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Flow Intermittence and Salinization Affect Life-History Traits in the Relict Sahara Desert Blue Tilapia (*Oreochromis aureus***) (Teleostei: Cichliformes: Cichlidae) in**

Morocco

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The blue tilapia *Oreochromis aureus* is a native afro-tropical cichlid that occurs in Morocco. In the intermittent rivers and ephemeral streams (IRES), this species has been facing multiple environmental stressors, particularly flow intermittence and salinization that may affect their life-history traits. The current study addressed two relict sub-Saharan populations of *O. aureus* in the Draa basin, SE Morocco, from the permanent saline Tissint River and an intermittent highly salinized reach of the Draa River at Sidi El Mokhtar. Captured tilapias were weighed and measured for body length. Scaled-mass index (SMi) was calculated to assess body condition. Based on body length and scalimetry-determined age, von Bertalanffy growth parameters were estimated in addition to length and age at maturity, mortality rate and longevity using empirical relations. Fish were much smaller, lighter and in poorer condition in the intermittent salinized stream than in the permanent saline Tissint River. The asymptotic length was half, but the growth constant rate was double, with rather a lower growth performance compared to Tissint. Sexual maturation occurred earlier at a much smaller body length. The mortality rate was twice that observed in Tissint, and the estimated longevity was three times shorter. Overall, *O. aureus* adapts to the extreme conditions of flow intermittence and salinization in the Draa basin by exhibiting fast growth and earlier maturation, which likely contribute to higher fecundity and recruitment. These traits help compensate for the increased mortality rate and shorter longevity associated with these challenging conditions. Such information would be useful for conservation and management of these relict tilapias and their habitat. Moreover, fish farming can be potentially developed in the permanent flowing brackish waters.

INTRODUCTION

Investigations of vital life history traits, such as growth, reproductive patterns and survival, provide important information for the analysis and assessment of fish population structure **(Maceina & Sammons, 2006)**. Growth in body size (length and weight) is a coevolved key life history characteristic and is correlated with maturation, maximum age,

mortality, generation time and the population's intrinsic growth rate. Growth parameters are therefore required parameters in most of the assessment models used to improve the management of species targeted for fisheries management or sustainable conservation **(Froese, 2022)**.

In the Mediterranean climate region, intermittent rivers and ephemeral watercourses (hereafter IRES) are especially predominant and are marked by repetitive stream interference with an unsurprising (calm ranges) or unusual (subtropical) hydrological pattern of drying and flooding, which appears to be inconsistent in frequency and intensity from year to year, mainly due to rainfall fluctuations **(Bonada** *et al***., 2007; Datry** *et al.***, 2014**). During the dry season, the stream may dry up completely or may become an arrangement of detached pools, although during the wet season these patches of environment are reconnected after the stream recovers **(Datry** *et al***., 2014)**. In the expansion, the changeability of streams can also be spatial since these streams can present dry and wet segments at the same time, depending on the hydro-morphological conditions in the vicinity **(Bonada** *et al***., 2007; Cid** *et al***., 2017)**. As a result, biota in these highly dynamic environments face a wide range of natural and anthropogenic conditions, and therefore need to demonstrate distinct resistance and resilience strategies to survive in these unstable environments **(Bogan** *et al***., 2017)**.

The Mediterranean inland fish species that inhabit the IRES adapt to this high natural variability, exhibiting life-history traits such as high tolerance and resistance to abiotic stressors and, for a few species, the ability to migrate along the stream to find satisfactory subsistence pools before the onset of the dry phase **(Kerezsy** *et al***., 2017)**. In any case, the survival of fish is only possible if suitable pools are nearby to serve as potential refugia during the dry season **(Bond** *et al***., 2015)**. In a particularly dry year, or after a chronic drought lasting several years in which pools are not reconnected, the survival of fish populations will depend on the quality of these refugia (e.g. dissolved oxygen, water temperature, salinity, etc.) and the rate of recharge **(Magalhães** *et al***., 2007)**. The refugia are essential during the dry season and can act as a potential source of colonizing fish after the rivers have recovered, thus contributing to the dispersal of adults. The persistence of fish in IRES can also be influenced by anthropogenic impacts.

In addition to stream intermittence, water salinization—whether of geological origin (primary) or influenced by human activity (secondary)—is one of several abiotic factors (such as temperature, dissolved oxygen, and alkalinity) that impact fish growth and development. These factors can affect processes such as egg fertilization, embryonic development, yolk sac resorption, swim bladder expansion, and larval development **(Boeuf & Payan, 2001; Melody** *et al.,* **2018)**. When fish are constrained to cope with changing salinities, depending on whether they are marine or freshwater, they use more energy to sustain their homeostasis and they grow more slowly (i.e. with osmoregulation disrupted, fish expend extra energy to hold sodium and chloride ions in or withdraw them from the body).

Tilapia, the family Cichlidae, are among the numerous riverine fish that can survive the extreme conditions in the IRES using a combination of adaptable colonization (migratory behavior, but no specialized adaptation allowing persistence in water-limited habitats) and recruitment strategies as well as their tolerance to large changes in abiotic factors (temperature, dissolved oxygen, alkalinity, salinity, etc.). They are classified as secondary low-salinity tolerant fishes with a tolerance to salinity at or near that of normal seawater and which, probably used the sea in their dispersal process **(Bianco & Nordlie, 2008)**. Among these, the Afrotropical Blue and Redbely tilapia coexist in small relic populations in the Saharan Oued Tissint, a brackish tributary of oued Draa, where they live in sympatry with the only endemic cyprinid, the Draa barbel, *Luciobarbus lepineyi*, the rare relict European eel, *Anguilla anguilla*, and the introduced pumpkin seed, *Lepomis gibbosus* and Mosquito fish, *Gambusia holbrooki*, which are particularly abundant in the Lower-Middle Draa subasins **(Clavero** *et al***., 2014)**.

