Heliyon 10 (2024) e37369

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Evaluating the reproductive performance of Summan grouper, *Epinephelus summana* (Forsskal, 1775), in the Red Sea

Faozi S. Shamlol [a], Elsayed M. Younis [a,**], Mohamed H. Gabr [b], Shimaa A. Amer [c,*], Simon J. Davies [d], Doaa M. Elnagar [a], Khalid E. Ibrahim [a], Saad M. Alsaiad [e]

[a] *Department of Zoology, College of Science, King Saud University, P.O, Box 2455, Riyadh, 11451, Saudi Arabia*
[b] *National Institute of Oceanography and Fisheries, Suez and Aqba Gulfs Branch, Ataqa, Suez, Egypt*
[c] *Department of Nutrition and Clinical Nutrition, Faculty of Veterinary Medicine, Zagazig University, Zagazig, 44511, Egypt*
[d] *School of Science and Engineering, National University of Ireland Galway Republic of Ireland, H91 TK33, Galway, Ireland*
[e] *Department of Fish Production, Faculty of Agriculture, Al-Azhar University, Nasr City, Egypt*

ARTICLE INFO

Keywords:
Epinephelus summana
Reproduction
Red sea

ABSTRACT

Grouper fish are among the most important components of the fisheries of many countries because they are found in warm water throughout the world. There are 15 genera and 159 species known worldwide; 8 genera and 66 species are exclusively found in the western Indian Ocean, Red Sea, and Arabian Gulf. The Summan grouper, *Epinephelus summana*, constitutes a considerable portion of these fisheries; therefore, this study aimed to evaluate the reproductive strategy of this important fish species. The fish samples were collected monthly for one year (from November 2020 to October 2021), and 217 fish were collected from the Red Sea of Jeddah, Saudi Arabia. The sex ratio, sexual maturation process, and spawning season were analyzed. Across all samples, landing consisted of 36.2 ± 4.7 % males, 64.0 ± 5.0 % females, and 3.4 ± 1.8 % transitional-stage fish, with an overall significantly different male-to-female sex ratio of 1:3.3. Furthermore, males were larger than females. The maturation index (MI), gonadosomatic index (GSI), and ovarian maturation rate (OMR) values fluctuated throughout the year, indicating that *E. summana* has extended spawning and spawns in batches during different months of the year. However, April to May is the main spawning season, with the highest female GSI recorded. Based on the microscopic histological examination of gonads, the maturation process can be classified into five stages in both males and females. In conclusion, this fish species has a complex reproductive biology. It undergoes sexual transformation and protogynous hermaphroditism, during which individuals mature first as female and then change sex to male. The obtained data is essential for successful fishery stock conservation, management, and aquaculture development.

1. Introduction

Effective conservation approaches within limited resources and time are critical for wildlife management to have the best effect on population sustainability [1,2]. Unfortunately, the scarcity of demographic data for many species and groups leads to reliance on life history traits to develop conservation strategies [3]. Understanding the ecological biology of any species, particularly those that show

* Corresponding author.
** Corresponding author.
E-mail addresses: emyounis@ksu.edu.sa (E.M. Younis), shimaa.amer@zu.edu.eg (S.A. Amer).

https://doi.org/10.1016/j.heliyon.2024.e37369
Received 6 March 2024; Received in revised form 28 August 2024; Accepted 2 September 2024
Available online 3 September 2024

remarkable plasticity in response to the local environment, such as growth, fecundity, and reproduction, is of specific value for real fish conservation [4].

The Red Sea is classified as a tropical sea, characterized by a rich biodiversity, endemic to more than 1000 fish species and more than 50 species of hermatypic corals [5]. In the Red Sea, groupers (Serranidae: Epinephelinae) are the main goals of recreational, artisanal, and profitable fisheries. Groupers are under expanding fishing pressure worldwide for food, medical, and ornamental intentions [6–8]. Groupers of the family Epinephelinae are one of the highest predators on coral reefs. Their distinct characteristics make them particularly vulnerable: slow growth, large sizes, long lifespan, and late reproduction [9]. They are the main components of cliff edge and slope environments, where biotic connections greatly influence their habitats at depth [10]. Their burrowing behavior, which they have called "ecosystem engineers," likely affects the biogeochemistry of the ecosystem, breaks down and processes sedimented organic matter, and provides refuges for fish and other invertebrates, increasing their abundance [10].

Worldwide, continuing overfishing has reduced large populations of predatory coral reef fish and triggered unpredictable top-down fluctuations in coral reef ecosystems. Groupers are particularly vulnerable to overexploitation as they congregate to reproduce at certain spots and periods. Knowing these fishes' spatial dynamics is critical for managing and maintaining fisheries [11]. Groupers are a prevalent kind of fish found within the Red Sea, which has great ecological and economic importance in all subtropical and tropical seas. Groupers are known to be found across the Red Sea, occupying a range of depths from a few meters to approximately 200 m. They are a major target of fisheries due to their high commercial value [12]. There are 98 species of fish in the genus *Epinephelus*, of which 16 are present within the Red Sea [13]. The Summan grouper, *Epinephelus summana* (Forsskal, 1775), is found in the western part of the Indian Ocean, the Red Sea, the Gulf of Aden, and off Socotra. *E. summana* is a reef-associated species in shallow protected coral reefs and seaward reef slopes from 1 to 30 m.

The reproductive characteristics of fish include age, size at sexual maturity, sex ratio, and timing and duration of spawning, which exhibit variability within and between populations [14,15]. These characteristics help evaluate life history traits, different strategies of gamete production, reproductive potential, and the productivity of the population over time [15]. The variability of reproductive life history features is observed within and among fish species. Despite the importance of the reproductive biology of groupers, the species-specific data, as with most marine fish, are not completely known [16]. Groupers play a pivotal role in numerous fisheries throughout diverse coastal nations. Many groupers are harvested in fisheries within the Arabian Gulf and the Red Sea. Nevertheless, the existing data regarding their biology and population dynamics are minimal.

A group or species is considered functionally hermaphrodite if a significant proportion of individuals at some point during its life cycle function as two sexes, either concurrently, sequentially (sex changers), or serially (bidirectional and cyclical sex changers). Sequential hermaphroditism, specifically protogyny (i.e., a functional female to a functional male sex change), is very familiar in shallow tropical marine reef habitations and between the perciforms. Protogyny commonly arises in two shapes; the first is named monandry, or a single route of male evolution in which all males are drawn from functional females (i.e., secondary males). The second is called diandry, or two routes of male in which some males are male at first maturation (i.e., primary males), while others arise from a sex change in functional females (secondary males) [17,18]. Protogyny is a prominent characteristic of grouper reproduction (epinephelidae: epinephelinae) [19] and has been verified in the genera *Epinephelus, Cephalopholis, Plectropomus,* and *Mycteroperca* [17, 18].

There is no published information on the biology of *E. summana,* so the objective of the current investigation was to examine the reproductive biology of this fish species and determine its spawning season in Saudi Arabia's Red Sea coast. This study reveals essential information required for designing effective strategies for fishery management, enabling the sustainable use and maintenance of important natural resources.

