

Reproductive Investigations of Male and Female Blue Line Snapper, *Lutjanus coeruleolineatus* (Ruppell, 1838) from Salalah Coast, Sultanate of Oman

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Received: 18.10.2016 / Accepted: 14.11.2016 / Published online: 22.11.2016

Abstract:

Improves standard assessments of many commercially valuable fish species as Blue line snapper *Lutjanus coeruleolineatus*, is considered as an important commercial species harvested in the traditional fishery in the Sultanate of Oman. This will increase our knowledge in fish industry management. The present investigation of some biological aspects of Blue line snapper including male-female sex ratio, condition factor (Kn), gonado somatic index (GSI), length at first maturity for both sexes and developmental stages of ovary. Histological changes during the annual reproductive cycle and the stage of maturity of the ovary of Blue line snapper, *L. coeruleolineatus* are reported. Random collection of the species (449 males and 529 females) were taken monthly from Dhofar Governorate off the coast of the Arabian Sea at the period from March 2015 to February 2016. The results of this study reported male-female sex ratio was significantly different ($P < 0.05$) from 1:1 with dominate of females. The average monthly condition factor (Kn) values showed quite similar trend for both sexes with a slight higher values for females. The gonado somatic indices (GSI) data indicate that *L. coeruleolineatus* has prolonged breeding season from August to January with two peaks occurring in October and January. The length at first maturity was 28.1 ± 0.0 cm for males and 29.7 ± 0.0 cm for females. In this study five developmental stages of ovary of *L. coeruleolineatus* are recognized during development.

Keywords: Blue line snapper; Spawning season; Arabian sea; Maturity; Histological study

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Introduction

The blue line snappers *L. coeruleolineatus* (Rüppell, 1838) belongs to the family Lutjanidae which contains 13 genera and about 110 species (Eschmeyer and Fong, 2015). It is commonly known as snappers and called Neissar and Qalaya in Oman. However, there are many species under this family and subfamily found in the Indo-Pacific area (Allen, 1985). Lutjanidae also distributed worldwide in tropical, subtropical, and occasionally temperate waters (Hastings et al., 2014). Although, there are about 36 species of lutjanus are known from the Omani waters (Fouda, 1998). *L. coeruleolineatus* is a very colorful fish which characterized by yellow body, darker on back and whitish ventrally with distinguished blue (7-8) longitudinal stripes on the sides, a large black spot on the lateral line below the anterior portion of the soft dorsal rays and blue spots and broken lines on the head (Al-Abdessalaam, 1995).

Pervious study by Al-Abdessalaam (1995) reported that demersal species inhabits coral and rocky reefs at depths between 3 and 25 m occurs solitarily or in small groups and most of them feed on crustaceans or other fish. Lutjanids are harvested by both the traditional fishermen and commercial industrial fishermen. It is mostly caught by gillnets, handlines and traps. Even though this species is one of the most commercial marine fish in Oman. To date, no study on the biological aspects of this species has been carried out. This study aims to determine the reproductive aspects of *L. coeruleolineatus* specifically their male-female sex ratio, average monthly condition factor (Kn), the gonado somatic indices (GSI) and the length at first maturity for both sexes. The findings would be beneficial and important in order to effectively manage and conserve the fish in the future.

Material and Methods

Blue line snapper from commercial catch were sampled monthly for 12 months from March 2015 to February 2016. Random specimens of *L. coeruleolineatus* were obtained from the main fish market in Dhofar Governorate, Salalah (Figure 1). The fish samples for the biological studies were brought to the laboratory where the reproductive organs were recorded to the nearest 0.01g. The maturity stages were macroscopically identified following Saunders RJ (2009) (Table 1).

The sex ratios in different size groups were tested by chi-square test (χ^2) to find differences if any, between sexes using the formula:

$$\chi^2 = \sum (E-O)^2/E$$

Where O is observed number of males and females and E expected number of males and females.

