes s'atrésient mais ils sont continuellement remplacés pas des ovocytes en vitellogenèse.

Mots-clés : *Chrysichthys nigrodigitatus, Heterobranchus longifilis,* poisson-chat, cycle reproducteur, ovogenèse, vitellogenèse, œuf.

INTRODUCTION

The two catfish species studied are commonly distributed in West Africa (Daget and Iltis, 1965) and are highly prized fish by local populations (Chauvet, 1972). Since they are economically important species, the studies undertaken were mainly focused on artificial breeding and rearing methods in order to develop in Ivory Coast the aquaculture of these valuable species (Hem, 1986; Legendre, 1986; Otémé, 1993*a*). Previous studies of wild populations indicated the possibility of the existence of an annual reproductive cycle in *Chrysichthys nigrodigitatus* and a possible correlation with the rainy season (Chauvet, 1972) as well as for *Heterobranchus longifilis* (Micha, 1973). From studies realized on fish held in aquaculture facilities in Ivory Coast, it has been observed that even if both species were annual brooders in the wild, mature fish could be found at any time of the year for *Heterobranchus longifilis* (Legendre, 1986) while mature *Chrysichthys nigrodigitatus* females were only observed just after the rainy season during July and August (Chauvet, 1972); or the short rainy season during September and October (Otémé, 1993*a*).

To better understand the reproductive cycles of these species maintained in aquaculture cages in the Ebrié lagoon, we have undertaken the study of vitellogenesis during two successive cycles of reproduction for *Chrysichthys nigrodigitatus* and a one-year period for *Heterobranchus longifilis.* The determination of the time and duration of the exogenous vitellogenesis process in both species is reported and some considerations on vitellogenesis reproduction strategies are reported.

MATERIALS AND METHODS.

Fish

Adult male and female of *Chrysichthys nigrodigitatus* (3-year old) and *Heterobranchus longifilis* (2-year old) selected from a breeding stock, were maintained in lagoon enclosures at the Layo aquaculture station located on the central north side of the Ebrié lagoon in Ivory Coast. Fish were fed 6 days a week with pellets given at 6% of biomass during all the experiment.

Oocyte biopsy and histological techniques

In order to follow the vitellogenesis stage without sacrificing the females, the oocyte sampling was performed by intra-ovarian biopsy of the females with a catheter of the appropriate interior diameter (2 mm for *Heterobranchus* and 3.5 mm for *Chrysichthys*). At each biopsy date 25 to 50 oocytes per female were sampled and measured under a binocular microscope.

In order to follow individual variations, each female was identified by alcian blue spots performed with a dermojet inoculator on the ventral part of the fish.

Histological samples were obtained from sacrificed females and from ovarian biopsies. The ovarian samples were immediately immersed in Bouin's liquid (Martoja and Martoja, 1967) and allowed to fix for a minimum of one week. After dehydration and embedding in paraffin, 7 μm sections were stained with eosin dye and mounted.

Calculations of the average oocyte diameter for vitellogenesis stages analysis were obtained from the readings of a digitizer coupled to the microscope on only median oocyte sections through the nucleus. The oocyte diameter of ovoid shape oocytes was calculated by the formula $\sqrt{D \times d}$ were D represents the larger section and d the smaller section of the oocyte.

Oocyte maturation and ovulation induction

Mature *Heterobranchus longifilis* females were injected with 1.5 $IU.g^{-1}$ of HCG (Human Chorionic Gonadotropin) and stripped 12 hours after the injection as described by (Legendre, 1986).

RESULTS

Reproductive cycle of *Chrysichthys nigrodigitatus*

The variations of the oocyte diameter have been observed during a two-year period in a population of 60 females maintained in aquaculture cages with an equal number of males. Salinity and water temperature variations during 1992-1993 and average

diameter calculated from all the oocyte diameter readings obtained by biopsy at each sampling time are summarized in *figure* 1. The results show a clear seasonality of the vitellogenesis with a reproduction period around September and October. Some variability is observed among the females as shown in *figure* 2 *a*. The oocyte distribution pattern in the prespawning period (September), summarized in *figure* 2 *b*, indicates a unique group of vitellogenic oocytes. If the fish (males and females) are maintained in large cages the maturation and spawning is not possible and a massive atresia of all the oocytes is observed by the end of December. At this time the observation of ovarian sections indicates that the ovary contains mainly previtellogenic oocytes at different development stages with diameters ranging from 50 to 300 μm (*fig.* 3 *a*). The exogenous vitellogenesis stage (incorporation of vitellogenin by the oocyte) starts approximately 3 months after the end of the complete atresia of the ovary. The early vitellogenic oocytes with an average diameter of 320±70 μm (n=50) are characterized by small yolk granules at the periphery of the ooplasm (*fig.* 3 *b*). By the end of vitellogenesis the oocyte is surrounded by a thin zona radiata and all the ooplasm is filled with yolk globules (*fig.* 3 *c*). A unique micropyle is observed at the animal pole and is occupied by a micropylar cell until ovulation

