

# **RESEARCH ARTICLE**

# Analysis of reproductive biology and spawning season of the pink ear emperor *Lethrinus lentjan*, from marine ecosystem

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ABSTRACT. A total of 593 samples of *Lethrinus lentjan* (Lacepede, 1802) were collected from the Red Sea, Jeddah, Saudi Arabia, to study their productive biology and spawning season of the local population. Sampling was carried out on a monthly basis for a period of one year. The monthly sex ratios indicated that females were dominant throughout the study period, with an overall male:female sex ratio of 1:7.98, although males were larger than females. The highest monthly performance maturation index (PMI), as well as the male and female gonadosomatic index (GSI) and ovarian maturation rate (OMR) were observed in February and March. Histological examination of the gonads confirmed the process of sexual transformation in this fish species, wherein individuals mature first as female, and then change sex to male (protogynous hermaphroditism). Histological sections also showed that the sexual maturation of males of *L. lenjtan* comprised three main stages, while the sexual development of females could be classified into four main stages. Extended spawning in the form of batches released during different months throughout the year were recorded for this fish species, with the main spawning season in February and March, and an additional, shorter spawning season in September.

KEY WORDS. Lethrinus lentjan, Red sea, reproduction, spawning.

# **INTRODUCTION**

The Lethrinidae (emperors) are a family of about 39 species of fish, abundant in tropical and subtropical marine areas all over the world (Carpenter and Allen 1989). The pink ear emperor, *Lethrinus lentjan* (Lacepede, 1802) is considered a delicacy in many states in the Arabian Gulf, India and other South East Asian countries (Anil et al. 2019). The species is widely distributed in the Indo-west Pacific, Red Sea, Arabian Gulf, East Africa to Rykus and Tonga.

Previous studies have reported a wide diversity of life histories among different fish species and locations (Carpenter and Niem 2001, Kulmiye et al. 2002, Hamilton 2005). Knowledge of the life history of economically important fish is fundamental in the management of fisheries (Tracey et al. 2006). For example, fishery managers need to know the size at first maturity and the onset and duration of the spawning season (Trindade-Santos and Freire 2015) and other important information such as growth, reproductive strategy, size at maturity, spawning season, aging, estimated mortality population dynamics.

Previous studies of the reproductive biology of emperors have reported that the majority of these fish exhibit protogynous

hermaphroditism, in which they change their sex from female to male (Carpenter and Allen 1989, Ebisawa 1990, Wassef and Bawazeer 1992, Bean et al. 2003, Grandcourt et al. 2006, Sadovy and Liu 2008). Some studies concluded that both male and female tissues were present in the gonads of juvenile fish, since individuals matured first as females and then changed sex later to become males (Ebisawa 1997). Protracted spawning of L. lentjan was reported in Australian waters by Currey et al. 2013. Previous works focusing on maturation under captive conditions of L. lentjan have been described by Anil et al. (2019). Studying the reproductive biology of economically important, commercially exploited fish species is vital because this aspect of their life history impacts the effectiveness of fisheries management (Grandcourt et al. 2010). The phenotypic flexibility of the morphology of the gonads is an adaptation to environmental changes (Galvão et al. 2016). Further, the histological stages of oocyte and spermatogenesis development together with the macroscopic aspects of the gonads are used to characterize the reproductive phases (Brown-Peterson et al. 2011, Francisco et al. 2019).

Although lethrinids are among the most important components of many fisheries in various coastal countries (Mehanna

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et al. 2012) and are widespread in the coastal waters of Saudi Arabia, where considerable amounts of them are caught in fisheries in both the Red Sea and Arabian Gulf (Essat et al. 1994), the available information on their biology and population dynamics is limited. The present study aimed to study the reproductive biology and determine the spawning season of *L. lentjan* on the Red Sea coast of Saudi Arabia, where such information is a requirement for the assessment of this species for fisheries stock. This study will contribute to efforts to design appropriate plans for fisheries management to allow this important natural resource to be maintained and used sustainably.

#### MATERIAL AND METHODS

For one-year period, from January 2015 to December 2015, samples of L. lentjan were collected three times per month (593 in total) from the landing site for fishing boats operating in the Red Sea waters off Jeddah, Saudi Arabia (21°29′24″N; 39°10′23″E) (Appendix 1). The samples included all possible categories of fish lengths and sizes.