The condition factor was calculated separately for females and males according to size class and on a monthly basis as suggested by Le Cren (1951) using equations:

$$Kn_i = \frac{\bar{w}_i}{(\bar{W}_i)^3}$$

Where W_i is condition factor in size class i and \bar{w}_i is the mean weight in size class i

And for the monthly estimates:

$$Kn, m_i = \frac{W_{i,m}}{(W_{i,m})^3}$$

Where K_n, m_i the condition is factor of the fish in month m and $W_{i,m}$ is weight in grams of the fish in month m . To determine the spawning season of the species, percentage occurrence of different maturity stages of gonads during various months were calculated, plotted and analyzed together with data on gonad-somatic index (GSI). The GSI calculated by following Rodriguez- Gutierrez (1992) formula:

$$GSI = GW/TW*100$$

Where GW is the wet gonad weight (g) and TW is the total fish weight (g).

The length at first maturity (L_{m50}), at which 50% of both sexes were calculated by using logistic equation considered by Froese and Binohlan (2000):

$$P = 100/(1+\exp(-a*(L - L_{m50})))$$

Where P is the proportion of mature females or males in each 1-cm length class, L is the mid-class length, L_{m50} is length at first maturity and a is a constant. Non-linear least-squares fitting with Microsoft Excel Solver problem was used to obtain the best fit of L_{m50} and a .

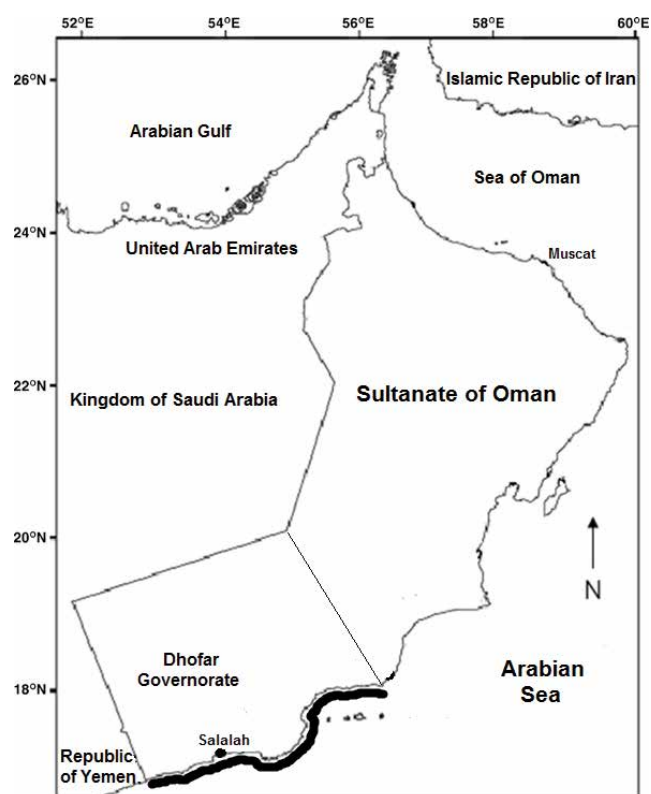


Figure 1: Map of Sultanate of Oman illustrate the landings sites of Dhofar Governorate where samples of the Blue line snapper, *Lutjanus coeruleolineatus* were collected from.

Table 1: The modified macroscopic maturity stages for female and male *Lutjanus coeruleolineatus* defined by Saunders.

Sex	Macroscopic stage	Macroscopic description
Female	Stage 1 (Immature)	Sexes almost indistinguishable, ovaries narrow thread, color variable.
	Stage 2 (Developing)	Ovaries small, opaque, pink in color, no oocytes visible
	Stage 3 (Ripe)	Ovaries medium to large, orange or yellow in color, large oocytes easily visible but not translucent.
	Stage 4 (Gravid/Running)	Ovaries large, orange or yellow and speckled with large translucent oocytes. Oocytes maybe ovulated.
	Stage 5 (Spent/Resting)	Translucent flaccid medium to small in size, generally red, particularly at posterior end, with some remnant oocytes visible.
Male	Stage1 (Immature)	Sexes almost indistinguishable, testis narrow threads, color variable but usually cream.
	Stage 2 (Developing/Resting)	Testis small to medium, white in color, no milt visible when gonad cut.
	Stage 3 (Ripe)	Testis large, creamy white, milt easily visible when gonad cut.