Few studies have reported a lower condition and/or reproductive success of fish in intermittent streams, possibly due to lower prey availability **(Oliva-Paterna** *et al***., 2003; Mas-Martí** *et al***., 2010)**. Other studies have nevertheless revealed that populations exposed to unstable environmental conditions may be made up of resilient individuals that can profit of variable local conditions, indicating life-history plasticity **(Glarou** *et al***., 2019)**.

In contrast to other parts of North Africa, especially Egypt, few recent studies were recently carried out in Algeria on growth and reproduction concerning the redbelly tilapia **(Zouakh** *et al***., 2016; Ghazi & Sidi Bachir, 2021)**. However, there were no investigations on life-history traits for the native tilapias in Morocco. Nevertheless, some life-history traits (age at sexual maturity and growth) have recently been studied in two invasive species in NW Morocco, namely *O. niloticus* and *O. aureus*, previously introduced for aquaculture and then released into the wild allowing the species to establish viable wild populations **(Ainou** *et al***., 2023)**.

In the present work, we aimed to assess differences in body condition, population age-structure, growth rate, maturation, longevity, and mortality rate between two small relict populations of the Saharan *O. aureus* from two contrasting reaches, differing by their flow regime (permanent vs. intermittent flow) and their salinity (saline vs. highly salinized) in the arid Draa river basin, southern Morocco. Based on our previous field observations, we assumed that individuals from suitable pools that may serve as potential dry season refugia after flow resumes, ensured the colonization of the intermittent reach. We hypothesized that during the drying episode, the refugia quality would have been negatively affected (both abiotic and biotic conditions) in the intermittent reach, with subsequent impacts on condition, growth, maturation and survival in the colonizers of these reaches. The idea is to provide baseline data for conservation and management, as well as for potentials of local farming of this economically important fish species.

MATERIALS AND METHODS

1. Sampling sites

Fish sampling was carried out at two contrasted sites in the arid Draa River basin, southeast Morocco: i) a permanent brackish upstream segment of Tissint river, a tributary of Draa river (lower Draa sub-catchment), and a salinized intermittent segment of the Draa River at Sidi El Mokhtar (middle Draa sub-catchment) (Figs. 1, 2). In this intermittent reach, average salinity, 2- 4 months after resumption, was higher than in Tissint. The description of the two study sites is shown in Table (1).

Fig. 1. Geographic location of the fish sampling sites (on the right) within the study area in the arid Draa River basin (on the left), SE Morocco (ArcGIS Online,Terrain Basemap, 2023)

Fig. 2. The fish sampling sites: Upstream Tissint (on the left), permanent naturally brackish stream (July 2021), and highly intermittent and more brackish (salinized) segment of the Draa River at Sidi El Mokhtar (on the right), here in drying phase, 4 months after resumption (July 2021)

Table 1. Description of fish two sampling sites (Tissint and Sidi El Mokhtar) in the arid Draa basin, SE Morocco. (*DDS: Decimal Degrees System)

2. Hydrological Physical and chemical characteristics

A hydrological impeller was used (SEBA Hydrometry) to measure the flow velocity and hence combined with the cross section area to estimate stream flow rate by taking three measurements across the width of the river for each site.

During sampling and surveying campaigns, water temperature, electrical conductivity (EC), pH, and dissolved oxygen (DO) were measured at each site using a multi-parameter probe (WTW MultiLine® Multi 3510 IDS) **(Kaczmarek** *et al***., 2023)**.

The ionic composition *in-situ* was determined (in the midstream) using a Macherey–Nagel Visocolor reagent case with the photometer PF-12Plus and Visocolor Eco colorimetric test kits. Nine parameters were then measured: chloride (Cl[−]), potassium (K^+) , ammonium (NH_4^+) , nitrites (NO_2^-) , nitrates (NO_3^-) , orthophosphates $(PO₄^{3−})$, sulfates $(SO₄ ^{2−})$, total hardness (TH) , and carbonate hardness (CH) . Concentrations of potassium, ammonium, nitrites, nitrates, orthophosphates, lower than 2, 0.1, 0.02, 4, and 0.6mg/ l, respectively, were below detection threshold. In this case, we used the mid-detection level **(Clarke, 1998)**. Other water samples from the midstream of each site were stored in clean PVC bottles and transported in a portable electric cooler to the lab for further lab chemical analyses.

Salinity (i.e., the total amount of dissolved salts that contribute to the salinity of water) was not directly determined but derived as practical salinity (expressed in

practical salinity units, PSU) from the $in-situ$ conductivity $(\mu S.cm^{-1})$ and temperature (°C) measurements. The conversion of conductivity into salinity (PSU) was performed online using the salinity unit calculator at [https://reefapp.net/en/salinity-calculator.](https://reefapp.net/en/salinity-calculator) The hydrological and physicochemical are shown in Table (2).

Table 2. Hydrological and physicochemical characteristics of the sampling sites (Indicated values are averages of three measurements)

3. Fish sampling and measurements

The blue tilapias were captured (under licence) in April and July 2021 (2 to 4 months after flow resumption in the case of the intermittent reach), using a cast net (diameter of 2.5m, and 15mm mesh size) $\&$ a gill net (15 x 2.5m, and 10mm mesh size). Upon their capture, fish were kept alive in a soft-mesh bag inside a plastic bucket halffilled with stream water. They were then weighed $(\pm 0.1g)$ and measured for their total (TL) and standard (SL) body lengths $(\pm 0.1$ mm). The presence of external parasites or visible abnormalities or infections were also recorded as part of another simultaneous investigation. Specimens were thereafter euthanized using a relatively high dose of clove essential oil (55mg/ L that equates to about 220µL of eugenol in 5L of water) following the American Veterinary Medical Association (AVMA) Guidelines for the Euthanasia of Animals (2020 Edition). They were then transported to the lab in a portable electric fridge-freezer (from 3-8 down to -20°C) for subsequent examinations. Few small specimens were selected for the reference collection in the Natural History Museum of Marrakech (Cadi Ayyad University at Marrakech, Morocco).

