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**Research Article**

# Reproductive biology of the blackchin tilapia (*Sarotherodon melanotheron*) in the marine protected area of Niamone-Kalounayes (Casamance estuary, Senegal)

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**Abstract**

Knowledge of the reproductive biology of fish is essential for the local fish population management in marine protected areas. The objective of the present study is to determine the sex ratio, reproductive period, and sexual maturity size classes of the blackchin tilapia (*Sarotherodon melanotheron*) in the marine protected area of Niamone-Kalounayes, located in Casamance, Senegal. A total of 471 individuals including 305 females and 166 males were sampled from June 2021 to July 2022, using an experimental beach seine (250 m long, 25 mm side mesh). The sex ratio (M: F = 1.0: 1.8) was in favor of females. The size at first sexual maturity ($L_m50$) was obtained 11.00 cm for males and 11.71 cm for females. The seasonal variation in the gonadosomatic index (GSI) indicates that this population has an extended reproduction period, ranging from March to September, with a clear peak in March, followed by a period of sexual rest (October-December), and a period of maturation of the gonads from January through March. Results of this investigation provided insights for better management practices for this specie.

**Keywords:** Reproductive biology, *Sarotherodon melanotheron*, Sex ratio, Maturity, GSI Senegal

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## Introduction

The government of Senegal has initiated the establishment of a network of marine protected areas (MPAs), spread out on the coastal regions and in many of the estuarine parts of its main rivers, through the Department of Community Marine Protected Areas (DCMPA), since 2004.

The objective of this network is to preserve the habitats and biological diversity of the coastal zone, fish restocking, and promote the improvement of the livelihoods of coastal populations. These areas are therefore considered great places for fisheries management and the conservation of marine and coastal biodiversity. Senegal's MPAs are essentially created in rich ecosystems of high interest from a commercial and livelihood standpoint. Indeed, most of these MPAs are in mangrove-filled estuaries. In Senegal, most of these MPAs correspond to multi-usage areas (tourism, conservation, and wildlife habitats), except for the MPA of Bamboung, which corresponds to a strict biodiversity reserve, *i.e* without permitting commercial fishing or livelihood activities. The three major foundational management axes of those MPAs are conservation, sustainable use, and participatory management of natural resources (DCMPA, 2015). It is in this context, the MPA of Niamone-Kalounayes was created in 2015 in the Casamance River estuary, in southern Senegal.

The estuaries of Casamance and Delta Saloum are described in the literature as species-rich. The number of fish species in both those estuaries are relatively high compared to other rivers in the country. Ichthyofaunal surveys have revealed the presence of 114 species of fish in the Saloum estuary (Diouf, 1996) and respectively 85 species and 59 species in the Casamance estuary (Albaret, 1987; Guèye *et al*., 2012). These two ecosystems are among the main local artisanal fishing areas in Senegal.

Tilapia species are one of the most abundant groups of fish in the Casamance estuary. Ubiquitous in all seasons in the Casamance River, the blackchin tilapia (*Sarotherodon melanotheron*) largely dominates fish communities (Albaret, 1987; Diedhiou *et al*., 2022). The species is highly valued by local populations as a source of protein and is the subject of particularly high fishing pressure. Its dominance in the Casamance system and its fishing pressure makes it a perfect model organism to consider a fisheries management program based on its reproductive biology.

Knowledge of the reproductive biology of a fish species is deemed essential for the establishment of effective fisheries management (Marshal *et al*., 2003). To assess the reproductive biology of bony fishes such as the blackchin tilapia, key parameters needed to assess fish stocks include sex ratio, breeding period, fecundity class and size at first sexual maturity (Sun *et al*., 2009). Despite its socio-economic importance, few studies have been devoted to the reproduction of the blackchin tilapia in the Casamance estuary, and in Western Africa. Albaret (1987) studied some aspects of the reproduction of *Sarotherodon melanotheron* in the Casamance River.