Table 2: Monthly sex-ratio of *L. coeruleolineatus* in Arabian sea during March 2015 to February 2016.

Month	No. Of fish	Male	Female	Sex ratio	Chi-square value	P-value
				Male : Female		
Mar-15	80	50	30	1:0.6	5.00	0.03
Apr-15	79	36	43	1:1.19	0.62	0.43
May-15	95	32	63	1:1.96	10.12	0.00
Jun-15	80	43	37	1:0.86	0.45	0.50
Jul-15	80	27	53	1:1.96	8.45	0.00
Aug-15	80	33	47	1:1.42	2.45	0.12
Sep-15	80	36	44	1:1.22	0.80	0.37
Oct-15	80	27	53	1:1.96	8.45	0.00
Nov-15	80	45	35	1:0.77	1.25	0.26
Dec-15	80	40	40	1:1	0.00	1.00
Jan-16	84	43	41	1:0.95	0.05	0.83
Feb-16	80	37	43	1:1.16	0.45	0.50
Pooled	978	449	529	1:1.17	6.54	0.01

Results

In present study the reproductive aspects of Blue line snapper, *Lutjanus coeruleolineatus* were investigated. The result of monthly sex-ratio during the period of March 2015–February 2016 is shown in **Table 2**. The male-female sex ratio was 1:1.17 which was significantly different from the expected sex ratio of 1:1 ($df=1$, $\chi^2=6.54$ and $P<0.05$). Monthly sex ratios were 1:1 for only the month December 2015. The females were more dominant in May 2015 ($df=1$, $\chi^2=10.1$, $P<0.01$), July 2015 ($df=1$, $\chi^2=8.45$, $P<0.01$), and October 2015 ($df=1$, $\chi^2=8.45$, $P<0.01$).

The females dominated in the length classes (33-35), (35-37) and (37-39) cm with a significant difference from the expected sex ratio of 1:1 ($P<0.05$) (**Table 3**). Moreover, the length classes (39-41), (41-43) and (43-45) cm were recorded only by females. On the other hand, males dominated the length classes (25-27), (27-29) and (31-33) cm but not significantly ($P>0.05$).

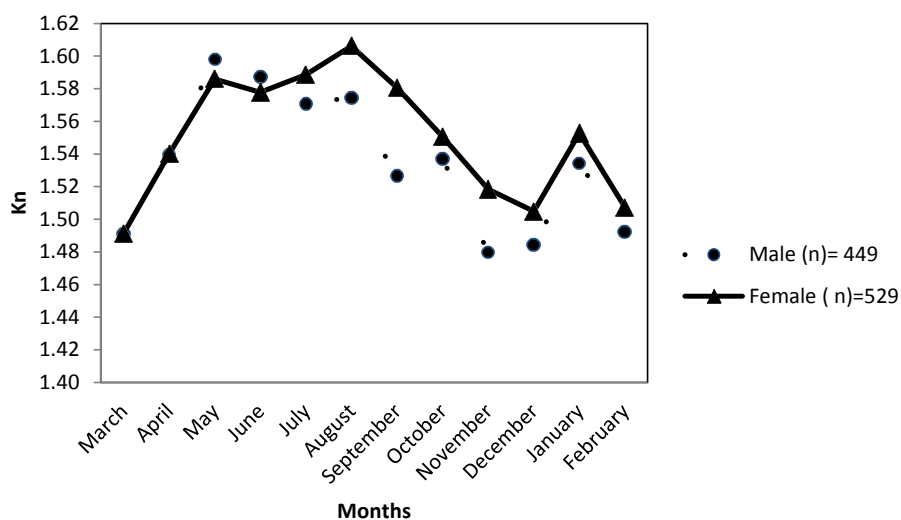
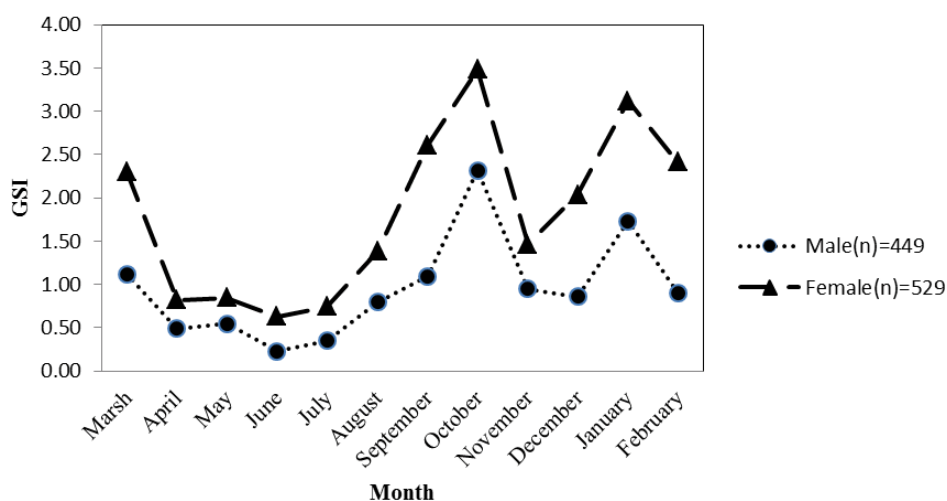
The monthly mean condition factor (K_n) for females was 1.55 quite similar to male which was 1.53. There was not significant different between female and male monthly mean condition factor (K_n) during the sampling period ($T_{0.05}$, $df=22=0.34$, $P=2.07$). The data indicates almost the same trend in the average monthly condition factor (K_n) values of males and female with a slight