The collected samples were euthanized by immersion in freezing water, placed in ice-box and transported to the fisheries research laboratory in zoology department, college of science, King Saud University where the total length (TL) of each fish was measured to the nearest 0.1 cm, and the total body weight (BW) was determined to the nearest 0.1 g. All animals were euthanized in accordance with the standards set forth in the guidelines for the care and use of experimental animals by the King Saud University, Riyadh, Kingdom of Saudi Arabia. Fish were then dissected and sexed, and their gonads were collected and weighed (to get the gonad weight, GW) to the nearest 0.1 g. The gonads were each assigned a maturity stage based on their external features, such as their size, color, shape, and texture, and were then fixed in neutral buffered formalin for the validation of their maturity stage assignment by subsequent histological examination. The fixed gonads were processed for routine histological evaluation (washed, dehydrated, cleared and embedded in paraffin wax). Sections of 4 µm thickness were cut by using microtome (LEICA RM2255) and stained with hematoxylin and eosin. Both morphological and histological assignments of gonad maturity were based on the protocols of Essat et al. (1994), Kulmiye et al. (2002), and Currey et al. (2013).

The sex ratio was calculated based on the percentage of the specimens that were females and males in each month and in each of the different length categories, according to the following equation: Percentage of males (or females) = (# males (or females)/total # samples) × 100.

The hermaphroditism of the species was diagnosed according to Sadovy and Shapiro (1987). The performance maturation index (PMI) was calculated monthly using the following equation (Newman et al. 2001): PMI =  $(\# \text{ mature fish/total } \# \text{ fish}) \times 100$ .

The gonadosomatic index (GSI) was calculated monthly using the following equation (Ebisawas 2006):  $GSI = (GW/BW) \times 100$ , where GW is the gonad weight and BW is the body weight, as defined earlier.

The ovarian maturation rate (OMR) was determined based on the percentage of the total number of ovaries that was classified to be in each of the second, third, and fourth maturation stages in each length category (Ebisawa 2006).

The spawning season was determined indirectly based on the inferred sexual maturity of the samples, as well as any patterns in the values of the GSI, PMI, and OMR determined by histological examination.

#### **RESULTS**

Sex ratios

The sex ratios calculated based on the 593 specimens of L. lentjan collected from fisheries in Jeddah waters of the Red Sea during the study period from January 2015 to December 2015 are presented in Table 1. The overall male:female sex ratio (1:7.98), as well as the monthly sex ratios, indicated that females were numerically dominant throughout the study period.

Table 1. Monthly sex ratios of the collected *L. lentjan* samples during the study period (January 2015 to December 2015).

Month	Samples collected	# males	# females	Male (%)	Female (%)	Male:female sex ratio
Jan	18	4	14	22.22	77.78	1:3.5
Feb	20	4	16	20	80	1:4
Mar	36	9	27	25	75	1:3
Apr	91	10	81	10.99	89.01	1:8.1
May	63	2	61	3.17	96.83	1:30.5
Jun	61	5	56	8.2	91.8	1:11.2
Jul	61	2	59	3.28	96.72	1:29.5
Aug	51	10	41	19.61	80.39	1:4.1
Sep	40	3	37	7.5	92.5	1:12.33
Oct	48	4	44	8.33	91.67	1:11
Nov	37	1	36	2.7	97.3	1:36
Dec	67	12	55	17.91	82.09	1:4.58
Total	593	66	527	11.13	88.87	1:7.98

Table 2 summarizes the sex ratios for males and females in different size classes. The sizes of the males found ranged from 18.0 to 43.5 cm, while the sizes of the females found ranged from 14.5 to 41.9 cm. The greatest abundance of females was recorded in the 24.0–24.9 and 23.0–23.9 cm length categories, while males were not particularly abundant in any length category. In general, these results showed that males were larger in size than females.

# Performance maturation index (PMI)

The monthly PMI values found are presented in Table 3. These results indicated that the mean PMI was 48.57%, the highest monthly PMI occurred in February (100%), and the second highest PMI occurred in March (97.22%). A noticeable decrease and fluctuation in PMI values was recorded through



Table 2. Sex ratio of *L. lentjan* in different size (total length, TL) categories.