The objective of this study is to improve the reproductive biology knowledge of blackchin tilapia. It specifically aims to determine the sex ratio, reproductive period, and sexual maturity size classes of the species, while using these metrics to propose sustainable management measures for the species within the MPA of Niamone-Kalounayes.

**Materials and methods**

*The study area*

The MPA of Niamone-Kalounayes covers the maritime, estuarine and freshwater parts of the Casamance River, in the Ziguinchor region, for a total area of 63 894 ha. It is composed of a complex and diffuse system of channels, commonly called bolongs, and bordered by mangroves, habitats that are characteristic of intertropical brackish wetlands. This MPA is bounded to the north by the classified forest of Kalounayes, to the east by the Soungrougrou River, to the west by the backwater of Bignona up to the Affiniam Dam, and to the south by the Casamance River (Fig. 1).

*Sampling method and reproductive parameters*

Data were collected monthly, over a one-year period ranging from July 2021 to June 2022. Fish were sampled in six different stations (Fig. 1), using a 250 m long beach seine with 25 mm side meshes. A total of 471 individuals, 305 females and 166 males individuals of blackchin tilapia were collected during this study. Total length of all fish sampled were measured to the nearest cm and then dissected. The total weight (TW), eviscerated weight (EVW), and gonads weight (GW) of each individual were taken to the nearest 0.1 g. The sex of each individual and the stage of sexual maturity were determined by macroscopic examination of the gonads. Seven different classes of sexual maturity were described using the Fontana scale (1969) and are outlined in Table 1.

The data collected made it possible to calculate a few reproductive biology metrics: the sex ratio (SR), the gonado-somatic index (GSI) and the size at first sexual maturity ($L_m50$).

The sex ratio (SR) was calculated in this study according to the formula of Kahn *et al.* (2021):

$$SR = \frac{M}{F}$$

Where F= number of females in the population and M= number of males in the population

The gonadosomatic index (GSI) was calculated using a formula from Analbery (2004):

$$GSI = \frac{GW}{EVW} \times 100$$

Where GW= weight of the gonads in g and EVW= weight of the eviscerated fish in g.

