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Optimizing tilapia *Oreochromis niloticus* production in Senegal by reducing financial cost and time-consuming of gender determination

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Abstract
Nile tilapia, *Oreochromis niloticus,* is the most important cultured fish species in Senegal, with male monosex populations being preferred over females because of their higher growth performance. Manual sorting of sexes is being used to obtain male monosex populations, but the technique is very laborious, wasteful, stressful to the fish and expensive, and it can be prone to considerable mistakes. The purpose of this pilot study was, therefore, to optimize the production of *O. niloticus* in the Maraye Farm (Saint-Louis, Senegal) by reducing the financial cost related to gender determination. This was achieved by orally administrating a dose of 6.02mg/ml of 17α-methyltestosterone (17α-MT) to sexually undifferentiated fry from the 4th to the 28th day post-hatching. The results showed that 12.5% of fry did not survive the hormonal treatment, probably due to changes in physicochemical parameters in the holding tanks, in particular sudden drops of the dissolved oxygen levels at the 10th day after the treatment. Although this study did not use a control group fed with commercial diet without hormonal supplements, control groups of previous studies exhibited around 50% male population. The hormonal treatments of this study showed a satisfactory sex reversal rate of 80%, indicating that the dose (6.02mg/ml) used is effective in inducing a sex reversal in *O. niloticus*. With such results, the hormonal sex reversal can be successfully used instead of the manual sexing to optimize the tilapia *O. niloticus* in the Maraye Farm. However, this can only be achieved by optimizing water quality and efficiency in feed conversion, as is evident from high mismanagement-related mortalities (40%) recorded before evaluating the effects of hormonal treatments.

Keywords: Sexual reversal; methyltestosterone; *Oreochromis niloticus*, optimization, aquaculture, production

Introduction
Fisheries depend on the productivity of exploited aquatic ecosystems, which have continued to decline in recent years as demand for fish continues to rise as a result of human population growth [1]. Thus, aquaculture has emerged as the main alternative to ensure an adequate fish product supply to the human populations [2]. As a result, the global production of fish from aquaculture has significantly increased during the last decades [3, 4]. The contribution of aquaculture to the world global fish production was 13.4% in 1990, 25.7% in 2000, 30.6% in 2003 and 42.2% in 2012, representing over 66.6 millions tones of food [5] [6]. Aquaculture provided half of the worldwide amount of fish consumed by the human population in 2012 [5]. The global aquaculture production is continuously growing and reached 97.2 million tons in 2013, representing an estimated value of 157 billion US dollars [3]. The sector of aquaculture is nowadays one of the fastest-growing food suppliers in the world and is expected to contribute about 50% of total fish consumption by 2020. Over 90% originates from Asia, 70% of which is from China [9]. The proportion of global production originating from African aquaculture farms is trivial but, currently, considerable efforts are being made to expand aquaculture into new regions, particularly in sub-Saharan Africa.
Among the most frequently cultivated fish species in Africa are the Nile tilapia, *Oreochromis niloticus* L., and the African sharptooth catfish, *Clarias gariepinus* [10]. The farming of tilapias, including Nile tilapia, constitutes the most widespread type of freshwater fish farming in the world, particularly in Africa [11]. The success of *O. niloticus* in aquaculture is due to its ability to grow and reproduce under a wide range of environmental conditions, its low trophic

level for feeding, and its high tolerance to stress induced by handling and poor water quality [14, 16, 18]. Despite these positive attributes, *O. niloticus* has a rapid and frequent reproduction that has a negative impact on growth performances because most of energy is invested in reproduction [20]. *Oreochromis niloticus* is a mouth brooder and a filter feeder, and females do not feed during the breeding period, which strongly impacts growth performance. Consequently, males of *O. niloticus* have higher growth rate due to better food conversion efficiency, making them the preferred sex for aquaculture [19, 21].

In order to meet the increased demand for food and for animal-based protein in particular, the Senegalese government has created a modern fish farm in Diama Maraye, located about fourty kilometers from the city of Saint-Louis in the Maraye village of the Diama municipality, in the department of Dagana. The promotion of fish production by the farm of Maraye contributes to reduce the risks of poverty and malnutrition, which is evident from the satisfactory production of *O. niloticus*. However, because of its reproductive particularities and sex-specific differences in growth rate, the breeding males of *O. niloticus* is economically more profitable compared to that of mixed (male/female) populations [22]. However, the Maraye Farm is confronted with the serious difficulties involved in the identification of the sex of the fry prior to their transfer into growing-out tanks. The sex identification of early life-history stages which was, so far, done manually is extremely time-consuming. It is also very expensive in terms of manpower and food supply since female fry were fed until they reached the stage where the gender can be determined manually, but were then discarded. There is therefore an urgent need to find a more efficient technique that allows producing only male offspring. To that end, we conducted this pilot study to produce *O. niloticus* male monosex sex populations by using 17-α-methyltestosterone, which is among the most effective and economic method to produce all male monosex [23, 24]. This hormone acts on undifferentiated fish gonads by overriding the expression of genotypically determined sex, allowing the establishment of all-male populations [25].