Length category (TL, cm)	Males	Females	Male (%)	Female (%)
14–14.9	0	2	0	100
15–15.9	0	0	0	0
16–16.9	0	4	0	100
17–17.9	0	8	0	100
18–18.9	2	12	14.29	85.71
19–19.9	4	25	13.79	86.21
20–20.9	5	27	15.63	84237
21–21.9	2	47	4.08	95.92
22–22.9	2	57	3.39	96.61
23-23.9	6	81	6.9	93.1
24–24.9	3	82	3.53	96.47
25–25.9	3	58	4.92	95.08
26–26.9	1	34	2.86	97.14
27–27.9	3	21	12.5	87.5
28-28.9	5	24	17.24	82.76
29–29.9	2	13	13.33	86.67
30-30.9	2	5	28.57	71.43
31-31.9	2	9	18.18	81.82
32-32.9	2	5	28.57	71.43
33-33.9	2	3	40	60
34-34.9	6	2	75	25
35-35.9	1	1	50	50
36-36.9	4	3	57.14	42.86
37-37.9	3	0	100	0
38-38.9	3	0	100	0
39-39.9	0	0	0	0
40-40.9	1	0	100	0
41–41.9	1	4	20	80
42-42.9	0	0	0	0
43-43.9	1	0	100	0
Total	66	527	11.13	88.87

Table 3. Performance maturation index (PMI) values for the *L. lentjan* samples collected over the study period (January 2015 to December 2015).

Month	Samples collected	Immature samples	Mature samples	PMI (%)
Jan	18	5	13	72.22
Feb	20	0	20	100
Mar	36	1	35	97.22
Apr	91	66	25	27.47
May	63	39	24	38.10
Jun	61	45	16	26.23
Jul	61	26	35	57.38
Aug	51	42	9	17.65
Sep	40	23	17	42.5
Oct	48	19	29	60.42
Nov	37	13	24	64.86
Dec	67	24	43	64.18
Total	593	305	288	48.57

the period from April to September, which was then followed by a period in which the PMI increased in October, November, and December.

The results presented in Table 4 represent the PMI values found in fish in the different length categories. The PMI found for the smaller groups (14.0 to 16.9 cm) was 0%, and the PMI then increased gradually from 19.0 to 32.9 cm. The PMI was 50% for the 34.0–34.9 cm length category, and then fluctuated around this value in the length categories from 33.0 to 38.9 cm, whereas the PMI in the larger length groups (40.0–43.9 cm) was recorded to be 100%.

Table 4. Performance maturation index (PMI) values for fish in different length categories.

		D. (1 (0/)		
Length category (TL, cm)	Collected	Immature	Mature	- PMI (%)
14.0–14.9	2	2	0	0
15.0-15.9	0	0	0	0
16.0–16.9	4	4	0	0
17.0–17.9	8	6	2	25
18.0–18.9	14	13	1	7.14
19.0–19.9	29	23	6	20.69
20.0–20.9	32	25	7	21.88
21.0-21.9	49	38	11	22.45
22.0–22.9	59	42	17	28.81
23.0-23.9	87	51	36	41.38
24.0-24.9	85	36	49	57.65
25.0-25.9	61	24	37	60.66
26.0-26.9	35	11	24	68.57
27.0-27.9	24	8	16	66.67
28.0-28.9	29	8	21	72.41
29.0-29.9	15	1	14	93.33
30.0-30.9	7	2	5	71.43
31.0-31.9	11	1	10	90.91
32.0-32.9	7	0	7	100
33.0-33.9	5	1	4	80
34.0-34.9	8	4	4	50
35.0-35.9	2	0	2	100
36.0-36.9	7	2	5	71.43
37.0-37.9	3	2	1	33.33
38.0-38.9	3	1	2	66.67
39.0-39.9	0	0	0	0
40.0–40.9	1	0	1	100
41.0-41.9	5	0	5	100
42.0-42.9	0	0	0	0
43.0-43.9	1	0	1	100
Total	593	305	288	48.57

Gonadosomatic index (GSI)

The monthly mean male and female GSI values determined herein are presented in Table 5. In these results, the GSI values of males and females showed similar seasonal trends,



wherein the highest GSI values occurred in February (0.39 for males and 1.54 for females) and March (0.22 for males and 0.83 for females). The GSI values of both sexes were then observed to fluctuate from April to August. However, the GSI values became high again in September (0.14 for males and 0.51 for females), before again declining gradually in October and November.

Table 5. Monthly mean gonadosomatic index (GSI) of the sampled males and females of *L. lentjan*.

