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Original Research

Studies on the reproduction of hybrids (OS) resulting from the intergeneric cross between *Oreochromis niloticus* (Linnaeus, 1758) female and *Sarotherodon melanotheron* (Rüppel, 1852) male in captivity

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ABSTRACT:

A study was conducted in a lagoon environment on second generation hybrids from the intergeneric cross between *Oreochromis niloticus* (female) and *Sarotherodon melanotheron* (male) in order to know certain reproduction parameters. The breeding was conducted in cages (3x2x1.5 m) installed in a pond bordering the Ebrié lagoon and in concrete tanks (2x2x1 m). The Gonado Somatic Index (GSI), the condition factor (K), the Hepato Somatic Index (HSI), the size of first sexual maturity, fecundity, the oocyte diameter and the sex ratio were determined. In females, the low values of HSI and the high values of GSI and 'K' recorded during vitellogenesis reflect the use of liver reserves as a source of energy. However, these parameters vary little in males. Males reach sexual maturity at larger sizes (11.61±0.08 cm) than females (10.11±0.09 cm). The absolute fertility is 1040±86 oocytes per female with a relative fecundity of 41.25±3.41 oocytes per gram of body weight. Egg diameter at egg-laying is 2.20 mm with a sex ratio at the hatch of 1: 1. These results suggest that these hybrids reproduce well in lagoon water and can be specimens of tilapia to be recommended to fish farmers.

Keywords:
Lagoon aquaculture, Oocyte diameter, Fecundity, Sexual maturity, Sex ratio.

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INTRODUCTION

Since the 1980s, two native lagoon tilapia (*Sarotherodon melanotheron* and *Tilapia guineensis*) have been studied for the control of their breeding (Legendre 1986; Koumi *et al*., 2008). The reported results indicated their low growth which does not encourage the extension of their breeding for an economic purpose. In order to contribute to the development fish farming which is still lagging, two tilapias (*Oreochromis niloticus* and *S. melanotheron*) commonly found in Ivory Coast have been crossed reciprocally to produce hybrids that would keep the good growth potential of *O. niloticus* and potential of adaptability to the lagoon environment of *S. melanotheron*. Preliminary data on growth and survival of the two hybrids allowed selection of those derived from *Oreochromis niloticus* female x *Sarotherodon melanotheron* male which showed the best performance (Amon *et al*., 2013).

Therefore, knowledge of the reproductive parameters of these hybrids in a controlled environment is essential for better control of their breeding. According to Poncin (1996), fish reproduction is the stage through which they survive and are maintained in the wild. It consists of a transfer of information from the parental generation to the offspring (Munro, 1990). This aspect of biology covers a set of parameters including fertility, size at first sexual maturity, breeding season, sex ratio, gamete size, etc., (Paugy and Lévêque, 1999). The present work proposes to study these reproductive parameters in the perspective of a control of the breeding of these hybrids in the lagoon environment.

MATERIAL AND METHODS

Field of study

The experiments took place at the Layo Experimental Aquaculture Station (Ivory Coast), located in Sector IV of the Ebrié Lagoon (5.18°N and 4.19° W). This lagoon, bounded on the west by the Assagny Canal and on the east by the Assinie Canal, stretches for 130 km along the Gulf of Guinea. Over the whole of this lagoon, the salinity is between 0 and 30% with temperatures ranging from 27.4 to 31.2°C (Durand and Guiral, 1994).

Measurement of physicochemical parameters of water

The physico-chemical parameters of the water tanks and cages were monitored daily between 6 a.m. and 7 a.m. throughout the experiment. Temperature and dissolved oxygen were measured with a Model WTW OXI 330 oximeter coupled to a thermometer. The pH of the water was monitored using a pH meter WTW pH 90. The salinity was measured using a salinometer of YSI33 type.

