The reproductive biology *of Torquigener flavimaculosus* (Hardy & Randall, 1983) along the Tobruk coast in the Mediterranean Sea, eastern Libya

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الملخص:

في الدراسة الحاليه تم تجميع عدد 161 سمكة ابونفاخ الصفراء من ساحل طبرق – البحر المتوسط – شرق ليبيا خلال الفتره من يناير الى ديسمبر 2022م.

وكان الشق الجنسى 1:1.30 للذكور والاناث على التوالي وكانت بداية النضج الجنسى L_{50} للذكور 8.51 cm والاناث 7.95 cm

وبدارسة معامل الدليل المنسلى ظهرت اعلى القيم في شهر أغسطس (11.34 ± 1.11% GSI) للإناث وكانت للذكور (5.12 ± 0.59%) مما يثبت بعد التأكد من معامل الدليل الكبدى ان للسمكة موسم تكاثر محدد في شهور يوليو واغسطس وسبتمبر (الصيف).

وبدارسة الخصوبة المطلقه لعدد 25 عينه من السمكه تبين انها تتراوح بين 844 بويضة الى 3214 بويضة للأسماك من طول كلى 7.5-17.4 cm. بمتوسط 1771±279.4.

Abstract

This study investigated the reproductive biology of the Yellow Spotted Puffer (*Torquigener flavimaculosus*). Fish were collected weekly from local fishermen along the Mediterranean Sea coast of Tobruk in eastern Libya between January and December of 2022. The male-to-female ratio was 1:1.30. Male fish reached sexual maturity (L_{50}) at a size of 8.51 cm, whereas female fish reached L_{50} at a size of 7.95 cm. The Gonadosomatic Index (GSI), which measures reproductive activity, peaked in June (4.22 for males, 10.22 for females), July (4.78 for males, 10.97 for females), and August (5.12 for males, 11.34 for females). This means the fish laid their eggs in the summer. To find out the total number of eggs (called absolute fecundity), 25 fully developed ovaries were checked. The number of eggs varied from 844 to 3214 for fish that were between 7.5 and 17.4 cm long, with an average of 1771±279.4 eggs.

Keywords: Reproductive biology - Yellow Spotted Puffer fish - *Torquigener flavimaculosus* - Tobruk shoreline - eastern Libya - Mediterranean Sea.

1- Introduction

The Suez Canal is largely to blame in the abundance of alien species in the Eastern Mediterranean today [14]. This is because the canal, which was constructed by humans, links the Mediterranean Sea, which is colder, with the Red Sea, which is warmer. Reports of exotic species in the Eastern Mediterranean have increased by 40% in the last decade [26]. Around the world, puffer fish come in about 200 varieties [13]. The *Torquigener* [25] group includes 29 of these species, all of which have Indo-Pacific origins.

The yellow spotted puffer is a species of puffer fish found in the western Indian Ocean [18]. This fish inhabits regions as far south as Madagascar and as far north as the Persian Gulf and the Red Sea. At depths of 3 to 57 meters, it is typically found close to coral reefs. It can reach a 13.0 cm then consumes small aquatic life, such as marine invertebrates. The yellow-spotted puffer's body can swell when it senses danger, and it has a spherical head with a blunt nose and two big, beak-like teeth in each jaw. Its side fins are broad at the sides, and it has tiny fins on the rear and close to the tail. It was initially found in 1987 in Israel's Haifa Bay [16]. Later, it was found in Egyptian coast [12], Syria, Greece, Turkey, and Libyan coast [2]. *Torquigener flavimaculosus* is actually the same species as *T. hyselogeneion*, according to recent studies on its genes and appearance [6]. Tetrodotoxin (TTX), a poison that is lethal to humans, is present in extremely high concentrations in *T. flavimaculosus*. According to [4], pufferfish with TTX levels more than 2.2 µg/g are deemed unsafe to consume, and *T. flavimaculosus* had TTX levels beyond this threshold is deadly to humans. According to [21], the fish's body is abundant in toxins, with over 15 µg/g found in its muscles, 5 µg/g in its reproductive organs, 12 µg/g in its intestines, 7 µg/g in its liver, and 35 µg/g in its skin. It is an extremely dangerous invasive species as a result. The majority of research in the Mediterranean focuses on various aspects of this fish, including its geographic range [15]; [3], Population studies [22], size and weight [11], diet and place in the food chain [8], development [7]; [23], and toxin levels [21]. New studies have been done to look at key biological features of the invasive fish **T. flavimaculosus** found in the Tobruk coast in the

