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**REPRODUCTIVE BIOLOGY OF ORANGE-SPOTTED GROUPER
Epinephelus coioides OF THE ARABIAN GULF AT SAUDI ARABIA
 BY**

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ABSTRACT

The orange spotted grouper *Epinephelus coioides* (Hamoor) is one of the prime fish species in Saudi Arabia. So, it was selected to study their reproductive biology in Saudi territorial waters of Arabian Gulf. The gonadal maturation, sex ratio, spawning season, size at first sexual maturity, hermaphrodites and fecundity were studied. Fish mature first as females and some of them become males later. The immature fishes that belong to age groups 1-3 years constitute about 53 %, while the mature fishes that belong to age groups 4-10 constitute about 47 % in the population. Sex ratio varied with fish length, where the males were gradually appeared and dominated in the oldest fishes. The overall mean sex ratio was 1 : 4 for males to females, and moreover about 6 % of the catch individuals were hermaphrodites. The estimated length at which 50% of fish in the population reached sexual maturity (L_{m50}) was 60 cm and 48 cm for males and females, respectively. The corresponded age at first sexual maturity (t_{m50}) was 4.90 and 3.25 years for males and females, respectively. The smallest size of fully mature was 76 cm for males and 64 cm for females. The GSI reached its maximum values during April, which is significantly higher ($p < 0.05$) for females than males, and the spawning season extends from March to May. The absolute fecundity ranged from 957, 270 to 3, 287, 515 eggs for the fish ranging from 40 to 80 cm total length, respectively. It can be concluded that it must be allowed the fishes to grow to sexual maturity (L_{m50}) before allowing substantial harvest to protect the stock and to improve the spawning stock biomass of Hamoor in the Arabian Gulf at the long run.

Keywords: Hamoor, *Epinephelus coioides*, Arabian Gulf fisheries, Saudi Arabia.

INTRODUCTION

Most species of grouper appear to be protogynous (change sex from female to male) and are generally considered to be long-lived and late-maturing (Huntsman *et al.*, 1999). There is considerable interest in the development of

grouper aquaculture particularly in the Asia-Pacific region. However, a main constraint to grouper culture development is the limited availability of fry that are obtained from the wild (Sugama *et al.*, 1998), as well as the limited supply of trash fish that is presently the main feed source for grow-out (Boonyaratpalin, 1993 and Serrano and Apines, 1996). Groupers of genus *Epinephelus* are widely distributed throughout the tropical and subtropical waters of the world. They are commercially important and highly regarded as a favorite marine food fish. Grouper farming appears to have great promise.

The orange-spotted grouper *Epinephelus coioides* (Hamilton, 1882) inhabits mainly in lower estuaries and around protected silty reef habitats. Adults are usually found along the bases of small drop-offs associated with large caves, or in shipwrecks (Kuitert, 1996), but they are also taken offshore to depths of 100m (Heemstra and Randall, 1993). This species usually occurs alone or in small groups (Yearsley *et al.*, 1999). They may occur on coral reefs in turbid areas and are often encountered in brackish environments (Randall *et al.*, 1997). Stomach contents include fishes, shrimps, crabs and cuttlefish (Heemstra and Randall, 1993). *E. coioides* (locally name Hamoor in Arabian Gulf) is an important food fish and a popular candidate for mariculture in Arabian Peninsula, owing to its fast growth, efficient feed conversion, high economic value and high demand in the local and the international markets. It is caught sporadically mainly using traps, seines, bottom set lines and by demersal fish trawlers. Some biological aspects for this species were studied in the Arabian Gulf (Sivasubramaniam, 1981; Samuel and Mathews, 1985; Mathews and Samuel 1987; Al Janhi *et al.*, 2002 and Tharwat, 2005), but most of them didn't deal with their reproductive cycle. Therefore, the present work was carried out to study the reproductive biology of *E. coioides* inhabiting Saudi territorial waters of the Arabian Gulf. That included the gonadal maturation, sex ratio, spawning season, size at first sexual maturity, hermaphrodites and fecundity studies.

