Int. J. Aquat. Biol. (2022) 10(6): 524-536
ISSN: 2322-5270; P-ISSN: 2383-0956
Journal homepage: www.ij-aquaticbiology.com

Original Article

Reproductive biology of greasy grouper, *Epinephelus tauvina* and coral hind grouper *Cephalopholis miniata* (Family: Serranidae) in the southern Red Sea, Shalatien, Egypt

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Abstract: Groupers are the main component of commercial fisheries in the Red Sea. The reproductive biology of *Epinephelus tauvina* and *Cephalopholis miniata* collected at the Shalatien landing site in the southern Egyptian Red Sea were investigated from January to December 2017. A total of 212 specimens of *E. tauvina* with a total length range of 23.6 to 70.3 cm and 243 specimens of *C. miniata* with a total length range of 17.4 to 42.1 cm were examined. Females of *E. tauvina* and *C. miniata* accounted for 61.7 and 61.9% of the total fish sampled, showing a 1:1.61 and 1:1.63 male-to-female sex ratio, respectively. The monthly distribution of maturity stages and gonadosomatic index values showed that the spawning season for both sexes was extended from April to October for *E. tauvina* and from April to September for *C. miniata*. The lengths at first sexual maturity of the males and females of *E. tauvina* were estimated at 49 and 48.1 cm, respectively, while those for *C. miniata* were estimated at 23.2 and 22.7 cm, respectively. All our results can help in the proper management of these valuable resources.

Article history:
Received 7 October 2022
Accepted 21 December 2022
Available online 25 December 2022

Keywords:
Gonadosomatic index
Spawning season
Grouper

Introduction

The Shalatien fishing area is a productive area in the Egyptian sector of the Red Sea. Coral reef terraces characterize the coastal area of Shalatien in many regions. These coral reefs help break the waves and form protected areas with abundant food sources for different fishes (Mahmoud et al., 2009). In 2020, groupers (Serranidae) were highly abundant in the Shalatien fishery, representing approximately 34.5% of the fish production from the Shalatien landing site (GAFRD, 2020). Because of their commercial importance, they suffer from strong fishing pressure, which extends to overexploitation (Morris et al., 2000), resulting in a decrease in their population worldwide (Sadovy de Mitcheson et al., 2013; Rajan, 2015; Bulanin et al., 2017; Ohta et al., 2017; de Mitcheson et al., 2020).

Many groupers form spawning aggregations at specific locations and times that are often associated with the lunar cycle (Sadovy de Mitcheson and Colin, 2012; Osman et al., 2021). At these locations and times, they are especially vulnerable to fishing pressure from artisanal and commercial fisheries because of increased catchability (lethal effects) and decreased reproductive output at lowered animal densities (Sadovy de Mitcheson, 2016; Ohta et al., 2017). Many studies have reported that the total catch of groupers has decreased and that the stock has been overexploited (e.g., Sadovy, 2005; Tharwat, 2005; Mohammad, 2007; Grandcourt et al., 2009; Mohammed-AbdAllah, 2015; El-Ganainy, 2017; Osman et al., 2021). The Red List assessments for all 163 grouper species show that 25% of the species are considered to be at risk of extinction or categorized as being near threatened, but 30% of all species are considered to be data deficient (Sadovy de Mitcheson et al., 2013; IUCN, 2021).

An understanding of fish reproductive strategies

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DOI: https://doi.org/10.22034/ijab.v10i6.1774
DOR: https://dorl.net/dor/20.1001.1.23830956.2022.10.6.9.2

Figure 1. Map of the study area (Shalatien).

is required to properly manage fisheries (Komolafe and Arawomo, 2007; Dinh, 2018). Knowledge regarding female maturity stages and spawning seasons is important to prevent catches during the spawning season (El-Ganainy, 1997; Osman et al., 2018). The sex ratio provides a useful tool for assessing fish's biological characteristics and estimating a population's reproductive potential (Sossoukpe et al., 2013; Sabrah et al., 2017). The gonadosomatic index (GSI) is widely used to measure the extent of reproductive investment, gonadal development and maturity of fish in relation to spawning (Ogunola et al., 2018; Khatun et al., 2021; Sabbir et al., 2021). The length and age at first sexual maturity are important parameters in fisheries research for evaluating the optimum age of the first capture and determining the minimum legal size that may be needed to preserve a suitable spawning stock of a species and to ensure the occurrence of at least one spawning event among mature fish (Lappalainen et al., 2016; Osman et al., 2018; Mawa et al., 2021). This work aimed to investigate the sex ratio, maturity, GSI and length at first sexual maturity of *Epinephelus tauvina* and *Cephalopholis miniata* in the Shalatien fishery to provide proper data for the conservation of their stocks.

Materials and methods

Random samples of *E. tauvina* and *C. miniata* were collected monthly from the Shalatien fishing port, which is one of the productive fishing grounds along the Egyptian Red Sea coast and located in the southern Red Sea, at Elba National Park, 520 km south of Hurghada, Egypt (Fig. 1).

