1. Introduction

The gray triggerfish *Balistes capriscus* (*B. capriscus*) (Teleostei: Balistidae) is a reef fish broadly distributed in temperate and tropical waters of the Atlantic basin[1], from Nova Scotia to Argentina and throughout the Gulf of Mexico in the Western Atlantic and, in the Eastern Atlantic, from Ireland to Southwestern Africa[2], including the Mediterranean Sea[3]. *B. capriscus* is a member of thermophilic fishes, typical from the southern sectors of the Mediterranean and their emergence is consistent with what we would expect from the climate warming[4]. Remarkably, the recent literature is rich in records of these species moving northward with respect to their previously known distribution[5].

This fish is considered as one of the most important species that are commonly associated with coral reefs, wrecks, outcroppings, artificial structure and hard bottom areas[6].

Recently, a considerable increase in the *B. capriscus* production was depicted along the Tunisian coast and particularly in the Gulf of Gabès. Catching is about six fold increase from 34 tons in 1993 to 219 tons in 2011[7].

Despite the increase of production of the gray triggerfish in the Gulf of Gabès added to both the high commercial value of this fish belonging to its appreciated quality of flesh, its traditional therapeutic practices[8-10], no peer-reviewed published data exist concerning the annual reproductive cycle of *B. capriscus* collected from the Southeastern Mediterranean Sea.

Previous studies on the reproductive biology have been focused...
on determining the spawning season and size at sexual maturity of *B. capriscus*. They have focused on certain distribution areas: Western Atlantic, Eastern Atlantic and Northeastern Mediterranean[11].

The aim of this study was to examine the annual reproductive cycle of *B. capriscus* using the gonadosomatic index (GSI) and gonad histology and to evaluate its spawning seasonality and sexual maturation in the Gulf of Gabès.

Obtained results could provide essential life history data for any future stock assessments and may serve as a guideline for future management decisions of the gray triggerfish population of the Gulf of Gabès.

2. Materials and methods

Biological data collected monthly from 756 specimens (480 females and 276 males) obtained commercial catches at different fishing ports at several fishing ports including Chebba, Kerkennah and Zarzis at respective GPS coordinates (34°14’ N, 11°06’ E), (34°45’ N, 11°17’ E), (33°41’ N, 11°48’ E) (Figure 1), was studied over 28 months (January 2008-April 2010). In the laboratory, the following data were recorded for all individuals: sex, total length, fork length and standard length to the nearest mm; total weights and eviscerated fish weights to the nearest 0.1 g and the weights of gonads and livers were recorded to the nearest 0.01 g.

*B. capriscus* is a gonochoristic fish. Females and males could be distinguished by the skin colour at the upper side. The former has a white colour, while the latter shows a purplish to bluish reflection. This dichromatism is confirmed with application of the squash mount technique which is more pronounced and more accentuated during the reproduction period. Sex-ratios (number of females / number of males) were calculated according to the size. Month and season were compared by applying the χ² test.

The monthly mean GSI, the hepatosomatic indices (HSI) and the condition coefficient (K) were calculated for all sexually identified specimens and for both sexes as follows:

\[
\text{GSI} (%) = \left( \frac{\text{GW}}{\text{EW}} \right) \times 100; \quad \text{HSI} = \left( \frac{\text{LW}}{\text{EW}} \right) \times 100; \quad \text{K} = \left( \frac{\text{EW}}{\text{FL}^3} \right) \times 100
\]

Where GW = gonads weight (g); LW = liver weight (g); EW = eviscerated weight (g) and FL = fork length (cm).

Correlation between GSI and temperature was tested using the Spearman rank correlation coefficient \( r_s \)[12]. This temperature is the sea water average temperature which was supplied by the National Institute of Meteorology in Tunisia.

Variations in the mean of these indexes during the reproductive period were tested using One-way analysis of variance. The post-hoc Turkey’s test was performed to determine the pair-wise differences.