MATERIALS AND METHODS

Specimens of Hamoor *Epinephelus coioides* were collected monthly from the commercial fishing boats at Tarout and Al-Jubail Fishing-Ports throughout the year of 2003. Specimens were subjected to sex determination, measured for total length (TL) to the nearest millimeter, weighed with a precision of 0.01g, then the gonads were removed, weighed and preserved in 10% formaldehyde for a week, and then transferred to 70% alcohol. Gonadal maturity stages were examined as well as macroscopic and microscopic gonad staging. Macroscopic (staged by eye) and microscopic (staged microscopically from a raw tissue smear) staging of fresh ovaries and testes followed Collins *et al.*, 1998. All 6 stages of ovaries and 4 stages of testes were found, along with transitional gonads adapted from Moe (1969) and Collins *et al.* (2002). Gonadosomatic index (GSI) that expressed gonad weight as a percentage of total body weight less gonad weight (Burgos, 2001) was calculated to delineate the spawning season and estimate reproductive potential. Sex ratio by length was determined to identify the transition from female to male. Length at which 50% of fishes reach sexual maturity (length at first sexual maturity, L_{m50}) was estimated by maturation curve

method (Brule *et al.*, 1999 and Burgos, 2001). Fish aging was estimated by applying the von Bertalanffy's parameters of *E. coioides* in the same localities according to Tharwat (2005). The hydrated oocyte method was used for batch fecundity estimates (Hunter *et al.*, 1985).

Statistical analysis:

One-way analysis of variance (ANOVA) was carried out by using SAS (1996) software computer program to compare differences in the sex ratios, size at first sexual maturity, gonado somatic index and fecundity ($P < 0.05$). Data were expressed as the mean \pm standard deviation of the mean (S.D).

RESULTS

Sex ratio and size at sex reversal:

The length frequency distribution, percentage of males, females and hermaphrodites of *E. coioides* and the sex ratio with the length class are shown in Table (1). It was found that sex ratio deviate significantly from 1:1 among the size classes. Females ranged from 26 to 86 cm in length and predominated the catch amongst the fish size ranging from 26 to 62 cm total length, where fish length less than 46 cm was found to be females only. While, males ranged from 46 to 94 cm and predominated for the size larger than 62 cm in length. Males were significantly ($p > 0.05$) longer (mean = 69 cm, SD=10.6, n=70) than females (mean = 46 mm, SD=10.8, n=277) in the catch. The total proportion of males, females and hermaphrodites in the catch were 19.4 %, 74.7 % and 5.9 %, respectively. The total length of transition from females to males ranged between 46-78 cm. On the other hand, the overall mean sex ratio was 1: 4 (Males: Females) during the year and differs significantly from 1:1.

Size of maturity:

First maturation curve (Fig 1) presented a characteristic shape of *E. coioides* that reached higher sizes and maturity. Its maturation process occurred along a vast amplitude of total length ranging 46-76 cm for males and 36-64 cm for females. The estimated length at which 50% of fish in the population reached sexual maturity (L_{m50}) was 60 cm and 48 cm for males and females, respectively. The calculated weight at first sexual maturity (W_{m50}) was 3.35 and 1.71 kg for males and females, respectively. The corresponded age at first sexual maturity (t_{m50}) was 4.9 and 3.25 years for males and females, respectively. The smallest size of fully mature was 76 cm for males and 64 cm for female.

Gonado-somatic index and spawning season:

The monthly average values of the GSI for males and females of *E. coioides* were graphically represented in Figure (2). The GSI nearly exhibited the same trend for both sexes, where its average values increased during the period from March to May and declined sharply during the period from June to February throughout the year, during which the gonads are inactive. The GSI reached its maximum values during April, it is significantly higher ($p > 0.05$) for females (2.52) than males (1.40). This was expected in view of yolk accumulation in female ovaries, which is much heavier than the spermatozoa in male testes. The present result indicated that *E. coioides* spawns once a year in the Arabian Gulf, and the spawning season extending from March to May.

Table (1): Proportion of males (M), females (F) and hermaphrodites (H) by length class and sex ratio (M:F) for Hamoor *E. coioides* collecting from the Arabian Gulf in Saudi Arabia.