4. Length-weight relationships (LWRs)

The relationship between total length (TL, mm) and weight (W, g) was estimated using a plotted power function $W = a.TL^b$ (Froese, 2006) with 95% confidence limits of the constants ("a" and "b") and log-transformed into $LogW = Log a + b.LogL$ (Froese *et al***., 2011)**, where a and b are the intercepts of the coefficient of regression, respectively. Preliminary analyses allowed to remove the outliers perceived in the log-log plots of all populations.

5. Somatic body condition

The relative condition index K_n was used as a proxy of <u>body condition</u>, which is widely utilized in [fisheries science](https://www.sciencedirect.com/topics/earth-and-planetary-sciences/fishery-science) (Gubiani *et al.*, 2020). For each specimen *i*, K_n was calculated as:

$$
Kn_i = 100(W_i/W_i) \qquad (1)
$$

Where, W_i is the actual weight and W'_i is the predicted weight from the length-weight equation $W' = a \times (TL)^b$, with TL being the total length.

The ordinary [least squares](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/least-square) (OLS) regression was employed for analysis. Preliminary analysis revealed no statistical differences in condition between the two populations of tilapias and thus condition indices were estimated for all individuals together. In addition to Kn, a second condition index was calculated by retrieving the residuals from the linearized regression of the length-weight relationship and standardising them by dividing each residual by the standard deviation of the predicted values (SRi = residual/standard deviation) **(Labocha** *et al***., 2014)**.

The scaled-mass index SMi was also used for analysis following the method of **Maceda-Veiga** *et al***. (2014)**:

$$
\mathit{SM}_i = [L_0/L_i]^{b\mathit{SMA}} \quad (2)
$$

Where, Wi is the fresh body weight; Li is the standard length of individual (i); L₀ is a constant length used to standardize body condition values estimated by the arithmetic mean of total lengths of all individuals captured, and b/SMA is the scaling component, which was obtained by dividing the slope of the least squares regression of weight on length (both of them ln-transformed) divided by the Pearson's correlation coefficient of that relationship.

6. Back-calculated body length

Five to ten intact scales were removed from the standard area between the lateral line and dorsal fin with a supposed low regeneration. They were cleaned with a 8% sodium hypochlorite (NaOCl) solution (for less than 3mn), dried, and then conserved in glycerin for further analyses. Scales were then washed and mounted dry between two microscope glass slides for reading under optical stereomicroscope (at magnification: x40). The scalimetric method for age determination was separately applied by two different operators **(Bagenal & Tesch, 1978)**. Length at age was back-calculated by means of the following formula:

Ln=
$$
a + Sn / S (L-a)
$$
 (3)

Where, Ln is the length of the fish at age 'n'; a is the constant; Sn is the radius of the annulus 'n'; S is the scale radius, and L is the standard length at the time of capture **(Bailey** *et al***., 2022)**.

7. Growth parameters

We expressed post-hatching growth curves using the von Bertalanffy 3 parameters model, one of the most frequent models used by fisheries biologists to investigate fish development and its interpretations. It was expressed as follows:

$$
L_t = L_{\infty} (1-\exp(-k(t-t_0)))
$$

Where, the expected or average total length L_t (mm) of the fish at age t (years) is estimated on the basis of three parameters: the asymptotic average length that fish could achieve $\text{L}\infty$ (mm), the growth constant rate (or so-called Brody growth rate coefficient) k (year⁻¹), which determines how fast the fish approach L∞, and t₀, which is a modelling artifact that represents the age at the average length of zero. These three parameters of the VBERT equation were calculated using Beverton-Holt's method **(Rafail, 1973)**.

An index of growth (*G*) (or Annual Specific Growth Rate) per age class between years was calculated on the basis of the mean length as follows, **(Karametsidis** *et al***., 2023)**:

$$
G_{(a\to a+1), t} = LnML_{(a+1), t} - LnML_{a, (t-1)} (4)
$$

Where, $ML_{(a+1), t}$, and $ML_{a,(t-1)}$ mean lengths of age $a+1$ at time t and age a at time t-1, respectively.

The overall growth performance indice (*prime-phi*, **φ**′) was calculated for the two studied populations based on the calculated VBGF parameters **(Gallucci & Quinn, 1979)**:

$$
\varphi' = \log k + 2\log L_{\infty} \quad (5)
$$

The mortality rate (M_{est}, yr^{-1}) was evaluated using the updated Pauly_{nls-T} estimator (when Tmax is not available), as recommended by **Then** *et al***. (2015)**:

 $M_{estP} = 4.118K^{0.73} L\omega^{-0.33}$ (6)

8. Size and age at maturity

Length at maturity (Lm) was estimated using the following relation established by **Froese and Binohlan (2000)** :

log*Lm* = 0·8979*log*L[∞]* - 0·0782 (7)

Age at first maturity (t_m , years), the average age at which fish of a given population mature for the first time, was calculated from the length at first maturity L^m by the relation of **Froese and Binohlan (2000)**:

$$
t_m = t_0 - \ln(1 - L_m/L_\infty) / K \qquad (8)
$$

Potential longevity (or life span) (t_{max} , years), which is the approximate maximum age that fish of a given population would reach, was calculated using the following relation **(Froese & Binohlan, 2000)**:

 $log_{tmax} = 0.5496 + 0.957 * log(t_m)$ (9)

9. Statistical analysis

Statistical analysis was performed using RStudio-2023.12.1 and SPSS Statistics 26.0.0. We used the Shapiro-Wilk test to confirm normal distribution of data **(Shapiro & Wilk, 1965)**, and homoscedasticity was verified by Levene's test. Analysis of variance (ANOVA) was used to evaluate the statistical significance of the fitted regression model, and the differences in SMi values among the three populations. The total length–weight relationship (LWR) for each population was estimated by the least-squares method, based on the logarithmic equation: $log_{10}W = a + blog_{10}L_t$ (cm). We calculated the standard error Sb for the slope (b), and isometric growth was tested through Student t-test using the equation: $t_s = b-3/5b$. Statistical differences were considered to be significant below the threshold $\alpha = 0.05$ (**Zar, 2009**).