The samples were collected from January to December 2017. For each specimen, total length (TL) was measured to the nearest cm, and total weight (TW) was determined to the nearest 0.1 grams (g). Specimens were sexed by macroscopic dissection, and the gonads were weighed to the nearest 0.01 grams.

The sex ratio was calculated from the formula of Sex ratio (M:F) = Number of Females/Number of males. This ratio was determined during different months and in different length groups. The Chi-square test at the 0.05 significance level was employed to determine the goodness of fit of the observed sex ratio to that of the theoretical sex ratio of 1:1 (M:F). The following formula of Wootton (1998) was used for the Chi-square test. $\chi^2 = [(F-S)^2/ S] + [(M-S)^2/ S]$, where χ^2 is the symbol for Chi-Square, F is the observed number of females, M is the observed number of males and S is the expected

Table 1. Descriptive statistics of means of *Epinephelus tauvina* and *Cephalopholis miniata* during (2017) from Southern Red Sea, Shalatien, Egypt.

Species	Sex	n	TL (cm)			Total weight (g)		
			Min	Max	Mean ± SD	Min	Max	Mean ± SD
E. tauvina	Males	77	24.4	62.2	40.6±11.7	206.1	3350.3	1226.5±1050.0
	Females	124	27.5	70.3	44.3±12.5	259.9	5015.3	1615.0±1339.3
	Unsexed	11	23.6	26.6	25.3±1.0	189.5	265.1	231.8±25.2
	Pooled	212	23.6	70.3	42.0±12.6	189.5	5015.3	1402.1±1245.8
C. miniata	Males	91	20.2	42	29.5±5.9	135.4	1286.9	474.3±282.8
	Females	148	17.4	42.1	27.5±5.0	75.6	1277.9	398.4±283.7
	Unsexed	4	22.6	23.5	23.2±0.4	190	214.9	205.7±11.4
	Pooled	243	17.4	42.1	28.1±6.1	75.6	1286.9	420.6±284.1

number of each sex (the hypothetical 1:1 ratio).

The monthly variation in the maturity stage was determined. Six maturity stages were identified according to the scale of Gunderson (1993), with some modifications, as follows: (1) *Thread*: Sex cannot be determined at this maturity stage, and the gonads in individuals are filamentous, (2) *Stage I* (Immature or inactive): The gonads have a translucent appearance, and the testes are smaller and thinner than the ovaries, (3) *Stage II* (Resting): The gonads are translucent and enlarged and the testes do not contain sperm, while the ovaries possess a few small eggs, (4) *Stage III* (Active or developing): Gonads are larger than Stage II, and the testes are opaque without sperm; ovaries are translucent with small eggs, (5) *Stage IV* (Active-ripe or developed): Testes are white, and some sperm are expelled from the core when cut, and ovaries are opaque and solid with fully formed eggs, (6) *Stage V* (Spawning or Ripe-running): Gonads are enlarged and occupy most of the body cavity; milt and eggs are expelled from the genital openings when we press slightly on the two sides of the body, and (7) *Stage VI* (Spent): Ovaries are flaccid with few eggs, while the testes are almost empty.

The GSI is an indirect method used to determine the spawning season of fish. It was calculated for each fish according to Sokal (1995) using the formula of GSI = (gonad weight/total fish weight) x 100. The length at first sexual maturity (L_m) represents the length at which 50% of individuals are mature during the spawning season. Individuals at stages III-VI were considered mature, and it was possible to determine their L_m values. After plotting the percentage of mature individuals against their mid-lengths (King, 1995), L_m was estimated as the point on the X-axis corresponding to the 50% point on the Y-axis. The equation used to estimate L_m from the logistic curve was as follows (Erisman et al., 2010): $P = 1/ (1 + \exp^{-r (Lt - Lm)})$, where P is the proportion of mature individuals in a length class, L_t is the total fish length, r is the intercept, and L_m is the slope.

Results

A total of 212 specimens of *E. tauvina* with a TL range of 23.6 to 70.3 cm, including 77 males, 124 female,s and 11 unsexed and 243 specimens of *C. miniata* with a TL range of 17.4 to 42.1 cm, including 91 males, 148 females and 4 unsexed were collected for reproductive biology studies (Table 1).