Mediterranean, Eastern Libya. My research aims to learn about the fish's breeding season, the sex ratio, the size when they start reproducing, and how many eggs they lay (fecundity). This information is important to better understand this harmful invasive species and its population in the region.

2. Materials and Methods

Samples were gathered on Brubeck's Mediterranean Sea beach. which is situated in eastern Libya at 32° 4′ 40.8″ N, 23° 58′ 58.8″ E. Samples were taken between January and December of 2022 (Fig. 1). The study included 161 *Torquigener flavimaculosus* fish. Each fish's total length (TL) and weight (gm) were measured in centimeters.

Sex ratio

The monthly percentage of ♂ to ♀ (M:F) was used to compute the sex ratio.

Gonado- somatic index (GSI)

The following equation, which was supplied by [10], the gonado-somatic index (GSI) was determined:

$$\mathbf{GSI = 100 \times (G / W)}$$

In this case, (W) is the entire body weight, and (G) is the weight of the gonads.

First sexual maturity length (L_{50})

Mature individuals to their mid-length, the length at which 50% of **Torquigener flavimaculosus** reach sexual maturity was determined using the methodology described by [19].

Fecundity

Fecundity refers to the number of eggs can lay during the breeding season, about 23 *Torquigener flavimaculosus* fish were carefully collected. Their ovaries were cleaned, weighed, then stored in 4% formalin solution. The samples were extracted from each ovary and weighed, with an accuracy of 0.01 grams. A microscope with a 16x magnification was

used to count the eggs in each sample, which was put in a small container with a small amount of distilled water. Using the procedure outlined by [3], Absolute fecundity was determined as follows:

A F is calculated as (the number of eggs in a sample × ovaries weight) / specimen weight

