



Genetic Characterization and Comparative Study of Two *Tilapia* Species and Their Hybrids Fecundity Reared in the South Eastern of Côte d'Ivoire

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Abstract: The present work was performed at Aboisso, in the Sud East of Côte d'Ivoire (West Africa). Broodstocks, *Tilapia zillii* and *Tilapia guineensis* were bred in cemented tank to study their fecundity and also that of reciprocal hybrids F1A [*Tilapia zillii* (Male) x *T. guineensis* (female)] and F1B [*Tilapia zillii* (female) x *T. guineensis* (male)] obtained. Before crosses, genetic characterisations were performed on parental strains and first generation hybrid to be sure that broodstocks are pure strain. This first investigation shows that the broodstocks used for reproduction are pure strain and all specimens hybrid F1A and F1B are clearly characterized by the presence of a composite profile, both constituted of RAPD specific fragments of *T. zillii* and *T. guineensis*. The reproduction shows that mortality rate of broodstocks [0% for all crosses except *T. zillii* (female) x *T. guineensis* (male)], average values of fecundity (4965 fingerlings/female kg/year - 6612 fingerlings/female kg/year), time between two successive spawns (45,67 d-70,36 d), duration before first reproduction (24 d-190 d) and sexe ration (1:1) obtained are satisfactory in the perspective of using hybrids in fish culture. The less satisfactory fecundity parameters are obtained with *T. zillii* (female) x *T. guineensis* (male) crosses.

Keywords: Genetic characterization, Côte d'Ivoire, Reproduction, RAPP, *Tilapia* hybridization.

I. INTRODUCTION

In Côte d'Ivoire, the gradual decrease of natural fish stocks is related to the strong pressure of fisheries, the rapid population growth, the industrial and anthropogenic activities. This phenomenon is one of the major reasons of the current fish farming development [1]. This livestock primarily concerns the cichlids family within which the systematics is often discussed. Indeed, the great richness of this group and the environmental factors, favor the emergence of hybrids ([2], [3], [4]). This is the case of the man made-lake Ayamé in the South Western of Côte d'Ivoire. The building of a hydroelectric dam in 1959 led to an artificial lake with wildlife and a aquatic flora.

Concerning the fauna, heterogeneous population of cichlids fish were observed. As species, *Tilapia zillii*, colonising continental water courses and *Tilapia guineensis*, subservient to lagoon. The third category of

fish, object of this study, was identified as a hybrid from the cross between these two species cited above ([5], [6]). From 1995 to 1998, the man made-lake Ayamé contained 39 species of fish [7]. The hybrids tilapia represent 24% of this production, against only 4% for *T. zillii* and 2% for *T. guineensis* [8]. According to [9], [10], [11]-[12], research works carried out on Ayamé man made-lake, showed that these hybrids create a great scientific economic and social interest. In addition, [13] believes that aquaculture in Africa must clearly develop from indigenous species. Hence the need to test their zootechnical performance in view of their popularization. However many problems related to the heterogeneous nature of the population constitute a brake to the continuation of studies ([14], [15], [5], [16]-[17]). That is the purity of the parental strains and the hybrids identification, because it is not possible to determine these fish stage of generations. These identification problems are clarified by [18], that make possible continuation of research.

The present work is in the context of Ivorian and Belgian project, entitled "experimental breeding of fish in floating cages". The main objective of this investigation is to study reproduction parameters among parent species and the hybrids, after the determination of these tilapia's genetic characteristics. This study has three parts. On the one hand, the mitochondrial DNA (DesoxyriboNucleic Acid) is analysed by the method of PCR-RADP to ensure of the parental purity. On the other hand, the physico-chemical characteristics of reproduction area are studied in order to check their variability. Finally, the comparative study of the fecundity is performed during the intraspecific, interspecific and introgressives reproductions.

II. MATERIALS AND METHODS

This study was carried out between June 2002 and May 2003 at the Aboisso Waters and Forests Office of Fish Culture. This city is located in the South eastern of Côte d'Ivoire, at 20 km from the Ayamé man-made lake (5° 36' N and 3° 10' W). It was built since 1959 on the Bia River, a 300 km of length small coastal basin.