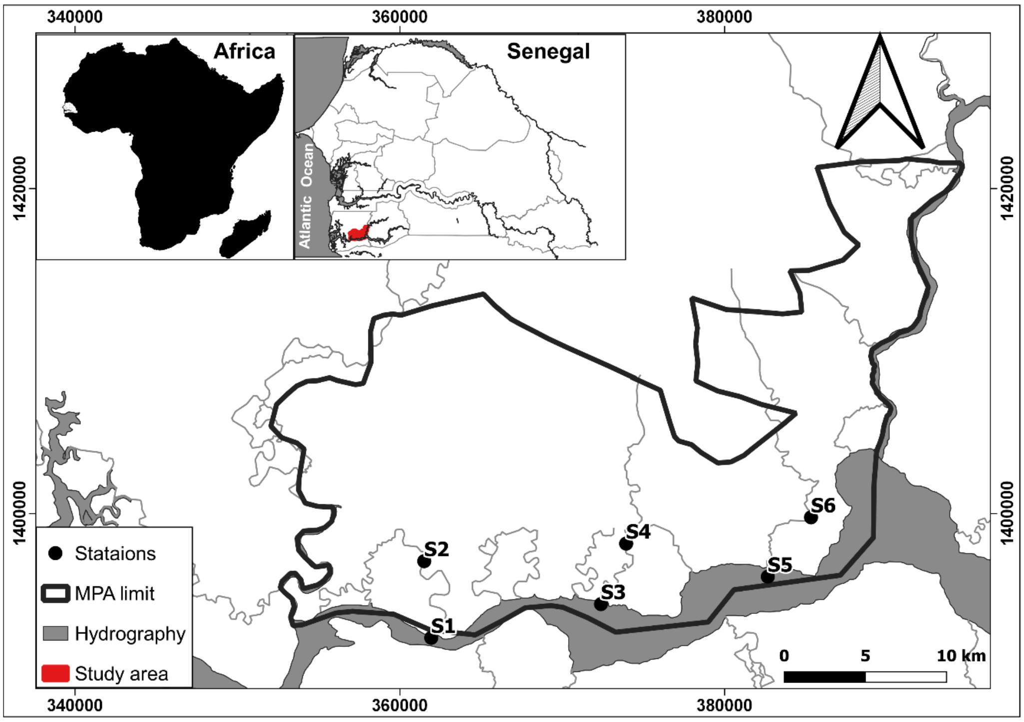


**Figure 1: Map of the MPA of Niamone-Kalounayes (Senegal) and sampling stations.**

**Table 1: Representation of the different stages of sexual maturity, according to Fontana (1969)**

| Stage | External macroscopic features | |
|---|---|---|
| | **Female** | **Male** |
| I | Immature: Firm gonad of small size, transparent or light pink, invisible oocytes. | Immature: White or slightly translucent gonad, very thin and in the form of a knife blade. |
| II | Sexual rest: Characteristics substantially identical to stage I. | Sexual rest: Characteristics substantially identical to stage I. |
| III | Ripening: Firm and colored gonad, varying from pale pink to light orange, some oocytes are visible through the ovarian membrane. | Ripening: Firm whitish gonad, no liquid flows if an incision is made. |
| IV | Advanced ripening: Larger and less firm gonad, usually light orange. The oocytes are visible through the ovarian membrane and make the surface of the ovary granular. | Advanced ripening: Softer and whiter gonad. A whitish fluid flows out as soon as an incision is made. |
| V | Ripe individual: Very large gonad occupying the entire abdominal cavity. Very thin ovarian membrane. Hyalin and large eggs are perfectly visible and are expelled at the slightest pressure exerted on the abdomen | Ripe individual: Large and soft gonad. Semen flows at the slightest pressure on the abdomen. |
| VI | Post-spawning: The ovary is very vascularized and flaccid. Its color varies from salmon pink to red. Through the ovarian membrane, the oocytes are not visible. Many hyaline spaces. | Post-spawning: Flaccid gonad and very fine vascularization, particularly in the posterior part. |
| VII | Spent: Ovary completely collapsed and very flaccid. Red color due to a very strong vascularization. At this stage, the ovary has the characteristic appearance of an empty sack. | Spent: Very flaccid gonad, exhausted and highly vascularized. |

The size at first sexual maturity ($L_m50$) was determined according to this equation from White *et al.* (2002):

$$\%P = \frac{100}{1 + e^{-\alpha(LT - LT_{50})}}$$

Where %P is the maturity percentage for a given length class, α = a constant, LT is the total length and $LT_{50}$ is the total length for 50% of the population.

Some physicochemical parameters of water, including temperature, salinity, pH, and dissolved oxygen, were recorded at each fishing station using an YSI probe (Model 63).

*Data analysis*

A Chi square test ($\chi^2$) was performed to compare the sex ratios between months against the theoretical sex ratio (1:1). An analysis of variance (ANOVA) was then used to compare the change in gonadosomatic index (mean GSI) between months for the same sex. The differences were considered significant at *p*-value<0.05. Statistical analyses were performed using R (version 4.3.1).

## Results

*Environmental parameters*

Monthly variations of the physicochemical parameters of the stations were recorded (Fig. 2). The highest temperature values were observed between April (34.1°C) and September (32.5°C) and the lowest between December (24.6°C) and February (25.2°C). In the Casamance River, salinity has a very marked temporal variation during the year. The highest salinities were recorded during the dry season, with a maximum in April (42 psu), and the lowest in the rainy season with minimum values in September (21 psu) and October (22 psu).