2. Materials and methods

2.1. Sample collection

All the protocols and procedures in this research were approved and permitted by the Research Ethics Committee (REC) at King Saud University, Saudi Arabia (Approval number: KSU-SE-20-85).

From November 2020 to October 2021, samples of *E. summana* were collected monthly from the landing spot of fishery ships operating within the Red Sea waters off Jeddah, Saudi Arabia (21°29′24″ N; 39°10′23″ E). The samples were collected from the fishermen within two days in the first week of each month. The samples encompassed a comprehensive range of fish lengths (27.46 ± 4.20 cm for females and 37.07 ± 7.55 cm for males) and sizes (348.56 ± 93.43 g for females and 956.58 ± 370.39 g for males) (mean length or size ± SD). The obtained specimens were promptly submerged in freezing water to induce euthanasia and, after that, stored in an ice box and transferred to the fishery research laboratory located within the Zoology Department of the College of Science at King Saud University.

2.2. Physical assessment and sample processing

The physical measurements of the fish included the determination of the total length (TL) with a precision of 0.1 cm and the determination of the total body weight (BW) with a precision of 0.1 g.

The fish were dissected, and the gonads were taken and weighed (GW) with a precision of 0.1 g. The size, color, and shape of the gonads assisted in the distinction between sexually mature and immature individuals. The gonads were subsequently immersed in neutral-buffered formalin to determine their maturity stage through further histological analysis. The immersed gonads underwent

routine techniques for microscopy, such as washing, dehydrating, cleaning, and embedding within paraffin wax. The tissue samples were sliced into sections with a thickness of 4 μm using a microtome (LEICA RM2255, Leica Biosystems, United States). These sections were then subjected to staining with hematoxylin and eosin.

2.3. Gonad development and sex change

The assessment of gonad developmental stages was based on histological investigation and classified according to the most advanced gametes present and their relative prevalence within the gonad (i.e., primary growth oocytes, cortical alveolar oocytes atresia vitellogenic and/or hydrated oocytes, and primary and secondary vitellogenic oocytes in females; primary spermatogonia, spermatozoa, and spermatid in males) [20,21].

The phenomenon of hermaphroditism or sexual transition in the given species (fish undergoing a sexual transition from female to male) was identified using the diagnostic criteria outlined by Erisman, Craig [22] and De Mitcheson and Liu [17], which include the presence of degenerating, vitellogenic oocytes or atretic follicles coincident with proliferating spermatogenic tissue.

2.4. Sexual measurements and calculations and spawning season

Determination of the sex ratio involved the computation of the percentage of males and females within the gathered samples for each month and across various length categories, according to the following equation:

The proportion of males (or females) = number of males (females)/total number of samples × 100.

The gonadosomatic Index (GSI) was used to explain the ovaries' ripeness and indicate the ovaries' stage and readiness for spawning [8]. A combination of data from the GSI, macroscopic observation of maturity stages, and microscopic analysis of gonads was carried out to estimate the spawning season. GSI was determined monthly employing the following equation:

GSI = (GW/BW) × 100, where GW is the gonad weight, and BW is the body weight [21].

The maturation index (MI) was computed monthly using the following equation:

MI = (number of matured fish/total number of fish) × 100 [23].

Determination of the ovarian maturation rate (OMR) was based on the proportion of mature ovaries in all maturation stages relative to the total number of ovaries observed within all length categories [21].

The body length at first sexual maturation (L_{50}) for the females was estimated based on the relationship between the percentage of mature fish ((number of mature females/total number of females) × 100) and length categories where the body length at first sexual maturation is the length at which 50 % of the females were distinguished as mature fish.

The total length (TL) at sex change (P_{50}) was calculated also based on the percentage at which 50 % of the females changed to male [24–26].

2.5. Data analysis

One-way Analysis of Variance (ANOVA) in SPSS 16.0 for Windows was used for the analysis of the data of the GSI in males and females during the investigation period spanning from November 2020 to October 2021(since failure to comply with the requirements may limit the use of the analysis of variance and its multiple comparison tests). Tukey's multiple range test was used to detect the significant difference between the means. The significant difference was detected at $P < 0.05$. The data of TL of female, male, and transitional fish during the investigation period spanning from November 2020 to October 2021 were analyzed using an independent sample *t*-test (since failure to comply with the requirements may limit the use of the analysis of variance and its multiple comparison tests). The results are presented as means ± standard deviation (SD). The significant difference between males and females was detected at $P < 0.05$.

Table 1
Monthly sex ratios of the collected *E. summana* samples during the study period (November 2020 to October 2021).

Month	% Male	% Female	% Transitional	Sex Ratio M: F
Nov	7.7	88.5	3.8	1:11.5
Dec	25.0	70.0	5.0	1:2.8
Jan	35.3	47.1	17.6	1:1.3
Feb	34.8	65.2	0.0	1:1.9
Mar	45.0	55.0	0.0	1:1.2
Apr	50.0	50.0	0.0	1:1
May	13.3	86.7	0.0	1:5
Jun	35.7	50.0	14.3	1:1.5
Jul	30.8	69.2	0.0	1:2.3
Aug	60.0	40.0	0.0	1:0.7
Sep	43.8	56.3	0.0	1:1.3
Oct	10.0	90.0	0.0	1:9

3. Results

3.1. Sex ratio

The calculated sex ratios of the 217 specimens of *E. summana* taken from fisheries across Jeddah waters of the Red Sea throughout the investigation period spanning from November 2020 to October 2021 are shown in Table 1. The mean percentage of male samples was 36.2 ± 4.7 %, whereas females constituted 64.0 ± 5.0 %, and 3.4 ± 1.8 % was in the transitional stage (hermaphrodite), with an overall significantly different male-to-female sex ratio of 1:3.3. The monthly sex ratios demonstrated that the females exhibited dominance during the investigation period. Table 2 shows the total length for the males and females during the investigation period. This study's findings indicate a significant difference in size between the males and females. Specifically, the recorded measurements for the males varied from 23 to 50 cm, while those for the females varied from 19 to 38 cm. The maximum number of males was recorded in the 27–30 and 31–34 cm length categories, whereas female samples were more abundant in the 23–26 and 27–30 cm length categories.

3.2. Histological findings of the ovaries

The ovaries of *E. summana* were subjected to histological investigation, revealing a distinct developmental sequence that can be classified into five stages (Fig. 1).

Stage I (immature stage): The immature ovaries are characterized by abundant primary growth oocytes.

Stage II (mature stage): Throughout the present stage, three types of oocytes can be distinguished, namely, primary vitellogenic oocytes, cortical alveolar oocytes, and secondary vitellogenic oocytes.

Stage III (spawning capable stage): Dominance of germinal vesicle breakdown and germinal vesicle migration, in addition to the postovulatory follicle complex, was found.

Stage IV (regressing stage): Distinguished via the prevalence of atresia vitellogenic and/or hydrated oocytes and germinal vesicle breakdown and postovulatory follicle complex.

Stage V (regenerating stage): The presence of primary growth oocytes, besides the existence of atresia vitellogenic and/or hydrated oocytes and postovulatory follicle complex.

