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Reproductive Aspects of Areolate Grouper (*Epinephelus areolatus*, Forsskal, 1775) from the Saudi Coast of Arabian Gulf

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Abstract - The increasing demand for fish and the declining supply anywhere in the world propelled aquaculture as an alternative source. In Saudi Arabia, despite the scarce resources, aquaculture production has been growing by volume and diversification of culture is taking place. The Areolate grouper, *Epinephelus areolatus* is one potential species, however, its full-cycle mariculture is not yet established. Thus, this study was conceptualized. The paper aimed to determine the reproductive aspects of *E. areolatus* from the Arabian Gulf. A total of 355 samples of *E. areolatus* collected over the 12-month sampling period revealed high proportions of females in the sample were observed throughout the year and in size class. Spawning individuals were found throughout the year, however, more pronounced during January to June. Males (29.3 cm, 358.44 g) were observed to be bigger and heavier than females (28.8 cm, 326.66 g). Minimum size observed at maturity was 27.0 and 19.1 cm for male and female, respectively. Female gonado-somatic index of 2.31 is highest during April which is an indication of peak spawning activity. The mean fecundity is 3,440,426 per spawn or 237,443 per gram of ovary for size class between 22.8 – 46.3 centimeters length and 196.84 – 1172.50 grams weight. The relationship between fecundity and the variables length, weight, eviscerated weight and gonad weight revealed positive correlations with p-value less than 0.0000 implying significant correlation among variables. Similarly, fecundity increases as gonado-somatic index increases. Despite the 20.77% variation in fecundity accounted to gonado-somatic index, significant relationship was observed at p-value of 0.0031.

Key words: Arabian Gulf, areolate grouper, fecundity, spawning, gonado-somatic index

INTRODUCTION

Groupers are fishes from the family Epinephelidae and subfamily Epinephelinae. They have an oblong or slender shape, thick or compressed body covered with small cycloid or ctenoid scaled skin, with a scaly head, large or moderate mouth, and moderately serrate pre-operculum with one to three spines in the operculum [1]. Their size ranges from a few centimeters to over 2 m and their weight reaches up to 400 kg. They have long life spans and late sexual maturation [2]. Most of them are sedentary and territorial in nature [3]. This group of fishes that is found in the tropics and sub-tropics is highly valuable in the seafood trade [4]-[6]. Annual grouper aquaculture production reached 9300 tons in 2000 and most stocks available in the market come from Taiwan, Thailand, Malaysia, and Indonesia [7]. Among the groupers produced, only 15-20% comes from fish farms and hatcheries [8]. In Saudi Arabia, two species groupers are commonly sold in the market namely: *Epinephelus coiodes* and *E. areolatus*. Their prices range from 15- 25$ and 8– 11$ per kg, respectively.

The increasing demand for groupers and all valuable food fishes is driving huge pressure on the fisheries sector to produce more supply [9]. In 2004, capture fisheries supplied the world with about 140.5 tons of food fish [10]. It provided an estimated 6.4 billion people their fish protein intake (105.6 metric tons) and other non-food uses (34.8 metric tons). It is observed that the annual fish consumption is continually increasing

at a fast pace that the sector cannot keep up on growing demand because the global sea fisheries capture reached its maximum sustainable levels already. To address the shortage, the aquaculture industries are being looked into for additional supply. As a result, the volume of mariculture is doubling each decade.

Aquaculture industry in the Middle East, particularly in Saudi Arabia is still at its early beginnings [11]. However, the fast development in this sector of fisheries in recent years gives a hint of a bright future thus investment in this business is good. Aquaculture production has been growing, in terms of volume, at an average rate of 13% [12]. Moreover, there is an increase of more than 100% in production in marine aquaculture every year despite of the extreme environmental condition.

Areolate grouper (*Epinephelus areolatus*, Forsskal, 1775) is perceived to be an ideal aquaculture organism for the area. It is a common grouper found in the waters of the Arabian Gulf. Research on the development of hatchery and nursery management techniques and rearing protocol on economically valuable species with a high market potential that are locally adaptive, such as the areolate grouper is timely.

Successful aquaculture of areolate grouper is attributed to a successful artificial propagation technique. Establishing a culture and rearing protocol requires knowledge of the life history and reproductive characteristics of your target culture organism. More so, data on the reproductive cycle, gonadal development, fecundity, and spawning frequency are essential in developing a breeding strategy for aquaculture. Likewise, information and a better understanding of the growth pattern of the culture organism are used in predicting aquaculture outputs. Thus, this study was conducted to consolidate such information that can help mold a strategy that is adaptive to the area with low operating cost and high business returns.