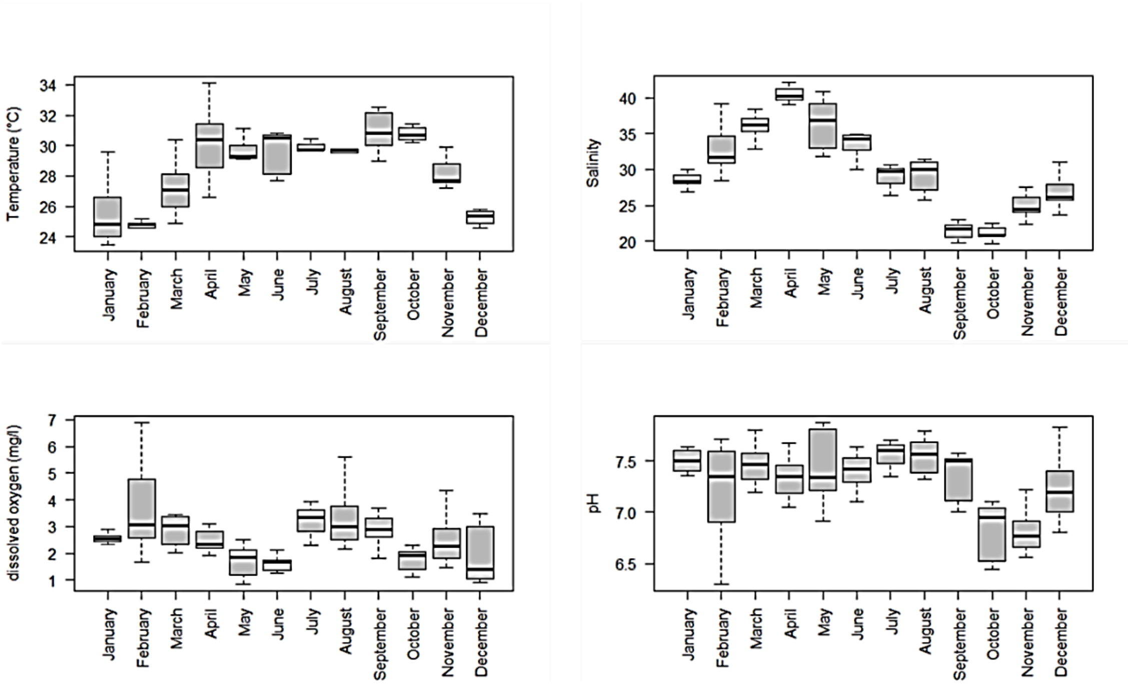


**Figure 2: Temporal variation of water physicochemical parameters in the MPA of Niamone-Kalounayes (Senegal), from June 2021 to July 202.**

The pH showed a relatively small temporal variation, with lower values recorded in October (6.44), November (6.56), December (6.80), February (6.30), and a peak observed in May (7.87). As for dissolved oxygen, temporal variation is a bit higher, with the highest concentration observed in February (6.89 mg/L) and the lowest in May (0.85 mg/L). The highest average values were recorded in February, March, July, August and September and the lowest in May, June, and October.

*Sex ratio*

The sex ratio (males: females) found (1:1.8) favors females and significantly differed from the theoretical sex ratio of 1:1, by $\chi^2$ test ($\chi^2$=38.53; *p*-value<0.05). Significant differences in the sex ratio in favor of females were recorded in March ($\chi^2$ =26.13; *p*-value<0.05), July ($\chi^2$=4.12; *p*-value<0.05), August ($\chi^2$=30.42; *p*<0.05) and September ($\chi^2$=33.92; *p*-value<0.05) (Table 2).