Month	Male GSI	Female GSI
Jan	0.12	0.26
Feb	0.39	1.54
Mar	0.22	0.83
Apr	0.09	0.32
May	0.20	0.36
Jun	0.11	0.32
Jul	0.08	0.42
Aug	0.08	0.35
Sep	014	0.51
Oct	0.13	0.28
Nov	0.05	0.25
Dec	0.12	0.41

#### Ovarian maturation rate (OMR)

Table 6 presents the monthly OMR values of *L. enjtan* determined during the study period from January 2015 to December 2015. The highest OMR were recorded in February and March (100%), after which the OMR then decreased dramatically in April (23.46%). The period from May to September showed fluctuating OMR values, and then a significant increase in the OMR was recorded from October (61.36%) to December (65.45%). The OMR values measured in fish in different length categories are presented in Table 7. These results indicated that the OMR in the smaller length groups (14.0 to 16.9 cm) was 0%.

Table 6. Ovarian maturation rate (OMR) values for the *L. lentjan* samples collected over the study period (January 2015 to December 2015).

Month	Female samples	Mature female samples	OMR (%)
Jan	14	11	78.57
Feb	16	16	100
Mar	27	27	100
Apr	81	19	23.46
May	61	22	36.07
Jun	56	12	21.43
Jul	59	34	57.63
Aug	41	9	21.95
Sep	37	16	43.24
Oct	44	27	61.36
Nov	36	24	66.67
Dec	55	36	65.45

The OMR values began to fluctuate in the length categories from 17.0 to 20.9 cm, and then steadily increased as the fish body length increased above this range, reaching OMR values of 100% in the length categories from 31.0 to 41.9 cm.

Table 7. Ovarian maturation rate (OMR) values of fish in different length categories.

Length category (TL, cm)	Female samples	Maturefemale samples	OMR (%)
14.0–14.9	2	0	0
15.0–15.9	0	0	0
16.0–16.9	4	0	0
17.0–17.9	8	2	25
18.0–18.9	12	1	8.3
19.0–19.9	25	5	20
20.0–20.9	27	5	18.5
21.0–21.9	47	9	19.1
22.0-22.9	57	17	29.8
23.0-23.9	81	32	39.5
24.0-24.9	82	47	57.3
25.0–25.9	58	35	60.3
26.0-26.9	34	23	67.6
27.0–27.9	21	16	76.2
28.0-28.9	24	18	75
29.0-29.9	13	12	92.3
30.0-30.9	5	4	80
31.0-31.9	9	9	100
32.0-32.9	5	5	100
33.0-33.9	3	3	100
34.0-34.9	2	2	100
35.0-35.9	1	1	100
36.0-36.9	3	3	100
41.0-41.9	4	4	100

## Sex change

Histological examinations (Figs 1–4) showed clear evidence for sex change in *L. lenjtan* fish. The presence of testicular tissues and ovaries in the same gonads and the presence of male sex cells spread out within female ovaries (Figs 1–4) was conclusive evidence that *L. lenjtan* fish mature first as females, and then change their sex to become male, which is known as protogynous hermaphroditism. More histological evidence of sex change in this fish is shown in Figs 1–4, such as the presence of a central cavity (the remnant of the oviduct) inside the testis, torsion of the wall of the ovary toward the inside to subsequently form the seminal canal, and the presence of brown masses representing ovary remnants in the gonads of males.

#### Spawning season

The results found for the monthly mean values of the performance maturation index (Table 3), male and female gonadosomatic index (Table 5), and ovarian maturation rate (Table 6)

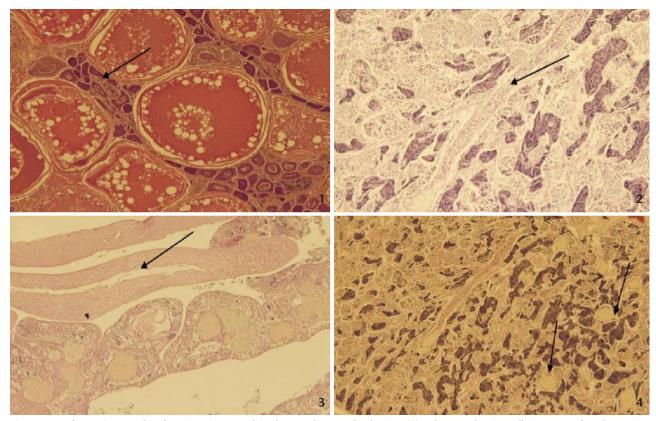


Figure 1–4. Photomicrographs of ovarian tissue explain the sex change of *L. lentjan*: (1)male reproductive cells in mature female ovaries; (2) the central cavity (remaining egg channel) inside the testicle; (3) torsion of the ovary wall to the inside to form the seminal canal; (4) brown masses (the porous layer of the remaining ovaries).

showed that *L. lenjtans* spawn eggs in extended batches during all the months of the year. However, the main spawning season was identified as occurring in February and March, when the highest PMI, GSI, and OMR values were recorded. A second, smaller main spawning season was also noticed in September, but with a magnitude smaller than that observed in February and March.