Sex ratio: The overall sex ratio indicated the occurrence of more females than males for the two studied species. The ratio of males to females of *E. tauvina* was 1:1.61, and females accounted for 61.7% of the total sampled fish. The sex ratio of males to females of *C. miniata* was 1:1.63, and females accounted for 61.9% of the total sampled fish. The chi-squared analysis indicated a significant difference compared to the expected 1:1 ratio (χ^2 = 10.53, *df* = 1, *P*<0.05 for *E. tauvina* and χ^2 = 13.12, *df* = 1, *P*<0.05 for *C. miniata*). The monthly changes in the percentages of males and females are represented in Figure 2 for *E. tauvina* and Figure 3 for *C. miniata*. The variation in the male-to-female sex ratios of *E. tauvina* and *C. miniata* according to

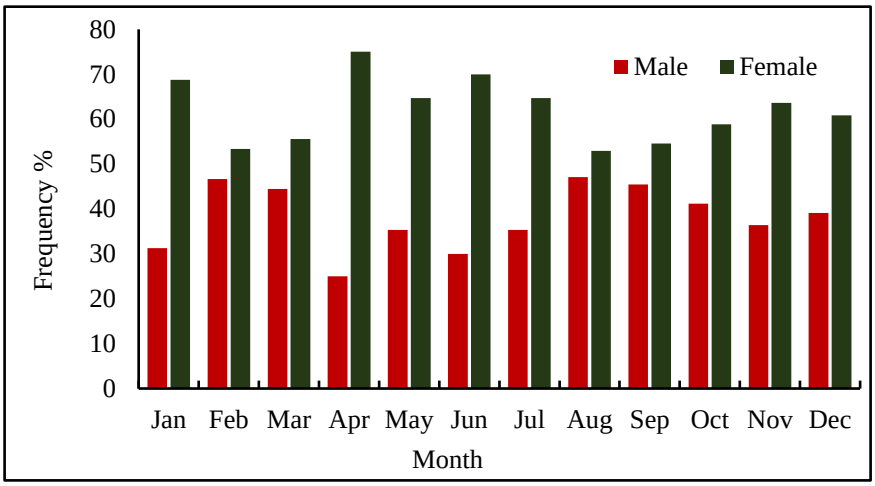

Figure 2. Monthly variation in the percentages of males and females of *Epinephelus tauvina* from the southern Red Sea, Shalatien, Egypt.

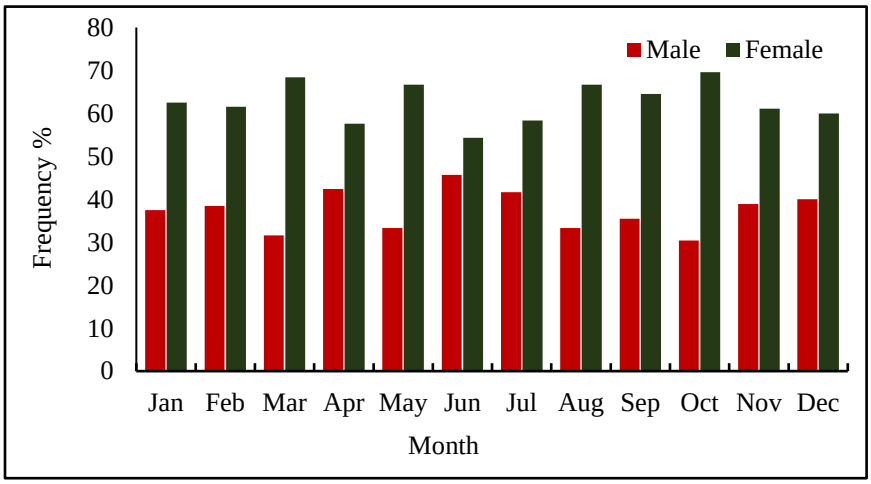

Figure 3. Monthly variation in the percentages of males and females of *Cephalopholis miniata* from the southern Red Sea, Shalatien, Egypt.

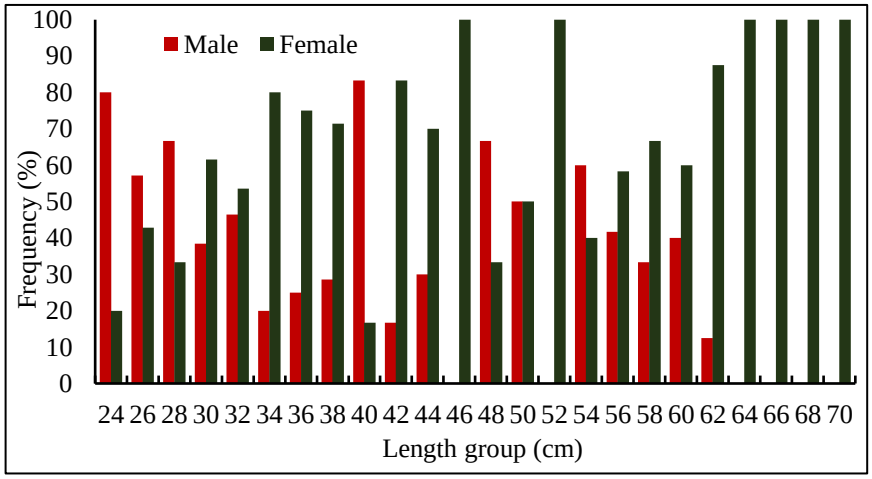

Figure 4. Percentages of males and females of *Epinephelus tauvina* according to length group.

length groups is presented in Figures 4 and 5, respectively.