The morphometric measures of eggs (Fig. 1) showed that the diameter of the yolk eggs ranged from 201 μm in primary vitellogenic oocytes, 361 μm in secondary vitellogenic oocytes, and 309 μm in tertiary vitellogenic oocytes. This gradient indicates the eggs' growth and the hydrated oocytes' diameter ranged from 370 to 455 μm.

The female maturation stages of *E. summana,* according to the monthly collected samples throughout the investigation period from November 2020 to October 2021, are shown in Table 3. The highest number of fish representing stages one and five were reported in October (14 and 13, respectively). In November and December, the fish specimens in the fifth stage accounted for the second largest number during the study period. Maturation stages 2, 3, and 4 were recorded irregularly throughout the study period.

3.3. Histological findings of the testes

The histological analysis of the testes of *E. summana* indicated that the maturation process can be categorized into the subsequent five stages (Fig. 2).

Stage I (immature stage): The seminiferous tubules are loaded with spermatogonia (SG). Spermatogonia are undifferentiated male germ cells. Spermatogonia undergo spermatogenesis to form mature spermatozoa in the seminiferous tubules of the testis.

Stage II (mature stage): The appearance of spermatocytes (SC) and spermatids (ST)are noted. The spermatocytes derive from the

Table 2
Total length (TL) of the collected *E. summana* samples during the study period (November 2020 to October 2021).

	Samples collected			TL (cm)			*P*-value
Month	M	F	T	M	F	T	
Nov	2	23	1	47.30 ± 0.98	26.94 ± 3.93	36.1	0.35
Dec	5	14	1	41.10 ± 3.22	27.45 ± 3.82	37.5	0.23
Jan	6	8	3	38.40 ± 8.28	31.32 ± 4.26	34.23 ± 10.42	0.008
Feb	8	15	0	35.07 ± 5.93	28.58 ± 3.80	–	0.045
Mar	9	11	0	39.93 ± 9.82	24.51 ± 3.13	–	0.000
Apr	4	4	0	38.37 ± 3.35	31.90 ± 1.15	–	0.044
May	2	13	0	39.80 ± 13.01	25.24 ± 1.86	–	0.000
Jun	5	7	2	28.16 ± 3.12	31.58 ± 2.48	30.5 ± 2.53	0.685
Jul	4	9	0	37.10 ± 4.68	26.77 ± 5.80	–	0.592
Aug	9	6	0	37.12 ± 10.28	25.96 ± 3.75	–	0.006
Sep	7	9	0	35.02 ± 2.96	29.94 ± 2.53	–	0.881
Oct	3	27	0	36.60 ± 7.10	26.57 ± 4.20	–	0.505

M: male, F: female, T: transitional. The data of TL are presented as mean ± SD. The data of transitional fish in November and December represented the length of the fish detected (only one fish). The significant difference between males and females was detected at $P < 0.05$.

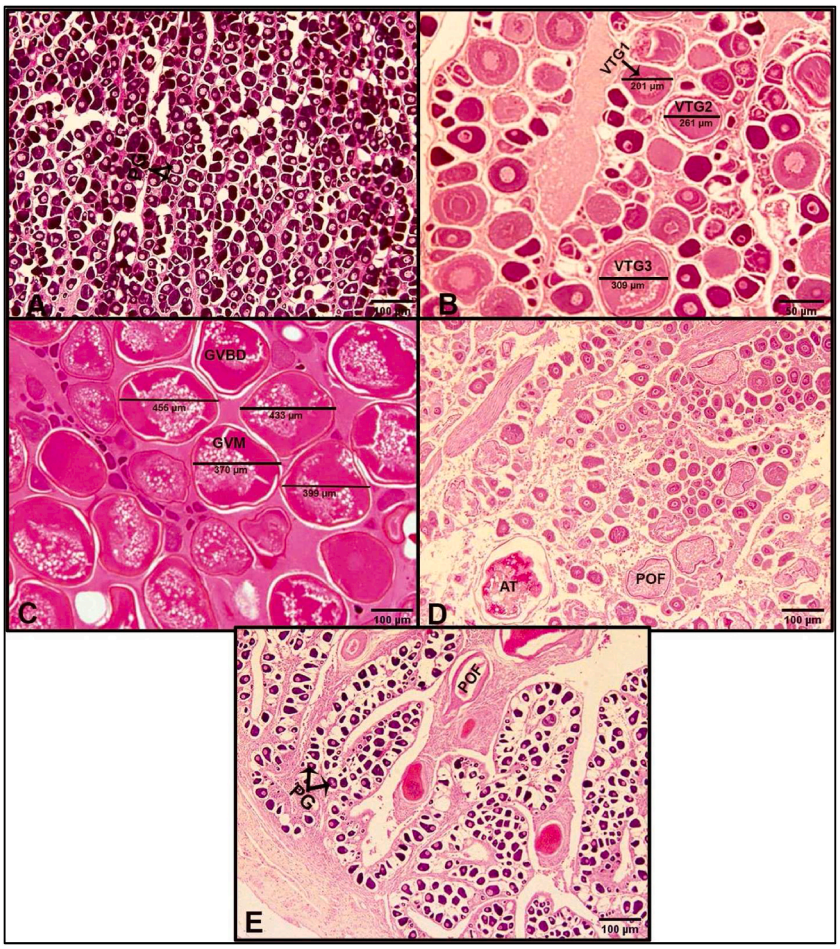

Fig. 1. Photomicrographs of *E. summana* ovary showing maturation stages of female (A) Immature stage, (B) mature stage (H&E−100X), (C) spawning capable (H&E−100X), (D) regressing stage (H&E−200X), (E) regenerating stage(H&E−100X). (PG) primary growth oocytes, (CA) cortical alveolar oocytes, (VTG1) primary vitellogenic oocytes, (VTG2) secondary vitellogenic, (VTG3) tertiary vitellogenic, (GVM) germinal vesicle migration, (GVBD) germinal vesicle breakdown, (At) atresia vitellogenic and/or hydrated oocytes, (POF) postovulatory follicle complex. (H&E−200X).

Table 3
Maturation stages of female *E. summana* detected throughout the study period (November 2020 to October 2021).

Months	Samples (n)	Stage 1	Stage 2	Stage 3	Stage 4	Stage 5
Nov	23	3	8	2	1	9
Dec	14	5	0	0	1	8
Jan	8	1	3	0	2	2
Feb	15	2	4	1	2	6
Mar	11	6	2	0	0	3
Apr	4	0	2	2	0	0
May	13	4	3	5	0	1
Jun	7	0	3	1	1	2
Jul	9	5	1	1	0	2
Aug	6	0	5	1	0	0
Sep	9	0	2	0	0	7
Oct	27	14	0	0	0	13

spermatogonia by a mitotic division through the spermatogenesis process. The spermatids are the haploid male games that results from a meiotic division of the spermatocytes.

Stage III (spawning capable stage): The seminiferous tubules are rich in spermatozoa (SZ). The spermatids develop into mature male gametes (spermatozoa) which also known as sperms.