OBJECTIVES OF THE STUDY

The study aimed to determine the reproductive patterns of areolate grouper, *E. areolatus* in terms of sex ratio, gonadal development, gonado-somatic index (GSI) and fecundity rate at maturity. Further, this study also intended to answer if there are associations between fecundity and some selected variables studied.

MATERIALS AND METHODS

Specimens of *E. areolatus* were collected every month from Jubail Fish Market and Auction Center, a major fish landing site in eastern province of Saudi Arabia, western part of the Arabian Gulf. A total of 355 samples were collected over the 12-month sampling period from February 2014 to January 2015. Total length (TL) and whole-body weight (BW) of each fish were measured.

The fishes brought from the landing site were dissected and gonad samples were collected and weigh. The sex of the fish was characterized, and maturity stage was assessed through gonadal morphology. The sexual maturity stage of the fish was assigned according to the presence and dominance of various germ cell stages as modified from Ozen and Balci [13] and Cushion et al. [14]. Stages III, IV and V ovaries were placed in plastic vials and immediately fixed in 10% formalin. After 24 hours, they were removed from the fixative material and were transferred in 70% ethanol for storage and further analysis.

The weight of the fish and its gonad were used to compute for the gonado-somatic index (GSI) with a formula as follows [21]:

$$GSI\ (\%) = \frac{\text{Weight of gonad}}{\text{Weight of fish}} \times 100$$

GSI was computed throughout the sampling period and was used as a tool to identify the spawning season. The highest GSI ratio will indicate the peak of spawning activities of *E. areolatus*.

For the fecundity, three subsamples from the preserved gonads were taken for counting. Sections were prepared from the front, middle and rear part of each ovary. Each subsample was placed in a Petri dish containing few drops of water. Clumps of adhering eggs were broken up and eggs were carefully separated using a dissecting needle. The number of mature eggs in the subsample was counted under a stereo microscope. Fecundity was calculated using the formula [15]:

$$\text{Fecundity (F)} = \frac{\text{Total wt. of ovary}}{\text{Wt. of sub-sample}} \times \text{No. of mature eggs (ova) in sub-sample}$$

RESULTS AND DISCUSSION

The reproductive organs of male and female *E. areolatus* were similar in morphology. Both consisted of two lobes, anatomically located at the posterior portion of the abdominal cavity of the fish. In some specimens, the lobes of the gonads were not identical. Both the testes and the ovary looked the same during the early stage of maturity. However, as it grew old, differentiation of the two reproductive organs was more evident even through visual inspection. Milky and smooth substance filled the testes, while ovaries had granulated eggs.

Size and color vary depending on the sexual maturity of the sample. Young gonads are usually small, at times transparent. The color of testes ranges from yellow, red, reddish yellow, off white, light to dark brown. Similarly, ovaries are usually reddish yellow, yellow, and light red.

After the macroscopic visual examination of the gonads and later confirmed under the microscope, 74.4% were identified as females (n=264) and 25.6% as males (n=91) in the samples collected. The data gathered revealed a mean ratio of 1:2.9 between male and female. High proportions of females in the sample were observed throughout the year except during the month of June 2014 (Table 1) where a 1:0.9 male – female ratio was noted. Similar findings were found in terms of size classes where females were from 17 to 40 cm in length (Table 2).

The data gathered on mean ratio between male and female where high proportions of females observed throughout the year and in terms of size classes from 17 to 40 cm in length can be an indication of fishing intensity. The scarcity in number of males in the samples implies that most of them are being caught in fishing before they reach sexual maturity. In the study of Coleman, et al. in 1996 [16] on reproductive styles of shallow-water groupers in the eastern Gulf of Mexico and the consequences of fishing spawning aggregations, they emphasized that the loss of males as a result of intense fishing pressure would not reduce female reproductive capacity but could have considerable effects on female reproductive potential. The excessive removal of males can lead to distorted sex ratios [17] which can result to sperm limitation in the population [18]. Most experts agree that most fisheries remove the biggest fish [19] which can result to a severe change in population structure [16], [17], [20]-[24] because of the compromised overall stock reproductive output [25], [26].