Material and methods

Presentation of study area

The farm of Maraye, which is managed by the National Agency for Agricultural Integration and Development (ANIDA), covers an area of 25 hectares with a production capacity of 30 thousand tons of fish per year. The farm has a hatchery with 18 tanks including twelve of 10 m^3 and six of 30 m^3 with a production capacity of five million quality male fry per year. The farm has 44 production ponds including four breeding ponds of 300 m^2, 20 nursery ponds of 25000 m^2 and another 20 growing out ponds of 10000 m^2. In the Maraye area, the climate is favorable for fish production. The average maximum temperature in the region is around 25°C from March to October. Such temperatures are favorable for reproduction and growth of *O. niloticus*.

Experimental design

In this study, sex reversal experiments were carried out over a period of 42 days (6 weeks), during which individuals of *O. niloticus* were kept in three different breeding environments corresponding to the three phases of the experiment. These phases were the reproduction of the broodstock (first phase) which was conducted over a period of two weeks in a fiberglass tank (2.90 m long, 1 m wide, 0.90 m high and 2.90 m2 surface) installed inside a breeding pond; the sex reversal of the larvae (second phase), which was done in two different tanks of the same dimensions (5m long, 2m wide, 1m deep) and lasted four weeks and the evaluation of the treatment efficiency (third phase), which was obtained by examination of the genital papilla.

Given that large variation in weight and size can increase aggressive interactions and lead to variability in batch fecundity in *O. niloticus*, the broodstocks used in this study were selected according to their weight in order to have a homogeneous batch and avoid any aggressiveness between individuals. They were maintained in a 2.90 m^2 hapa nets in the breeding ponds with a sex ratio of 1:3 at a density of 500g/m^2. Thus, three males weighing 150 g each and nine females weighting 100 g each were used. These spawners were fed with a feeding rate of 2% of the total biomass/day (2.90 $m^2 \times 0.5$ m = 1.45 kg). The optimal time to harvest the eggs was estimated over a period of 15 days. Recently hatched larvae were harvested from the mouths of females. A total of 400 larvae were placed directly into two different hatchery tanks (10 m^3 volume each) referred to hereafter as T1 and T2, with 200 larvae per tank.

The physico-chemical parameters of the water (temperature, pH and dissolved oxygen) were monitored daily during the experimental period. These parameters were recorded twice a day, in the morning and in the afternoon. Although tilapias have wide tolerance ranges to physical parameters, but exceeding these may result in impaired growth and reproduction. The water quality variables were recorded with a Hach HQ30D portable multi-meter (Hatch Company, Loveland, Colorado, US) that simultaneously measures pH, conductivity, salinity and dissolved oxygen.

Feed preparation and feeding for sexual reversion

A high quality feed is needed for an active hormone/feed response and an efficient sexual reversion. The feed used in this study contains a high level of protein (48%) and lipid (5%), but the particles are too large for young fish, and they were thus crushed for a better assimilation by the young fish. Given that the 17-α methyl testosterone is a steroid hormone that is not water soluble, it is necessary to dissolve an appropriate quantity in alcohol [26]. Thus, a stock solution was initially prepared by dissolving 1.00 g of hormone in 166 ml of absolute alcohol. This stock solution of 6.02 mg/ml concentration was stored in a 4°C refrigerator to protect it from light, which can degrade the hormone during prolonged exposure. For the feed preparation, one kg of feed was placed in a bowl. Ten ml of 17-α-methyl testosterone stored stock solution were mixed with 490 ml of absolute alcohol for a final volume of 500 ml solution, which was then poured over one kg of feed contained in a plastic container. After being thoroughly mixed, the moist feed saturated with the 17-α-methyl testosterone solution was spread until dry and then stored under dark and dry conditions in a 4°C refrigerator for further use.

Usually, during treatment with the hormone, young tilapia fry consume 20% or more of their body weight per day [26]. This rapid growth requires a daily adjustment of the quantity of feed provided. This adjustment was done by using a feeding chart based on the method of Phelps and Popma [26]. Weekly corrections were then made based on the assumed growth rate. The hormone-treated feed was administered to sexually undifferentiated fry from the 4th to the 28th day post-hatching.