Broodstocks of *Tilapia zillii* and *T. guineensis*, come from Ayamé man-made lake. They were selected according to the criteria of mature individuals choice established by [19], [20]. The fish are caught using traps or nets. After the capture of 60 broodstocks composed of 30 males and 30 females of each species, they are introduced separately in oxygenated water and immediately routed in 2 acclimatization cemented tanks of Aboisso for 15 days. So that they can adapt to the captivity conditions.

Measurement instruments used are microprocessor type. A conductivity meter model WTW LF 340, was used to measure the conductivity and TDS (Total Dissolved Solids). The values obtained are expressed respectively in micro-siemens per centimeter ($\mu\text{s}/\text{cm}$) and in milligrams per liter (mg/l). An oximeter model WTW OXI 330, combined with a thermometer, were used to assess simultaneously dissolved oxygen values in mg/l and temperature in Celsius degrees ($^{\circ}\text{C}$). A model WTW pH 330 pH-meter allows us to measure the acidity. Instruments are turned on for a duration of 5 minutes. Each probe is then driving in 50 cm from the surface of water. Finally, the values of the physico-chemical characteristics are automatically displayed on each screen.

A Secchi disk with a 30 cm diameter allowed us to determine the transparency of the water in millimeters (mm). After immersion in the water until completely disappearing, it is gone back very slowly until it is visible. The depth at which the disk appears constitutes the visual measurement of the water transparency [21]. The measurements are carried out on a weekly basis, 2 times per day, specifically in the morning between 6:30 and 7 a.m. and the afternoon between 15:30 and 16:00 p.m.

The genetic characterization has focused on muscle. It concerned 30 samples from each parental strain and reciprocal hybrids of first generation. On each specimen, 0.5 cm^3 muscle is taken on dorsal right side of fish and then kept in ethanol (100%). They are submitted to PCR-RAPD (*Random Amplified Polymorphic Dexoyribonucleic Acid*) for the DNA mitochondrial analysis. From each collected tilapia species and hybrid, DNA extraction and purification were carried out according to [22].

A. Muscle mixing

After the mixing of 50 to 100 mg tissue muscle of *Tilapia zillii*, *T. guineensis*, hybrids tilapia F1A or F1B, 3 ml of PBS buffer sterile (Phosphate-Buffered-Saline) are added. The mixture obtained is incubated at $+4^{\circ}\text{C}$ under stirring during 12 h, and then centrifuged for 2 minutes at 13000 tr/min. The supernatant containing low density particles is centrifuged for 15 min at 13000 tr/min. The gall obtained is suspended in 500 μl of sterile water DEPC containing 100 mM Tris-HCl (pH = 7.5), 5 mM EDTA, 1% of SDS and 200 $\mu\text{g}/\text{ml}$ of proteinase K. The mixture is then agitated with a magnetic stirrer vortex and incubated at 65°C in a water bath for 1 hour. Then 50 μl of CTAB/NaCl are added to the incubated solution. This mixture is incubated again until 10 minutes. Subsequently,

two extractions by phenol-chloroform method were performed [22].

B. Extraction of mitochondrial DNA with phenol-chloroform

That is performed with a 250 μl phenol mixture saturated with water and a 250 μl chloroform-isoamyl alcohol which are added to the gall of mitochondria lysed. After homogenization, centrifugation at 13000 tr/min at 4°C is performed for 10 min in a refrigerated centrifuge (JOUAN type). The aqueous phase above undergoes a second extraction with phenol-chloroform as described previously. It is collected for the DNA precipitation. The gall is washed twice with 75% ethanol and dried.

C. PCR-RAPD reaction

DNA is amplified by using 2 different primers: OPA17 and OPB8 [23]. The PCR reactions were carried out in a mixture of 50 μl containing a suspension of 3.5 mm MgCl₂, 1.2 μm of each primer, 40 to 90 ng of total DNA, 0.4 mm of each dNTP (dNTP-Mix, Hybaid) and 1.5 unit of a mixture of Taq polymerase (Proof Sprinter, Hybaid).

The amplified DNA was then analyzed by electrophoresis on an agarose gel to 1.5% (QualexGold, Hybaid). The electrophoretic migration has been carried out in a buffer TBE (Tris-Borate-EDTA) for 1 hour at 100 volts. The amplification products were then visualized by illumination on a UV transilluminator (Bioblock).