higher values for females (**Figure 2**). The monthly relative condition factor (K_n) values for female were higher from May to September ranging from 1.4–1.6. The maximum condition factor was in August (Peak) with a value of 1.6 while the minimum value was 1.4 in March. The condition factor increased steadily from March and reached the highest value in July and August. Then it declined to a value of 1.5 in December. The monthly condition factor (K_n) values for male were higher from May to August similar to the female condition factor pattern season. It was between 1.4 and 1.59. The maximum condition factor was in May (Peak) with a value 1.59 while the minimum value was 1.4 in November. The condition factor increased steadily from March and reached the highest value in May and August. Then it declined to a value of 1.4 in November.

The monthly gonado somatic index (GSI) shows the same trend for both sexes (**Figure 3**). It is obvious that female recorded higher GSI value than male. GSI values fluctuated between 0.22 and 3.5, with highest in October and lowest in June. From June the gonado somatic index values for female increased dramatically and reached its maximum values in October and January (3.4 and 3.1 respectively). The trend then decreased sharply from January to minimum value in June (0.64). Male gonado somatic index values had a gradual rise from June and reached its maximum

Table 3: Sex-ratio in different size groups of *L. coeruleolineatus* in Arabian Sea during March 2015 to February 2016.

Size group	No. Of fish	Male	Female	Sex ratio	Chi-square value	P-value
				Male : Female		
19-21	5	2	3	1:1.5	0.20	0.65
21-23	68	34	34	1:1.0	0.00	1.00
23-25	141	67	74	1:1.10	0.35	0.56
25-27	208	116	92	1:0.79	2.77	0.10
27-29	156	79	77	1:0.97	0.03	0.87
29-31	164	70	94	1:1.34	3.51	0.06
31-33	92	48	44	1:0.92	0.17	0.68
33-35	59	20	39	1:1.95	6.12	0.01
35-37	32	10	22	1:2.20	4.50	0.03
37-39	24	3	21	1:7.00	13.50	0.00
39-41	14	0	14		14.00	0.00
41-43	8	0	8		8.00	0.00
43-45	7	0	7		7.00	0.01
Pooled	978	449	529	1:1.2	6.54	0.01

**Figure 2:** Average monthly condition factor (Kn) for female and male *L. coeruleolineatus* sampled from Dhofar Governorate coast of the Arabian Sea.**Figure 3:** Monthly Gonado somatic index (GSI) of male and female *L. coeruleolineatus* in the Dhofar Governorate coast of the Arabian Sea from March 2015 to February 2016.