Ovaries from 280 individuals were preserved in Bouin’s solution in order to allow for histological analysis and were transferred to

![Figure 1. Map of the Gulf of Gabès describing the location of fishing port which collected *B. capriscus*.](image)
50% ethanol after 24 h. The preserved ovaries were embedded in paraffin, sectioned into 6 μm thick slices, and stained with hematoxylin and eosin[13]. Six maturity phases were established for females as follow: I-immature, II-developing, III-capable, IV-actively spawning, V-regressing, VI-recovering.

Ovarian developmental stages were assessed microscopically using a light microscope. In addition, the relative frequency of the different sizes of oocyte was estimated[14]. The diameters for each oocyte development stage were measured with an ocular micrometer. The terminology used for describing the different oocyte stages and ovarian phases is based on Brown-Peterson et al[15].

The mean size at first sexual maturity was estimated for both sexes by using a logistic function connecting the proportion P of mature individuals and fork length. This function was calculated as follow[16]:

\[ P = \frac{1}{1 + e^{r(L - L_{50})}} \]

Where P is the proportion of mature individuals; L is the fork length corresponding to the proportion (P); r is constant and L_{50} is the mean size at first sexual maturity; it was taken as the size at which 50% of individuals were mature.

3. Results

Out of 765 specimens B. capriscus sampled during this study, there were 480 females and 276 males. The fork length size ranges were 11.30-42.90 cm and the weight varied from 39.86 to 1858.37 g for female whereas for male the fork length size and weight ranged between 11.90-45.60 cm and 37.97-2318.07 g respectively.

Furthermore, sexual dimorphism was observed. The mean size of males (27.09 cm fork length) was significantly larger than females (26.74 cm fork length) based on the entire dataset (P < 0.05). An overall sex ratio (females / males) of (1: 1.74) which significantly deviated from the hypothetical distribution of 1:1, was significantly in favor of females (\( \chi^2, P < 0.05 \)) (Table 1).

The curves of the GSI monthly values were unimodal for both sexes (females and males) and sea surface temperature (T (°C)). Significant correlations were obtained between the average GSI values of both sexes (females and males) and sea surface temperature (r_s = 0.582; n = 12; P < 0.05 for females) (r_s = 0.628; n = 12; P < 0.05 for males) (Figure 2). Based on these correlations, we noted that the complete gonad maturity occurred at the highest sea surface temperature (27 °C).

The HSI of females (8.92 ± 2.01) and males (9.05 ± 1.75) were not significantly different (Mann-Whitney U-test, Z = -0.89, P > 0.05). HSI of both sexes showed a clear seasonal pattern, demonstrating a significant variation across months (females: Kruskal-Wallis test, \( H = 161.13, P < 0.001 \); males: Kruskal-Wallis test, \( H = 162.37, P < 0.001 \)).

The HSI cyclic evolution for both sexes was slightly synchronous with that of GSI showing a peak in June (female HSI: 11.89 ± 0.62 and male HSI: 11.65 ± 0.18), while the condition factor K showed no fluctuation along the year (Figure 3).

The ovarian lamellae inside the ovarian stroma indicated the presence of germinative cells at different oocyte development stages, according to the stage of gonadal maturity. The germinative cells or oocytes developed gradually inside the ovary. Eggs began

| Table 1 The fork length of B. capriscus in the Gulf of Gabès. |
|------------------|------------------|------------------|
| Parameter        | All fish (cm)    | Females (cm)     | Males (cm)     |
| Min              | 11.30            | 11.30            | 11.90          |
| Max              | 45.60            | 42.90            | 45.60          |
| Mean ± SD        | 26.87 ± 8.03     | 26.74 ± 7.55     | 27.09 ± 8.91   |
| Mode             | 18.00            | 18.00            | 28.50          |

Min: Minimum; Max: Maximum.
as oogonia and went through similar stages of oocyte development, which was commonly divided into primary growth and secondary growth beginning with the CA stage and then proceeding through vitellogenesis (Vtg), which could be broken into substages associated with the extent of yolk globules or platelets in the ooplasm [primary (Vtg1), secondary (Vtg2), and tertiary (Vtg3)]. Oocyte maturation occurred after the appropriate trigger and could include germinal vesicle migration, yolks coalescence and hydration. At ovulation, the follicle ruptures and the oocyte was released. POFs remained in the ovary, where they were resorbed.