D. Rearing structure and reproduction

Ponds and concrete tanks used for reproduction have a 6.24 m^3 surface area and receive a freshwater from a small river. The inputs and outputs of these trays are fitted with protective grilles of 1 mm mesh to avoid any intrusion of foreign fish from the restraint. The broodstock selected were subjected to intraspecific and interspecific crosses. Hybrids obtained after the first crosses are used for introgressive crosses. Whatever the cross, 6 pairs of corresponding broodstock were put together in concrete tank in order to reproduce. The stocking density varied between 0.35 and 1.06 individual/ m^3 [24]. Whatever the type of reproduction, females of average weight ranging between 79 g and 98 g were used. With regard to males, their weight varied between 128 g and 133 g ([25], [26], [27]). The reciprocal interspecific crosses, between *T. zillii* (Male) and *T. guineensis* (female) and between *T. zillii* (female) and *T. guineensis* (male) were performed to obtain first generation hybrids named respectively F1A and F1B. With regard to introgressive crossings, the F1A were crossed each other to get second generation hybrids called F2A. The same is true for the F1B which have F2B for descendants.

After the broodstock stocking, the sexes were revised for three times, with seven days of interval. This precaution has the benefit to confirm the sex of each individual. Each group of fish stocked for the reproduction was followed for a year [28]. Broodstock were fed *ad libitum* with a 28% protein pelleted commercial food (T4GF) manufactured by FACI [Société de Fabrication

d'Aliments en Côte d'Ivoire: 18 BP 686 Abidjan 18] (Table 1). Food is a 4 mm diameter for the broodstock and floury for fish fry.

The fertility expression among the tilapia was defined in several ways ([29], [30]). Thus, [31] defines fertility as the numbers of fingerlings obtain after breeding, during an individual existence. In experimental breeding condition, [28] limit the period of fertility to a year. In the present investigation, the number of fingerlings from breeding is therefore expressed in the following way:

$$\text{Number of fingerlings produced (fing / fe kg / y)} = \frac{\text{Fingerlings in the concrete tank}}{\text{Weight of females (kg) x 1 year (365 days)}}$$

Fing = Fingerlings ; fe kg = female kg ; y = year.

Mean period between successive spawn (PSS) and time before the first reproduction (TR) were also determined.

Mortality rates (MR): The mortality rate is the proportion of dead fish during the farming.

$$\text{MR (\%)} = \frac{(\text{Ni} - \text{Nf}) \times 100}{\text{Ni}}$$

Ni = initial number of fish, Nf = final number of fish.

Table 1. Flour and 4 mm pellet foods composition; Data indicated by the commercial notice of FACI (Food Manufacturing Company in Côte d'Ivoire).

Components	Flour and T4GF
Protein gross	28 %
Fat gross	7 %
Cellulosic material	7 %
gross	
Vitamin A	8000 UI/kg
Vitamin D3	2000 UI/kg
Vitamin E	100 mg/kg
Vitamin C	130 mg/kg

III. RESULTS

E. Genetic characterization of parental strains and first generation hybrid

The electrophoregram obtained following RAPD of these 4 specimens revealed profiles with migration bands with sizes between 200 and 2000 pb (Figure 1). The *Tilapia guineensis* samples are characterized by the RAPD fragments of a 830 pb sizes with OPA 17 primer and 980 pb with OPB 8 primer. Similarly, the samples of *Tilapia zillii* can easily be identified by the presence of RAPD fragments which the bands of migration are 220 pb and 1100 pb with the OPA 17. With the OPB 8, they are 580 pb. All specimens hybrid F1A and F1B are clearly characterized by the presence of a composite profile, both

constituted of RAPD fragments specific of *T. zillii* and *T. guineensis*. No difference to the interior of the RAPD profiles was noticed between the two groups of reciprocal hybrids.