Sampling and measurement of breeding parameters

The determination of the size of the first sexual maturity, the absolute fecundity and the oocyte diameters took place on fish raised in floating cages, in mixed breeding. Before the transfer of the different batches of fish in cages, the gonads of about 30 males and females with an initial average weight of 7.80±0.10 g and length 7.50±0.20 cm were taken to characterize their initial state. Subsequently, three batches of 120 fish (60 females and 60 males) were constituted and each were transferred into a cage at a density of 13.33 ind./m^3.

These fish were fed twice a day (morning and evening) with a meal following a ration representing 5% of the biomass. At the end of each month, a sample of 10 fish per sex and per cage was taken and transferred to the laboratory for dissection. On each fish, the total length was measured to the nearest millimeter, the total and eviscerated masses were determined using a scale of Sartorius type (model LP 6200S) with a precision of 0.01 g and a range of 6200 g. The masses of the gonads and liver were weighed using a Sartorius scale (model BP 221S) with a precision of 0.0001 g and a range of 220 g. The determination of sexual maturity stages was made on the basis of the sexual maturity scale of

Duponchelle and Legendre (2000): stage 1 includes immature individuals, stage 2 represents the beginning of sexual maturity, stage 3 indicates mature individuals, stage 4 characterizes individuals that reproduce (spawning or spermiation) and stage 5, post-laying for females.

Since the fish used in this study come from the same cohort (second-generation individuals), the size of first maturity was determined by taking the average standard length at which all subjects are able to reproduce. The Gonado Somatic Index (GSI) and the Hepato Somatic Index (HSI) of mature individuals were calculated at each sampling from the following formulas (Bougis, 1952; Analbery, 2004)

$$\text{Hepatosomatic Index} = \frac{\text{Liver weight}}{\text{Eviscerated weight of the fish}} \times 100$$

$$\text{Gonado Somatic Index} = \frac{\text{Weight of the gonad}}{\text{Eviscerated weight of the fish}} \times 100$$

To verify the possible role of muscle reserves in gametogenesis, the condition factor (K) was determined according to the following formula (Laleye, 1995):

$$K = \frac{\text{Total weight of fish (g)}}{LS^3} \times 100$$

where, LS = Standard length (cm)

Potential fecundity was determined by counting stage 3 oocytes. Oocyte diameters (30 oocytes per female) were measured using a micrometer (Model Wild M3C) binocular microscope. Regarding the determination of the sex ratio of hybrids at hatching of eggs, a group of broodstock was put for reproduction in a concrete tank. After 45 days during which the fish received a granulated feed containing 30% protein at 5% of the biomass ration, the parents were removed. The fry obtained were followed for two months at the end of which they were counted and sexed according to the observations of Bard *et al.* (1974). After the sexing operation, the percentages of males and females were determined according to the following formulas (Albaret, 1982):

$$\text{Percentage of males (\%)} = \frac{\text{Number of males}}{\text{Number of sexed fish}} \times 100$$

Percentage of females (%) = 100 - Percentage of males

Statistical analysis

The one-way analysis of variance (ANOVA) was applied to look for differences between GSR, HSR and K from one stage of sexual maturity to another, and on the other hand, differences between the sizes of first sexual maturity were recorded in males and females. The Tukey test was used for posterior comparisons when the effects are significant. The differences are considered significant at $P<0.05$. Statistical treatments were performed with the STATISTICA 7.1 software.

RESULTS

Physicochemical parameters

During the work, the physicochemical parameters (Table 1) of the water in the tanks and cages varied from 2.30 to 2.40 g/l for salinity, 4.20 to 4.22 mg/l for dissolved oxygen, 27.83 to 27.90°C for temperature and 6.82 to 6.91 for pH.