**Table 2: Monthly changes of the sex ratio of Sarotherodon melanotheron in the MPA of Niamone-Kalounayes (Senegal), between July 2021 and June 2022.**

| Month | Males | Females | Total | Sex ratio (M: F) | $\chi^2$ | *p*-value | Significance |
|---|---|---|---|---|---|---|---|
| January | 13 | 16 | 29 | 1:1.2 | 0.31 | 0.57 | $p > 0.05$ |
| February | 9 | 15 | 24 | 1:1.7 | 0.47 | 0.49 | $p > 0.05$ |
| March | 1 | 29 | 30 | 1:29 | 26.13 | 3.18e-07 | $p < 0.001$ |
| April | 11 | 22 | 33 | 1:2.0 | 3.66 | 0.05 | $p > 0.05$ |
| May | 18 | 12 | 30 | 1:0.7 | 1.2 | 0.27 | $p > 0.05$ |
| June | 20 | 25 | 45 | 1:1.2 | 0.55 | 0.45 | $p > 0.05$ |
| July | 14 | 27 | 41 | 1:1.9 | 4.12 | 0.04 | $p < 0.05$ |
| August | 4 | 41 | 45 | 1:10 | 30.42 | 3.47e-08 | $p < 0.001$ |
| September | 5 | 47 | 52 | 1:9.4 | 33.92 | 5.73e-09 | $p < 0.001$ |
| October | 29 | 30 | 59 | 1:1.0 | 0.01 | 0.89 | $p > 0.05$ |
| November | 35 | 30 | 65 | 1:0.8 | 0.38 | 0.53 | $p > 0.05$ |
| December | 7 | 11 | 18 | 1:1.6 | 0.88 | 0.34 | $p > 0.05$ |
| **Total** | **166** | **305** | **471** | **1:1.8** | **38.53** | **5.38E-10** | $p < 0.001$ |

*Gonadosomatic index (GSI) and reproductive period*

GSI increased steadily from February and reached a maximum in March in females and males (Fig. 3). Maturation of the gonads occurred between January and March. The peak of sexual maturity was recorded in March for both sexes, with a mean GSI of 11.00 (± 0.12) for males and 7.78 (± 1.76) for females. For each of the two sexes, the breeding season covered both the dry and rainy seasons. However, most of the reproductive activity based on GSI is observed in the dry season (March, April, May and June). Based on this metric, the reproductive period of *S. melanotheron* in that population seems to be from March to September (ANOVA, *p*-value<0.05), followed by a period of sexual rest from October to December.

*Size at first sexual maturity*

The size at first sexual maturity ($L_m50$) was 11.00 cm for males and 11.71 cm for females (Fig. 4). No significant differences were observed between the size of the first sexual maturity of males and females, by $\chi^2$ test ($\chi^2$=0.04; *p*-value>0.05). All male and female *S. melanotheron* individuals were considered sexually mature at a total length of 14 cm.

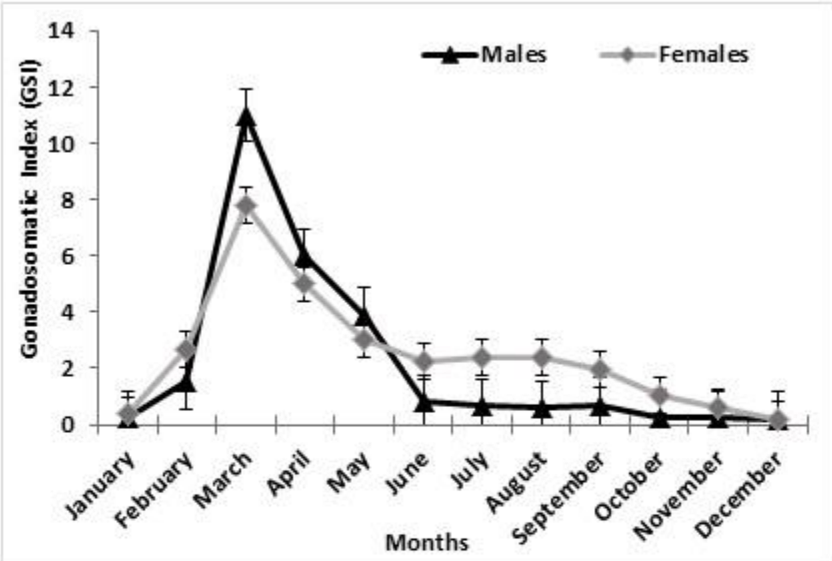


**Figure 3: Monthly variation of the GSI of Sarotherodon melanotheron in the MPA of Niamone-Kalounayes (Senegal), between July 2021 and June 2022.**