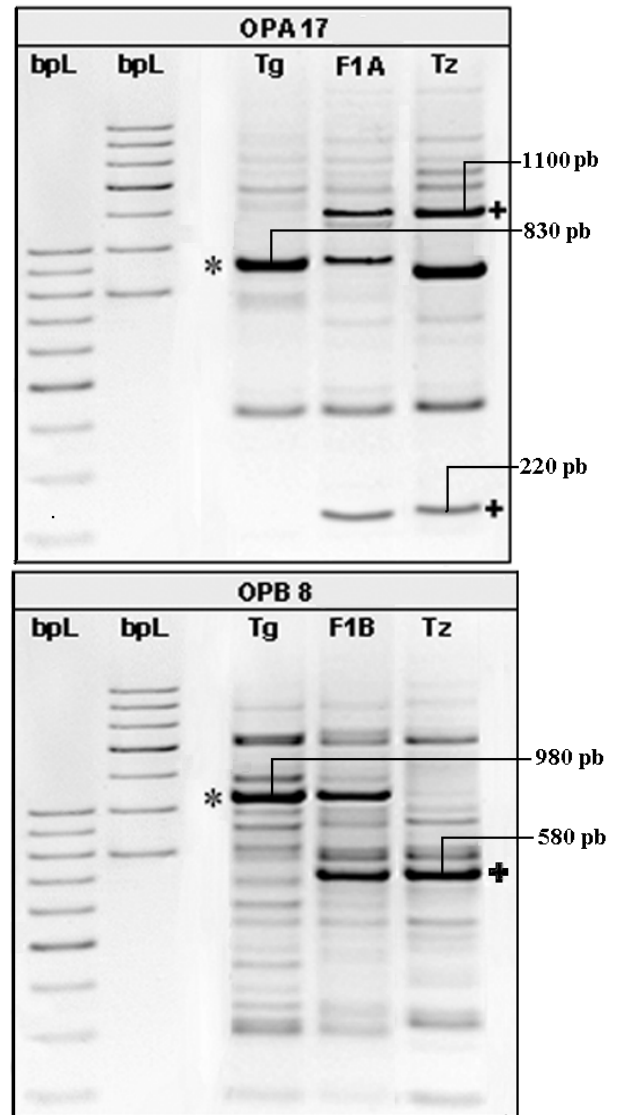


Fig. 1. Electrophoregram of Random amplified Desoxyribonucleic Acid polymorphic (RAPD) of *Tilapia zillii* (Tz), *T. guineensis* (Tg) and hybrids of F1A and F1B. OPA 17 and OPB 8: profiles of three different RAPD primers; + and *: RAPD specific fragments respectively of *T. zillii* and *T. guineensis*, present in the set of reciprocal hybrids. bpl: molecular weight marker (200 - 2000 pb).

F. Physicochemical parameters

Morning water temperature varies between 25.7 °C in August and 28.5 °C in March (Figure 2a). Those of the afternoon remain high throughout the year and acquires maximum values of 32.4 °C in May and minimum of 27°C in August. The difference observed between morning and afternoon temperature is significant (p = 0.001). The mean monthly dissolved oxygen rate show little variation on

morning and it is located between 1 mg/l and 1.5 mg/l (Figure 2b). Afternoon values drop in January until to reach 2 mg/l and then undergoes a significant increase of 9 mg/l in March. A significant difference ($p = 0.001$) between the oxygen rate of morning and afternoon is noticed. The mean monthly values of transparency fall on July and March morning. They are respectively 260 mm and 300 mm. The peaks are observed in January and April, they are respectively 430 mm and 360 mm. The afternoon values seem to follow the same trend, however, a peak of 420 mm is recorded in January, as well as two minima. One of 260 mm in July and the other of 280 mm in march (Figure 2c). No significant difference was noticed between

morning transparency values and those of the afternoon ($p = 0.13$). The water pH is slightly acid or neutral. Morning values are located between 6.8 and 7.7 (Figure 2d). The afternoon values fluctuate during the annual cycle, they are between 6.6 and 8.2. Differences observed are not significant ($p = 0.126$)

The conductivity increased from July (105 $\mu\text{s/cm}$) to February (145 $\mu\text{s/cm}$). Then it decreased up to 100 $\mu\text{s/cm}$ in April (Figure 2e). The TDS shows fluctuations during the annual cycle (Figure 2f). The lower values are recorded in October (70 mg/l) while maximum value of 130 mg/l, is reached in March.