RESULTS

1. Length-weight relationship (LWRs)

The body length (SL) of the captured *O. aureus* ranged from 66 to 200mm and from 45.30 to 132mm, respectively, in Tissint and Sidi EL Mokhtar; the corresponding mean values being 113.25 ± 37.12 (n=48) and 80.22 ± 22.08 mm (n=42). The respective body weight ranges were 12.20-273.40 and 3.7-71.60g, and the means values were 66.96 \pm 66.99g (n=48) and 21.56 \pm 17.13g (n=42), respectively.

The estimated length-weight relationships (LWR) for all individuals (both males and females) of *O. aureus* from the two studied localities are represented in Table (3). The *t*-test for equality of two regression coefficient revealed no significant difference in relative growth (P -value > 0.05). The descriptive statistics for the LWR of the two populations are given in Table (3). Significant correlation coefficients (r^2) indicate linear log-scaled length-weight relationships in both cases. 'b' values (2.79 and 2.80, respectively) are statistically non-significant from $b = 3$ (*P*-value > 0.05), which is indicative of an isometric growth for the two studied populations.

Table 3. The descriptive statistics of length-weight relationships (LWRs) in *Oreochromis*

aureus from Tissint and Sidi EL Mokhtar in the arid Draa River basin, SE Morocco

N= sample size; SD=standard deviation.

2. Body condition

The average individual somatic body condition of the blue tilapias, expressed by the SMi, was very significantly higher in Tissint of about threefold that in Sidi El Mokhtar $(52.24 \text{ vs. } 18.10)$ (*t*-test, t= 44.15, *P*-value <0.001). It was also significantly higher in Tissint at all age-classes (t-tests, *P*-value <0.001 in all cases) (Fig. 3). In contrast, Le Cren's condition factor (Kn) showed no significant difference between the two studied populations in (*t*-test, $t = 3.7$, *P*-value > 0.05) (Table 4).

Fig. 3. Scaled-Mass index (SMi) at each age class of *Oreochromis aureus* from a permanent saline reach (Tissint) and intermittent salinized segment of the Draa River (Sidi El Mokhtar, SM) in the arid Draa River basin, SE Morocco

Table 4. Body condition expressed by scaled-mass index (SMi) and Le Cren's condition factor (Kn) in the the blue tilapia *Oreochromis aureus* from a permanent saline reach (Tissint) and intermittent salinized segment of Draa River (Sidi El Mokhtar), in the arid Draa River basin, SE Morocco

Locality	N	$SMi \pm SD$	Min Max	$K_n \pm SD$	Min	Max
Tissint		48 52.24 ± 4.47 *** 44.59 62.03 0.97 \pm 0.08			0.82	1.14
Sid El Mokhtar		$42 \quad 18.10 \pm 1.31 \quad 15.6 \quad 20.79$		0.99 ± 0.09 0.77		1.21

***Highly significant (*P*-value<0.001).

3. Age and body size of sampled fish

The estimated age ranged from $0+$ to $4+$ years and from $0+$ to $3+$, respectively, in Tissint and Sidi EL Mokhtar. The age 1+ was the most numerous in both populations for the total population (40.5 and 46.7%, respectively) and 18.9, 18.9, 13.5 and 8.1% for ages 0+, 2+, 3+, and 4+, respectively, for Tissint, and 30, 16.7, and 6.7% for ages 0+, 2+, and 3+, respectively, for Sidi EL Mokhtar.

The respective mean sizes of fish at the time of formation of the first annulus were 22.8 and 40.1mm.

Fish of class size 80-100mm dominated in Tissint with a frequency of more than 45% while the other size classes 60-80 and >100mm ranged from 5 to less than 15% of the sample size. In Sidi EL Mokhtar, the two size classes 60-80 and 80-100mm were the most represented with an average of 30%, followed by the smallest size class 40-60mm with 20% and then the last two largest size classes of 100-120 and 120-140mm, with around 13 and 3%, respectively.

4. Back-calculated lengths

The relationship between the fish length (L, mm) and the scale radius (S, mm) of *O. aureus* fitted to a linear model (Fig. 4), which were:

L= 4.01+47.33*S, r^2 = 0.998 and L= 2.28+63.61*S, r^2 = 0.995, respectively in Tissint and Sidi El Mokhtar.

Fig. 4. Standard body length (SL, mm) to scale radius (mm) relationships in the blue tilapia *Oreochromis aureus* from: (A) a permanent saline reach (Tissint), and (B) intermittent salinized segment of the Draa river (Sidi El Mokhtar), in the arid Draa river basin, SE Morocco

The average back-calculated lengths of the pooled data estimated for Tissint at ages 0+ to 4+ years were 81.73, 97.16, 114.79, 115.54, and 157.80mm, respectively (Table 5). The estimated values for Sidi El Mokhtar at ages 0+ to 3+ years were 62.09, 77.56, 104.29, and 122mm, respectively (Table 6). The back-calculated length at annulus formation suggested that *O. aureus* in Tissint increased in length by 40.93% in age 0+ and 19% at age 4+. In Sidi El Mokhtar, the increase in length was of 59.81 and 17.70%, respectively, at ages $0+$ and $3+$ (Tables 5 & 6).

Table 5. Mean back-calculated total lengths at age of the blue tilapia *Oreochromis aureus* from the permanent saline reach Tissint in the arid Draa River basin, SE Morocco

Table 6. Mean back-calculated total lengths at age of the blue tilapia *Oreochromis aureus* from the intermittent salinized segment Sidi El Mokhtar of the Draa River

5. Growth parameters

5.1 von Bertallanfy growth parameters

Using back-calculated lengths at age (Table 2), the estimated von Bertalanffy growth equation parameters for the two populations were as follows:

> Tissint: $L_t = 275.4(1-exp(-0.19(t+1.19)))$ Sidi El Mokhtar: $L_t = 141.3(1-exp(-0.41(t+0.96)))$

L[∞] in Tissint is about twofold that in Sidi El Mokhtar (275.4 *vs.* 141.3mm). On the other hand, the growth constant rate k is more than two times greater in Sidi El Mokhtar (0.410 *vs.* 0.190 year⁻¹)(Table 7).