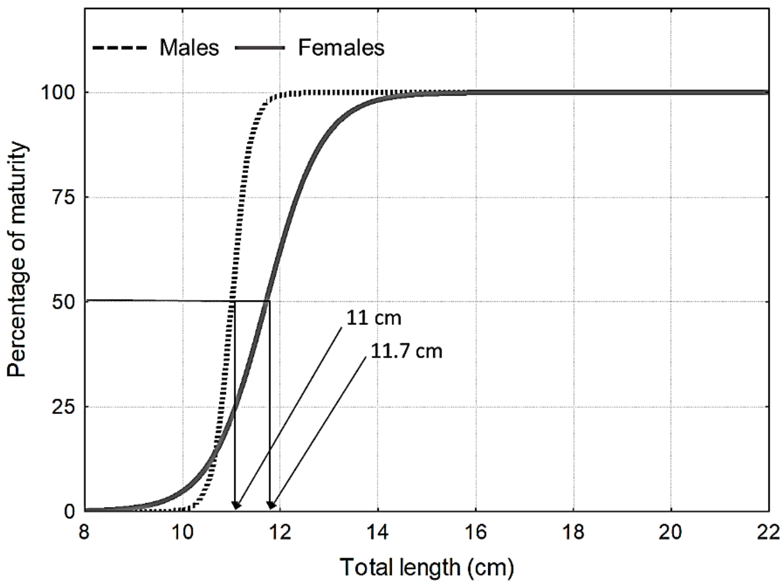


**Figure 4: Size determination curve at first sexual maturity ($L_m50$) for males and females of Sarotherodon melanotheron in the MPA of Niamone-Kalounayes (Senegal).**

## Discussion

Reproduction remains one of the most important aspects in the study of the biology of fish species (Hislop *et al.*, 1978). It is influenced by several factors, including the nutritional and physiological status of the species but also ecological factors (Mensah *et al.*, 2015). The blackchin tilapia (*S. melanotheron*) is an important and characteristic species of estuarine ecosystems in West Africa, for the fact that it is an abundant species and coastal populations highly depend on this resource as a source of revenue or protein.

*S. melanotheron* has significant adaptability to different habitat types (Amoussou *et al*., 2016). Analysis of the variation in physicochemical parameters showed some variations during the year in the study stations. This fish is highly tolerant to low levels of dissolved oxygen (Ouattara *et al*., 2003; Ouattara *et al*., 2009). Like most tilapia species, *S. melanotheron* does not encounter any metabolic difficulties if dissolved oxygen in the water is low (Ouattara *et al*., 2009). The dissolved oxygen values recorded in the study zone vary between 0.85 mg/l and 6.89 mg/L, in the range of what the blackchin tilapia can support. In addition, studies by Chikou *et al.* (2013) in Beninese rivers such as Porto-Novo Lagoon and Lake Ahémé indicate that the species can live comfortably in environments where dissolved oxygen levels range from 0.35 mg/L to 9.74 mg/L. The monthly variations of pH and water temperature in the study area showed that this species also tolerates minor pH variations and can survive in a wide temperature range, from 23°C to 34°C as outlined in the study of Mélard (2014) in laboratory essays and Philippart and Ruwet (1982) in Philippines. The highest temperatures were recorded in March and April, corresponding to the peak of reproduction of the species. These results are also consistent with the work of Philippart and Ruwet (1982), showing an optimal temperature range for the reproduction of the species which extends between 17°C and 32°C. Also, to note, one of the particularities of this species is its high euryhalinity (Panfili *et al.*, 2006), allowing it to survive at a wide range of salinities (Chikou *et al*., 2013). High salinity levels were observed during the breeding season of the species in the study area (March-September), with a peak between March (38.38 psu) and April (42.13 psu). This result is consistent with the work of Pauly (1976) who showed that *S. melanotheron* probably develops normal gametogenesis in the study area and can reproduce even at high salinities. Thus, in terms of physicochemical parameters, water in the MPA is within the limits associated with good survival, growth, and reproduction of *S. melanotheron*. However, the species, known for its resistance and plasticity, may have differences in life traits in other areas with contrasting environmental conditions imposed by humans (Guèye *et al*., 2012). These findings are applicable to mostly pristine coastal habitats in tropical rivers, *i.e* Marine Protected Areas.