Evolution according to the stages of sexual maturity, Gonadosomatic Index (GSI), Hepatosomatic Index (HSI) and condition factor (K) ratios of females and males

Variations in Gonadosomatic Index (GSI), Hepatosomatic Index (HSI) and condition factors of females and males at different stages of sexual maturity are presented in Figure 1. In females, great variability of GSI is seen from stage 1 to stage 5. Stage 1 and 2 individuals have low GSI values ranging from 0.64 ± 0.20 to 1.73±1%. This GSI increases considerably to reach the stages 3 and 4, with the values of 10.42 ± 2.10% and

Table 1. Average values of the physicochemical parameters of water recorded in tanks and cages during the reproduction of second-generation OS hybrids

S. No	Parameters	Livestock environment	
		Concrete tanks	Cages
1	Temperature (°C)	27.83±0.11	27.90±0.10
2	Dissolved oxygen (mg/L)	4.20±0.01	4.22±0.02
3	pH	6.91±0.14	6.82±0.12
4	Salinity (g/L)	2.40±0.02	2.30±0.01

14.73 ± 3%, respectively. At stage 5, the GSI falls to 2.11 ± 1%. The statistical comparison of the GSI values shows a significant difference ($P<0.05$) between stages of sexual maturity. In males, these ratios ranged from 0.11±0.01% in stage 1 to 0.67±0.03% in stage 4. Stages 2 and 3 showed intermediate values of 0.23±0, 0.02% and 0.50±0.01% respectively. A significant difference ($P<0.05$) between stages of sexual maturation was observed.

With respect to the Hepatosomatic Index (HSI) and the condition factor K, they vary sllightly from one stage to another in males. In females, however, these parameters vary considerably from stage 1 to stage 5. The maximum K values at oviposition (0.24±0.02) and minimal K at stage 5 (0.02±0.00) vary in the same meaning as those of the GSI. In stages 1, 2 and 3, they are 0.14±0.03, 0.16±0.03 and 0.19±0.04. As for the HSI, it evolves in the opposite direction of the GSI. The recorded values were maximum in stage 5 (7.50±0.30%) and minimal in stage 4 (1.70±0.51%). The values obtained in stages 1 and 2 were 6.31 ± 0.10% and 6.20±0.22% respectively while those in stage 3 was 4.92±0.30%.

Size of first sexual maturity

The size of first sexual maturity was 11.61±0.08 cm in males and 10.11±0.09 cm in females. Females reached sexual maturity at a smaller size than males ($P<0.05$).

Fecundity and diameters of oocytes

A female average height of 10.47 cm with an average weight of 25.21 g, produced approximately 1040±86 oocytes or a relative fecundity of 41.25 oocytes per gram of body weight. The largest recorded value is 1210 oocytes while the smallest is 711 oocytes. The oocyte diameters (Figure 2) were between 0.2 and 0.6 mm in stage 2 (only one mode = 0.4 mm), between 1.4 and 1.8 mm in stage 3 (only one mode = 1.6 mm), between 2.0 and 2.3 mm (only one mode = 2.2 mm) at stage 4 and between 0.3 and 0.7 mm (only one mode = 0.6 mm) at the stage 5.

DISCUSSION

In general, the physico-chemical parameters recorded during the different reproduction phases are within the recommended limits for tilapia (Balarin and Haller, 1982; Watanabe *et al*., 1985). The Gonadosomatic Index (GSI) of females, observed during the experiments, vary from one stage of sexual maturity to another. In stages 1 and 2, the GSI were weak. This is the stage of previtellogenesis where no reproductive activity is observed. In stages 3 and 4, the GSI values were high; which is the vitellogenesis phase. It corresponds to all the processes by which the oocyte synthesizes and accumulates essential reserves (lipid, proteins) at hatching and embryonic development (Stéquert and Ramcharrum, 1996). The fall of the GSI at stage 5 could be due to the weight loss of the gonads, following the expulsion of the mature oocytes. At the male level, the GSI were weak compared to those of the females. This observation is in agreement with that of Paugy and Lévêque