Frequency of *E. areolatus* at different stages of maturity is shown in Figure 1. Spawning individuals (mature, ripe and spent) were found throughout the year except during the month of September and October. However, it more pronounced during January to June. The greatest number of individuals with mature, ripe and spent reproductive organs was observed during April. The minimum size observed at maturity was 27.0 and 19.1cm for male and female, respectively.

Table 1. Sex ratio of E. areolatus from February 2014 to January 2015, n=355.

Month	Male				Female				Sex Ratio
	N	%	Mature*	%	N	%	Mature*	%	Male:Female
February 2014	10	33.3	4	40.0	20	66.7	7	35.0	1:2
March	4	13.3	1	25.0	26	86.7	17	65.4	1:6.5
April	4	13.3	2	50.0	26	86.7	24	92.3	1:6.5
May	5	16.7	2	40.0	25	83.3	21	84.0	1:5
June	16	53.3	2	12.5	14	46.7	11	78.6	1:0.9
July	13	48.1	0	0.0	14	51.9	4	28.6	1:1.1
August	5	16.7	0	0.0	25	83.3	2	8.0	1:5
September	13	43.3	0	0.0	17	56.7	0	0.0	1:1.3
October	5	16.7	0	0.0	25	83.3	0	0.0	1:5
November	1	3.3	0	0.0	29	96.7	2	6.9	1:29
December	8	28.6	0	0.0	20	71.4	1	5.0	1:2.5
January 2015	7	23.3	6	85.7	23	76.7	18	78.3	1:3.3
TOTAL	91	25.6	17	18.7	264	74.4	106	40.2	1:2.9

* Number of individuals who reached maturity stage. This includes individuals with stage IIb, III, IV, V gonads.

Table 2. Sex ratio of E. areolatus at different size ranges, n=355.

Month	Male				Female				Sex Ratio
	N	%	Mature*	%	N	%	Mature*	%	Male:Female
17-20	9	22.0	0	0.0	32	78.0	1	3.1	1:3.6
21-24	17	27.0	0	0.0	46	73.0	5	10.9	1:2.7
25-28	21	29.6	5	23.8	50	70.4	22	44.0	1:2.4
29-32	18	18.9	5	27.8	77	81.1	45	58.4	1:4.3
33-36	13	25.5	2	15.4	38	74.5	18	47.4	1:2.9
37-40	7	30.4	3	42.8	16	69.6	9	56.2	1:2.3
41-44	4	50.0	1	25.0	4	50.0	4	100.0	1:1
45-48	2	66.7	1	50.0	1	33.3	1	100.0	1:0.5
TOTAL	91	25.6	17	18.7	264	74.4	105	39.8	1:2.9

* Number of individuals who reached spawning stage. This includes individuals with stage IIb, III, IV, V gonads.

The monthly gonado-somatic index of *E. areolatus* over the entire period of study was presented in Figure 2. Results revealed that male *E. areolatus* exhibited an almost the same index throughout the year, with values ranging from 0.06 to 0.42. Whereas males exhibited less fluctuation in GSI, year-long observation for females revealed a varied result. An increasing trend of GSI values were observed from February 2014 and peaks in April. It gradually declines in the following months and starts to increase again by January 2015. In general, peak spawning occurred in April as indicated by the highest mean GSI of 2.31.

The maturation of the female genital products in groupers, as in other fish, follows a cyclic pattern [27]. This can happen several times in a year [28]. Duration varies depending on the species and observed to be relatively longer in smaller species [29] such as *Cephalopholis boenak* [30], *Cephalopholis fulva* [31] and *Epinephelus rivulatus* [32].

Studies made by different fisheries experts on other Epinephelid fishes from the Arabian Gulf

found out that they spawn during the same period as the species presently studied. *Epinephelus tauvina* in Qatar and Kuwait spawns from May-December [33] and March – May [34], respectively. *Epinephelus coioides* spawning season extends from March to May [35]. The findings above verify the observation of Hussain and Abdullah [36] that most of the fish in the northern Arabian Gulf breed during springtime which is from February to May.