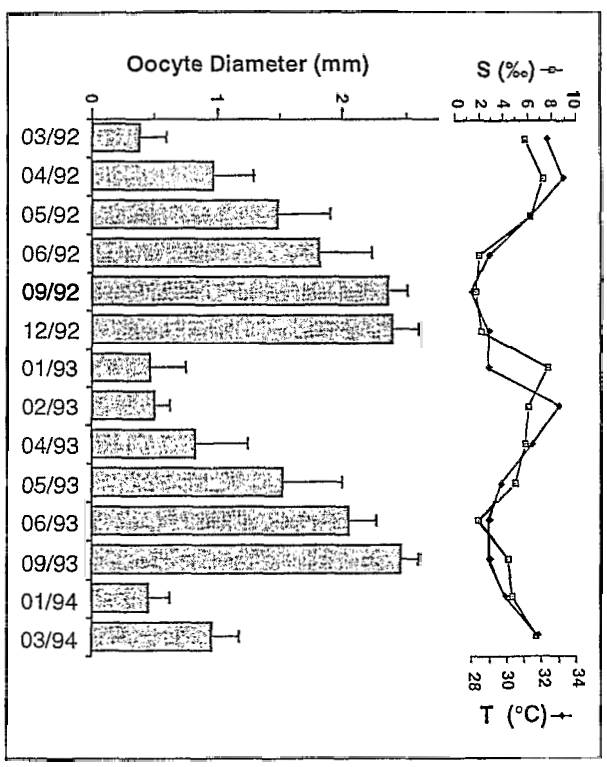

Figure 1. – Variations of salinity, temperature and oocyte diameter of *Chrysichthys nigrodigitatus* females reared in Ebrié lagoon during 1992-1993 at the Layo aquaculture station. Oocyte diameter mean±SD, (n=1200, for each point).

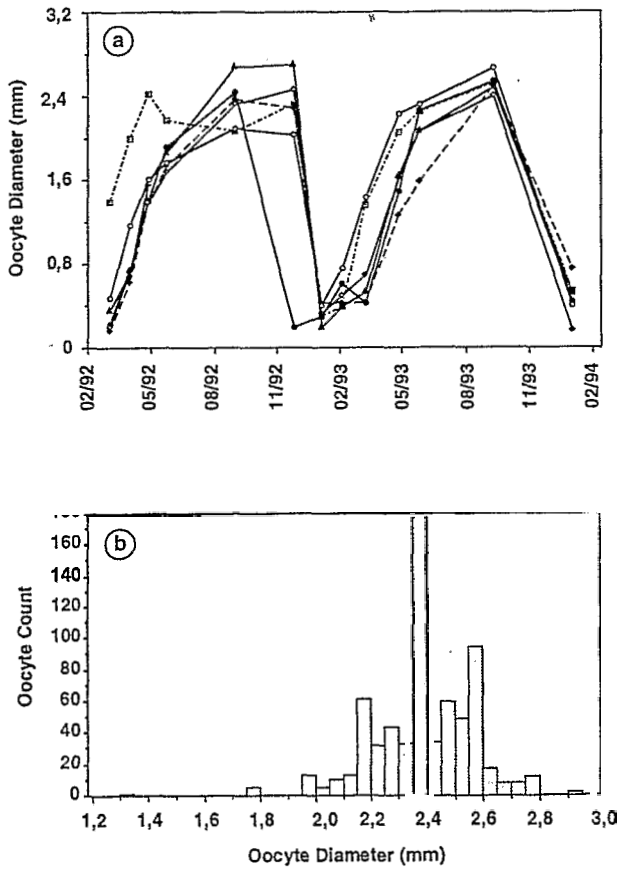

Figure 2. – a. Individual variations of oocyte development patterns of six *Chrysichthys nigrodigitatus* females reared in Ebrié lagoon during two successive reproduction cycles. Values represent the mean oocyte diameter obtained from the readings of ovarian biopsies. b. Oocyte diameter distribution in *Chrysichthys nigrodigitatus* ovaries in the prespawning period (September).