# Male maturation stages

The histological sections examined showed that the testis of *L. lenjtan* is of a radial type and consists of many convoluted seminiferous lobules, which increase in size as sexual maturity progresses. The maturity of the testicle can be classified into the following three stages (Figs 5–7).

Stage I (immature): Since each spermatozoon passes through a series of phases until it reaches its final form, seminiferous lobules containing cells in all of these phases, including spermatogonia (0.8–4.1  $\mu m)$ , primary spermatocytes (1.9–3.6  $\mu m)$ , and secondary spermatocytes (0.9–1.8  $\mu m)$ , can be classified as immature.

Stage II (mature): In this stage, the seminiferous lobules appear to be filled with spermatids (0.63–0.90  $\mu$ m) and mature

spermatozoa as a result of the completion of the process of spermatogenesis.

Stage III (spawning/running): Discharge of sperm cells during the spawning process occurs in this stage, which is thus distinguished by the presence of sperm cells outside of the lobules.

# Female maturation stages

The histological examination of the ovaries of *L. enjtan* found a developmental pattern that can be classified into four stages, each with their own features as outlined in the following section (Figs 8–11 and Table 8).

Stage I (immature): The immature ovaries possess numerous oocytes, which can be differentiated into three generations: pre-perinucleolar oocytes, early perinucleolar oocytes, and late perinucleolar oocytes.

Stage II (early maturation): In this stage, there are oocytes in three dominant phases: late perinucleolar oocytes, primary yolk vesicle oocytes, and secondary yolk vesicle oocytes.

Stage III (mature): This stage is characterized by the predominance of coalesced secondary oocytes and hydrated oocytes.



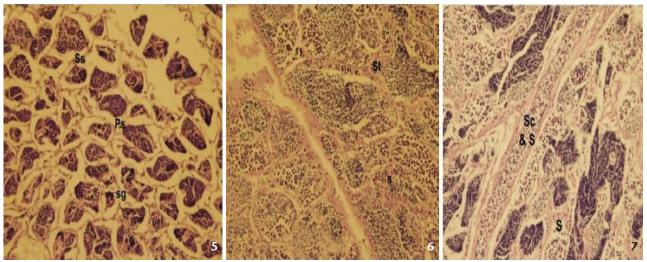


Figure 5–7. Histological sections in the testis of *L. lantjan* showing the maturation stages of male: (5) immature; (6) maturity; (7) spawning. (Ps) primary spermatocytes, (Ss) secondary spermatocytes, (S) sperm, (St) spermatids, (Sg) spermatogonia, (Sc& S) sperm cells and sperms outside the seminal vesicles

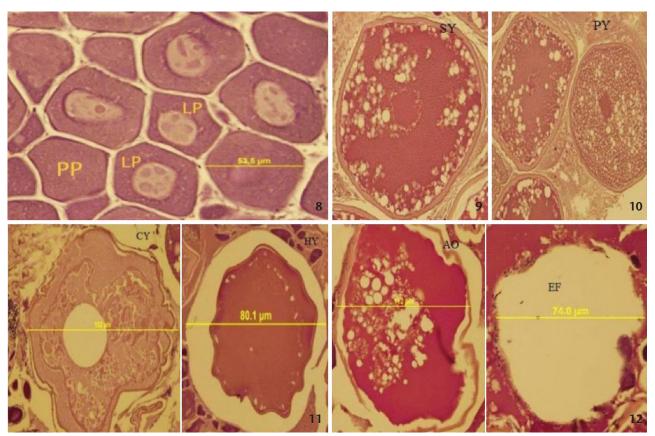


Figure 8–11. Histological sections in the ovary of *L. lantjan* showing the maturation stages of female: (8) immature; (9) early maturation; (10) maturity; (11) spawning. (AO) atretic oocytes, (CY) coalesced secondary oocytes, (EF) empty follicles, (HY) hydrated oocytes, (LP) late perinucleolar, (PP) pre-perinucleolar, (PY) primary yolk vesicle oocytes, (SY) secondary yolk vesicle oocytes.