Total length (cm)		Males		Females		Hermaphrodites		Total	Sex Ratio (M : F)
Range	Mean \pm SD	Freq.	%	Freq.	%	Freq.	%		
26-30	28 \pm 0.9	0	0.0	17	100.00	0	0.00	17	0.00 : 1
30-34	32 \pm 0.7	0	0.0	20	100.00	0	0.00	20	0.00 : 1
34-38	36 \pm 0.8	0	0.0	29	100.00	0	0.00	29	0.00 : 1
38-42	40 \pm 0.6	0	0.0	37	100.00	0	0.00	37	0.00 : 1
42-46	44 \pm 0.2	0	0.0	46	100.00	0	0.00	46	0.00 : 1
46-50	48 \pm 0.3	2	4.7	40	93.02	1	2.33	43	0.05 : 1
50-54	52 \pm 0.4	3	8.1	32	86.49	2	5.41	37	0.09 : 1
54-58	56 \pm 0.5	7	23.3	21	70.00	2	6.67	30	0.33 : 1
58-62	60 \pm 0.3	6	28.6	12	57.14	3	14.29	21	0.50 : 1
62-66	64 \pm 0.6	10	41.7	9	37.50	5	20.83	24	1.11 : 1
66-70	68 \pm 0.6	11	52.4	6	28.57	4	19.05	21	1.83 : 1
70-74	72 \pm 0.7	9	60.0	4	26.67	2	13.33	15	2.25 : 1
74-78	76 \pm 0.9	8	61.5	2	15.38	3	23.08	13	4.00 : 1
78-82	80 \pm 1.0	5	66.7	1	16.67	0	16.67	6	5.00 : 1
82-86	84 \pm 1.2	6	71.4	1	14.29	0	14.29	7	6.00 : 1
86-90	88 \pm 1.5	3	100.0	0	0.00	0	0.00	3	100%M
90-94	92 \pm 1.6	2	100.0	0	0.00	0	0.00	2	100%M
Total	-	72	19.41	277	74.66	22	5.93	371	0.25 : 1 or (1:4)
Mean \pm SD	60 \pm 11.73	6.00 \pm 2.97	-	18.47 \pm 14.71	-	2.75 \pm 1.20	-	21.82 \pm 13.21	-

\pm SD means standard deviation.

Fecundity:

Egg characteristics of *E. coioides* were found to be spherical; smooth; not sticky and transparent. The egg diameter distributions of the ovaries of ripe and prespawning females ranged 0.2 - 0.5 mm and most of them (about 80% of eggs) were around 0.4 mm. The relationship between the absolute fecundity and fish total length of *E. coioides* is graphically represented in Figure (3). The absolute fecundity ranged from 957,270 to 3,287,515 eggs for the fish ranging in total length from 40 to 80 cm. It appeared to be an exponential relationship, and can be expressed mathematically by the following exponential equation: $F = 1347 (L)^{1.78}$, $r = 0.975$, where F is the absolute fecundity, L is the total length of fish (cm), r is the correlation coefficient of the regression. However, the absolute fecundity for the successive age groups of *E. coioides* are shown in Figure (4). It was observed that within any age group, the number of eggs varied greatly, and this variation was significantly greater within age group than within length group. The regression relationship between absolute fecundity (F) and fish age (g) can be expressed and estimated by the exponential equation as follow: $F = 213045 (g)^{1.27}$, $r = 0.987$. Generally, the mean number of eggs increased, as the fish got older. Thus, it increased from 859,835 eggs in fishes of third year to 3,470,239 eggs in fishes of nine year.