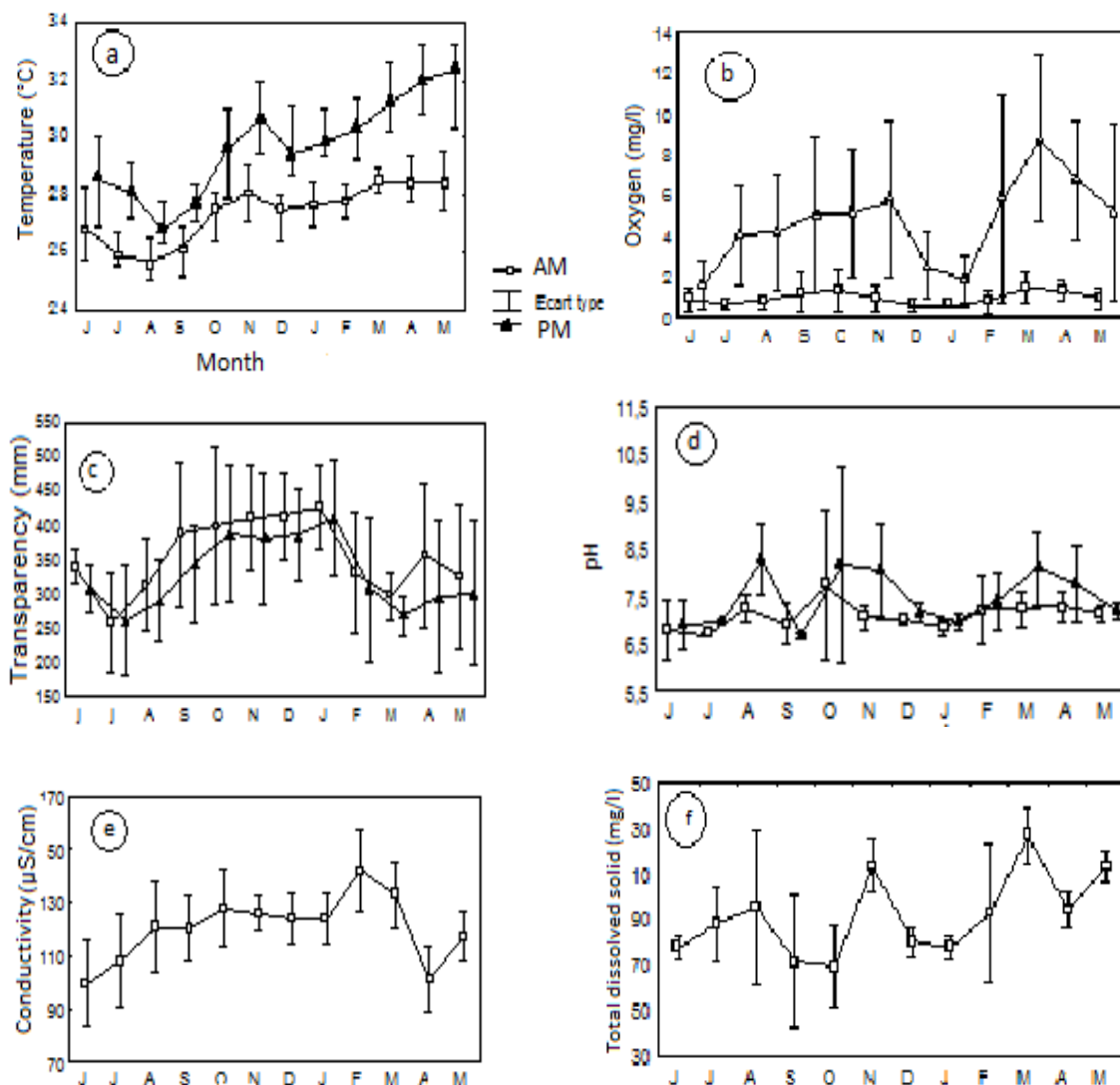


Fig. 2. Mean monthly variations of the physico-chemical characteristics recorded in the morning and afternoon in breeding concrete tanks of the Fish Culture Station of waters and forests of Aboisso, between June 2002 and May 2003. a: water temperature; b: dissolved oxygen; c: transparency; d: pH; e: conductivity and f: rate of dissolved solids.

G. Fecundity

The highest mean fecundity of *Tilapia zillii* is 8020 fing/fe kg/y and the lowest is 7650 fing/fe kg/y (Table 2). The average elapsed time between broodstock stoking and

first reproduction is 22 days. The average period between two spawn is 55 days. No Mortality is recorded among the brood stock of *T. zillii*.