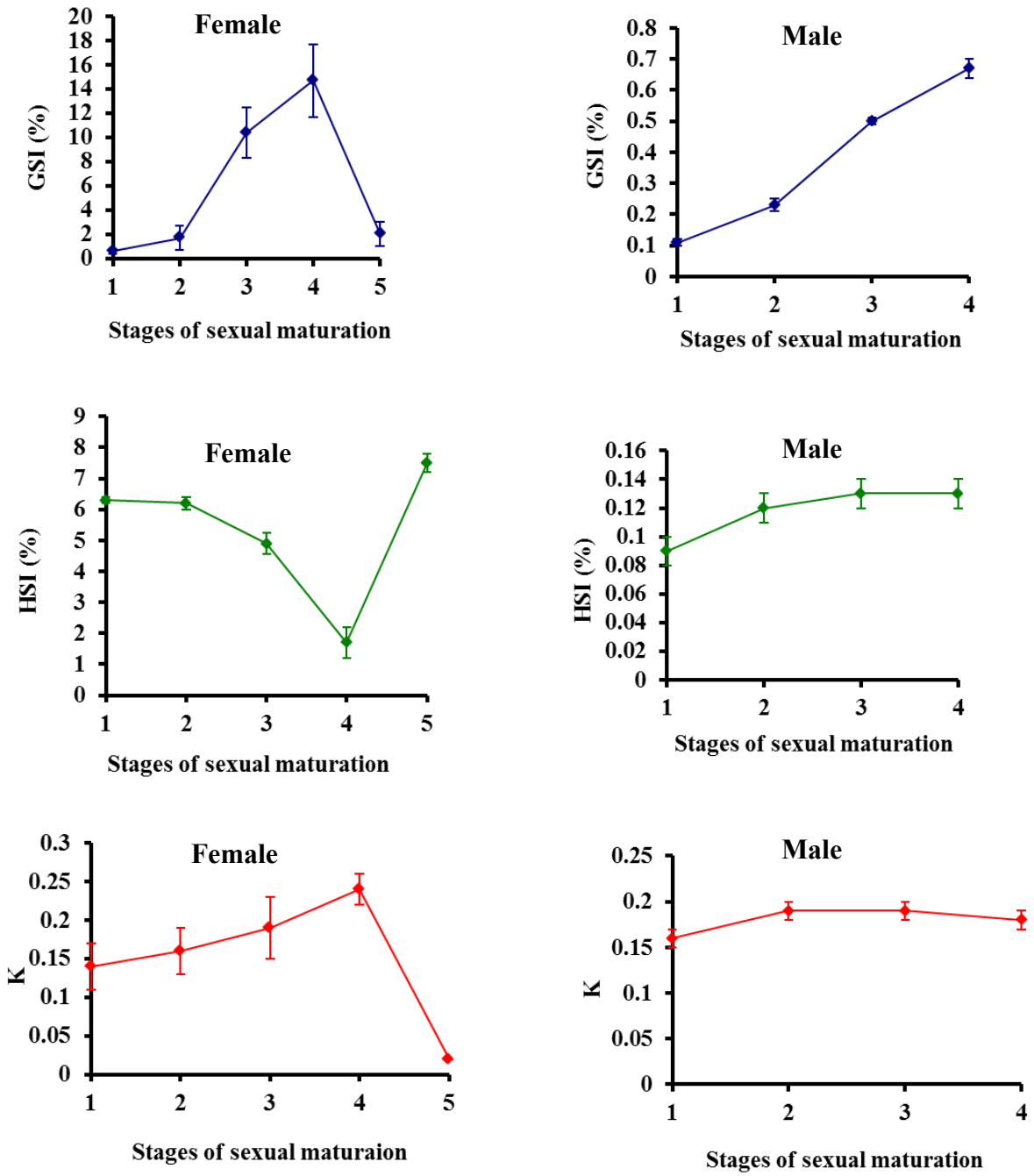

Figure 1. Variations of GSI, HSI and K of male and female hybrids by the stages of sexual maturity

(1999) who reported that in the tropics, the testes develop much less than the ovaries and, for African species, the GSI of the males only exceptionally exceed 2%. When the GSI and K were at their maximum value, the HSI was lower level. This observation was reported by Djadji *et al*. (2010) in the mugilidae species, *Mugil cephalus* in Ebrié and Grand-Lahou lagoons (Ivory Coast). According to them, the decrease in HSI and the increase in GSI and K during the maturation period is explained by the fact that fish uses liver reserves as energy sources for vitellogenesis.