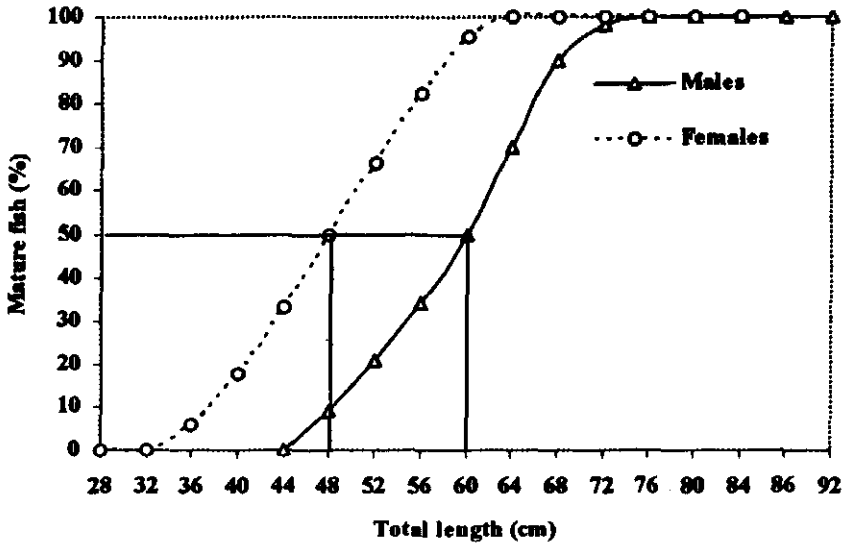


Fig. (1); The percentages of mature males and females of Hamoor *E. coioides* collecting from the Arabian Gulf at Saudi Arabia [the

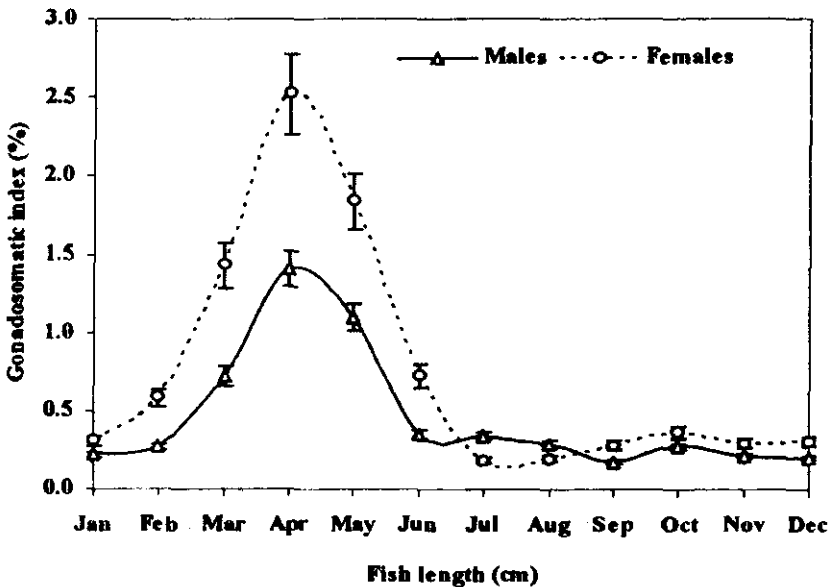


Fig. (2): Monthly variations of gonadosomatic index (GSI, %) for males and females of Hamoor *E. coioides* collecting from the Arabian Gulf at Saudi Arabia.

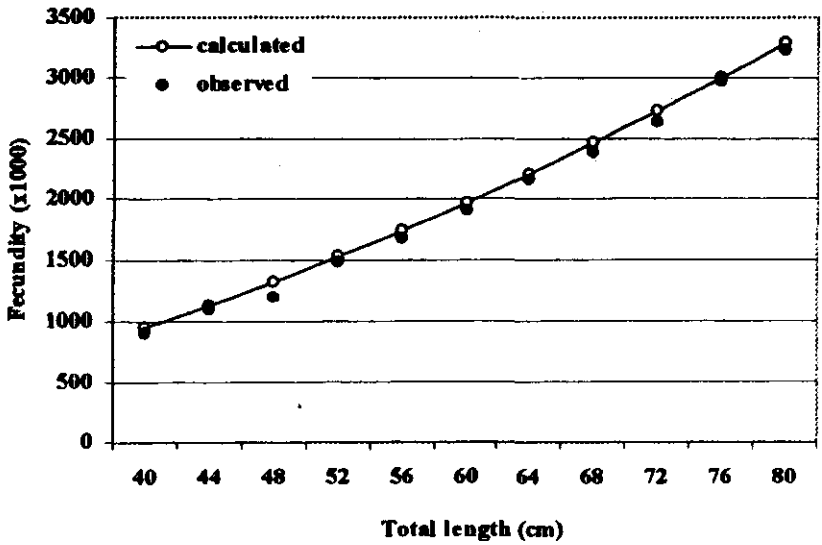


Fig. (3): The relationship between batch fecundity and total length of Hamoor *E. coioides* collected from the Arabian Gulf of Saudi Arabia.