values in October and January (2.3 and 1.7 respectively) similar to female trend. The trend then decreased abruptly from January reaching its lowest value in June (0.22). The occurrence of mature and ripe males and females together with GSI data indicate that *L. coeruleolineatus* has prolonged breeding season from August to January with two peaks occurring in October and January while approximately 80% of spent and rest stages were dominated in May and June and is therefore considered as inactive time for spawning. Monthly average Gonado somatic index (GSI) for female and male were 1.8 and 0.94 respectively ($T_{0.05}$, $df=22=2.6$, $P<0.05$).

The minimum size of maturity observed for the Blue line snapper for current study was 22.4 cm for males and 24.0 cm TL for females. The length at which 50% of maturity (L_{m50}) of the

blue line snapper was estimated at 28.1 cm TL \pm 16.1 for males and 29.7 cm TL \pm 10.3 for females (Figure 4) indicating that males mature at a slightly earlier length than females.

The maturity keys for the identification of different stage of gonads in females *L. coeruleolineatus* are given (Table 4).

The observation via light microscopy in this study revealed different histological structure of each oocyte developmental stage.

Stage I

Only primary growth of oocyte and oogonia. They are gathered in the ovigerous lamellae. The cytoplasm was very scarce during this development stage which were mostly appeared in the months between February and June (Figure 5A).

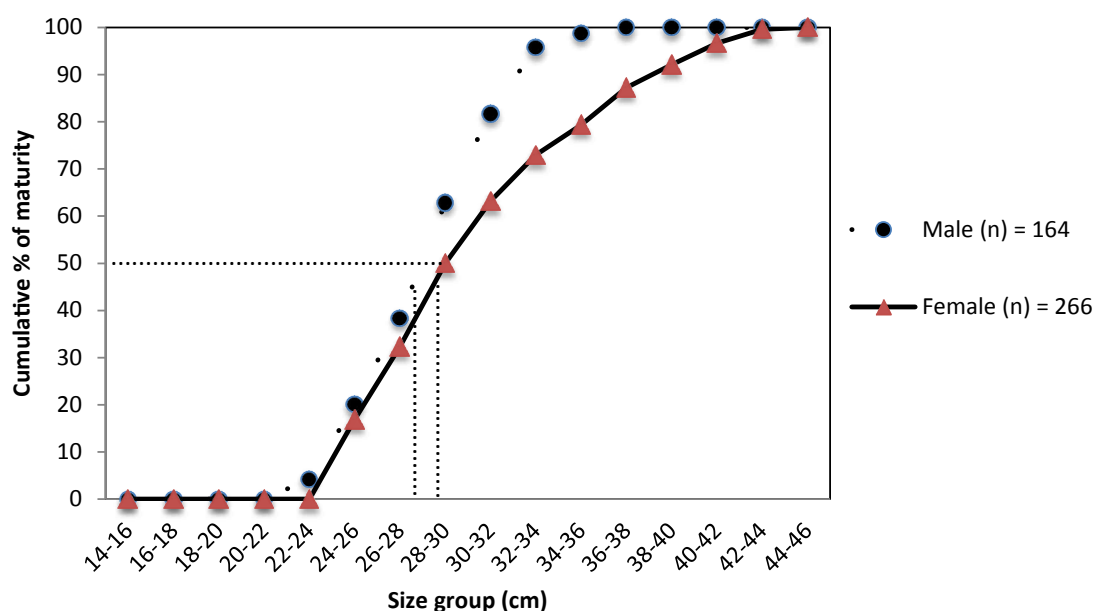


Figure 4: Cumulative percent of ripening and mature (stage III, IV, V) females and males of *L. coeruleolineatus* in 1cm size classes and length at first maturity (L_{m50}).

Table 4: Macroscopic and Microscopic characteristics of various maturity stages of female (F) gonads of *L. coeruleolineatus*. CAO: Cortical Alveolar Oocytes; FOM: Final Oocyte Maturation; POF: Post Ovulatory Follicles.