This stage was subdivided in three different steps:

- Vtg1: Oil droplets occupied more cytoplasmic area than yolk granules (Figure 4E).
- Vtg2: Oil droplets occupied similar cytoplasmic area than yolk granules (Figure 4E).
- Vtg3: Oil droplets occupied less cytoplasmic area than yolk granules (Figure 4E).

4. Germinal vesicle migration: The nucleus started to migrate to the animal pole and the oil droplets fused to coalescence into a unique oil globule.

5. Germinal vesicle breakdown: Oocyte measuring between 370 and 489 μm (mean = 457.5 μm). The nucleus completed its migration to the animal pole and the unique oil droplet was clearly evident at central part of the oocyte (Figure 4F).

6. Hydration: Yolk granules fused in yolk plates, and eventually form a homogeneous mass. The nucleus had disintegrated and the cortical alveoli and cytoplasm were restricted to a thin peripheral layer. The oocyte significantly increased in size due to the uptake of fluids. Hydrated oocyte had a translucent appearance. At ovulation, the follicle ruptured and the oocyte was released. POF remained in the ovary, where they were resorbed.

The gonad maturation phases for females are described in Table 2. Monthly distribution of proportional female maturity phases showed that the period of spawning activity occurred mainly between July and mid-September with a peak in July (Figures 2 and 5).

The characteristic of oocyte development stage observed on histological section of ovaries grey triggerfish B. capriscus in the Gulf of Gabès, Tunisia were described as following:

1. Chromatin nuclear: Small oocyte ranging between 53 and 75 μm, which were round and/or oval. The nucleus was large and centrally located, surrounded by a thin layer of cytoplasm and containing a large and single nucleolus (Figure 4A).

2. Perinucleolar: Round and/or oval cells measuring between 75 and 94 μm (mean = 84.5 μm) with basophilic cytoplasm, with slightly more color than during the previous stage. Nucleus increased in size and multiple nucleoli appeared at its periphery (Figure 4B).

3. Cortical alveoli formation: Cell measuring between 220 and 242 μm (mean = 231 μm), spherical vesicles started to appear at the periphery of the cytoplasm. They increased in size and number to form several peripheral rows and give rise to cortical alveoli. Oil drops began to accumulate in the cytoplasm. At this stage chorion and follicle layers seemed to be apparent (Figure 4C).

Oocyte measuring between 324 and 374 μm (mean = 350 μm) emerge of some yolk vesicles in the cytoplasm. Besides, the separation of the chorion in two different layers occurred: inner and outer zona radiate (Figure 4D).
Figure 5. Monthly distribution of the maturational phases for female *B. capriscus* collected from commercial catches at several fishing ports between January 2008-April 2010 (*n* = 280).

Figure 6. Size at first sexual maturity for *B. capriscus* in the Gulf of Gabès. A: Males; B: Females.

The logistic curves showed the estimated relationship between the maturity proportion in each length interval and the fork length. The results obtained by the application of the logistical function showed that the size at first sexual maturity was 20.26 cm for females and 21.30 cm for males (Figure 6). The $\chi^2$ test did not show any significant differences between the theoretical proportions and the observed ones and this non-linear logistic regression model had an excellent fit ($r^2 = 0.98$).

4. Discussion

Sexual dichromatism concerning the color of the upper side was already observed in *B. capriscus*, collected from the Gulf of Gabès. This character has been described by several authors in other sites. Garnaud proved that during the period of reproduction, the male has more pronounced color[19]. Gerlotto and Stéquert reported that *B. capriscus* has a sexual dichromatism in which males and females were different in skin color at the sub-opercular region[20]. Mackichan and Szedlmayer showed that the dominant males could be identified by coloration and behavior[21].