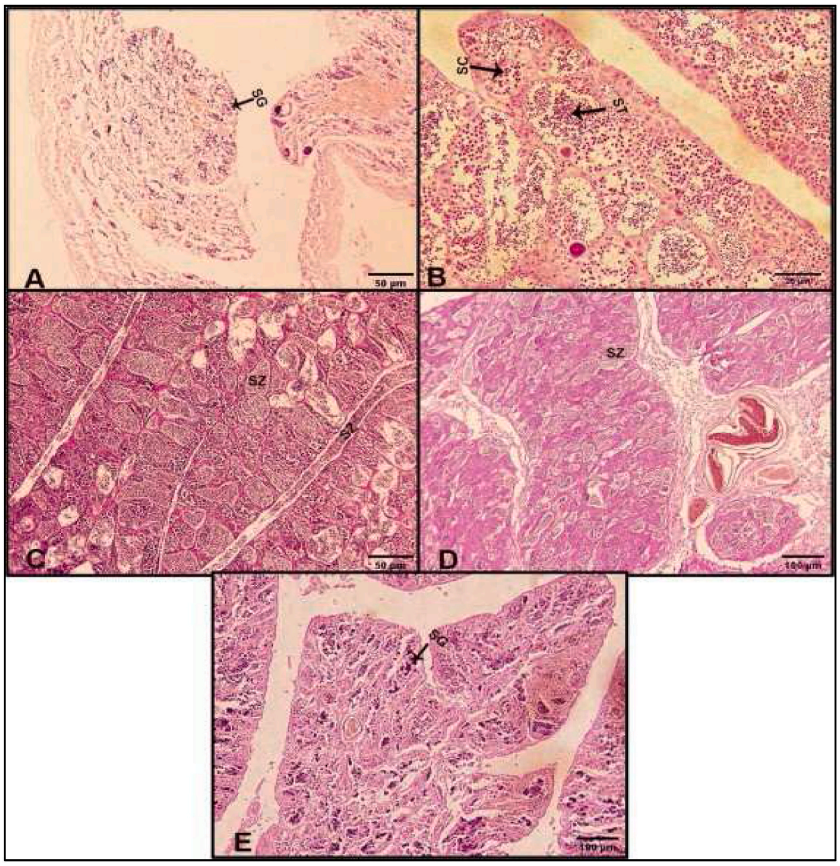

Fig. 2. Photomicrographs of *E. summana* testes showing maturation stages of male. (A) Immature (H&E−200X): the seminiferous tubules are loaded with spermatogonia (SG). (B) mature (H&E−400X): the appearance of spermatocytes (SC) and spermatids (ST). (C) spawning capable (H&E−200X): the seminiferous tubules are rich in spermatozoa (SZ). (D) regressing stage (H&E−100X): characterized by a few spermatozoa. (E) regenerating stage (H&E−100X): the prevalence of primary spermatogonia can be observed.

Stage IV (regressing stage): This stage is characterized by a few spermatozoa.

Stage V (regenerating stage): The prevalence of primary spermatogonia can be observed.

The male maturation stages of *E. summana,* according to the monthly collected samples throughout the investigation period from November 2020 to October 2021, are shown in Table 4. The highest number of fish representing stage two was reported in January and August.

3.4. Transitional stages

The histological sections analyzed provided clear evidence of sex change in *E. summana* (Fig. 3). The coexistence of testicular tissues

Table 4
Maturation stages of male *E. summana* detected throughout the study period (November 2020 to October 2021).

Month	Samples (n)	Stage 1	Stage 2	Stage 3	Stage 4	Stage 5
Nov	2	0	2	0	0	0
Dec	5	1	4	0	0	0
Jan	6	0	6	0	0	0
Feb	8	0	2	4	2	0
Mar	9	0	2	6	1	0
Apr	4	0	0	4	0	0
May	2	0	1	1	0	0
Jun	5	0	4	1	0	0
Jul	4	0	0	2	0	2
Aug	9	0	6	3	0	0
Sep	7	2	4	0	0	1
Oct	3	0	3	0	0	0

and ovaries within the same gonads, coupled with the observation of male sex cells in the ovaries, provides compelling evidence supporting the notion that *E. summana* undergoes a developmental process wherein it initially matures as a female and subsequently changes to a male state. This phenomenon is commonly referred to as protogynous hermaphroditism. The histological investigation revealed more proof of sex alteration, as indicated by the identification of a central cavity (the residual oviduct) within the testes, inward torsion of the ovarian wall leading to the formation of the seminal canal, and the existence of brown masses indicating remnants of ovaries in the male gonads.

3.5. Maturation index and ovarian maturation rate

The results show that the MI values fluctuated throughout the study, and the mean female MI was 93.26 %. The greatest female MI value was recorded in April, June, August, and September (100 %), while the second greatest female MI was recorded in January, November, and February (87 %). In contrast, the lowest female MI occurred in March and July (45 %) (Fig. 4-A). In males, the MI was 100 % in all months except December (80 %), September (71.42 %), and October (0 %). The greatest OMR was documented in September (77.78 %); after that, the OMR decreased and fluctuated (48.15 %–64.29 %) from October to February. Furthermore, the OMR showed a decline and fluctuation (33.33 %–57.14 %) during the period from April to July, whereas the lowest OMR was recorded in August (16.67 %) (Fig. 4-B).

The OMR and MI values corresponding to the various length categories are shown in Fig. 5. The findings indicate that the OMR was 0 % in the smallest length category (19–22 cm) and in the LCs from 39 to 50 cm. The highest OMR (77.67 %) was found in the 27–30 cm length category (Fig. 5-A).

The highest female MI (100 %) was recorded in the LCs 23–38 cm. The female MI was 4.5 % in the LC (19–22 cm) and 0 % in the LC (39–50 cm). In the male, the highest MI (100 %) was observed in the LCs 23–26 cm, 31–34 cm, and 43–50 cm. The MI was 0 % in the LC 19–22 cm (Fig. 5-B).

3.6. Length at sexual maturity and sex change

For female *E. summana* grouper, the length at sexual maturity (L50) was established as the body length at which 50 % of the fish were mature. This determination was based on the percentage frequency of all ovaries that showed signs of current (Existence of vitellogenic oocytes and hydration oocytes) or past (Existence of post-ovulatory follicles) spawning activity (from maturity stage 2–5). The length at first sexual maturation for females (L50) was 24.8 cm (Fig. 6-A). For male *E. summana* grouper, the length at sex change (P50) was established as the body length at which 50 % of the fish were sex changed. This determination was based on the percentage frequency of all mature male specimens (from maturity stage 2–5). The length at first sex change for males (P50) was 31.5 cm (Fig. 6-B).

3.7. Gonadosomatic index

Fig. 7 displays the monthly mean GSI values for both the males and females. The male GSI showed a gradual increase from November (0.02 %) to April (0.28 %), then it decreased and fluctuated from May to October. A significant reduction and variability in female GSI was observed from November to March. A distinct increase was found in the female GSI in April and June (0.85 %), whereas the highest value was found in May (0.91 %%). However, the female GSI values declined again in July (0.32 %) and increased in August (0.85 %) before declining again in September and October.