(*fig.* 3 *d*). The exogenous vitellogenesis was completed in approximately 3 months and the average oocyte diameter reaches 2.6±0.2 mm (n=50) just before oocyte maturation.

Effects of confinement on vitellogenesis of *C. nigrodigitatus*

In lagoon cages, *C. nigrodigitatus* females mixed with males undergo a complete atresia of the ovary by the end of the reproductive period in December (*fig.* 4) when water temperature starts rising (*fig.* 1).

If females are confined with males in spawning receptacles the average oocyte diameter is maintained at the level reached at the end of vitellogenesis for more than 3 months after the reproduction period (*fig.* 4). The larger oocytes become atretic but vitellogenic oocytes continuously replace the atretic ones. Nevertheless the spawning never occurred out of the reproductive season (September to November).

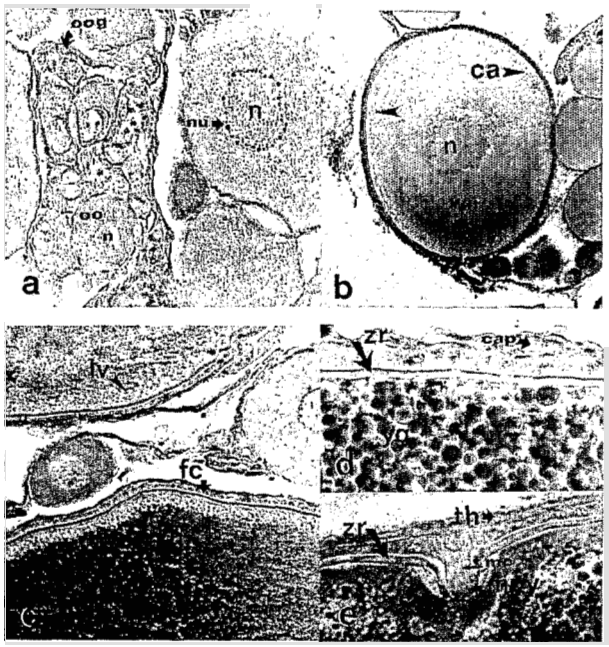

Figure 3. – Photomicrographs of histological sections of *Chrysichthys nigrodigitatus* ovary during vitellogenesis, stained in eosin. a: previtellogenic oocytes (× 200); b: Early vitellogenic oocyte (× 50); c: mid-vitellogenic oocytes (× 75); d: Fully vitellogenic oocyte (× 100) e: animal pole and micropyle (× 200).

ca: cortical alveoli, cap: capillary, fc: granulosa cells, n: nucleus, nu: nucleolus, mc: micropylar cell, mpy: micropyle, oo: previtellogenic oocyte, oog: oogonia, th: thecal layer, yg: yolk globules, ygr: yolk granules, zr: zona radiata.

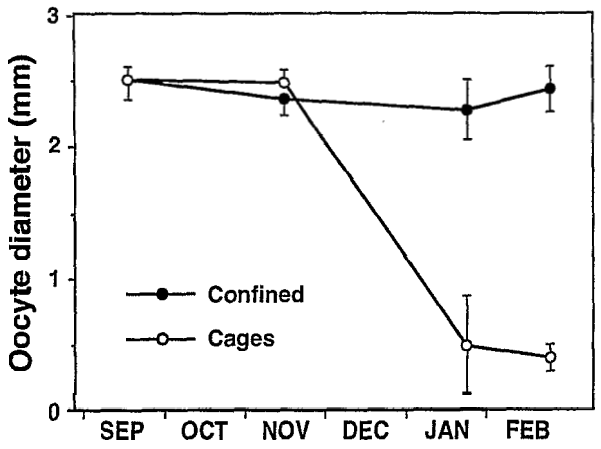

Figure 4. – Effect of confinement on vitellogenesis of *Chrysichthys nigrodigitatus* females. Two groups of 20 fish (10 males and 10 females) were selected from the breeding stock and one group is maintained in 5 m × 5 m lagoon cages (solid circles) and in the other group 1 male and 1 female are confined together in 10 closed spawning receptacles immersed in circular tanks with running lagoon water (open circles). Mean ± SD.