Monthly distribution of maturity stages: The seasonal variation in the maturity stage was determined for each individual. The *E. tauvina* spawning period began in April because ripe gonads could be observed in the samples at that time, and the percentages of spawning individuals were 40 and

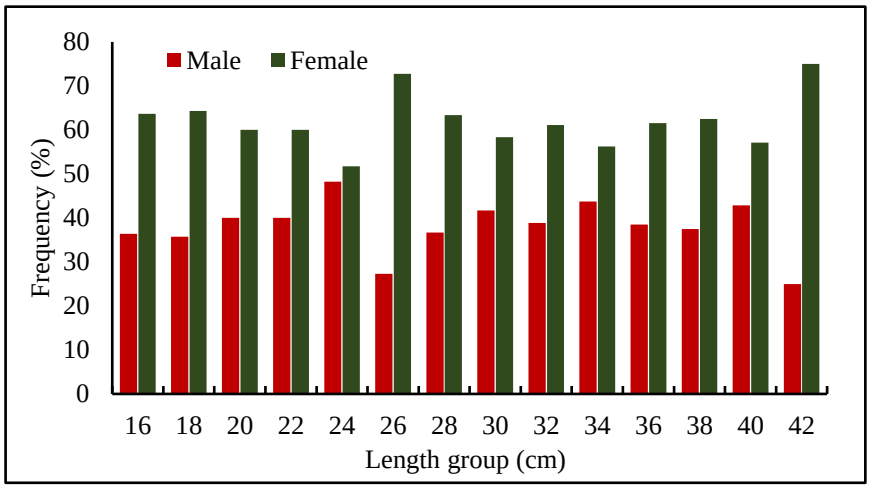

Figure 5. Percentages of males and females of *Cephalopholis miniata* according to length group.

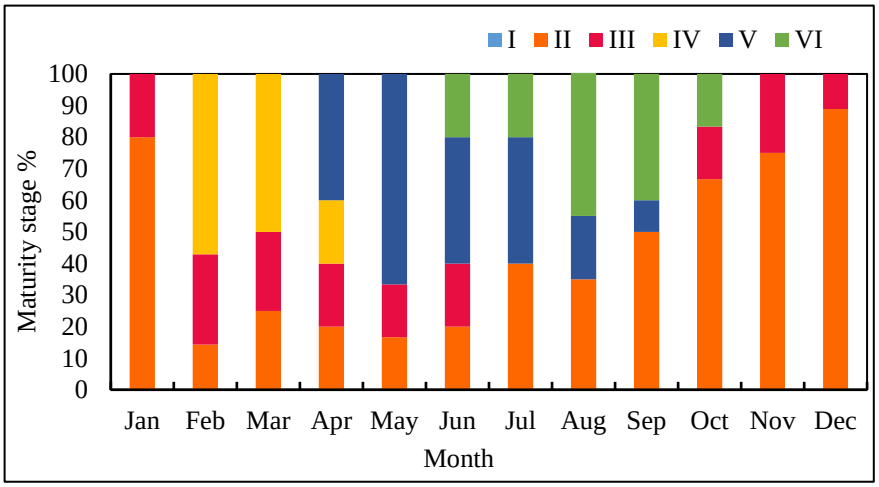

Figure 6. Monthly distribution of maturity stages (I: Inactive, II: Spent, III: Active, IV: Active-ripe, V: Ripe-running, VI: Spent) in male *Epinephelus tauvina*.

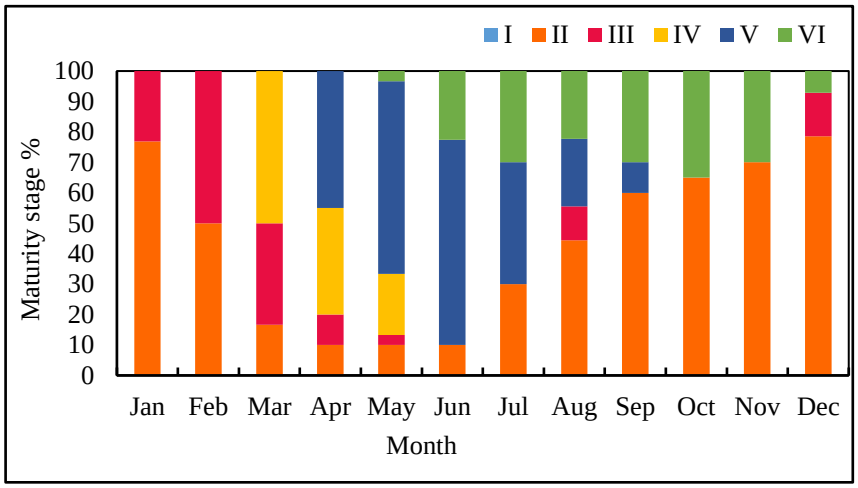

Figure 7. Monthly distribution of maturity stages (I: Inactive, II: Spent, III: Active, IV: Active-ripe, V: Ripe-running, VI: Spent) in female *Epinephelus tauvina*.