Table 7. The estimated von Bertalanffy growth parameters, maximal growth rate, and growth performance in *Oreochromis aureus* from Tissint and Sidi El Mokhtar, the arid Draa River basin, Morocco

5.2 Growth index and growth performance

The growth index (**G**) (annual specific growth rate) was relatively higher in Sidi EL Mokhtar at each age class (from 0+ to 3+ years), but the overall growth performance (**φ'**) was lower in Sidi El Mokhtar than in Tissint (1.91 vs. 2.15) (Tables 7, 8).

Table 8. Growth index (or annual specific growth rate) per age class of *Oreochromis aureus* from a permanent saline reach (Tissint) and intermittent salinized segment of the Draa River (Sidi El Mokhtar), in the arid Draa River basin, SE Morocco

Locality	G_{0-1}	G 1-2	G 2-3	G 3-4	
Tissint	0.212	0.225	0.232	0.253	
Sidi El Mokhtar	0.243	0.304	0.245		

6. Length and age at maturity

The estimated length at maturity *Lm* (mm) of the blue tilapia in Sidi El Mokhtar was almost half of that in Tissint (71.2 vs. 129.6mm). These body lengths were achieved at respective ages of 1.25 years (15 months) and 2.16 years (26 months) (Table 9).

Table 9. Length (L_m) and age (t_m) at maturity, mortality rate (M) and potential longevity (tmax) in *Orechromis aureus* in the saline permanent stream (Tissint) and intermittent salinized reach in the arid Draa basin SE Morocco

7. Mortality and longevity

The estimated annual mortality rate $(M_{estP}, year^{-1})$ in Sidi El Mokhtar was twice that of Tissint $(0.89 \text{ vs. } 0.41)$ (Table 9). Furthermore, the estimated longevity (t_{max} , years) in Sidi El Mokhtar was three times shorter than that in Tissint (4.4 vs. 12.6) (Table 9).

DISCUSSION

This study is the first to examine the growth and life history traits of the native relict Sahara tilapia, *Oreochromis aureus*, in the arid Draa River basin in southeastern Morocco. It aimed to assess the potential effects of flow intermittence and salinization on body condition, growth patterns, maturation, longevity, and mortality rates. The only other sympatric tilapiine relict species found at one of the study sites is the redbelly tilapia, *Coptodon zillii*.

Recent research by **Ainou** *et al.* **(2023)** investigated the life-history traits of introduced tilapia in northwestern Morocco. Previous studies on subtropical populations have been conducted in Egypt **(Bakhoum, 1994, 2002; Mehanna, 2004; Mahmoud & Mazrouh, 2008)** and Mexico **(Jiménez-Badillo, 2006; Messina** *et al.,* **2010)**, with more recent studies in Iraq **(Al-Wan & Mohamed, 2019; Mohamed & Salman, 2020)**.

1. The slope 'b' of the LWR

Regardless of their sex, the relative growth of *O. aureus* in the two studied populations was isometric, although a nearly positive allometry was found by **Mehanna** **(2004)** for the same species in Wadi El-Raiyan Lakes, Egypt $(b = 3.109)$. In NW Morocco, the values were 2.97 (negative allometry) and 3.43 (positive allometry), respectively, for females and males **(Ainou** *et al.,* **2023)**. The 'b' value from other studies for this species revealed a probable negative allometric growth, such as 2.51 sexes combined in the Aguamilpa Reservoir, Mexico **(Messina** *et al***., 2010)**. However, most of the authors do not test for the statistical significance of the difference of the isometry (b= 3). The positive allometric type of growth (b>3) implies that the fish becomes relatively stouter or deeper-bodied as it increases in length **(Riedell** *et al***., 2007)**. **Messina** *et al***. (2010)** stated that allometric growth is often common in species of the genus *Oreochromis*, which may be attributed mainly to problems of food, fish density, sexual dimorphism and behavior. However, such variations in the 'b' value may depend upon various factors like sample size, sizes of fish examined, sex variation, stomach fullness, stage of maturity, parasite loads, disease, environment and season, food abundance and fishing pressure **(Cuardado** *et al.,* **2019)**.

2. Somatic condition

The calculated SMi in the blue tilapia in the Draa basin performed better in explaining spatial variation in body condition than the Le Cren's relative condition factor Kn (or other similar basic indexes such as Fulton's condition factor). This has been previously shown in four different inland freshwater fish species in Northeastern Spain **(Maceda-Veiga** *et al***., 2014)**. Kn did not present a significant difference between the two studied population but may vary among seasons within each population. In this regard, **Messina** *et al***. (2010)** found that the highest values of Kn of *O. aureus* in the Aguamilpa Reservoir, Mexico, occurred in the months with the largest percent of spawning and postspawning females. The fluctuations in the condition factor of many fish were observed concerning their reproductive cycle, feeding rhythms and other environmental and physiological factors **(Ahmed** *et al***., 2012; De Giosa** *et al***., 2014)**.

The blue tilapia from the intermittent salinized reach in Sidi El Mokhtar showed a much lower mean and age-specific SMi values than those in the permanent brackish segment of Tissint. These differences may be related to lower food availability and growth performance and higher competition in Sidi EL Mokhtar where individuals were smaller and lighter. Therefore, incorporating SMi into fish monitoring is likely to improve the value of ichtyological studies as indicators of river quality and ecological change. As suggested by **Maceda-Veiga** *et al***. (2014)**, future investigations should compare the response of SMi to specific fish health indicators such as parasite load, haematological assays and pollutant bioaccumulation to improve our understanding of the value of SMi as a non-lethal diagnostic procedure.