Reproductive cycle of *Heterobranchus longifilis*

After HCG-induction of the oocyte maturation and ovulation and stripping of the females we followed the evolution of the vitellogenesis by sampling a group of 30 females every 3 days. The histological observations of the ovaries indicated that the recrudescence of previtellogenic oocytes (*fig.* 5*a*) starts immediately after the stripping of the mature oocytes. Previtellogenic oocytes are present in the ovary between the numerous post-ovulatory follicles (*fig.* 5*c*). In the larger oocytes around 350 μm in diameter (*fig.* 5*b*), large cortical alveoli are observed at the periphery of the ooplasm (*fig.* 5*d*). Vitellogenesis was observed in oocytes of 380 ± 55 μm (n = 50) in diameter only three days after the stripping.

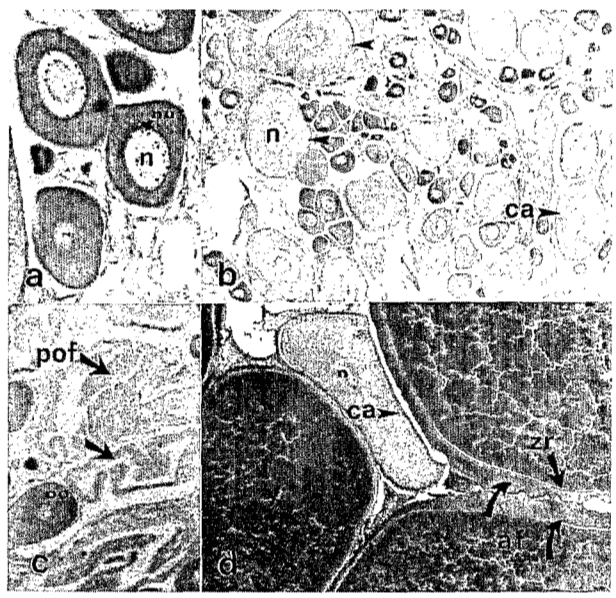

Figure 5. – Photomicrographs of histological sections of *Heterobranchus longifilis* ovary, stained with eosin. a: previtellogenic oocytes (× 200), b: early vitellogenic oocytes 4 days after the stripping (× 50), c: post-ovulatory follicles and previtellogenic oocytes 1 day after the stripping (× 75), d: Fully vitellogenic oocytes 15 days after stripping (× 100).

af: attaching filaments, ca: cortical alveoli, n: nucleus, nu: nucleolus, pof: post-ovulatory follicles, oo: previtellogenic oocyte, y: yolk, zr: zona radiata.

The calculation of the average oocyte diameter of the growing oocytes indicated that fully vitellogenic oocytes were present in the ovaries 12 to 15 days after the stripping (*fig.* 6). These oocytes are characterized by a dense ooplasm filled with yolk globules and they exhibit at the animal pole an attachment disk formed by the secretions of the granulosa cells (*fig.* 5*d*).

In an other group of 100 females intra-ovarian biopsies have been performed on 10 females randomly chosen every 7 days during a one-year period. The average diameter was calculated on a batch of 30-50 oocytes per female. The average oocyte diameter varied only in the range 1.2-1.6 mm indicating that the females present fully vitellogenic oocytes all year round (*fig.* 7).

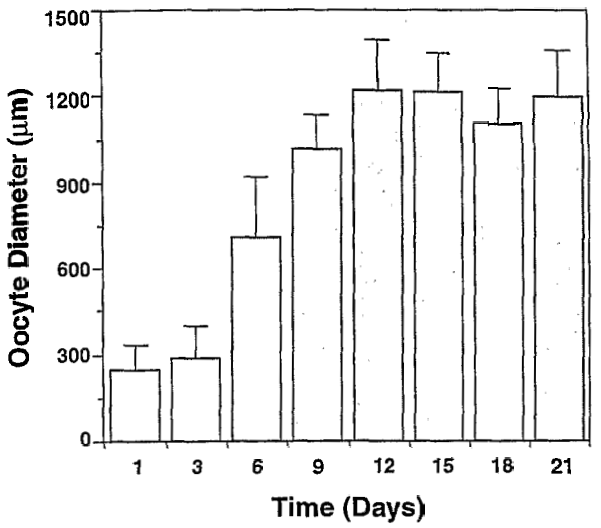

Figure 6. – Vitellogenesis of *Heterobranchus longifilis* females after HCG-induced maturation and stripping. Values are calculated from the measurements of oocyte diameters on histological sections of the ovaries. Mean ± SD.