Furthermore, sexual dimorphism in the length and weight has been also observed for this species in the Gulf of Gabès. In fact, mean lengths of males were significantly larger than that of females. Similar findings have been reported for gray triggerfish in the Southeastern US, Atlantic coast[22], and Gulf of Mexico[23]. The larger size of male gray triggerfish could be advantageous, allowing them to more adequately defend the nests in order to optimize survival of the eggs.

In the Gulf of Gabès, the sex ratio of *B. capriscus* is generally favorable to females. It is also the case in the Northeastern Gulf of Mexico[24] and in South Atlantic[25]. Only the populations of Alexandretta (Southern Turkey) have a male-dominated ratio[26]. The imbalance of the sex can be attributed to longevity, a differential catchability of the sexes or a spatio-temporal segregation of the sexes[27]. In the Gulf of Gabès, female dominance is probably related to a spatio-temporal distribution of the sexes.

Dependent upon location, *B. capriscus* population exhibited different reproduction period, which occurs mainly during the warmer months (July to mid-September) in the Gulf of Gabès, during November-December on the coast of Ghana[28] and during June-July in the Gulf of Mexico[29]. The water temperature probably plays a major role in the oocytes emission.

In general, this period coincides with favorable environmental
conditions for the survival and growth of larvae. In fact, low temperature may substantially decrease the chance of survival for fish larvae[30]. In the Gulf of Gabès, the reproduction period of *B. capriscus* begins in May, corresponding to the time when the water temperature increases and the zooplankton productivity is the highest in the Mediterranean[31] and particularly in the Gulf of Gabès[32]. Mediterranean subtidal plant communities (algae and seagrasses) begin to develop at this time[33]. This temporal coincidence allows the development of young triggerfish which find food resources in addition to the maintenance of rapid growth, development and protection within the subtidal seagrass and algae.

Like many fish species, the gonad index of *B. capriscus* is lower in males than females[34]. In fact, these index values for *B. capriscus* females in Tunisian coasts are higher than those found in the Gulf of Guinea[35]. These differences may be related to genetic potential variations and also to food resources availability. This suggests important fertility among individuals in the Gulf of Gabès.

Fish may store the energy required for spawning, in the liver or the muscles. However, the fluctuations of the HSI are relatively synchronous with those of the GSI for both females and males. However no relationship between K and spawning were clearly depicted. This suggests that the required energy for spawning might be derived from *B. capriscus* liver accumulating lipid reserves to be spent during laying. However, the muscles do not take part in the energy supplying for both sexes reproduction.

The obtained results (GSI) are agreed with gonad histology analysis. Effectively the period of spawning activity occurred mainly between July and mid-September.

Based on the method of the logistic curve, we estimated the size at maturity to be 20.26 cm fork length and 21.30 cm fork length for females and males, respectively. In São Paulo, Brazil, the length of the first sexual maturity is 16.9 cm fork length for females and 20.0 cm fork length for males[36]. However in South Atlantic sexual maturity is reached at 17.3 cm fork length for females and 18.1 cm fork length for males[25]. These differences are explained by the fact that the size of mature individuals depends on the biological factors and/or the ecological environment[37]. Human activities such as overfishing (increased fishing effort) can also explain the differences in size[38].

Fish subjected to these pressures may have ecophysiological adaptations related to the growth or the reproduction, the dwarfism or the early sexual maturity, in order to ensure their survival[39].

Male *B. capriscus* attained 50% sexual maturity (21.30 cm) almost 1 cm larger than that of female (20.26 cm). The maturation of female at smaller size than that of male is consistent with findings for triggerfish *Balistes vetula* in Brazil (fork length = 23.5 cm female, male 26.5 cm)[40]. This could be explained by the differential growth rates between the sexes[41].

**Conflict of interest statement**

We declare that we have no conflict of interest.

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