3. Age determination

Modeling somatic growth requires precise age estimations of captured fish, typically using calcified structures such as scales or otoliths **(Abouelfadl** *et al.,* **2020)**. For more accurate studies, it is recommended to use otoliths for a better assessment of growth parameters, as these structures allow for the examination of development and age from the larval stage through the analysis of scale rings. However, scales may be relatively inaccurate for estimating the age of fish older than four years **(Abouelfadl** *et al.,* **2020; Ainou** *et al***., 2023)**. Nevertheless, they are easy to collect and process without lethal effects, making them a widely used method for age estimation **(Mehanna, 2004; Jiménez-Badillo, 2006; Maceina & Sammons, 2006; Al-Wan & Mohamed, 2019; Abouelfadl** *et al.,* **2020)**. **Abecasis** *et al***. (2008)** found that otoliths may be better structures for ageing some marine fish species, but scales can also be used as a nondestructive technique and with satisfactory results or even better results in other species.

The use of scales for ageing and growth studies in *O. aureus* has been shown to be valid **(Mehanna, 2004; Jiménez-Badillo, 2006; Mahmoud & Mazrouh, 2008; Attee** *et al***., 2018)**. The total length-scale radius relationship of *O. aureus* revealed a strong linear correlation. This confirms the validity of using scales for growth assessment **(Bagenal & Tesch, 1978)**. The maximal ages determined in the two studied populations in southern Morocco are similar (3 to 4) to those reported for the *O. aureus* from Egypt **(Mehanna, 2004 ; Mahmoud & Mazrouh, 2008)**. These are similar to the maximal age determined by otolithometry in *O. aureus* in NE Morocco (3-4 years) **(Ainou** *et al***., 2023)**. In contrast, *O. aureus* can reach an advanced age of 7 or 8 years in other populations **(Jiménez-Badillo, 2006; Attee** *et al***., 2018)**. The observed differences can be related, among others, to fishing pressure and sampling methods. The population age-structure reflect a high recruitment in both populations, in both permanent and intermittent reaches.

4. Maximal body size

The maximal body length of the blue tilapias from Tissint (200mm) was lower than those reported in NW Morocco (274 and 291mm, respectively, for males and females) **(Ainou** *et al***., 2023)**. The body lengths observed in this study are also lower than those reported for many other populations **(Ramos-Cruz, 1995; Mehanna, 2004; Jiménez-Badillo, 2006; Mahmoud & Mazrouh, 2008; Messina** *et al.,* **2010; Attee** *et al.,* **2018; Jawad** *et al.,* **2018; Al-Wan & Mohamed, 2019)**. In contrast, the maximum body length recorded in Tissint (200mm) is relatively longer than that reported for the El-Salam Channel in Egypt (185mm), as reported by **Badr El-Bokhty and Fetouh (2023)**. However, the length reported in Sidi El Mokhtar (132mm) is the lowest ever recorded for blue tilapia. As for maximal body weight, the values reported for *O. aureus* from other previously studied populations ranges from 288 to 1800g according to locality (Table 10). In NW Morocco, males were heavier than females (640 vs. 316g) **(Ainou** *et al***., 2023)**.

The blue tilapia from Tissint were much heavier than in Sidi El Mokhtar (273.4 vs. 72.4g), in which they showed the lowest ever-reported maximal body weight among all studied populations. The differences in sizes (length and weight) of the fish across different geographic regions may partly be attributed to various factors including genetic differences among stocks, and local ecological conditions (e.g. temperature, salinity, restricted habitats, food availability, population density, competitors, predators, parasites), fishing pressure and fishing gears **(Riedel** *et al***., 2007)**. The smallest body size in Sidi El Mokhtar may be due to local constraints related to flow intermittence and increased salinization, which induce a fast growth and an earlier sexual maturation leading to a reduced adult body size.

Table 10. Maximum weight (W_{max}), maximum length (L_{max}), coefficient of allometry (LWR) (b), von Bertalanffy growth parameters (L∞: asymptotic length, K: growth coefficient, to: age at length 0 mm), T_{max} : longevity and growth performance indice (phiprime: φ'), for native and non-native populations of *Oreochromis aureus* worldwide. (* Estimated)

Locality,	W_{max}	$L_{\rm max}$	$\mathbf b$	Lm	\mathbf{L}^{∞}	K	t ₀	$\boldsymbol{\varphi}$	$t_{0.99}$	M	Reference
country	g	$\mathbf{C}\mathbf{m}$	LWR		cm	year ¹	year		year	year ¹	
				mm			S		S		
Shatt Al-Arab	311	25.0	3.05	\overline{a}	27.8	0.49	-0.27	2.58	13.3	1.08	Mohamed
River,											and Abood
Iraq											(2020)
Garmat Ali	356	26.3	3.07	139	29.9	0.25	-1.29	2.35	13.9	0.61	Mohamed
river,											and Salman
Basrah, Iraq											(2020)
Lake Mariut,	\blacksquare	\overline{a}	L.	\overline{a}	30.40	0.23		2.34	\overline{a}	\equiv	Bakhoum
Egypt											(1994)
El-Raiyan	297	23.9	3.109	$\overline{}$	27.2	0.56	-0.32	2.62	15.3	0.25	Mehanna
Lakes,											(2004)
Egypt											
Rosetta	288	24.5	2.87	\equiv	26.4	0.40	-0.21	2.45	10.5	0.83	Mahmoud
branch,											and
Nile River,											Mazrouh
Egypt											(2008)
El-Salam	\sim	18.5		÷,	19.14	1.90	\overline{a}	2.84	\overline{a}	2.78	El-Bokhty
channel,							0.001				and Fetouh
Egypt							9				(2023)
Nozha	356	26.3	2.97	÷,	38.1	0.21	-0.25	2.48	13.2	0.51	Mahmoud et
Hydrodrome,											al. (2013)
Egypt											
Aguamilpa	1800	40.3	2.56	$\frac{1}{2}$	43.2	0.37	-0.40	2.84	7.7	0.83	Messina et
Reservoir,		SL(M)	(M)	\overline{a}		0.35	-0.43	2.79	8.1		al. (2010)
Nayarit,		37.0	2.42		41.8						
Mexico		SL(F)	(F)								
Aguamilpa,			$\overline{}$	$\overline{}$	36.5	0.60	$\overline{}$	2.90			Hernández-
Nayarit,											Montaño
Mexico											and Orbe-
											Mendoza