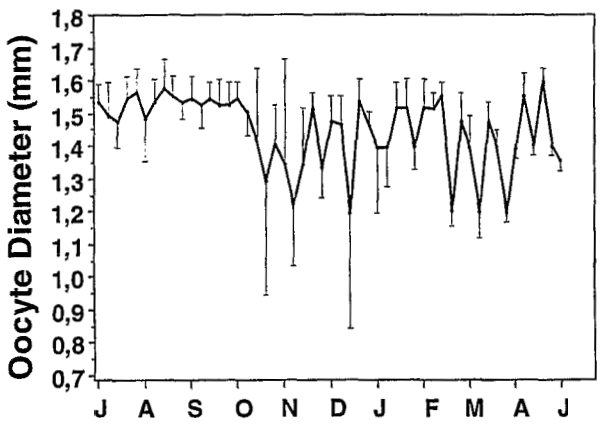

Figure 7. – Variations of the mean oocyte diameter of *Heterobranchus longifilis* females reared at the Layo aquaculture station during a one-year period. Mean diameters are calculated from oocytes obtained by ovarian biopsies. Mean ± SD.

DISCUSSION

We have characterized the different steps of the reproductive cycles in two African catfish species, maintained in artificial breeding conditions in brackish lagoon waters of Ivory Coast. The reproductive cycle of *Chrysichthys nigrodigitatus* is annual with a spawning season around September and November and a vitellogenesis duration of 4 months, nevertheless due to the spawning behaviour of this species, the oocyte maturation and spawning never occurred in cage breeding conditions without spawning receptacles. The spawning is only achieved by placing the fish in receptacles for 2 to 4 weeks as previously described (Hem, 1986; Otémé, 1993 *b*).

For *Heterobranchus longifilis,* in our rearing conditions the reproductive cycle was completed in less than a month but as in *C. nigrodigitatus* spontaneous spawning is not observed, and oocyte maturation and ovulation must be induced by HCG (Legendre, 1986). Continuous vitellogenesis as in *H. longifilis* has also been reported in other Clariidae species (Ali, 1993; De Leeuw *et al.*, 1985; Hogendoorn and Vismans, 1980; Rinne and Wanjala, 1983). Furthermore the induction of the oocyte maturation and ovulation is possible all year round in *H. longifilis* (Legendre, 1986). The higher variability observed in oocyte diameter during the dry season might be explained by the rise of the water temperature which seems to be also involved with a decrease of fecundity as reported by Legendre (1986).

The histological observations and the oocyte samplings allow us to conclude that these two species present two different strategies in terms of vitellogenesis. *H. longifilis* ovaries are all year round in final vitellogenesis stage with continuous atresia and oocyte recruitment, while *C. nigrodigitatus* oocytes develop synchronously till the end of vitellogenesis and spawning but they become completely atretic if spawning conditions are not found. The oocyte development pattern of both species are comparable and exhibit similar characteristics to those of other teleost species studied (Selman and Wallace, 1989; Wallace and Selman, 1990 for review). Histological studies generally describe several staging series defined according to size and other cellular events. In this study we considered only the oocyte size and its relation to vitellogenesis. This allowed us to characterize previtellogenic oocytes (from oogonia to the first appearance of yolk granules) and exogenous vitellogenic oocytes from the first formation of yolk granules to oocyte maturation.

The exogenous vitellogenesis is the most important part of the oogenesis process since almost 80% of the oocyte yolk proteins (mainly vitellogenin synthesized in the liver and released in the bloodstream) is accumulated during exogenous vitellogenesis (Wallace, 1985).

According to our observations exogenous vitellogenesis is completed much more rapidly (15 days) in *H. longifilis* than in *C. nigrodigitatus* (120 days). The comparison of the total amount of vitellogenin incorporated in both species taking into account exogenous vitellogenesis duration, fecundity and the different oocyte size by the end of vitellogenesis in both species (Legendre, 1986; Otémé, 1993 *b*), shows that the effectiveness of VTG incorporation is three to four times higher in *H. longifilis* when expressed as vitellogenin incorporated per mm^2 of oocyte surface.

These characteristics clearly indicate two strategies of reproduction, *C. nigrodigitus* being more adapted to annual variations of the environmental conditions which probably trigger vitellogenesis and spawning concomitantly with other behavioural and pheromonal

cues as described in *Clarias gariepinus* (Van Weerd and Richter, 1991). On the other hand *H. longifilis* is able to maintain vitellogenesis all year round even with the variations of environmental parameters observed in lagoon waters. Thus spawning would be potentially possible in these conditions at any time of the year if the behavioural stimuli were present. Nevertheless these potentialities might not be expressed in natural environmental conditions due to the higher variability of the water parameters observed in the habitat of this species, particularly in the lakes and rivers with wide fluctuations of water level and water flow.

Acknowledgments

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