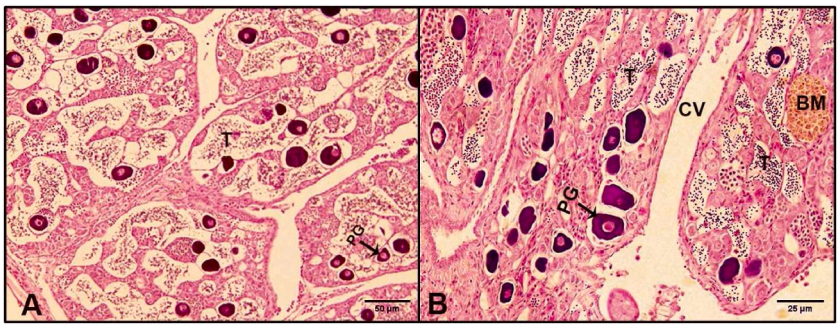

Fig. 3. Photomicrographs of *E. summana* ovarian tissue revealing sex change to testicular view, (A) presence of testicular foci (T) among different ovarian stages and primary growth oocytes (PG) within the same gonads, coupled with the observation of male sex cells in the ovaries, (H&E−200X). (B) developed testicular foci (T) in ovarian tissue, primary growth oocytes (PG), central cavity (CV) (remaining egg channel) inside the testicle, brown masses (BM) (the porous layer of the remaining ovaries), (H&E−400X).

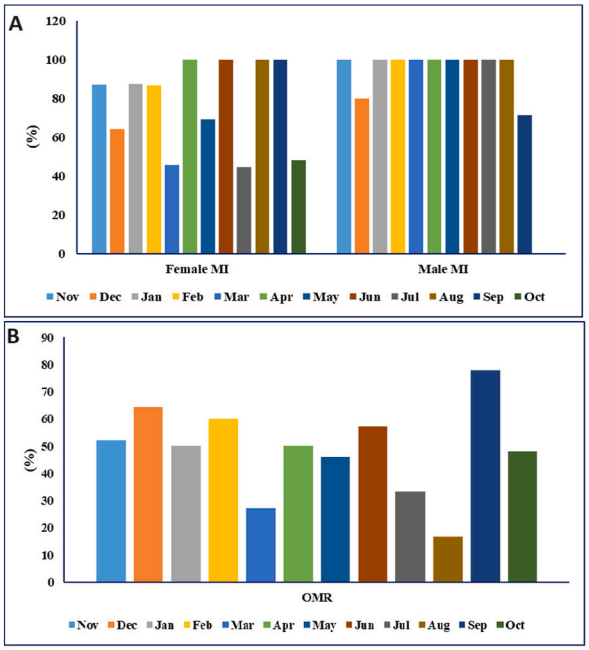

Fig. 4. (A) Maturation index (MI) and **(B)** ovarian maturation rate (OMR) for the *E. summana* samples collected over the study period (November 2020 to October 2021).

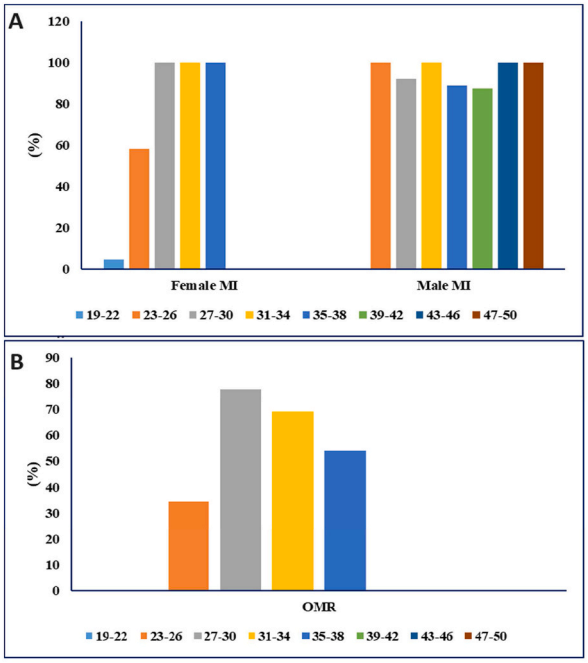

Fig. 5. (A) Maturation index (MI) and (B) ovarian maturation rate (OMR) for the *E. summana* samples in the different length categories (LCs). The MI is zero in males in the LCs from 19 to 22, while in females in the LCs 39–50 cm. The OMR in the LCs from 19 to 22 and 39–54 is zero.

3.8. Spawning season

The results of the monthly GSI values for males and females (Fig. 7) and recording the different maturation stages of the gonads during the months of the year (Table 3) indicated that *E. summana* has a protracted spawning season. However, the primary spawning season was found to occur in April and May, when the greatest female GSI was recorded.

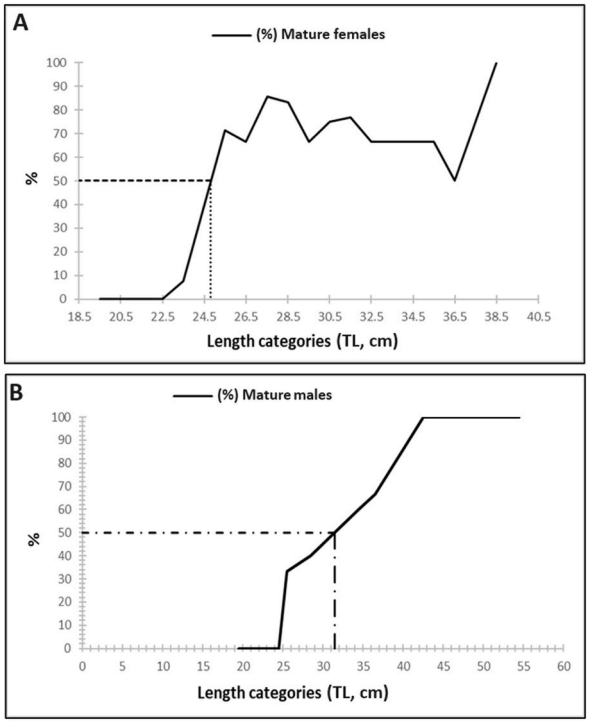

Fig. 6. (A) The total length (TL) of the female at first sexual maturation (L_{50}): At which 50 % of the female fish samples were distinguished as mature fishes. **(B)** The total length (TL) at sex change (P_{50}): At which 50 % of the females changed to male.

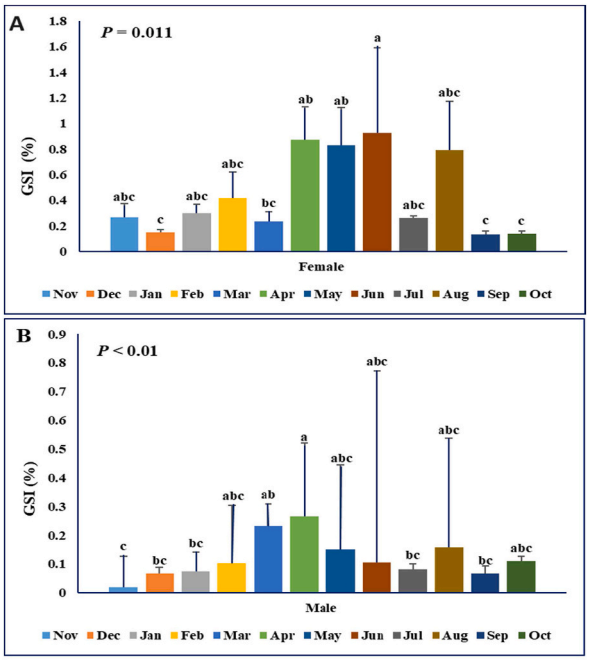

Fig. 7. Female (A) and male (B) gonadosomatic index (GSI) values for the *E. summana* samples collected over the study period (November 2020 to October 2021). (a,b,c) means carrying different letters are significantly differed ($P < 0.05$). Tukey's multiple range test was used to detect the significant difference between the means. The number of samples for each month is presented in Table 2.