Stage	Macroscopic characteristics	Microscopic characteristics
Immature	Ovaries very small, translucent, ribbon-like and pinkish in color.	Primary growth of oocytes only; Lamellae organised well.
Maturation	Ovaries ranging from small to medium (<25% of body cavity); light orange in color; no opaque.	Dominated by CA and yolk granule oocytes and yolk globular oocytes. Atresia and POF present.
Spawning	Ovaries ranging from medium to large (25-75% of body cavity); clear oocytes have been ovulated and are visible as a collective clear strip among the vitellogenic oocytes; some may have been extruded; occasionally no opaque oocytes present.	Oocytes undergoing FOM or ovulated. POF and atresia may be present. CA and vitellogenic oocytes may be present.
Spent	Ovaries quite flaccid and small (<20% of body cavity); mustard yellow to orange, occasionally maroon; often contain clear fluid; can detect a few opaque oocytes.	Widespread atresia of vitellogenic and some CA oocytes. POF may be present.
Regressed	Ovaries very small; dark orange to maroon in color; no opaque oocytes present; ovarian membrane thickened and more opaque than immature fish.	Only primary growth oocytes. Late stage atresia present. Muscle bundles present.

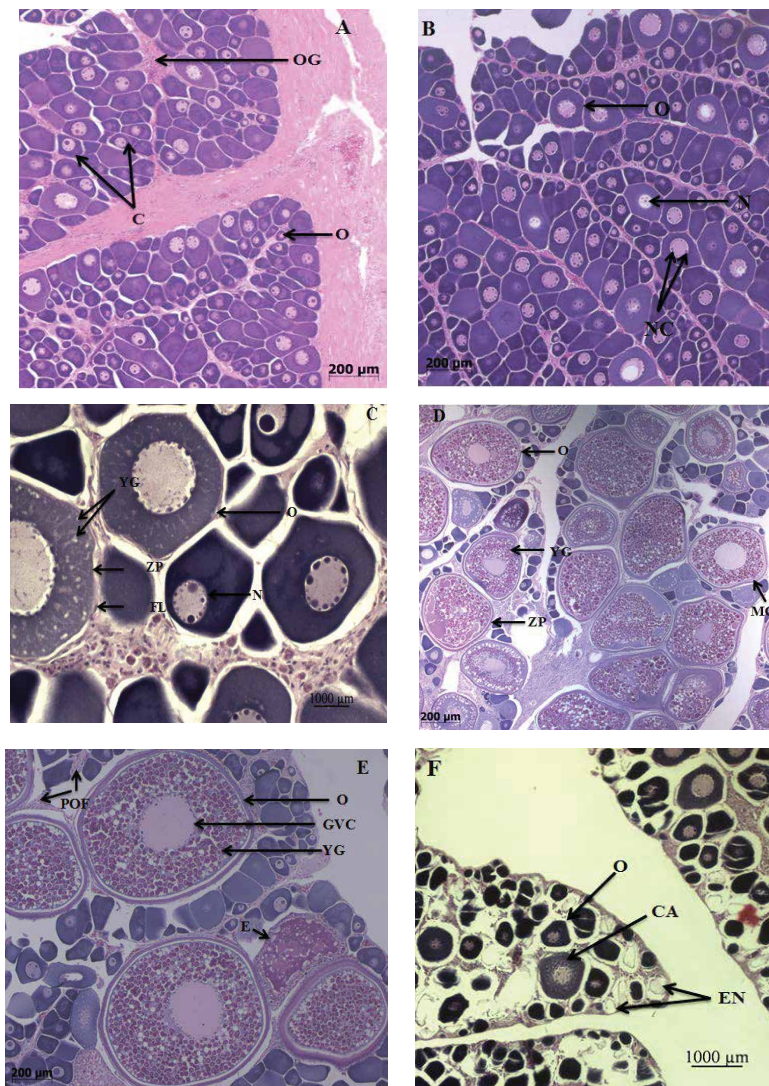


Figure 5: The appearance of ovaries sections of blue line snappers at different development stages. (A) Immature stage (B) early maturation, (C) middle maturation, (D) Late maturation, (E) spawning and (F) Regressed. Abbreviations: Oogonia (OG), Cytoplasm (C), Oocytes (O) Nucleus (N), Nucleoli (NC), Cortical Alveoli (CA), Yolk Granule (YG), Eggs (E), Empty Nets (EN), Zona Pellucida (ZP), Mature Oocyte (MO), Post Ovulatory Follicles (POF), Germinal Vesicle (GVC) and Follicular Layers (FL).