The amount of feed to provide was distributed to young fish five times a day, which were administered daily. At the end of the treatment, the fish stopped receiving the hormone because it affects the development of the gonads.

Evaluation of physico-chemical parameters and survival rate

The mean and standard deviation of water temperature, pH and dissolved oxygen were calculated for each tank. Correlations between water temperature and dissolved oxygen levels, and between pH and dissolved oxygen, were estimated using Spearman rank correlation [27].

The mortality rate is the number of deaths occurring within a given time period. In this study, the mortality rate (expressed in %) is equal to fraction of fish that died during the experiment period. The mortalities of the larvae in each tank were recorded by the end of the hormonal treatment (day 28) by manual counting. The mortalities of juveniles just before the gender determination (3 months after the end of hormonal treatment) were also evaluated. Both mortality rates were estimated using the following formula:

$$MR = \frac{Ni - Nf}{Ni} \times 100$$

Where MR is the mortality rate, Ni the initial number of fish, and Nf the final number of fish.

The survival rate (SR) of the larvae after hormonal treatment as well as that of the juveniles was deduced from the mortality rate used the following formula:

$$SR = 100\% - MR$$

The significance (probability, *P*) of difference between means of mortality and survival rates was determined from the t-test.

Evaluation of hormone treatment efficiency

After hormonal treatment (from the 4th to the 28th day post-hatching), the evaluation of the treatment efficiency should be based on the histological examination of the gonads after dissection of a representative sample of individuals. Since the acetocarmine was not available, fish were manually sexed based on the appearance of the genital papilla. Thus, three months after the completion of the hormonal treatment, fish were manually sexed.

Results

Physico-chemical Parameters

The water temperature, dissolved oxygen levels and pH of both tanks T1 and T2 were recorded during the experimental period. The results obtained in this study are compared with the literature data to determine if the variations in these parameters remained within the species' tolerance range limits during the experimental period. Water temperature varied between 28°C and 30.4°C for T1 and between 28°C and 30.7°C for T2 (Figure 1), with an average of 29.01°C and 29.18°C, respectively. The dissolved oxygen variations ranged from 4.1 to 0.6 mg/l for T1 and from 5.1 to 0.6 for T2 (Figure 2), with an average value of 1.65 and 1.97, respectively. A significant decrease in dissolved oxygen levels was recorded over the length of the experiment (Figure 2). The decrease in oxygen levels was more marked between the first and the 10th day, and then change only very little from the 10th day until the end of the experiment. The variations in oxygen concentration in the both tanks during the experiment were significant, but they were always within the tolerance range limits (0.1-0.65 mg/l) of the species [28]. These variations reached a value of 0.6 mg/l of dissolved oxygen (Figure 2). The pH levels ranged from 7.1 to 7.4 for T1 from 7.1 to 7.5 for T2. The average value of the pH was 7.2 and 7.3 for T1 and T2, respectively. The pH was near to neutrality (pH = 7) during the whole period of the experiment (Figure 3). Despite the fluctuations observed during the 28 days of hormonal treatment, the maximum and minimum variations of these parameters were always within or close to the tolerance limit of the species. There were no significant differences in water temperature, dissolved oxygen and pH between the tanks T1 and T2.

There was no significant correlation between the dissolved oxygen levels and water temperature in both tanks T1 (R^2 = -0.021; $P > 0.5$) and T2 (R^2 = -0.009; $P > 0.5$). Likewise, the dissolved oxygen content was not correlated with water pH in both tanks T1 (R^2= 0.17; p > 0.5) and T2 (R^2= 0.24; p > 0.5).

Mortality and survival rates after hormonal treatments

The mortalities in each tank were recorded at the end of the hormonal treatment (day 28) by manual counting, and was 15% for T1 and 10% for T2 (Figure 4). The survival rate was high in both tanks and was 85% (170 individuals) for T1 and 90% (180 individuals) for T2 (Figure 4). The morality rates as well as the survival rates are not significantly different between the tanks T1 and T2 (Figure 4). Of the 350 individuals that survived to the hormonal treatment, 40% (140 individuals) died before the evaluation of the hormonal treatment efficiency.