The average fecundity of *T. guineensis* is respectively 6-

900 fing/fe kg/y. No mortality is observed among the broodstock. In this parental strain, the average time elapsed before the first reproduction is 26 days. The average period between two spawn is 58 days.

The fecundity of F1A crossing is 6612 fing/fe kg/y. No mortality was recorded after broodstock stocking. The average period elapsed before the first reproduction is 24 days. The average duration between two successive spawn is 63.3 days. When at the second crossing F1B, the average fecundity is of 4965 fing/fe kg/y. An average mortality rate of 33.33% is recorded. The average period between two successive spawn is 45.67 days. The average time of 190 days elapses before the first reproduction.

Within the introgressive crossovers between F1A hybrids, the average fecundity is 6146 fing/fe kg/y. No mortality was observed. The average period between two successive spawn is 70.36 days. The mean duration observed before the first reproduction is 56 days.

The average fecundity of the second crossing F1B is 6253 fing/fe kg/y. No mortality was recorded among the broodstock. The average period between two successive spawn is 66.34 days. The mean period elapsed before the first reproduction is 38 days.

H. Sex ratio of the progeny

In the intraspecific crosses, respectively 50.81% and 51.05% male sex of *Tilapia zillii* and *T. guineensis* were registered, corresponding to respective 1.62: 0.97 and 1.04

: 0.96 sex ratios (Table 2). Male Hybrids F1A and F1B of 55.26 % and 52.85% resulting from interspecific crosses were recorded, corresponding to respective sex ratios of 1.23: 0.81 and 1.12: 0.89. In the second generation F2A hybrids, 56.03% of males were produced. Concerning F2B hybrids, 51.69% of males were noticed. These results give respectively sex-ratios of 1.27: 0.79 and 1.07: 0.93.

Whatever the crossing, the proportion of males doesn't differ significantly ($p>0.05$) from that of the females (Table 3). Therefore, the different sex ratios recorded are statistically in the same order of magnitude.

Table 2. Growth and reproduction parameters as females mean weight (FMW), males mean weight (MMW), mean fecundity (MF), mean period between successive spawns (PSS), time before the first reproduction (TR) and mortality rate of broodstock (MR) observed during the intraspecific, interspecific and introgressive crossbreeding of Aboisso Waters and Forests Fish Culture Station.

Tz : *Tilapia zillii*; Tg : *Tilapia guineensis* ; Tzm x Tgf: *Tilapia zillii* (Male) x *T. guineensis* (female) ; Tzf x Tgm: *Tilapia zillii* (female) and *T. guineensis* (male); F1Am: hybrid from the dipped headlights Tzm x Tgf ; F1Af : hybrid from the dipped headlights Tzm x Tgf ; F1Bm: hybrids derived from crosses of the Tzf x Tgm; F1Bf: hybrids derived from crosses of the Tzf x Tgm ; Fing = Fingerling ; fe kg = female kg ; y = year.

	FMW (g)	MMW (g)	MF(fing/fe kg/y)	PSS (d)	TR (d)	MR (%)
Tz x Tz	94.66±0.50	123.67±11.61	7690±203.06	55±46.45	22±3.05	0±0.00
Tg x Tg	96.67±7.51	124.33±6.00	6900±87.36	58±51.07	26±3	0±0.00
Tzm x Tgf	92±10.15	128.67±4.00	6612±797.49	63.33±45.05	24±3	0±0.00
Tzf x Tgm	88.33±3.01	130.33±2.01	4965±493.50	45.67±11.00	190±12.59	33.33±0.00
F1Am x F1Af	96±6	126.67±4.52	6146±208.89	70.36±46.49	56±7.00	0±0.00
F1Bm x F1Bf	94.67±8.51	135.67±4.55	6253±266.66	66.34±42.23	42±0.00	0±0.00

Table 3. Sex ratios of fingerlings obtained during the intraspecific, interspecific and introgressive crosses. The values of a same line sharing a letter in common do not differ significantly. Tz: *Tilapia zillii*; Tg: *Tilapia guineensis*; Tzm x Tgf: *Tilapia zillii* (Male) x *T. guineensis* (female); Tzf x Tgm: *Tilapia zillii* (female) and *T. guineensis* (male); F1A: hybrid from the dipped headlights Tzm x Tgf; F1B: hybrids derived from crosses of the Tzf x Tgm; F2A: hybrid derived from crossbreeding F1A x F1A.