Stage II

Early maturation. This stage is characterized by the appearance of clear vesicles in the cytoplasm. The vesicle began to accumulate from the periphery of the oocyte. The nuclei were perinucleolar. In this stage, a thin acidophilic zonaradiata which look like primary envelope became visible for the first time. Follicular layers were also seen for the first time. A nucleus (N) was also being seen in the cytoplasm with or more than two nucleoli (**Figure 5B**).

Stage III

Mid maturation. Oocyte was increase in size. Small yolk granules were visible as a ring of deep eosinophilic in the cytoplasm. The nucleus was still convoluted. The zona radiata was clearly visible as a non-cellular deep eosinophilic band. Follicular layers were also be seen (**Figure 5C**).

Stage IV

Late maturation. During this phase of development, Zona

pellucida (zona radiata externa and interna) is much more defined and visible. Yolk globules and Oocytes were no longer organized in ovigerous lamellae. The nucleus has migrated toward the periphery of the cell and is in the process of dissolution (**Figure 5D**).

Stage V

Spawning. The oocytes were characterized by large mass of yolk. Eggs are hydrated and the appearance of flowing sexual products is noted, commencement of spawning is ready to begin. Germinal vesicle breakdown and post-ovulatory follicles were dissolved. Histologically, large size oocytes with coarse yolk granules scattered in the cytoplasm are presented (**Figure 5E**).

Stage VI

Regressed. This stage is characterized by low numbers of only young cells previtellogenic oocytes and cortical alveoli oocytes comparing with previous stages. High number of empty nets presented (**Figure 5F**).

Discussions

Current study was conducted to investigate reproductive parameters of Blue line snapper, of *L. coeruleolineatus*. Such parameters like sex ratio, condition factor (Kn), gonado somatic indices (GSI) and length at first maturity.

In this study male-female sex ratio was 1.00:1.17. This result was significantly different from the ratio expected for the family Lutjanidae, which is 1.00:1.00 (Garcia-Cagide et al., 2001). However, it was observed that males were found in small length classes while females in large length class similarly to the observation conducted by Garcia-Cagide et al. (2001). Similar results were also obtained by various scientists Heupel et al. (2010) and Kritzer (2004). Study on *L. carponotatus*, *L. gibbus* and *L. vitta* from Australia by Heupel et al. (2010) showed differences characteristics of lengths variation where females reaching larger maximum sizes than males but not significant. Kritzer (2004) also mentioned females of *L. carponotatus* were larger size than males.

The reason could be explained why females become bigger than males is the females behavior during spawning seasons. During this time female escape from catch area which gives them the opportunity to get bigger than males.

On the other hand, previous investigations by Fernandes et al. (2007), Torres, (1996) and Rojas, (1997) figured that male dominance in some snappers for instance male- female sex ratio was 1.60:1.00 for Brazilian snapper *Lutjanus alexandrei* (Fernandes et al., 2007). Male-Female sex ratio was 2.00:1.50 for *L. argentiventris* from Colombia (Torres, 1996) and 1.30:1.00 for *L. guttatus* from Costa Rica (Rojas, 1997).

The length at first maturity of *L. coeruleolineatus* showed that male start to mature slightly at an earlier length and age (28.1 cm TL-2.9 years) than females (29.7 cm TL-4.3 years). Similar to this results were observed by various scientist (Emata et al., 1999 and Russell et al., 2008) where the length at first maturity of male *L. argentimaculatus* were 496.0 mm FL which was significantly lower than females 570.0 mm FL and males matured earlier at 4 years than females at 5 years (Philippines-Emata et al., 1999) and also males mature at length of 471.0 mm FL compare to the females which mature at 531.0 mm (Australia - Russell et al., 2008). Recently, the differences recorded probably due to the different growth rates for both sexes as supported by Head et al. (2014).