Stage IV (spawning/running): This stage is characterized by the presence of predominantly empty follicles and vitellogenicatretic oocytes.

Table 8. Maturation stages of *Lethrinus lentjan* (females) detected throughout the study period (January 2015 to December 2015).

Month	Female samples	Sage 1	Stage 2	Stage 3	Stage 4
Jan	14	3	10	1	0
Feb	16	0	8	2	6
Mar	27	0	14	11	2
Apr	81	62	13	2	4
May	61	39	13	7	2
Jun	56	44	8	1	3
Jul	59	25	27	3	4
Aug	41	32	8	0	1
Sep	37	21	12	2	2
Oct	44	17	22	3	2
Nov	36	12	24	0	0
Dec	55	19	27	8	1

#### DISCUSSION

Assessing the reproductive biology of fish species is essential. Reproductive parameters such as sex ratios, size at sexual maturity, length of the reproductive period, and spawning season can be determined by the examination and classification of gonads into developmental stages. The assignment of gonads to macroscopic maturation stages based on their external appearance, including their size, color, shape, and texture, is an inexpensive and fast method, and may be especially suitable for samples that are not fresh enough for histological examination. While the microscopic investigation of histologically prepared sections of gonads is a more accurate and detailed way to assess gonad maturity, it is also costly and time consuming. This study used both macroscopic and microscopic strategies, and the results obtained for the monthly sex ratios using both methods indicate that females were dominant throughout the study period, with the overall male:female sex ratio being 1:7.98. The examination of male and female sex ratios in different fish size classes showed that males of this species are larger than females. The dominance of females may be due to the fact that these fish are protogynous hermaphrodites: they mature first as females, and then later change their sex and become males. Similar results, wherein overall sex ratios were biased towards females, were obtained in many previous studies of Lethrinus species, although different specific ratios were recorded; for example, Kulmiye et al. (2002) reported a male:female sex ratio of 1:1.10, Ayvazian et al. (2004) reported ratios of 1:1.19, 1:1.93, and 1:2.05 in different study areas, Abu Degoon and Ali (2013) reported a ratio of 1:1.7, and Restiangsih and Muchlis (2019) reported a ratio of 1:2.06. The difference between the overall sex ratio found in the current study and those found in these related studies can likely be attributed to differences among the *Lethrinus* species examined, environment of the study area, and fishing periods sampled.

The mean gonadosomatic index values generally remained similar between the periods when they rose and fell with respect totheir major peak in February and lower peak in September, especially in females. It is possible that the stability of the GSI values between these peaks indicates the nature of the spawning process of this fish species, wherein it lays eggs in extended batches throughout the year. The histological sections examined demonstrated the presence of different maturation stages in the gonads throughout the year, which provides clear evidence that this fish species has an extended spawning process during all months of the year, which occurs in the form of successive batches. However, based on the GSI, PMI, and OMR results, the months of February and March were concluded to comprise the main spawning season for this fish species, in addition to it having a second, smaller spawning season in September.

Many previous studies have pointed out that Lethrinus species spawn their eggs over the course of long seasons, and that they release their eggs in the form of batches during different months of the year, with different spawning peaks in different environments. Toor (1964) reported that L. lentjan mostly spawns from December to February. Nzioka (1979) postulated that there are two spawning seasons, in September/October and January/ February, for some lethrinid species in East Africa. Kuo and Lee (1990) concluded that there is a prolonged spawning season extending from September to February for Lethrinus nebulosus (Forsskål, 1775) along the Northwestern Shelf of Australia, although the peak spawning season of *L. nebulosus* in the coastal waters of Hormozgan Province, Iran occurrs in March according to Taghavi Motlagh et al. (2010), and Grandcourt et al. (2006) found that spawning by L. nebulosus in the southern Arabian Gulf mainly occurs from April to May. Wassef and Bawazeer (1992) reported that L. elongates has a protracted spawning season spanning from May to August in the Red Sea. Kulmiye et al. (2002) reported that Lethrinus harak (Forsskål, 1775) has a prolonged spawning season throughout the year along the Kenyan coast, with two peaks in October and February. Abu Degoon and Ali (2013) mentioned that the sky emperor, Lethrinus mahsena (Forsskål, 1775), has a prolonged spawning season, with one main peak in January.