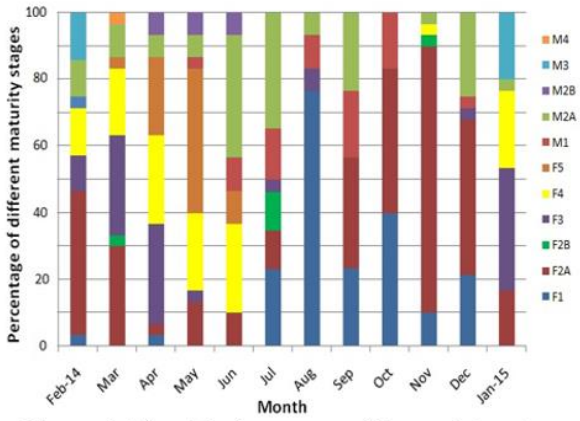

Figure 1. Monthly frequency of E. areolatus at different stages of maturity (M=Male, F=Female, 4=Ripe, 3=Mature, 2B=Recovering, 2A=Developing, 1=Immature).

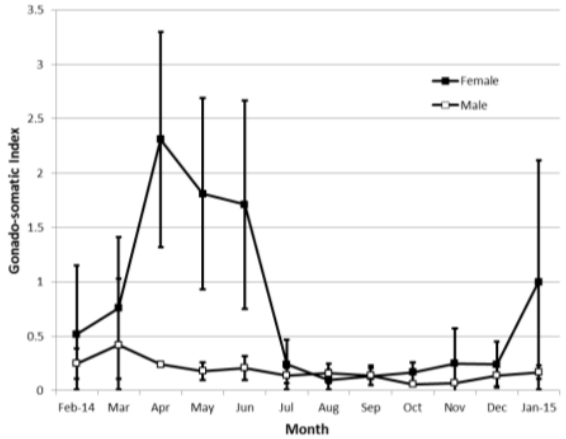

Figure 2. Monthly gonado-somatic index of *E. areolatus* from February 2014 to January 2015.

In a similar study conducted by Mahmoud [37] on the same species in Red Sea reveals that spawning of this particular species happens in May and June, which matches the result of this study.

While Ephinephelids are known to exhibit sex reversal, no degenerating gonads in the fish samples were observed that can be a sign of a transitional stage. However, the presence of immature stage in bigger class size of males can be an indication that areolate groupers demonstrate a protogynous hermaphroditic characteristic. According to Charnov [38], hermaphrodites change sex when net future reproductive success would be higher for the opposite than for the existing sex. It is further emphasized by Devlin and Nagahama [39] that such characteristic provide fish with maximum reproductive output through increased high-quality eggs produced, enhanced competitive fertilization or improved progeny.

Reproductive biology, together with physiology processes, water quality parameters, habitat and other external environmental factors determine fish population [40]. Changes in population's productivity or reproductive potential over time is a result of life-history trait plasticity [41]. Information on spawning seasonality and duration, size at sexual maturity, and sex ratio provide a deeper understanding on how various strategies influence gamete production [42].

The uniform GSI index of male *Epinephelus areolatus* throughout the study imply that there is enough supply of male reproductive gametes even there is less males found in the population as implied by the earlier results. According to Sadovy and Colin [43], a high GSI in male is an indication of sperm competition among the fishes. A similar study conducted by Tharwat et al. in 2005 [44] on *E. coioides* population on the same area reveals that GSI reached its maximum values during April.

Fecundity Rate at Maturity of *E. areolatus*. A total of 40 gonads of mature *E. areolatus* from Arabian Gulf were collected throughout the duration of the study. Fecundity and morphometric measurements were given in Table 3. The minimum and maximum total fecundity observed were 3,329 eggs and 38,702,445 eggs, respectively. The mean fecundity was 3,440,426 eggs per spawn or 237,443 eggs per gram of

ovary for size class between 22.80 – 46.30 cm long and 196.84 – 1172.50 g weight.

Table 3. Fecundity and morphometric measurements of mature female *E. areolatus* from Arabian Gulf, n=40.

Measurement	Sample Size	Minimum	Maximum	Mean
Fecundity per ovary weight	40	8537	958864	237443
Total Fecundity	40	3329	38702445	3440426
Length (cm)	40	22.8	46.3	31.4
Weight (g)	40	196.84	1172.50	412.28
Eviscerated Weight (g)	40	179.66	1083.92	382.01
Gonad Weight (g)	40	0.39	40.36	9.56

The results of the present study are higher than the results of the study of Kandula et al. [45] on the same species, with fecundity values ranging from 22,128 to 93,607 eggs per spawn for length and weight range of 14.2-33.0 cm and 78-430 g, respectively (n=16).