From current study, the occurrence of mature and ripe males and females together with gonado somatic index (GSI) data indicate that *L. coeruleolineatus* has prolonged breeding season from August to March. The peak spawning activities of fish occur in October and January (after SW monsoon) when seawater temperature decline to around 23°C. At this time (October - January) the oocytes were characterized by large mass of yolk and eggs are hydrated and the appearance of flowing sexual products is noted. Furthermore, the relationship between annual variations in GSI and sea temperature were observed in *Cheimerus nufar* (Al-Marzouqi, 2012), in *Lethrinus nebulosus* and *Argyrops spinifer* (Al-Mamry et al., 2009) during the same period in the

Arabian Sea. A similar pattern was figured by Russell et al. (2008) reported spawning season of *L. argentimaculatus* in northeastern Queensland, Australia began around October peaked in December and then failed over summer from January through March in Vanuatu. On the other hand, reproductive development for *L. argentimaculatus* also occurred in spring between October and November (Brouard and grandperrin, 1984). While, *L. argentimaculatus* in Thailand had two spawning seasons started from late September (peak) to November when sea water temperatures were dropping and rainfall was highest and second seasons occurs in late March and April when temperatures were greater than normal (Doi and Singhagruiwan, 1993).

Meanwhile, McPherson et al. (1992) reported that some lutjanids in Great Barrier Reef waters spawn during the spring and summer months. There were some environments factors affect the timing of reproduction for lutjanus like temperature, photoperiod and the lunar cycle (Grimes 1987, Head, et al., 2014). Previous study by Grimes and Huntsman (1980), Everson (1984) and Arnold et al. (1978) remarked that temperature was correlated with gonad development and preparation for spawning in lutjanids. Another study on reproductive development of *Lutjanus argentiventris*, was tested in captivity and figured that spawning period extended from April through November with a peak in the summer (Muhlia-Melo et al., 2003).

Due to scarcity of information, an integrated concept of the impact of environmental factors on the reproductive process of fishes has not yet emerged. Nevertheless, it is known that fishes integrate their physiological functions with environmental cycles. Brown et al. (2009) indicated that endogenous periodicities of physiological processes are responsible in part for seasonal reproduction. Additionally, Lutjanid species are generally ready to spawn when environments are suitable. Previous researches observed that tropical reef fishes spawn over longer periods within the year than do cooler-water species (Lowe-McConnell, 1979).

The states of marine ecosystems are also influenced by increasing pressure on exploitation marine resources and human activities. The interactions of all these factors contribute to decline fish population and affect to control the fishery management system. The seasonal monsoons and the occurrence of extensive oxygen minimum zones are the main two distinguishing features of the Arabian Sea that significantly influence productivity in the region (Wishner et al., 1998). Seasonal monsoon can be classified to northeast monsoon (NE) which occurs from November to mid-February and the SouthWest monsoon (SW) monsoon with sustained strong winds typically occurs from June to mid-September (Weller et al., 1998). Wishner et al. (1998), Lee et al. (2000) and Weller et al. (2002) have studied the effect of the monsoon periods on water temperatures and documented that warm sea surface temperature were typically seen offshore during the SW monsoon period.

Conclusions

The results of the present study concluded that Blue line snapper *Lutjanus coeruleolineatus* has prolonged breeding season and recognized that using reproductive histological methodology

in assessment the development fish was an accurate tools and provide useful predictive information.

Acknowledgment

My kindly appreciation goes to my supervisors, Dr.Laith A.Abdul Razzak, Dr Rumeaida Binti Mat Piah, Dr Abdulaziz Said Al-Marzouqi and Dr. Mikhail Chesalin who definitely discussed my views for current study, providing me valuable comments and advices. My great thank to Ministry of Agriculture and Fisheries Wealth, Sultanate of Oman for provides me all facilities to make this research.

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