45% of the males and females, respectively (Figs. 6, 7). This stage extended until September, revealing a long spawning season, with a peak in May for males (66%) and in June for females (67%). The first appearance of ripe gonads of *C. miniata* occurred in April, indicating the beginning of the spawning

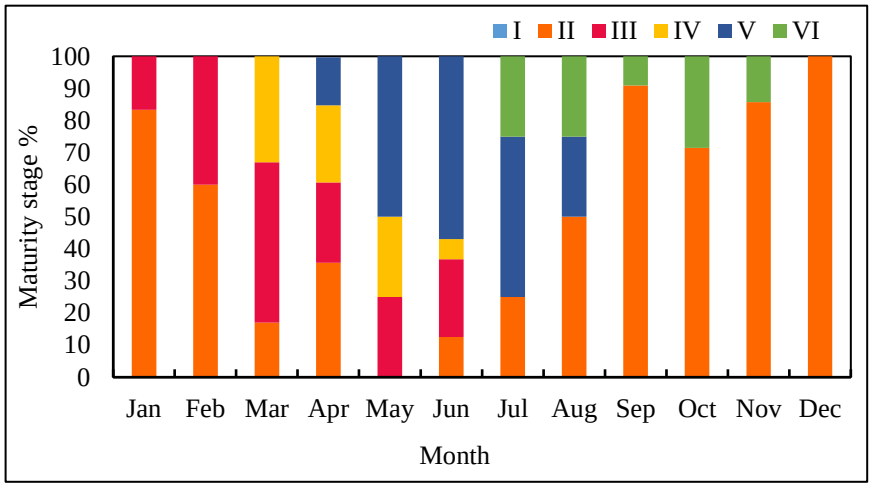

Figure 8. Monthly distribution of maturity stages (I: Inactive, II: Spent, III: Active, IV: Active-ripe, V: Ripe-running, VI: Spent) in male *Cephalopholis miniata.*

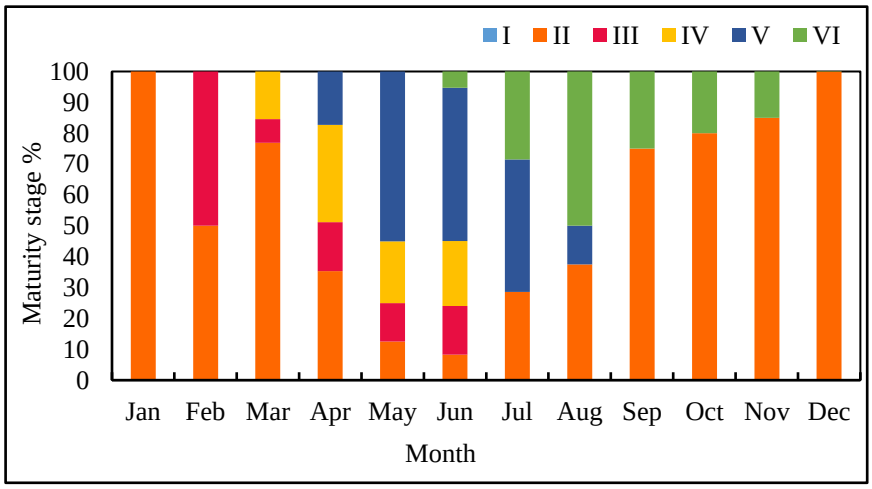

Figure 9. Monthly distribution of maturity stages (I: Inactive, II: Spent, III: Active, IV: Active-ripe, V: Ripe-running, VI: Spent) in female *Cephalopholis miniata.*

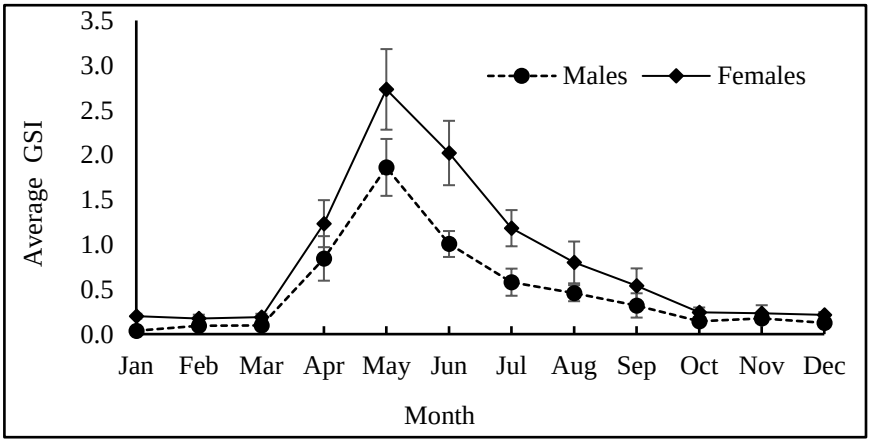

Figure 10. Monthly variation in GSI values for *Epinephelus tauvina* from the southern Red Sea, Shalatien, Egypt.

period. The percentages of individuals in this stage during this month were 15% and 17.3% of the males and females, respectively (Figs. 8, 9). This stage extended until August, revealing a long spawning season, with a peak in June for males (57%) and in May for females (55%).