In this study, males reach sexual maturity at higher sizes than females at the same age. This would be due to the differential growth observed in these hy-

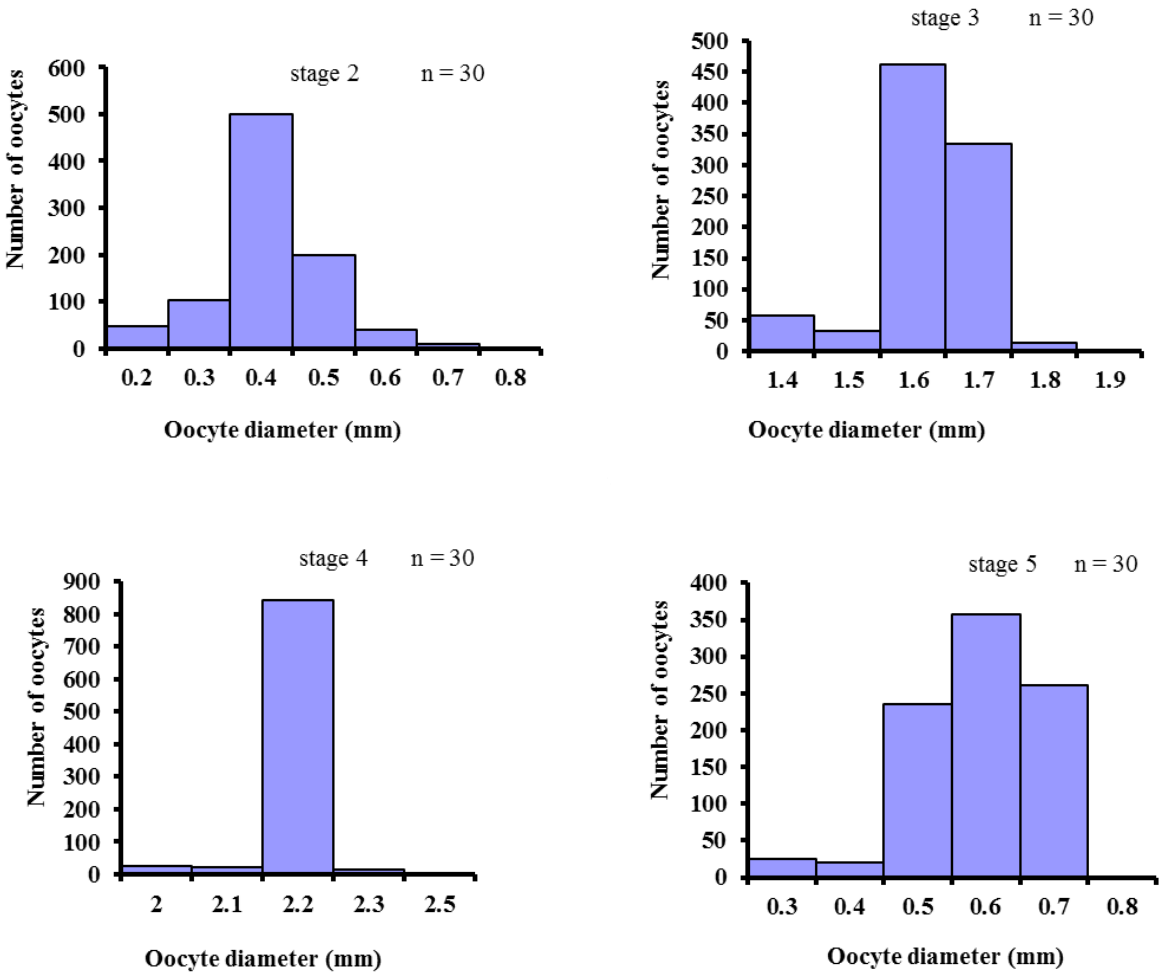

Figure 2. Frequency distributions of oocyte sizes in the gonads at different macroscopic stages in OS hybrids ((n) = number of females analyzed)

brids as in most tilapia (Toguyeni *et al*., 1997). The values obtained could increase in a more open environment as suggested by L'Anson *et al.* (1991). In this regard, Duponchelle and Panfili (1998) showed that the size of first sexual maturity in *Oreochromis niloticus* was higher in the lakes of Ayamé (14,000 ha) and Kossou (80,000 ha) than in small impoundments of agro-pastoral water (6 to 620 ha). Indeed, according to these researchers, the energy used for activities such as foraging and other movements in large environments, as in captivity was invested in the reproductive activities, hence the precocity of sexual maturity.

The absolute fertility achieved in hybrids is high overall. Indeed, when relativizing this fertility, the values obtained (41.25 per gram of female) were much higher than those recorded by some authors, in the parental species. In *Oreochromis niloticus,* Moreau (1979) and Duponchelle and Legendre (2000) recorded values of 3.43 to 12 oocytes per gram of female whereas in *Sarotherodon melanotheron* values of in between 3.30 and 10 oocytes per gram of female were obtained by Peters (1963) and Legendre et al. (1990). In some species of highly fertile Cichlids (*Tilapia zillii*, *Tilapia guineensis*, *Hemichromis fasciatus*, etc.), values of 20.6 to 100 oocytes per gram of female were obtained by Albaret (1982). As a result, the hybrids obtained in this work could be considered high fertility species.

The oocyte diameter noted in hybrids at laying