4. Discussion

Even though groupers are biologically diverse and commercially important in fisheries worldwide, a comprehensive understanding of the ecology and biology of all grouper categories is limited [27–29]. Fishery management requires the study of the life span, reproduction, mortality, and other life history characteristics [30,31]. Reproduction is the most important biological activity of fish that must be studied for fishery management and maintenance of stock sustainability, as it can provide a future vision for fish production and add new births to the fish community [32–34].

The sex-change process is curiously changeable and arises over a wide age and size range, indicating that it is subject to social control [29]. In the current study, the reproductive biology of *E. summana* was described in terms of their sex ratio, maturation stages, size at sexual maturity, and spawning season. The results of the monthly sex ratios showed that females exhibited dominance during the investigation period, and females outnumbered males within the small length categories but were missing in the large categories.

Many previous investigations conducted on different grouper species recorded dominance of females with varying sex ratios, such as Vicente [35], who reported a male-to-female sex ratio of 1:2.9 for *Epinephelus areolatus*, areolate grouper, in the Saudi Coast of the Arabian Gulf. Frisch, Cameron [29] reported that sex ratios of Plectropomus are usually female-biased (up to 14F:1M); however, some (typically unused) populations may be male-biased (up to 3M:1F). Osman, El-Ganainy [36] recorded 1:6.8 male-to-female sex ratio for *E. areolatus* in the Gulf of Suez. Brulé, Renan [37] mentioned a male-to-female sex ratio of 1:4 for the black grouper, *Mycteroperca bonaci*, in the southern Gulf of Mexico [38]. concluded a 1:3.5 male-to-female ratio for the dusky grouper, *Epinephelus marginatus*, in the southern Mediterranean. Mackie [39] indicated that the male-to-female sex ratio for the half-moon grouper, *Epinephelus rivulatus*, at the Ningaloo Reef, Western Australia, was 1:5.5. *E. summana*, like other grouper fishes, is a protogynous hermaphrodite fish where they mature first as females and then undergo a transitional stage and finally become males. This phenomenon may explain the tendency of their sex ratios towards females, as most of them are caught before they transform from female to male.

This study's findings indicate a significant difference in size between the males and females. Specifically, the recorded measurements for the males varied from 23 to 54 cm, while those for the females varied from 19 to 38 cm. The maximum number of males was recorded in the 27–30 and 31–34 cm length categories, whereas female samples were more abundant in the 23–26 and 27–30 cm length categories. Condini, García-Charton [40] recorded that the body length (L_{50}) of female *E. marginatus* extended from 62.2 cm TL in South Africa to 39.1 cm TL in Turkey at first maturation. This parameter is critical for maintenance and management targets and can vary between populations from different geographical areas under various environmental conditions and fishing pressures [41]. Abdul Kadir, Piah [42] documented that the TL for *E. areolatus* from Kuala Dungun and Pulau Kambing was 35.70 cm and 32.60 cm, respectively. Meanwhile, the TL for E. sexfasciatus from Kuala Dungun and Pulau Kambing was 22.80 cm and 24.00 cm, respectively.

Fish growth and gonad development are closely related. The GSI has been used to elucidate the degree of ovarian maturity and indicate the phase and readiness of the ovary for spawning [8]. The gonad size increases during the ripe condition and spawning season, so the GSI increases until the gonads ripen and then declines sharply after spawning [43]. Therefore, the GSI is widely employed as the primary utilized index for quantifying the spawning period [44]. According to Mackie [39], the spawning season is determined based on the combination of data of the GSI, macroscopic observation of maturity stages, and microscopic analysis of the gonads. The current study used this protocol, and the results indicated that *E. summana* has an expanded spawning season. However, the primary spawning season was found in April and May, when the greatest female GSI was recorded.

This result is consistent with the outcomes outlined within other investigations on different grouper fish species because it showed that fish exhibit year-round spawning, but with more pronounced spawning during certain periods that vary based on the species of grouper and the environment. Mahmoud [45] studied the areolate grouper, *Epinephelus areolatus*, across the Halaieb and Shalatien region of the Red Sea and reported that the spawning of this grouper species happens across May and June. Vicente [35] reported that *Epinephelus areolatus,* found across the Saudi Coast of the Arabian Gulf, exhibits year-round spawning; however, this is more considerable from January to June. Nor, Rumeaida [43] reported that two grouper species, areolate grouper *Epinephelus areolatus* and six-barred grouper *Epinephelus sexfasciatus*, in the Terengganu waters, Malaysia, exhibit an extended spawning season from January to May. Meetei, Haq [46] reported that *Epinephelus malabaricus* from the Mandapam Coastal waters, Southeast coast of India, exhibits spawning from January to April. Chan and Sadovy [47] reported that the spawning season for the chocolate hind grouper, *Ceph3alopholis boenak*, in Hong Kong is from April to October. In dusky groupers, *Epinephelus marginatus,* the spawning season was from late spring to summer [48,49], while peak spawning in the populations from the southern hemisphere was found to be from November to January [49,50], whereas, in the northern hemisphere, the peaks appeared from July to September [48,51]. The spawning season of *E. areolatus* and *E. sexfasciatus* extends from January to May [42].

The findings obtained from the histological examination of gonads revealed the maturation process of *E. summana* as protogynous hermaphroditism, indicating that individuals of this grouper fish species initially mature as females and subsequently undergo a sex alteration to become males. The aforementioned findings are consistent with those of various earlier investigations on different grouper fish species, such as Ohta and Ebisawa [52] for the white-streaked grouper, *Epinephelus ongus*; Hwang, Min [53] for the red spotted grouper, *Epinephelus akaara*; DeMartini, Everson [24] for the Hawaiian grouper, *Hyporthodus quernus*; Özen and Balci [44] for the dusky grouper E*pinephelus guaza*; Brulé, Renan [37] for the black grouper *Mycteroperca bonaci;* Chan and Sadovy [47] for the chocolate hind grouper, *Cephalopholis boenak*.

Microscopic analysis of the gonads revealed the presence of the following five sexual maturity stages for both males and females of *E. summana*: Stage I (immature), stage II (developing stage), stage III (spawning capable), stage IV (regressing), and stage V (regenerating stage). These outcomes are aligned with the findings of Nor, Rumeaida [43], who reported the maturation stages of the areolate grouper *Epinephelus areolatus*. They classified the maturation process of males into four stages, where stages I and II were considered the immature and developing stages, and stages III and IV were the ripe and regressed stages but with the capacity of spawning. For

female *E. areolatus*, Nor, Rumeaida [43] reported the following six maturation stages: Stages I and II were considered the immature and developing stages; stages III, IV, and V were considered the mature and spawning stages; stage VI was classified as a regressed stage.