Our results indicate a greater proportion of females in the study area. The sex ratio is in favor of women except in May and November. Our results corroborate the work of Arizi *et al.* (2015) in Dominli Lagoon in Ghana. In Nigeria, in the Lagos lagoon, Fagade (1979) observed similar sex ratios in favor of women, with sex ratios of 1:2.0, 1:2.4, and 1:1.6. Similar results for the same species were reported by Koné and Teugels (1999), with sex ratios of 1:2.7 and 1: 2.0 for two following years in the Ayamé reservoir in Côte d'Ivoire. Major differences in fish sex ratios are not uncommon, especially for Cichlids, and may *be* related to seasonal migration during the breeding season or be linked to fish movement for foraging (Albaret and Legendre, 1985; Ameur *et al*., 2003).

According to the present study, it was observed that *S. melanotheron* has a continuous reproductive cycle over the year with an intense breeding period from March to June for males, while it extends into September for females. This breeding period is then followed by sexual rest that extends from October to December. Maturation of the gonads seems to be occurred between January and March, when the gonad stages reach from III to VI sexual maturation (Fontana, 1969). This increase in the GSI corresponds to the maturation phase of the gonads (Blackwell *et al*., 2000). Prolonged fish reproduction can be attributed to the reproduction of various size groups in succession during the spawning season (Diedhiou *et al.*, 2022). These results apply well with those reported by Panfilie *et al.* (2004) in the Saloum and Gambia estuaries, with a peak of reproduction at the beginning of the rainy season (May-July). Legendre and Ecoutin (1996) also report a continuous year-round breeding cycle of *S. melanothron* in the Ebrié lagoon (Ivory Coast), with intense breeding activity in the dry season. Again, the same results were reported by Koné and Teugels (1999) in the Ayamé reservoir in Côte d'Ivoire. This is also consistent with the results obtained by Guèye *et al.* (2012) in the Sine-Saloum estuary for *S. melanotheron*, with a breeding season covering both the dry and rainy seasons with greater sexual activity in the dry season. The concomitant increase in the gonadosomatic index (GSI) with the increase in certain environmental parameters such as temperature and salinity observed in the study area, also reported by Panfili *et al*. (2004), Diouf *et al.* (2009) and Tine *et al.* (2007) in the Sine-Saloum estuary suggests a potential impact of these parameters on the reproduction of the species in the study area. The largest GSI were recorded between March and June, right when tropical ecosystems in West Africa undergo a dramatic seasonal change between the dry and rainy seasons, thus enticing changes in the physicochemical properties of the water.

The sizes at first sexual maturity ($L_m50$) obtained in male (11.00 cm) and female individuals (11.71 cm) are relatively low for a species that can grow up to about 250 mm in total length (Teugels and Thys Van den Audenaerde, 2003). These sizes corroborate the results reported by Panfili *et al.* (2004) in the Sine Saloum estuary, a river with similar characteristics as the Casamance River (*i.e* higher salinity upstream), where $L_m50$ of 13.1 cm is observed in females and 11.3 cm in males. On the other hand, according to the same author, the sizes at first sexual maturity were higher in the Gambia River estuary (higher salinity downstream) for both females (17 cm) and males (16.2 cm). Similar results were observed in the Brimsu reservoir in Ghana with $L_m50$ of 11.26 cm and 11.34 cm respectively in males and females (Mireku *et al*., 2016). Interestingly, several other studies report smaller $L_m50$ for this species in the Sine-Saloum estuary (10.2 cm; 7.1 cm) by Diouf (1996), in Lake Taho in Benin (6.9 cm and 7.7 cm) by Lederoun *et al*. (2016) and more recently in the Sine Saloum again, according to Guèye *et al*. (2012) with sizes of less than 8 cm for both sexes. Nevertheless, fish species in tropical river systems are known to become sexually mature very rapidly in response to rapidly changing seasonal conditions in these systems (Lowe-McConnell, 1982).