Crossing	Salmon obtained	Male (%)	Female (%)	Sex-ratio
Tz x Tz	Tz	50.81 a	49.19 a	1.62
Tg x Tg	Tg	51.05 a	48.95 a	1.04
Tzm x Tgf	F1A	55.26 a	44.74 a	1.23
Tzf x Tgm	F1B	52.85 a	47.15 a	1.12
F1A x F1A	F2A	56.03 a	43.97 a	1.27
F1B x F1B	F2B	51.69 a	48.31 a	1.07

IV. DISCUSSION

The analysis of the mitochondrial DNA of parental strains that are *T. zillii*, *T. guineensis* as well as that of the hybrid F1A and F1B by the technical RADP, just as the study of the full sequence of the cytochrome b conducted by [32], demonstrates that the specimens used in the present work are of pure strain. The ability to successfully conduct reciprocal crosses of *T. zillii* and *T. guineensis* in an artificial environment such as the basins in concrete is demonstrated in the present investigation. In addition, genetic studies carried out, such as the analysis of mitochondrial DNA confirms the identity of the parental strains and their hybrids. They also indicate that these latter are intermediate between the two parents.

According to [33], the rapid fluctuations of the temperature and the rate of dissolved oxygen during the day severely stress the fishes. Apart from this disadvantage, the values of the physico-chemical parameters saved in the concrete basins show that this environment is favorable to the mass production of fry.

During the crossing the *T. zillii* (female) x *T. guineensis* (male), the values the less significant, concerning the reproduction settings have been saved, in the sense that it has been observed a relatively long period before the first reproduction. One of the important characteristics of this crossover is that the female is *T. zillii*. The reproduction within this type of low beam seems to be very strongly influenced by the female deemed very aggressive according to the observations of [24]. The reproductive parameters above cited appear to be strongly influenced by the aggressiveness of the female. In addition, faced with the observations of [34], the results obtained at the reproductions within this crossover *T. zillii* (female) x *T. guineensis* (male) are better than those of incubators mouthwashes such as *Oreochromis niloticus*.

In the present investigation, the periods between two successive spawn reaching 128 days, were noted as well in the intraspecific, interspecific and introgressive crossings. Among *T. guineensis*, [27] attributed the rest periods long enough for 126 days of the adverse conditions in pens. According to [35], the temperature affects the duration of interpones by inhibition or slowing down of the vitellogenese. In effect, in the basins in concrete for the Fish Culture Station of waters and forests of Aboisso, the differences often pupils between the temperature of the water in the morning and the evening can severely strain the fish ([36]). According to [37] -[30], females of tilapia tend to spawn so desynchronised, depending on environmental conditions, are verified in the present study. In addition, tilapias in general are renowned for their low fertility, which may constitute a limiting factor, in the process of mass production of fingerlings ([38], [37], [39], [40]). These constraints are all the more important when the process of reproduction must involve two different species, which is not the case in the present investigation.

Any time, the fertility values obtained at the end of this researches are satisfactory in the perspective of an extension of these results.

According to [14] - [15], this species is naturally subservient to water courses continental. The significant production of fingerlings by the *T. zillii* seems to reflect an adaptation of these fishes to the fresh water, therefore the lake of Ayame I.

Sex ratio near 1:1 were reported by [41] with hybridization between *T.zillii* x *T. guineensis*. The sex ratio is not static. It is a dynamic parameter of the population able to change in such a way as spatial and temporal ([42]; [43]). [34]-[44] agree with these authors. [45] notices that the sex ratio may be a function of cross-species and of the meaning of the low beam. According to these authors, the dipped beam headlights between *Oreochromis niloticus* (female) and *O. aureus* (male) gave 30 per cent to 100 per cent of fingerlings of the male sex, while the reverse low beam product of individuals of sex ratio neighbor of 1: 1. According to [46]-[47], determinism polygenetique sex tilapia would be an

additional factor in the variability of the sex-ratio of hybrid populations. Of after [48], the factors such as temperature, photoperiod, the quality of the water, the stress would act on the determinism of the sex. In the case of the present study, the sex ratio is independent of the direction of the beam and of the lineage that is to say, the first or the second generation.

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