Similar results have been reported in many previous studies, such as by Özen and Balci [44], who reported the following seven stages of maturation for the female dusky grouper E*pinephelus guaza*: Immature female stage, resting female stage, developing stage, maturing stage, mature stage, ripe and partially running, and post-spawning stage. Meanwhile, they classified the maturation of males into the following four stages: Immature male stage, developing stage, mature stage, and ripened stage. Cushion, Cook [16] reported the maturation cycle of three grouper species, *Epinephelus striatus* (Nassau grouper), *E. guttatus* (red hind), and *Mycteroperca venenosa* (yellowfin grouper), from the Bahamas as comprising six stages for females (immature, developing, mature, spawning, spent, and regressed) and only four for males (immature, developing, spawning capable, and regressed).

5. Conclusion

Reproductive biological studies are very important for fishery management and conservation. This study aimed to investigate the reproductive strategy of the Summan grouper, *Epinephelus summana,* in terms of the sex ratio, maturation stages, size at sexual maturity, and spawning season. The results indicated that *E. summana,* like other grouper fishes, is a protogynous hermaphrodite fish, where it initially matures as a female and subsequently changes to a male. Therefore, the sex ratio showed a tendency toward females, while males reached larger sizes than females. The maturation process passes through five stages that can be described as the immature, developing, spawning capable, regressing, and regenerating stages in both males and females. The spawning season extends throughout the year; however, April and May are the main spawning seasons.

Ethical approval

All the protocols and procedures in this research were approved and permitted by the Research Ethics Committee (REC) at the King Saud University, Saudi Arabia.

Consent for publication

Not applicable.

Data availability statement

The datasets generated or analyzed during the current study are not publicly available but are available from the corresponding author upon reasonable request.

CRediT authorship contribution statement

Faozi S. Shamlol: Writing – original draft, Software, Methodology, Formal analysis, Data curation, Conceptualization. **Elsayed M. Younis:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Funding acquisition, Conceptualization. **Mohamed H. Gabr:** Writing – review & editing, Validation, Supervision, Methodology, Formal analysis, Conceptualization. **Shimaa A. Amer:** Writing – review & editing, Writing – original draft, Formal analysis, Data curation. **Simon J. Davies:** Writing – review & editing, Visualization. **Doaa M. Elnagar:** Writing – review & editing, Software, Methodology, Data curation. **Khalid E. Ibrahim:** Writing – review & editing, Methodology, Data curation. **Saad M. Alsaiad:** Formal analysis.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This work was supported by the Researchers Supporting Project (RSP2024R36), King Saud University, Riyadh, Saudi Arabia.

References

[1] F. Akhter, et al., Reproductive seasonality and the gonadal maturation of silver pomfret Pampus argenteus in the Bay of Bengal, Bangladesh, J. Mar. Biol. Assoc. U. K. 100 (7) (2020) 1155–1161.
[2] M.A. Islam, et al., Comprehensive study on the reproductive biology of black pomfret (Parastromateus Niger) in the Bay of Bengal, Bangladesh, Regional Studies in Marine Science 41 (2021) 101556.
[3] M. van de Kerk, et al., Carnivora population dynamics are as slow and as fast as those of other mammals: implications for their conservation, PLoS One 8 (8) (2013) e70354.
[4] R. Mann, Growth and production, in: Cyprinid Fishes: Systematics, Biology and Exploitation, Springer, 1991, pp. 456–482.
[5] M.L. Berumen, et al., The status of coral reef ecology research in the Red Sea, Coral Reefs 32 (2013) 737–748.
[6] D. Pauly, et al., Towards sustainability in world fisheries, Nature 418 (6898) (2002) 689–695.