Gonadosomatic index (GSI): The overall GSI values were higher for females than for males in both

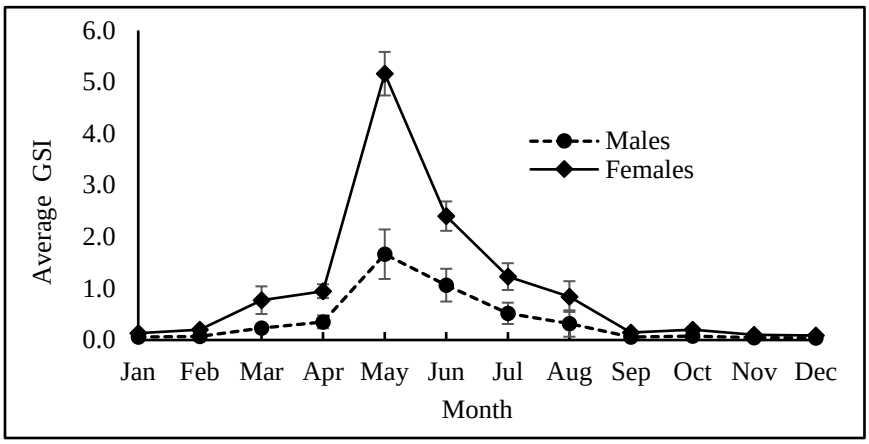

Figure 11. Monthly variation in GSI values for *Cephalopholis miniata* from the southern Red Sea, Shalatien, Egypt.

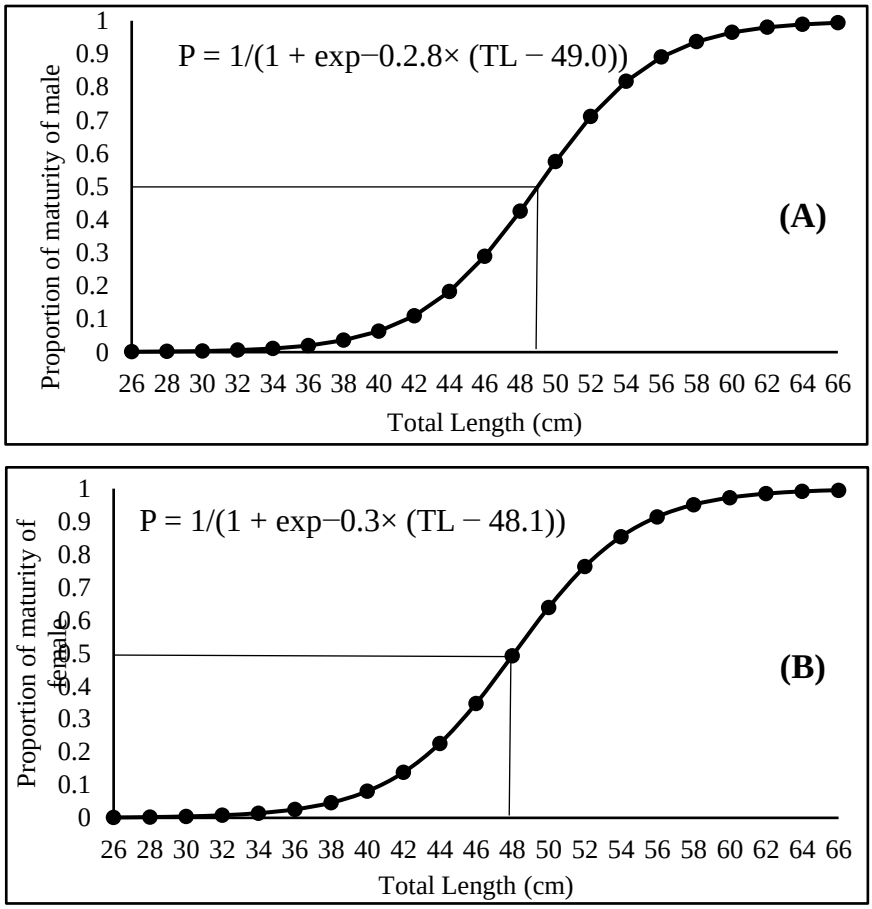

Figure 12. Logistic curves for the proportions of mature males (A) and females (B) of *Epinephelus tauvina* from the southern Red Sea, Shalatien, Egypt.

species. The spawning season of *E. tauvina* was extended from April to October, while it spanned only from April to September for *C. miniata*. The highest value of this index was recorded in May for males and females of *E. tauvina* and *C. miniata* (Figs. 10, 11), and the lowest values for both sexes were recorded in January for *E. tauvina* and in December for *C. miniata*.

Length at first sexual maturity (L_m): The L_m values for the males and females of *E. tauvina* were estimated at 49 and 48.1 cm (Fig. 12), respectively, while those for the males and females *C. miniata* were estimated at 23.2 and 22.7 cm (Fig. 13), respectively.