The results of the microscopic investigation of gonads in this study described the maturation process of *L. lenjtan* as being protogynous hermaphroditism, which means that this fish matures first as a female, and then change its sex to become male. These results agree with many previous studies, such as Currey et al. (2009), who investigated the gonads of four lethrinid species (*L. nebulosus, Lethrinus atkinsoni* Seale, 1910, *Lethrinus olivaceus* Valenciennes, 1830, and *L. lentjan*) and concluded that all male individuals therein developed from females; however, these species did not conform to the typical trends displayed by other protogynous hermaphroditic lethrinids. Further, according to



Ebisawa (2006), the sexual development of *L. harak, Lethrinus miniatus* (Forster, 1801), and *Lethrinus ornatus* Valenciennes, 1830 was also considered to represent protogynous hermaphroditism.

The examination of histological sections of the gonad showed that the sexual maturation of males of *L. lenjtan* can be classified into three main stages (immature, mature, and spawning/running), while that of females can be classified into four main stages (immature, early maturation, mature, and spawning/running). These results differ from those of previous studies done on the same fish species, which described more maturation stages. Essat et al. (1994) mentioned that there were six such stages for both males and females of *L. lenjtan* collected from the Red Sea. Kulmiye et al. (2002) also described the maturation process in the gonads in both males and females of *L. harak* in Kenyan coastal waters as comprising six stages, while Abu Degoon and Ali (2013) concluded that *L. mahsena* in the Sudanese Red Sea passed through five gonadal maturation stages.

Lethrinus lenjtan has a somewhat complex reproductive biology, in that it undergoes sexual transformation, protogynous hermaphroditism, and both extended and seasonal spawning. Therefore, the description of sexual maturity in terms of many stages in this species will be characterized by much overlap and uncertainty between stages. The present study adopted only three main stages for defining the maturity of male gonads and four main stages for that of female gonads to avoid this potential source of confusion and lack of clarity, in contrast to previous studies that described more maturation stages. Indeed, some of the previously described stages could instead be considered sub-stages. It should also be noted that environmental conditions could play an important role in the maturation process, and thus environmental variation may have led to differences among the results of different studies.

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# **APPENDIX**

Appendix 1. The details of collected specimens of *Lethrinus lentjan* deposited at Department of Zoology, King Saud University, Riyadh are given in Table. Samples were preserved in 10% formalin solution followed by transfer to alcohol for long-term preservation, maximum 10 fishes per jar segregated according to their total length (cm).

Length category (TL, cm)	Number of samples	Voucher number
14.0–14.9	2	0115LL
15.0–15.9	0	-
16.0–16.9	4	0215LL
17.0–17.9	8	0315LL
18.0–18.9	14	0415LL, 0515LL
19.0–19.9	29	0615LL, 0715LL, 0815LL
20.0–20.9	32	0915LL, 1015LL, 1115LL, 1215LL
21.0-21.9	49	1315LL, 1415LL, 1515LL, 1615LL, 1715LL
22.0–22.9	59	1815LL, 1915LL, 2015LL, 2115LL, 2215LL
23.0–23.9	87	2215LL, 2315LL, 2415LL, 2515LL, 2615LL, 2715LL, 2815LL, 2915LL, 3015LL
24.0–24.9	85	3115LL, 3215LL, 3315LL, 3415LL, 3515LL, 3615LL, 3715LL, 3815LL, 3915LL
25.0–25.9	61	4015LL, 4115LL, 4215LL, 4315LL, 4415LL, 4515LL, 4615LL
26.0–26.9	35	4715LL, 4815LL, 4915LL, 5015LL
27.0–27.9	24	5115LL, 5215LL, 5315LL
28.0-28.9	29	5415LL, 5515LL, 5615LL
29.0–29.9	15	5715LL, 5815LL
30.0-30.9	7	5915LL
31.0-31.9	11	6015LL, 6115LL
32.0-32.9	7	6215LL
33.0-33.9	5	6315LL
34.0–34.9	8	6415LL
35.0–35.9	2	6515LL, 6615LL
36.0–36.9	7	6715LL
37.0–37.9	3	6815LL
38.0–38.9	3	6915LL
39.0–39.9	0	-
40.0–40.9	1	7015LL
41.0–41.9	5	7115LL
42.0–42.9	0	7215LL
43.0–43.9	1	7315LL
Total	593	