[7] A. Vincent, Y. Sadovy, Reproductive Ecology in the Conservation and Management of Fishes, Behavioural Ecology and Conservation Biology Tim Caro, 1998.
[8] A.C. Rao, L. Krishnan, Studies on the reproductive biology of the female spiny cheek grouper, Epinephelus diacanthus (Valenciennes, 1828), Indian J. Fish. 56 (2) (2009) 87–94.
[9] A.V. Morris, C.M. Roberts, J.P. Hawkins, The Threatened Status of Groupers (Epinephelinae), vol. 9, Biodiversity & Conservation, 2000, pp. 919–942.
[10] F.C. Coleman, S.L. Williams, Overexploiting marine ecosystem engineers: potential consequences for biodiversity, Trends Ecol. Evol. 17 (1) (2002) 40–44.
[11] R.M. Starr, et al., Spatial dynamics of the Nassau grouper Epinephelus striatus in a Caribbean atoll, Mar. Ecol. Prog. Ser. 343 (2007) 239–249.
[12] E. Abd-Allah, A. El-Ganainy, A. Osman, *Age and growth of the areolate grouper Epinephelus areolatus from the Gulf of Suez.* American, J. Life Sci. 3 (2015) 7–12.
[13] F.S. Mehanna, et al., Age and growth, mortality and exploitation ratio of Epinephelus summana (Forsskål, 1775) and Cephalopholis argus (Schneider, 1801) from the Egyptian Red Sea coast, Hurghada fishing area, Egyptian Journal of Aquatic Biology and Fisheries 23 (4) (2019) 65–75.
[14] H. Murua, F. Saborido-Rey, Female reproductive strategies of marine fish species of the North Atlantic (2003).
[15] M.J. Morgan, Integrating reproductive biology into scientific advice for fisheries management, J. Northwest Atl. Fish. Sci. 41 (2008).
[16] N. Cushion, et al., Reproductive classification and spawning seasonality of Epinephelus striatus (Nassau grouper), E. guttatus (red hind) and Mycteroperca venenosa (yellowfin grouper) from the Bahamas, in: Proceedings of the 11th International Coral Reef Symposium, WorldFish Center ReefBase Project Penang, 2008.
[17] Y.S. De Mitcheson, M. Liu, Functional hermaphroditism in teleosts, Fish Fish. 9 (1) (2008) 1–43.
[18] Y. Sadovy, D.Y. Shapiro, Criteria for the diagnosis of hermaphroditism in fishes, Copeia (1987) 136–156.
[19] W.L. Smith, M.T. Craig, Casting the percomorph net widely: the importance of broad taxonomic sampling in the search for the placement of serranid and percid fishes, Copeia 2007 (1) (2007) 35–55.
[20] N.J. Brown-Peterson, et al., A standardized terminology for describing reproductive development in fishes, Marine and Coastal Fisheries 3 (1) (2011) 52–70.
[21] A. Ebisawa, Reproductive and sexual characteristics in five Lethrinus species in waters off the Ryukyu Islands, Ichthyol. Res. 53 (3) (2006) 269–280.
[22] B. Erisman, M. Craig, P. Hastings, Reproductive biology of the Panama graysby Cephalopholis panamensis (Teleostei: epinephelidae), J. Fish. Biol. 76 (6) (2010) 1312–1328.
[23] S.J. Newman, Stock Assessment of the Outer-Shelf Species in the Kimberley Region of Tropical Western Australia, Fisheries Western Australia, 2001.
[24] E.E. DeMartini, A.R. Everson, R.S. Nichols, Estimates of body sizes at maturation and at sex change, and the spawning seasonality and sex ratio of the endemic Hawaiian grouper (Hyporthodus quernus, F. Epinephelidae) (2011).
[25] E. Nolan, et al., Life-history strategies of the rock hind grouper Epinephelus adscensionis at Ascension Island, J. Fish. Biol. 91 (6) (2017) 1549–1568.
[26] D.K. Boddington, et al., Age, growth and reproductive life-history characteristics infer a high population productivity for the sustainably fished protogynous hermaphroditic yellowspotted rockcod (Epinephelus areolatus) in north-western Australia, J. Fish. Biol. 99 (6) (2021) 1869–1886.
[27] S. Kandula, K.V. Shrikanya, V. Iswarya Deepti, Species diversity and some aspects of reproductive biology and life history of groupers (Pisces: serranidae: Epinephelinae) off the central eastern coast of India, Mar. Biol. Res. 11 (1) (2015) 18–33.
[28] Y. Sadovy de Mitcheson, et al., Fishing groupers towards extinction: a global assessment of threats and extinction risks in a billion dollar fishery, Fish Fish. 14 (2) (2013) 119–136.
[29] A.J. Frisch, et al., Key aspects of the biology, fisheries and management of Coral grouper, Rev. Fish Biol. Fish. 26 (2016) 303–325.
[30] E.M. Younis, et al., Analysis of reproductive biology and spawning season of the pink ear emperor Lethrinus lentjan, from marine ecosystem, Zool. 37 (2020).
[31] H.S. Al-Khalaifah, et al., Optimizing the growth, health, reproductive performance, and gonadal histology of broodstock fantail goldfish (Carassius auratus, L.) by dietary cacao bean meal, Animals 10 (10) (2020) 1808.
[32] M. Osman, Hanan, et al., Reproductive biology of the two commercially important grouper species Epinephelus summana and E. polyphekadion in the Egyptian coast of the Red Sea, Egyptian Journal of Aquatic Biology and Fisheries 25 (1) (2021) 665–679.
[33] M. Osman, Hanan, et al., Characterization of the reproductive biology of the sparid fish: Rhabdosargus haffara in Suez bay, Red Sea, Egyptian Journal of Aquatic Biology and Fisheries 24 (1) (2020) 83–90.
[34] A.A. Khalil, et al., Effects of dietary Hyphaene thebaica powder on reproductive performance, physiological parameters, growth, and disease resistance in Clarias gariepinus, Aquacult. Int. (2024) 1–17.
[35] J.A. Vicente, Reproductive aspects of areolate grouper (Epinephelus areolatus, Forsskal, 1775) from the Saudi coast of Arabian Gulf, Journal of Natural and Allied Sciences 4 (1) (2020) 40–51.
[36] A.G. Osman, A. El-Ganainy, E. Abd-Allah, Some reproductive aspects of the areolate grouper, Epinephelus areolotus from the Gulf of Suez, The Egyptian Journal of Aquatic Research 44 (1) (2018) 51–56.
[37] T. Brulé, et al., Reproduction in the protogynous black grouper Mexico (Mycteroperca bonaci (Poey)) from the southern Gulf of Mexico (2003).
[38] G. Marino, et al., Reproduction in the dusky grouper from the southern Mediterranean, J. Fish. Biol. 58 (4) (2001) 909–927.
[39] M. Mackie, Reproductive biology of the halfmoon grouper, Epinephelus rivulatus, at Ningaloo Reef, Western Australia, Environ. Biol. Fish. 57 (2000) 363–376.
[40] M.V. Condini, J.A. García-Charton, A.M. Garcia, A review of the biology, ecology, behavior and conservation status of the dusky grouper, Epinephelus marginatus (Lowe 1834), Rev. Fish Biol. Fish. 28 (2018) 301–330.
[41] J.A. Gulland, Fish Stock Assessment: a Manual of Basic Methods, vol. 1, 1983.
[42] N.H. Abdul Kadir, et al., Reproductive aspects of areolate grouper, Epinephelus areolatus and six-barred grouper, E. sexfasciatus from Terengganu waters, Malaysia, Aquaculture, Aquarium, Conservation & Legislation 9 (6) (2016) 1372–1379.
[43] H. Nor, Abdul Kadir, et al., Reproductive aspects of areolate grouper, Epinephelus areolatus and six-barred grouper, E. sexfasciatus from Terengganu waters, Malaysia (2016).
[44] M.R. Özen, B.A. Balci, Histological study on reproductive pattern and sex reversal of dusky grouper Epinephelus guaza in natural environment of Antalya Bay of Mediterranean in Turkey, Turk. J. Fish. Aquat. Sci. 12 (1) (2012).
[45] H.H. Mahmoud, Gonadal maturation and histological observations of Epinephelus areolatus and Lethrinus nebulosus in Halaieb/Shalatien area “Red Sea”, Egypt, Global Vet. 3 (5) (2009) 414–423.
[46] K.B. Meetei, et al., Maturation and spawning habits in Epinephelus malabaricus (Bloch and Scheneider, 1801) from Mandapam coastal waters, southeast coast of India, Solid State Technol. 63 (2020) 8127–8147.
[47] T.T. Chan, Y. Sadovy, Reproductive biology, age and growth in the chocolate hind, Cephalopholis boenak (Bloch, 1790), in Hong Kong, Mar. Freshw. Res. 53 (4) (2002) 791–803.
[48] O. Reñones, et al., Reproductive pattern of an exploited dusky grouper Epinephelus marginatus (Lowe 1834)(Pisces: Serranidae) population in the western Mediterranean (2010).
[49] M.V. Condini, et al., Reproductive biology of the dusky grouper (Epinephelus marginatus) at the southern limit of its distribution in the south-western Atlantic, Mar. Freshw. Res. 65 (2) (2013) 142–152.
[50] L.C. Gerhardinger, et al., Collaborative approach in the study of the reproductive biology of the dusky grouper Epinephelus marginatus (Lowe, 1834) (Perciformes: Serranidae), Acta Sci. Biol Sci. 28 (3) (2006) 219–226.
[51] M.R. Özen, B.A. Balcı, Determinations of reproductive characteristics of dusky grouper,(Epinephelus guaza, Linnaeus, 1758) in Antalya bay of eastern Mediterranean, Pak. Vet. J. 31 (3) (2011) 215–218.
[52] I. Ohta, A. Ebisawa, Age-based demography and sexual pattern of the white-streaked grouper, Epinephelus ongus in Okinawa, Environ. Biol. Fish. 99 (10) (2016) 741–751.
[53] I.J. Hwang, B.H. Min, H.J. Baek, Mature and Bi-sexual phase gonad occurrence in cultured red spotted grouper, Epinephelus akaara, Development & Reproduction 24 (3) (2020) 225.