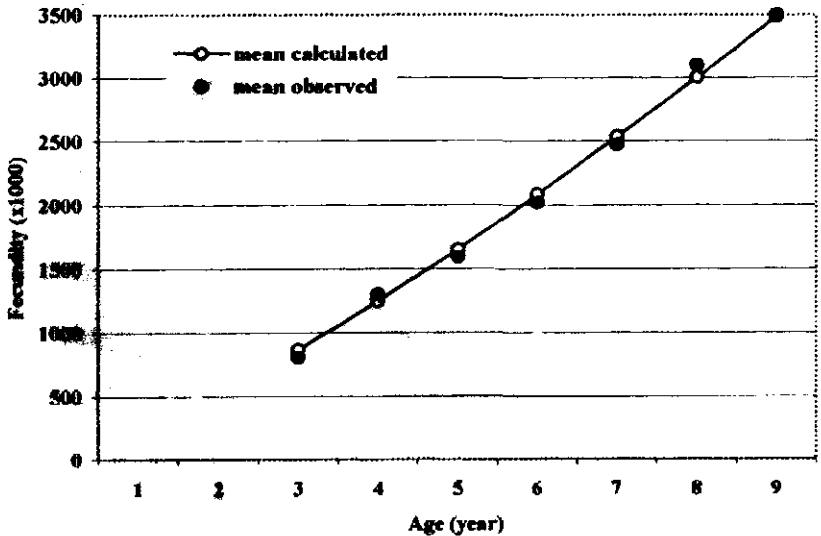


Fig. (4): The relationship between fecundity and age of Hamoor *E. coioides* collected from the Arabian Gulf of Saudi Arabia.

DISCUSSION

The groupers are protogynous hermaphrodites, where most individuals mature first as females and some of them become males later (Chen *et al.*, 1977; Tan-Fermin *et al.*, 1994 and Sadovy and Colin, 1995). Some of those species, as a rule, change from female to male, as they grow older; others might change only if there is a shortage of males. The present result on grouper *E. coioides* is in agreement with other studies on *Epinephelus* (Moe, 1969; Brule *et al.*, 1999; Burgos, 2001 and Collins *et al.*, 2002) that males increased in proportion with increasing length, so a high sex ratio (1:4 males to females in the present result) is found. FAO (1981) found 1:7.5 ratio between male and female of this species in the Arabian Gulf. This may be due to sampling the population during the spawning season. Moe (1969) indicated that sex ratio of red grouper *Epinephelus morio* in the west Florida shelf was 1 male/transitional: 5 females. A study from the Campeche Banks showed a male to female ratio of 1:3.4 (Brule *et al.*, 1999) and a survey from the U.S. south Atlantic showed a male to female ratio of 1:6.6 (Burgos, 2001). Collins *et al.* (2002) found that 69% and 71% of *E. morio* were females during 1996 and 2001, respectively; the remaining fish were males (28.6% and 27.9%, respectively) and transitionals (2.8% and 1.1%, respectively), 1 male/transitional: 2.3 females. In the estuarine grouper, *Epinephelus tauvina*, the transition from female to male begins at the age of 7 years, and the proportion of sex-inverted males increases thereafter up to 100% at the age of 10 years (Chen *et al.*, 1977; Chao and Lim, 1991 and Tan-Fermin *et al.*, 1994). Moreover, first maturation curve presented a characteristic shape typical of species that reached higher sizes, higher longevity and slower growth rate, as was the case of *E. coioides* in the present study. Its maturation process occurred along a vast amplitude of total length up to 76 cm for males and 64 cm for females. The estimated size and age at which 50% of fish in the population reached sexual maturity (L_{m50}) revealed highly significance ($p>0.05$) between both sexes (length 60 and 48 cm, weight 3.35 and 1.71 kg and age 4.9 and 3.25 years for males and females, respectively). The value of $L_{m50}=47$ cm was within the amplitude found for Tunisian groupers *E. guaza* by Chauvet (1988), where the population studied reached sexual maturity between 45 and 55 cm total length, at an average age of 5 years. Tunisian groupers had an inversion size between 80 and 90 cm (Chauvet, 1988 and Barreiros, 1998). In our samples the total length of *E. coioides* ranged between 46-94 cm for males, 26-86 cm for females and 46-78 cm for hermaphrodites. Most groupers that have been studied will mature within 2 to 6 years. Females are mature at 25-30 cm standard length (2-3 years old), and protogynous sexual transition occurs at a length of 55-75cm (Heemstra and Randall, 1993) or slightly smaller (49cm) according to Lau and Li (2000). However, Lee *et al.* (1980) studied the size and age of maturity else where in the Arabian Gulf and they indicated that Hamoor sexually mature at a length of 61 cm and probably at 5-6 years old, with 95% confidence limit of the mean size at maturity of 57.4-65.0 cm, and the minimum size at maturity of 44 cm. Among the widely accepted measures of sexual activity in fishes is the Gonado-somatic index (GSI). Comparing the 3-month spawning period for grouper (higher values of GSI), it was obvious that March, April and May are the peak months in the Arabian Gulf. Similar results were obtained for the same species inhabits the