Similar studies on fecundity of other species of grouper in the area revealed a wide range of values. *E. tauvina* releases 367,000 to 627,000 eggs per/female/spawn [45]. *E. coioides* with size ranging from 40 to 80 cm total length lays 957,270 to 328,7515 eggs/spawn [47]. Studies revealed that disparity in fecundity is typical in fishes [46]. It is largely dependent on the organism's size [29], [47], age [29], environment [47]-[52] and sex change [53].

Relationship between Fecundity and Some Selected Morphometric Characteristics of *E. areolatus*. The relationship between fecundity and the variables length, weight, eviscerated weight and gonad weight are presented in Figure 4. Regression results revealed positive correlations as described by equations below:

Length - Fecundity	$F = 841758L - 2E+07$
Weight - Fecundity	$F = 24548W_T - 7E+06$
Eviscerated Weight - Fecundity	$F = 26356W_E - 7E+06$
Gonad Weight - Fecundity	$F = 793878W_G - 4E+06$

Results of the analysis indicated that the coefficients of determination (r^2) of 0.5035 (F to L), 0.6794 (F to W_T), 0.6730 (F to W_E) and 0.8234 (F to W_G). It further revealed a p-value less than 0.0000. Such results implied a significant correlation among variables.

Regression results revealed positive significant correlations between fecundity and the variables length, weight, eviscerated weight and gonad weight. The results of this study are similar to observations of Bouain and Siau [27] on the female reproductive cycle and fecundity of *E. aneus* from the southeast Tunisian seashores. They found out that fecundity increases in relation to length, to the weight of the eviscerated fish and to the weight of the gonads. Moreover, fecundity is very closely related to the weight of the gonads.

Similar studies have been conducted on the relationship of fecundity and growth. Beverton [54], He and Stuart [55], [56] and Charnov [57] explained that growth is directly associated to the age of sexual maturity of a fish. Similarly, Whiteman et al. [58] revealed that a reduction in the mean size of sex change in groupers may significantly decrease fecundity. Significant results given above implies that change in growth rate may result to variation in reproductive output thus may change the population structure of a species.

Relationship Between Fecundity and Gonado-somatic Index of *E. areolatus*. Relationship between gonado-somatic index and fecundity of *E. areolatus* is described in Figure 5. Based on the figure below, fecundity increases as gonado-somatic index increases. Regression analysis revealed a very low coefficient of determination (r^2) of 0.2077. Analysis of variance revealed a p-value of 0.0031 lower than the standard 0.05 which infers significant relationship among the variables.

The data on relationship between gonado-somatic index and fecundity of *E. areolatus* in this study showed a significant positive correlation. Despite this, regression analysis revealed a very low coefficient of determination (r^2) which implied that only 20.77% variation in fecundity was accounted to gonadosomatic index.