5. Growth parameters and growth performance

The theoretical growth in length (L∞, mm) of *O. aureus* in Tissint is in the range of those recorded for the same species from water bodies in other areas (170-480) and close to those reported from Iraq, most of studied populations in Egypt (264-304), and Oaxaca in Mexico, but much lower than most values from other populations of Mexico (365-479), and higher than those from Nador channel, Morocco and El Salam channel, Egypt (275 vs. 141 and 190, respectively) (Table 10). On the other hand, L∞ of *O. aureus* from Sidi El Mokhtar is the lowest ever-reported value among all the studied populations. **Jiménez-Badillo (2006)** indicated that fluctuations of L∞ in *O. aureus* among populations in different reservoirs in Mexico may be related to the environmental differences more than genetics for maturation and growth, in addition to nonrepresentative sampling and erroneous methodological applications. K values (year⁻¹) reported for *O. aureus* vary extremely among populations and habitats (lake, channel, or river) (range: 0.12 to 1.90) (Table 10). The recorded value in Tissint is slightly higher than that reported in the Nador channel (0.19 vs. 0.12 sexes combined) **(Ainou** *et al***., 2023)**, but much lower than that in the intermittent and salinized stream of Sidi El Mokhtar (0.41). The relatively higher growth rate in Sidi El Mokhtar, where tilapia reach a lower maximum body size, might be due to accelerated growth in order to reach sexual maturity as quickly as possible in the conditions of unpredictable drought, intermittent

flow and increased salinity and other stressors. In the present work, the values of growth performance index (φ') in *O. aureus* (2.15 and 1.91, respectively, in Tissint and Sidi El Mokhtar, Morocco) are relatively higher than the average value recorded for the same species (1.50) in the Nador channel, NE Morocco **(Ainou** *et al***., 2023)**. However, all these values are lower than those in some other populations **(Mahmoud & Mazrouh 2008; Messina** *et al.* **2010; Mohamed & Salman, 2020)**.

The lower growth performance of *O. aureus* in Tissint, Sidi El Mokhtar and other populations are probably due to local conditions such as temperature, food availability, metabolic and reproductive activities, among other differences as reflected in parameters L∞ and k **(Isaac, 1990; Jiménez-Badillo, 2006)**.

6. Longevity and mortality rate

The tmax (years) of *O. aureus* in Tissint is in the range of those reported for other populations (8 to 15.3), except for Nador channel, Morocco, with an exceptional high value in females (40) **(Ainou** *et al***., 2023)**. The very short lifespan in Sidi El Mokhtar can be attributed to the high mortality rate, which is in the range of those reported for other studied populations (0.25-2.78) (Table 10). This may be related to the combined effects of environmental multiple stressors including flow intermittence, salinization, and high competition for food, along with anthropogenic and climate change impacts.

7. Size and age at sexual maturity

The length at sexual maturity of *O. aureus* from Tissint is within the range of those reported for other populations including those from NE Morocco **(Ainou** *et al***., 2023)** (Table 10). On the other hand, L^m in Sidi El Mokhtar, which is only half of that in Tissint, is the lowest value ever reported in *O. aureus*. **Ainou** *et al.* **(2023)** reported an age at sexual maturity (t_m) of 1.75 year (sex combined). This value is lower than the estimated value for Tissint (2.16 years), which is twofold that in Sidi EL Mokhtar (1.26 years). However, and as was indicated by **Prince** *et al***. (2015)**, L^m based on histological analysis of gonads in fishes, is generally 15% smaller than the actual values. Size at first sexual maturity is a variable parameter that changes according to environmental conditions **(Adams, 1980; Gunderson, 1980)**. **Trippel (1995)** pointed out that the decrease in size may be an effect of density dependence, and it is an indicator that a fish population is under stress due to high exploitation (mortality) rates. This can be interpreted as a strategy to maximize reproductive success at the expense of growth, as a population's response to overfishing or high natural mortality. In the case of Sidi El Mokhtar population, the precocious maturity with a higher growth rate would constitute an adaptive response to the unpredictable environmental conditions (flow intermittence, drought and flooding) along with high salinity stress.

8. Effect of flow intermittence and salinization

In the present study, there were highly significant differences in the somatic condition between the perennial saline and the intermittent salinized sites, 2-4 months after flow resume. Regardless of the condition index used, these are in accordance with other studies that reported lower condition in various freshwater species in intermittent river sections, mostly from Iberian Peninsula, obtained either from field investigations **(Oliva-Paterna** *et al***., 2003; Mas-Martí** *et al***., 2010)** or experimentally **(Harvey** *et al***., 2006)**. On the other hand, other works indicated similar or higher condition of cyprinids in intermittent stream **(Merciai** *et al***., 2018; Glarou** *et al***., 2019)**. **Merciai** *et al***. (2018)** suggested that this was related to reduced competition upon rewetting or to colonization by better-adapted individuals. Fish living in unstable conditions of the IRES may exhibit higher requirements of energy reserves to increase their reproductive success **(Merciai** *et al***., 2018; Glarou** *et al***., 2019)**. Investigations on reproduction and food habits of the blue tilapia are needed to test for such differences.

The observed variations in growth parameters and life-history traits between the two studied populations are probably caused by differences in environmental variables. In *O. aureus*, the upper salinity tolerance limit is 40 PSU **(Suresh & Lin, 1992)**. The best growth is observed in tilapia at salinities from 0 to 35 PSU according to species, with 10 to 15 PSU for *O. aureus* **(Suresh & Lin, 1992)**. Other factors may be involved in the salt tolerance, such as flow intermittence, hypoxia and hyperthermia, along with population genetic and demographic characteristics.