Arabian Gulf (Heemstra and Randall, 1993 and AL-Janhi *et al.*, 2002) and for *E. morie* inhabits the Gulf of Mexico (Moe 1969, Brule *et al.*, 1999 and Burgos, 2001). Moreover, Collins *et al.* (2002) indicated that GSI varied by month with the greatest values occurring from February through June. They pointed that histological assessment indicated a relatively similar seasonality; that spawning occurred from March 23 to June 20 in 1996 (a period of 89 days) and from February 6 to June 26 in 2001 (140 days). It can be concluded from the present investigation of the macro/microscopic ripe gonads and GSI values that the spawning season of *E. coioides* extends through March to May, and the peak of sexual activity occurs during April in Saudi territorial waters of the Arabian Gulf.

Concerning the fecundity study, it is well known that oocytes attain their maximum developmental size just before or during the breeding season (Shenouda, 1988). The estimated fecundity and its exponential relationship were in a good agreement with previous studies for the same species inhabiting the Arabian Gulf (Heemstra and Randall, 1993) and for *E. morie* inhabiting the Gulf of Mexico (Collins *et al.*, 2002). It is obvious that fish of *E. coioides* did not reach the senility age in the Arabian Gulf, where, the increase in age is usually accompanied by an increase in length and weight, so fecundity increases with them. According to Nikolsky (1963), this increase continues until the senility age at which it stops increasing or decreasing. However, the commercial fish species, which has an economic importance, does not reach this age. On the other hand, it is assumed that adults of *E. coioides* populations leave estuarine areas to spawn offshore, so the protection of specific offshore areas is important for conserving mature individuals of this species. The reproductive characteristics of three hermaphrodite grouper species studied by Sluka and Sullivan (1988) and Coleman *et al.* (1996) showed that these characteristics may affect stocks sustainability. Nowadays it is known that some protogynous stocks may be far more vulnerable to fishing than are comparable gonochoristic stocks (Schaaf and Huntsman, 1994; Coleman *et al.*, 2001 and Musick *et al.*, 2001). Fishing that focuses on large fish may therefore result in a significant reduction in the proportion of males. Size at maturity information is often used in stock assessments to define the size or age at which individuals are reproductively active and producing offspring. It is a principal of fisheries management to allow individuals to grow to maturity before allowing substantial harvest. From the present work, the following items can be concluded:

- 1) The spawning season of Hamoor occurs during March - May with a high peak in April. The length at which 50% sexual maturity (L_{m50}) was 48 cm. There is an urgent need to ban fishing of under sized L_{m50} to protect the stock and to allow the fish to grow above so as to improve the spawning stock biomass of Hamoor.
- 2) Fecundity increases with increasing length, weight and age of the fish. It exhibited an exponential relationship with fish length, fish weight and fish age.
- 3) Further study on the taxonomy of this species is also recommended to accurately assess its distribution and abundance, due to the confusion with other closely related *Epinephelus* species mentioned above.