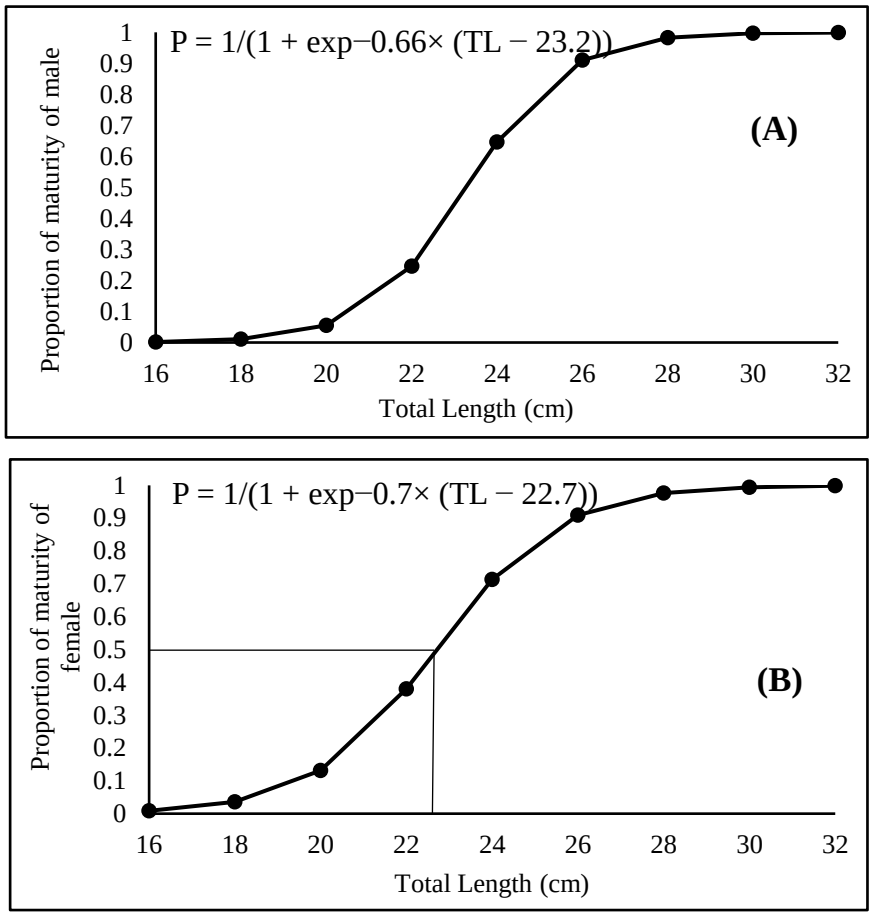

Figure 13. Logistic curves of the proportions of mature males (A) and females (B) of *Cephalopholis miniata* from the southern Red Sea, Shalatien, Egypt.

Discussion

The application of the sex ratio is useful in studying spawning populations (Faltas, 1983; Sabrah et al., 2017). In the present study, the sex ratio of *E. tauvina* and *C. miniata* revealed that females dominated the catch during the whole period of study. Our results agree with those recorded by Ohta et al. (2017) for *E. tauvina* in the Yaeyama Islands and Wahbeh (2005) for *C. miniata* in the Aqaba Gulf, Jordan. According to Wahbeh (1992), Cherif et al. (2007) and Sabrah et al. (2017), the predominance of females may be connected with the higher catchability of females than males or due to the high mortality of females, as they are more vulnerable to fishing pressure. Chakroun-Marzouk and Ktari (2006) reported an unbalanced sex ratio favouring females in some species, revealing that natural mortality may be higher among males, while females may present high vulnerability to fishing. According to the length group's evaluation, males of *E. tauvina* were dominant at smaller fish sizes and absent among larger fish sizes, while females of *C. miniata* were dominant at all sizes. The results are supported by Nikolsky (1963), who stated that males predominate in fish populations at early life stages, while females predominate later due to probable adaptive mechanisms. The sex ratio in these species could vary greatly; however, no studies have explicitly addressed the potential mechanisms explaining those patterns.

The monthly distribution of maturity stages showed that the ripe stage for males and females of *E. tauvina* began in April, ended in September, and increased to its highest peak in May for males and in June for females. On the other hand, the ripe stage for males and females of *C. miniata* began in April, ended in August, and increased to its highest peak in May for both males and females. These results

Table 2. The spawning season, sex ratio and the length at first sexual maturity (L_m) of *Epinephelus tauvina* and *Cephalopholis miniata* found in the present study and in various regions by different authors.