Food ingestion and stimulation of food conversion depend on the environmental salinity **(Boeuf & Payan, 2001)**. In this regard, **Küçük** *et al***. (2013)** showed experimentally that the optimum condition for the blue tilapia fish farming, with respect to growth rate and metabolic parameters occur at salinities lower than 12 PSU (between 8 and 12 PSU). At a high salinity level of 24 PSU, specific growth rate, weight increament and food intake were significantly highly reduced to only 20 to 25% of their mean values at lower salinities (8 to 12 PSU). Scientists have attempted to understand how saltinity influences fish development and metabolism and whether it modifies fish energetics or not **(Mohamed** *et al***., 2021)**. Osmoregulation is one of the most vital functions to all euryhaline fish, which provide hyper-osmoregulatory and hypo-osmoregulatory abilities to maintain osmolality of body fluids within a narrow homeostatic physiological range in freshwater (FW) and seawater (SW) fish, respectively **(Kang** *et al***., 2013)**.

There is an accepted assumption that isosmotic water diminishes utilize of energy for osmoregulation compared to fresh water and salt water. This spared energy is utilized for growth. Some investigations indicate that increasing salinity in fresh water improve growth rate in tilapias: from 8 to 24 PSU **(Vonck** *et al***., 1998)** or from 5 to 10 PSU **(Kang'ombe & Brown, 2008)**. Energy expenses for osmoregulation were assumed to be nil in isosmotic environments.

It is estimated that the energy consumption for osmoregulation in salinity hypertonic environments ranges between 10 and 50% of the total energy budget of the fish **(Bœuf & Payan, 2001; Hwang, 2008)**. In other words, to adapt to a high salinity environment, the consumption of energy for growth must be cut down. While there have been several studies on this topic, there is some confusion about how to estimate or calculate the ion regulation cost. In the past, the energetic cost of osmotic regulation was found to be very high (20-30%) for the rainbow trout, tilapia, and 50% for the catfish in freshwater and salt water compared to isomotic waters.

In an experimental study, **Mohamed** *et al.* **(2021)** found that the high salinity stress affected the respiratory, acid-base, hematological, biochemical (including hormonal) parameters, expression of some genes (of Na⁺-K⁺-ATPase and cytosolic carbonic anhydrase) and histological (gills, liver, kidney tissues) features of the Nile tilapia to adapt to the environmental changes adverse effects of high salinity stress on the health status of the Nile tilapia. This is useful for the management practices in aquaculture and its impact on the health status of fish.

Water temperature is another environmental factor that influences the body function, food consumption and growth performance of fish **(Pandit & Nakamura, 2010)**. Low lethal temperatures range from 7 to 11°C for *O. aureus* **(Rezk & Kamel, 2011)**. This species is also able to cope with high temperatures as high as 34°C **(Desprez & Melard, 1998)**. In the Draa valley, spring temperatures were of 26°C, but can be much higher in summer (above 30°C). Thus, water temperatures at both study sites are within a favorable range of temperatures for growth parameters of these species, and it is therefore difficult to state whether temperature is a limiting factor for these life-historical traits. The relationship between temperature and salinity is complex. Many hormones play a role in osmoregulation as well as growth regulation, such as controlling food intake. As is often the case, multiple causes may be at play and the interaction between salinity and physiology and behaviour must be considered **(Boeuf & Payan, 2001)**.

9. Implications for conservation and valorization

The native desert relict blue tilapia populations in the arid Draa basin of Morocco face various constraints, including increased habitat fragmentation, pollution, drought, flow intermittence due to river regulation (such as the flow management from the Mansour Eddahbi Dam), secondary salinization, water abstraction, and climate change (characterized by prolonged droughts or unpredictable floods). These factors exacerbate anthropogenic water demand and create unsuitable conditions that negatively affect small, isolated populations.

Conservation of these relict blue tilapia populations in the intermittent river ecosystems (IRES) of the Draa basin depends on preserving their habitats to maintain periodic flow connectivity and facilitate regular or irregular wetting episodes. Areas with natural or near-natural flow regimes, particularly those unaffected by river regulation and water extraction, should be preserved to maintain their ecological integrity. Recovery efforts for IRES should focus on restoring areas where flow regimes and connectivity have been altered, along with native fish species. This can be achieved by reducing the effects of fragmentation (e.g., installing fishways or fish ladders on weirs to facilitate movement), managing invasive species, reshaping river channels, and restoring natural flow regimes through an effective water management.

Additionally, it is crucial to prevent the introduction of new non-native species and to control the expansion of already established species (e.g., *Lepomis gibbosus* and *Gambusia holbrooki*) by avoiding their introduction into basins where they are not yet present **(Clavero** *et al.,* **2014)**. The Nile tilapia, introduced to Moroccan waters in 2004, has recently been found in a few coastal rivers in northwestern and southwestern Morocco **(Louizi** *et al.,* **2019)**. Its introduction through aquaculture poses a potential threat to native tilapia species, particularly the relict *O. aureus* in the Draa basin, through hybridization, ecological competition, and disease transmission.

Finally, due to its larger body size—up to 20cm and a maximum weight exceeding 300g—the blue tilapia in the Draa basin has great potential for fish farming, especially in permanent naturally brackish waters like those in Tissint. This could provide an affordable source of protein for low-income local populations living in resource-poor arid environments in the Draa basin.

CONCLUSION

It is concluded that the blue tilapia in the intermittent and highly salinized reaches of the Draa River basin are smaller, lighter, and exhibit lower body condition compared to those in saline streams with permanent flow and lower salinity. To compensate for high mortality rates, these fish demonstrate faster and more pronounced growth, earlier maturity at smaller body lengths, and shorter longevity. Given that life-history traits are influenced by extreme conditions such as flow intermittence and high anthropogenic salinization, and are critical in determining population growth rates, they may serve as important indices of population viability.

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Ethical statement

The handling and use of experimental animals in this research work were under European animal welfare laws and guidelines. We conducted all procedures according to the American Veterinary Medical Association (AVMA) Guidelines for the Euthanasia of Animals (2020 Edition) and Directive 2010/63/EU of the European Parliament and the Council on the protection of animals used for scientific purposes.

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