(2.2 mm) is lower than those (3.4 and 3.5 mm) obtained by Legendre and Trebaol (1996) and Duponchelle and Panfili (1998) respectively, in *Sarotherodon melanotheron,* and *Oreochromis niloticus* at the same stage. Our results are similar to that of Albaret (1982) which obtained in some Cichlids (*Hemichromis fasciatus* and *Tilapia zillii*), a mean ovocyte diameter of 1.65 mm at laying stage. At the laying, the diameters of the oocytes present only one mode. This would mean that these hybrids lay once and it is after this spawning that another generation of oocytes is formed.

The average absolute fecundity obtained is 1040 oocytes per female. However, with this high fecundity, the number of sexed fish per female was an average of 434 fry. This low value could be due to a change in the breeding strategy of these hybrids as mentioned above. It would also be due to a low hatching rate. The size of the eggs determining the size of the larvae could also justify the number of sexed fry. In fact, the size of the eggs has an influence on the development, survival and growth of the larvae. According to Beacham and Murray (1985) and Goto (1990) larvae from large eggs can survive longer without food and are more able to avoid predators than those from small eggs.

Hybrids breed throughout the year with a sex ratio of 1: 1 as similar to their parents (Marche-Marchad, 1981, Legendre and Ecoutin, 1989, Baroiller and Toguyeni, 1996, Eknath *et al*., 1996). This sex ratio was also observed by Toguyeni et al. (2009) after artificial insemination between *Oreochromis niloticus* and *Sarotherodon melanotheron* in freshwater.

CONCLUSION

This study showed that the size of first sexual maturity of hybrids was 11.61 cm in the male against 10.11 cm in the female. The sex ratio at egg hatch was 1: 1. The high relative fecundity recorded in these hybrids (41.25 oocytes / g female) as well as their reproductive potential throughout the year could be considered as advantages at the lagoon fish farm.

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