- 4) Reduce or eliminate the use of destructive habitat or particularly wasteful fishing gears or methods for grouper seed. Develop management approaches to protect key seed settlement and nursery habitats, such as mangrove areas and sea grasses, and protect the production of those seed by safeguarding the spawning adults.
- 5) Provide governmental assistance in terms of incentives or low-interest loans to enable small-scale fishers to enter the culture sector to produce low intensity, high quality, cultured grouper, in suitable grow-out areas. Provide assistance in breaking relationships involving indebtedness.

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بيولوجيا التكاثر لأسماك الهامور ذات البقع البرتقالية

Epinephelus coioides

في المياه الإقليمية السعودية بالخليج العربي

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تعتبر أسماك الهامور ذات البقع البرتقالية *Epinephelus coioides* من أهم أنواع الأسماك الفاخرة المصادة في المملكة العربية السعودية، ولذلك تم اختيارها لدراسة دورة تكاثرها في المياه الإقليمية السعودية بالخليج العربي. حيث تضمن البحث دراسة نضج الغدد الجنسية، والنسبة الجنسية للذكور والإناث في العشيرة السمكية، وتحديد موسم التكاثر، وحجم الأسماك عند بداية النضج الجنسي، والأفراد الخناث، وأيضا خصوبة الأسماك. وقد وجد أن أسماك الهامور تنضج أولا كإناث ثم بعض أفرادها تتحول للذكورة فيما بعد مع الكبر في الحجم. وأن الأسماك التي أعمارها لا تزيد عن ثلاث سنوات تكون غير ناضجة جنسيا وتمثل ٥٣% في العشيرة، بينما الأسماك الناضجة جنسيا يتراوح أعمارها ما بين ٤ و ١٠ سنوات وتمثل حوالي ٤٧% من العشيرة السمكية. وقد لوحظ إختلاف في النسبة الجنسية للذكور و الإناث مع الزيادة في طول الأسماك، حيث يبدأ ظهور الذكور تدريجيا مع الزيادة في أطوال الأسماك حتى تصود في أعدادها في الأسماك الكبيرة الحجم، وكان المتوسط العام للنسبة الجنسية هو ١ ذكر : ٤ إناث، وأن الأفراد الخناث تمثل حوالي ٣% من العدد الكلي للأسماك المصادة. وتم تقدير طول الأسماك عند بداية النضج الجنسي (٥٠% من النضج الجنسي للأفراد، L_{m50}) وكان ٦٠سم للذكور و ٤٨ سم للإناث (طول كلي)، وأن عمر الأسماك عند بداية النضج الجنسي (t_{m50}) يعادل ٤,٩ سنة للذكور و ٣,٢٥ سنة للإناث. وقد وجد من الدراسة أن أصغر حجم للنضج التام لجميع أفراد الأسماك هو ٧٦ سم في الذكور و ٦٤ سم في الإناث. ومن دراسة قيم معامل المناسل الجسمي تبين أنها تصل إلى أقصى قيم لها خلال شهر أبريل وترداد فيه القيم معنوياً للإناث عن الذكور، وأن موسم تكاثر أسماك الهامور يمتد خلال الفترة من مارس إلى مايو في المياه الإقليمية السعودية بالخليج العربي. ومن دراسة الخصوبة لأسماك الهامور إتضح أن أعداد البيض تتراوح ما بين ٨٧٢٧٤٠ إلى ٣٤٣٥٢٢٣ بيضة في مبايض الإناث التي يتراوح أطوالها ما بين ٤٠ - ٨٠ سم طول كلي. ومن خلال الدراسة يمكن التوصية بمنع صيد أسماك الهامور قبل وصولها للحجم عند بداية النضج الجنسي (L_{m50}) على الأقل لحماية أسماك الهامور الناضجة جنسيا وإعطائها الفرصة للتكاثر ولو لمرة واحدة على الأقل قبل صيدها من أجل حماية وتجديد المخزون السمكي لها في الخليج العربي على المدى الطويل.