Indeed, Tilapias are a group of species capable of allocating a large part of their energy reserves to reproductive activity at the expense of growth, if environmental conditions are not favorable (Fryers, 1972; Lowe-McConnell, 1982). The species is also well known for its high tolerance to temperature variations (Panfili *et al*., 2004; Panfili *et al*., 2006; Diouf *et al*., 2006; Labonne *et al*., 2009) and dissolved oxygen at the scale of daily and seasonal fluctuations. However, tilapia living in the hyper-salty areas of Sine Saloum and Casamance rivers have been shown to exhibit altered growth and early reproduction performance, mirroring the same pattern in other species such as *Ethmalosa fimbriata* in similar settings (Labonne *et al*., 2009). Indeed, due to climate change, hypersaline conditions are increasingly observed in some Sahelian estuaries in West Africa (Guèye *et al*., 2012). In these estuaries, freshwater inputs, mainly from underground discharges, but also from precipitation, are largely exceeded by evaporative losses (Pagès and Citeau, 1990; Savenijie and Pagès, 1992). This has led to a reversal of the salinity gradient in some estuaries such as the Sine-Saloum and the Casamance rivers in Senegal, with salinities that can increase from downstream to upstream where they can exceed 130 psu (Guèye *et al*., 2012). Salinity levels in these estuaries can also change between the dry and rainy seasons (Panfili *et al*., 2004; Panfili *et al*., 2006). Such spatio-temporal variations in salinity are serious abiotic obstacles that could profoundly impact the normal biological functions of species such as growth and reproduction. Thus, some species, to cope with this particularly stressful situation, have developed adaptation strategies including regulation of growth and reproduction (Stewart, 1988; Duponchelle *et al*., 1999). The blackchin tilapia is clearly one of them.

Low size of first sexual maturity is a very common phenomenon in cichlids. It is well known that cichlids can exhibit dwarfism and breed at relatively small sizes depending on the local conditions (Plisnier, 1990). Food availability, poor physicochemical conditions and fishing pressure are factors most often cited to explain this phenomenon in these species (Leonardos and Sinis, 1998; Panfili *et al*., 2004). In line with most of the results obtained, the early maturity observed in this study is probably related to fishing activity, in view of the high consumption of the species in the area, but also its socio-economic importance for the population. Because food availability and physicochemical conditions are supposed to be sufficient in the MPA of Niamone-Kalounayes, considering its protected area status, these two factors might not play as huge a role as the overfishing in sizes at first maturity.

The results of this study are similar to other studies conducted in West Africa on the same species. Indeed, the seasonal variation of the GSI in both sexes indicates that the species has a reproductive peak from March to September. In this population, the sex ratio was largely in favor of females, with significant monthly fluctuations showing the seasonality of the reproductive cycle for this population. For all individuals identified in the study area, males and females reach their first sexual maturity ($L_m50$) at almost the same size, 11.00 cm and 11.71 cm, respectively. The results presented in this study could be beneficial to the management of fisheries resources of the MPA of Niamone-Kalounayes. Indeed, all the metrics used to describe the reproductive biology of the blackchin tilapia could be used to develop best management practices for fisheries in the MPA, like a seasonal threshold for fishing activities, define a minimum catch size, and establish biological rest periods in certain parts of the MPA for this species.

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