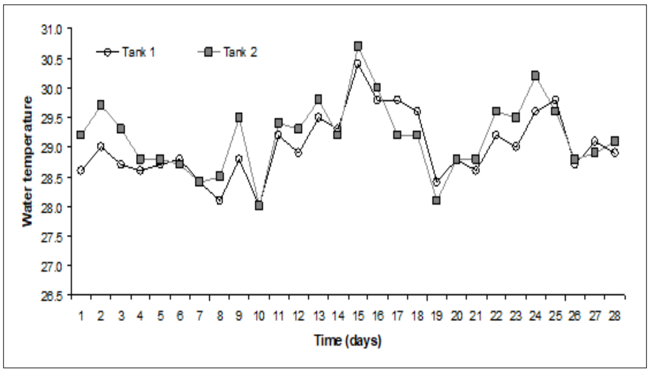

Fig 1: Evolution of water temperature in tanks during the period of the hormonal treatment

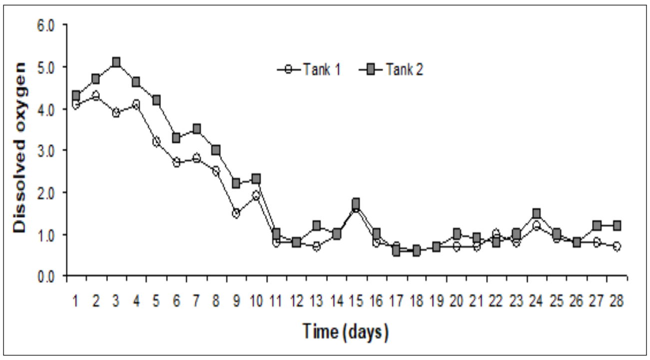

Fig 2: Evolution of dissolved oxygen levels in the tanks during the hormonal treatment experiment

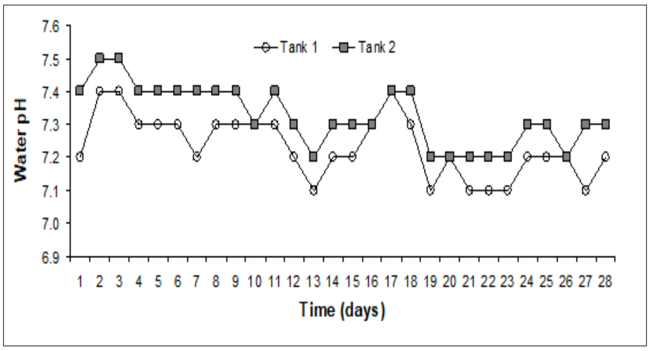

Fig 3: Variations of water pH in the experimental tanks during the hormonal treatment

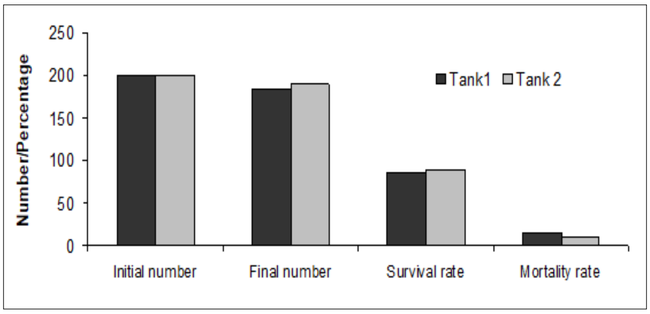

Fig 4: Survival and mortality rates recorded in the tanks after the hormonal treatment

Evaluation of hormone treatment efficiency

A total of 210 individuals, each weighing approximately 30g and being 3 months old were manually sexed. Of these, 168 were males and 42 were females, which corresponds to an average sex reversal rate of 80%.

Discussion

The results of the hormonal treatment for the sex reversal are very positive since an average survival rate of 87.5% was obtained at the end of the experiment. This high survival rate is comparable with the results of previous studies [29-33], which showed that tilapia fry have a high survival rate when treated with masculinizing than feminizing hormones. It has been demonstrated that both the timing and duration of hormonal treatment are extremely important for an efficient sex reversal in *O. niloticus* [34]. The satisfactory results of sex reversal observed in this study could be explained by the efficient timing and duration of the hormone treatment. However, another experiment with a different timing and duration of hormonal treatment would allow to better support this assertion. The main percentage of males obtained in this study (80%) could also be explained by a good monitoring of the fry during the whole hormonal treatment experiment. The absence of parasitism in both tanks is also a favorable factor for a high survival rate [35], which may explain the 85% and 90% survival rates recorded in T1 and T2, respectively. The average mortality rate of 12.5% observed after hormonal treatment could reflect the natural mortality rate of fry of tilapia. Indeed, it has been demonstrated that tilapia fry of the same age can undergo significant mortalities due to cannibalism, which can contribute up to 35% of total fry mortality [36]. The mortalities in both tanks cannot be explained by variations in water pH and temperature since values recorded for both parameters during the experiment are within the optimum range of *O. niloticus* and should not impact the survival of the fry [37]. However, the lowest dissolved oxygen levels are close to the species' lower tolerance and may be responsible for some mortalities.