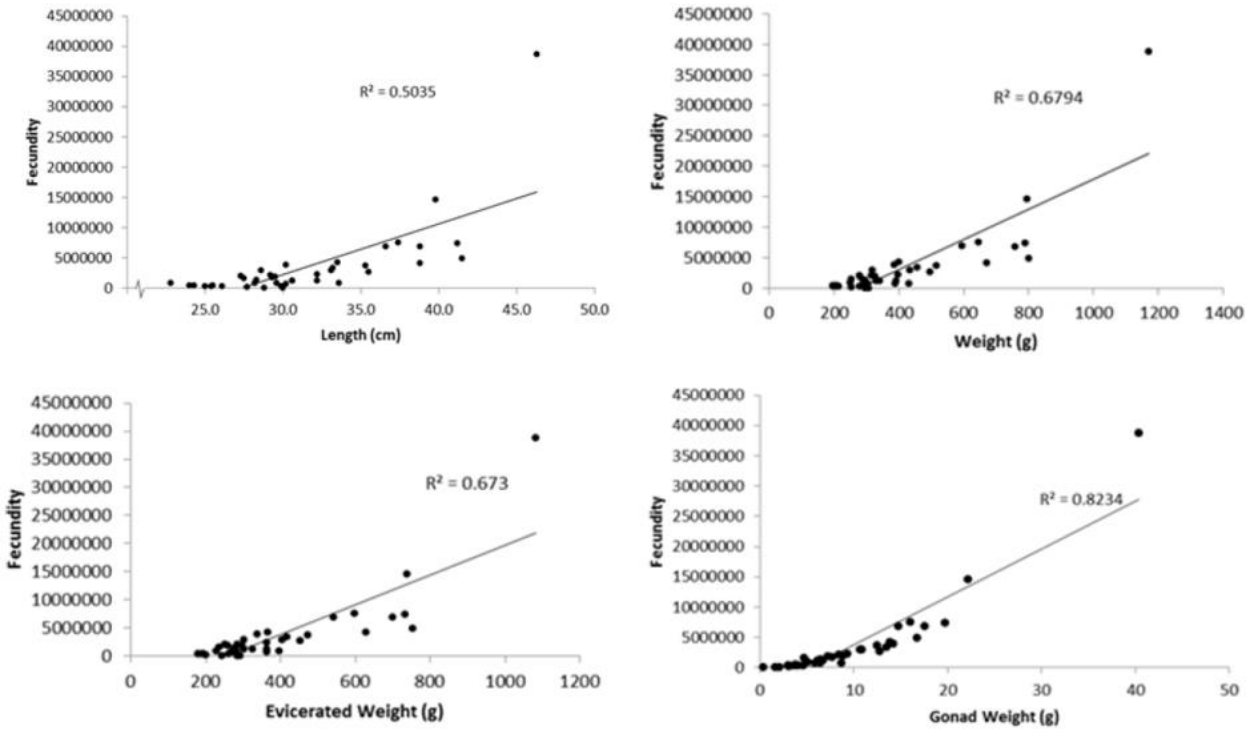

Figure 4. Fecundity related to length (cm), weight (g) eviscerated weight (g) and gonad weight (g).

In a similar study conducted by Buragohain and Goswami [59], Gundersen et al. [60] and Gundersen et al. [61]; they found out that fecundity and GSI has a positive correlation. The determination of the relationship between fecundity and GSI gives us a perspective on the reproductive capacity and the spawning season of fish.

Gonado-somatic index is the proportion between the weight of the gonads and the weight of the fish. Studies revealed that fecundity has a linear relationship with both the fish weight and gonad weight [62]-[64]. In the study conducted by Jonsson and Jonsson in 1997 [65], they found out that increase of body size in fish will increase the body cavity to accommodate more eggs and more energy available to produce many eggs. However, Devlaming et al. [66] emphasized that GSI is not an accurate indicator of gonadal activity.

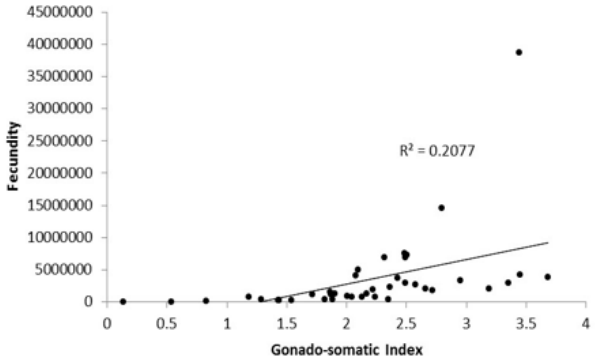

Figure 5. Relationship between gonado-somatic index and fecundity of *E. areolatus*.

CONCLUSIONS AND RECOMMENDATIONS

The following conclusions were drawn based on the analyses and interpretation of the findings of the study.

1. The high proportions of females in the sample were observed throughout the year and in size class (sex ratio of 1:2.9 male to female) indicates of fishing intensity that most males are being

caught in fishing before they reach sexual maturity.

2. *E. areolatus* from Arabian Gulf spawns from January to June, peaking in April. Minimum size observed at maturity was 27.0 and 19.1cm for male and female, respectively.

3. The mean fecundity was 237,443 per gram of ovary for size class between 22.80 – 46.30 centimeter long and 196.84 – 1172.50 grams weight.

4. Fecundity and the variables length, weight, eviscerated weight and gonad weight have positive significant correlations.

5. Fecundity increases as gonado-somatic index increases. There is significant association between the two variables.

Based on the findings and conclusions of the study, it is recommended that an in-depth study must be conducted to understand important mechanisms and gather more data on the behavioral aspect of reproduction, i.e., sex change, pairing, and courtship of the Areolate grouper, *E. areolatus*. Such knowledge can also be used in setting-up strategies and designing plans for setting up culture facility for this species.

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