Species	Author	Region	Spawning season	Sex ratio (M:F)	L_m
E. tauvina	Abu-Hakima et al. (1982)	Kuwait water	March to June	-	61.1
	Mathews and Samuel (1987)	Kuwait water	March to May	-	-
	El-Sayed (1999)	Persian Gulf off Qatar	May to June	-	53
	Shakeel and Ahmed (1996)	Maldives	-	-	37.5
	Abu-Hakima (1987)	Kuwait water	April to May	-	-
	Ohta et al. (2017)	Yaeyama Islands, Japan	March to June	1:1.8	37.1
	El-Ganainy (2017)	Red Sea, Egypt	May to June	-	36.6
	Present study	Red Sea, Shalatien, Egypt	April to October	1:1.61	48.55
C. miniata	Shakeel and Ahmed (1996)	Maldives	-	-	20
	Wahbeh (2005)	Aqaba, Jordan	May to October	1:1.28	-
	El-Ganainy (2017)	Red Sea, Egypt	May to June	-	28.2
	Present study	Red Sea, Shalatien, Egypt	April to September	1:1.63	22.95

indicate that both *E. tauvina* and *C. miniata* in the southern Red Sea, Shalatien, have a prolonged spawning season that extends from April to October for *E. tauvina* and from April to September for *C. miniata*.

The GSI refers to the relationship between the gonadal weight and the fish somatic weight (Wootton, 1991), and the seasonal timing of reproduction is often identified based on changes in the GSI (Biswas, 1993; King, 2013; Ogunola et al., 2018). Our results revealed that the GSI differed between males and females of *E. tauvina* and *C. miniata*; the GSI for males was lower than that for females. These results suggest that the energy invested in gamete production by females is likely more than that invested by males (Patimar, 2008). The results showed that the GSI increased in both sexes of *E. tauvina* from April to October and in both sexes of *C. miniata* from April to September, indicating the timing of the spawning season in these species. These findings for *E. tauvina* agree to some extent with those found by Ohta et al. (2017) in the Yaeyama Islands, Japan, Abu-Hakima et al. (1982) in Kuwait and El-Ganainy (2017) in the Red Sea, Egypt, whose investigations of the spawning season of *E. tauvina* indicated that it occurs from March to June. Abu-Hakima (1987) stated that *E. tauvina* collected from Kuwait spawned in April and May. El-Sayed (1999) recorded that *E. tauvina* in the Persian Gulf spawns in May and June, while members of this species from Kuwait spawn from March to May (Mathews and Samuel, 1987). The observed decrease in the GSI values during January for *E. tauvina* and December for *C. miniata* indicated the end of the spawning season and the beginning of the spent and resting periods. Likewise, the spawning season of *C. miniata* in the Aqaba Gulf, Jordan, was found to occur between May and October (Wahbeh, 2005). El-Ganainy (2017) stated that *C. miniata* collected from the Red Sea, Egypt, spawned in May and June. The spawning season of *C. miniata* is relatively long, which is consistent with the general pattern of longer spawning seasons among smaller groupers (Sadovy, 1996; Wahbeh, 2005). Differences between our results and the previous studies about the spawning seasons of investigated species may be attributed to differences in water temperature among locations (Jansen and Gislason, 2011; Osman et al., 2018), sampling techniques, geographic disparity and the sampling year of the studies (Soykan et al., 2020).

Knowing the Lm of fish helps predict their harvestable size and hence has great value for fishery management (Sabrah et al., 2017). In the present study, *E. tauvina* reached first sexual maturity at 48.55 cm TL. The L_m values of *E. tauvina* found in the present study were longer than those reported by Ohta et al. (2017) in the Yaeyama Islands, Japan,

Shakeel and Ahmed (1996) in the Maldives (37.3 cm) and El-Ganainy (2017) in the Red Sea, Egypt (36.6 cm), but smaller than those reported by El-Sayed (1999) in the Persian Gulf (53 cm) and Abu-Hakima et al. (1982) in Kuwait waters (61.1 cm) (Table 2). Our results revealed that *C. miniata* reached first sexual maturity at 22.95 cm. These findings roughly correspond to those obtained by Shakeel and Ahmed (1996) for *C. miniata* collected in the Maldives (20 cm) but are smaller than those estimated by El-Ganainy (2017) in the Red Sea, Egypt (28.2 cm). These differences in length and age at the first sexual maturity of investigated species in the present study and in the previous studies may be attributed to the length and age at first sexual maturity of fishes influenced by environmental factors like abundance and seasonal availability of food, predation, temperature, photoperiods and also the locality (Osman et al., 2018; Adekoya et al., 2019; Hossen et al., 2019; Soykan et al., 2020).

It can be concluded that, both sexes of *E. tauvina* and *C. miniata* in the Shalatien landing site in the southern Red Sea, Egypt, showed a prolonged spawning season that extended from April to October in *E. tauvina* and from April to September in *C. miniata*. *E. tauvina* reached sexual maturity at 49 cm for males and 48.1 cm for females, while *C. miniata* reached sexual maturity at 23.2 cm for males and 22.7 cm for females. Fishing of spawning aggregations leads to a reduction in the average size of the individuals caught and a remarkable decline in the sex ratio. Establishing protected areas (or no-take zones) in the Red Sea is a valuable management option for protecting the size and the breeding populations of coral reef fish, especially groupers that aggregate to spawning.

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