Although water temperature can influence dissolved oxygen levels [38], the decrease in dissolved oxygen levels during the experiment is not due to changes in water temperature or pH because there is no significant correlation of both parameters with oxygen content. By contrast, the drops in oxygen content may be explained by an increase of oxygen consumption due to greater feed digestion/absorption as fish are growing up [39, 40]. Although the relationship between oxygen consumption and growth rate was not evaluated in this study, an increased energetic metabolism as fish grow is the more plausible explanation of the observed decreases in dissolved oxygen concentration [41]. Indeed, the circulating system that supplied oxygen did not change and was correctly functioning throughout the experimental period. This decrease in oxygen levels may limit feed utilization capacity and consequently lead to impaired growth [42]. It has been demonstrated that low oxygen supply may cause metabolic depression, and therefore, constrain fish growth [39, 41]. Depressed metabolic rates associated with impaired growth performances have been reported in many aquatic organisms, including teleost fishes [43, 44].

Studies on hormonal sex reversal using 17-α-MT to produce a male monosex population generally use control group where fish are not fed with hormonal treated feed. Here, we did not use a control group but previous studies have shown that control groups exhibited around 50% male population [26, 45, 46]. The results of this study showed a sex reversal rate of 80%, suggesting that the dose (6.02 mg of 17-α-MT/ml solution) used is very effective for inducing a sex reversal in *O. niloticus*. These results are consistent with those obtained by Rouf *et al*. [47] (93.33%-96.66% of sex reversed male with four different doses: 40, 50, 60 and 65 mg of MT hormone), The percentage of females that was not sexually reversed may be reduced by increasing the dose of 17-α-MT or by changing the timing and duration of the hormonal treatment. Further studies are needed to confirm such an assumption.

With an average rate of 80%, we believe that the hormonal sex reversal can be used instead of manual sexing to optimize the production of the tilapia *O. niloticus* in Maraye farm. The average sex reversal rate obtained in this study is satisfactory and can be optimized by using different hormonal doses and by taking care that fish consume only the hormonally treated feed supplied. This can be fulfilled by preventing the growth of planktonic or benthic organisms in the tanks that might represent an alternative food source. The manual sexing based on the dimorphism observed in the urogenital papilla has long been used to produce tilapia monosex males [12, 48, 49]. Although manual sexing is simple and gives good results, this method is expensive in terms of time and manpower and wasteful in terms of fish feed supply. Indeed, this technique requires a skilled personnel and rearing the female fry for 3 to 4 months, after which they have to be discarded. It also implies the utilization of large farming infrastructures for rearing both males and females until they reach the age that the gender can be manually determined.

Conclusion

The main objective of this study was to reduce the costs resulting from the manual separation of males and females using hormonal treatment, which could allow improving the aquaculture production of tilapia *O. niloticus* in Senegal. By orally administrating a dose of 6.02mg/ml of 17α-methyltestosterone (17α-MT) to sexually undifferentiated fry from the 4th to the 28th day post-hatching, we obtained an average sex reversal rate of 80%. The physico-chemical parameters recorded in the different phases of breeding of the broodstock and larvae throughout the experiment seem to be suitable for *O. niloticus*. Indeed, almost all the recorded values are in the optimum range of the species. The survival of the fry at the end of the experiment with a rate of 85 and 90% in the hatchery tanks T1 and T2, respectively, shows that physico-chemical parameters such as oxygen play a crucial vital role for the survival and growth of fish. Although growth was not analyzed in this study, it has been shown that the androgen-treated Tilapia alevins generally show faster growth than untreated individuals [50-54]. Therefore, future studies are needed to better evaluate the effects of sex steroid treatment on growth performances of *O. niloticus*.

List of abbreviations

ANIDA: Agence Nationale d'Insertion et de Développement Agricole
17α-MT: 17α-methyltestosterone
NMA: Nouvelle Minoterie Africaine
MR: Mortality Rate
Ni: Initial number of fish
Nf: Final number of fish.
SR: Survival Rate

Consent for publication

All authors read and approved the final version of the manuscript.

Availability of data and material

All datasets supporting the results of this article are within the article and its Additional Files.

Competing interests

Eventual conflicts of interest (including personal communications or additional permissions, related manuscripts), sources of financial support, corporate involvement and patent holdings are disclosed.

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Authors' contribution

AN and CABF conceived the study. SW conducted the experiment under the supervision of AN and CABF. MT analyzed the data and wrote the manuscript. All other authors have contributed to the manuscript preparation. All authors have agreed to the submitted version of the manuscript.

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