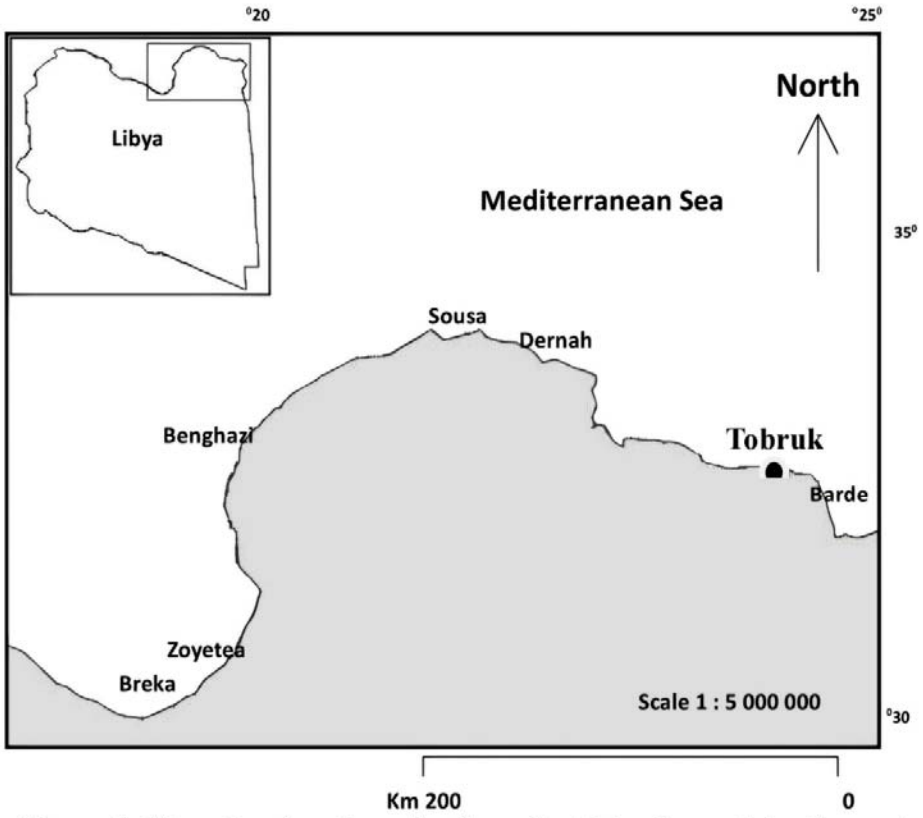


Figure 1. Map showing the collection site, Tobrok coast in the eastern Libyan Mediterranean coast

3. Results and Discussion

Sex ratio:

70 males, or 43.5% of the 161 *Torquigener flavimaculosus* specimens that were examined for sex, and 91 females, or 56.5% of the total, were found. This results in a ♂ to ♀ ratio of sexes of 1.30 to 1. Females were always more prevalent than males along the Tobruk shoreline from January to December 2022. One man for every 1.30 females was the general sex ratio (see Table 1). This difference was not significant when compared to a 1:1 ratio,

according to a statistical test ($\chi 2$) ($P>0.05$). In this study, the highest percentage of females was seen in January (60.0%), February (62.5%), May to October (62.5%), and December (60.0%). This outcome is consistent with that of [7], who discovered that in samples taken from the Turkey's southeast coast, ratio of sexes of **T. flavimaculosus** was 37.5% male and 62.5% female. These findings also align with those of [23] in Egypt. The trend of having more females than males has been observed in many previous studies on puffer fish. [1] discovered that the male : female ratio for **L. sceleratus** in Mediterranean populations in Egypt was 1:1.27, favoring females. This lack of difference might be due to changes in how many males and females are available for fishing, or because males and females live in different areas or have different eating habits [20].

Table 1. Monthly changes in the *Torquigener flavimaculosus* sex ratio from January to December 2022 at the Tobruk shoreline in eastern Libya.

		Males		Females		
Months	**No. of fish**	**No.**	**%**	**No.**	**%**	**Sex ratio**
Jan. (2022)	**10**	4	40.0	6	60.0	**1 : 1.51**
Feb.	**8**	3	37.5	5	62.5	**1 : 1.66**
Mar.	**11**	5	45.5	6	54.5	**1 : 1.20**
Apr.	**12**	5	41.7	7	58.3	**1 : 1.40**
May	**8**	3	37.5	5	62.5	**1 : 1.67**
Jun.	**20**	9	45.0	11	55.0	**1 : 1.22**
Jul.	**26**	12	46.2	14	53.8	**1 : 1.17**
Aug.	**32**	15	46.9	17	53.1	**1 : 1.13**
Sep.	**7**	3	42.9	4	57.1	**1 : 1.33**

Oct.	**8**	3	37.5	5	62.5	**1 : 1.67**
Nov.	**9**	4	44.4	5	55.6	**1 : 1.25**
Dec.	**10**	4	40.0	6	60.0	**1 : 1.50**
Total	**161**	**70**	**43.5**	**91**	**56.5**	**1 : 1.30**

Length of first sexual development (L_{50}):

The time of sexual maturity, or L_{50}. It helps decide the best net size and the smallest legal catch size needed to protect the breeding population and ensure that mature fish can spawn at least once. As seen in Figures 2 and 3, female *Torquigener flavimaculosus* reach sexual maturity at a size of 7.5–8.4 cm, with 49.9% mature at this stage. Males reach maturity at a slightly larger size of 8.5–9.4 cm, with 52.7% mature. In the current study the size at which 50% of the fish reach spawning is approximately 7.95 cm for ♀ and 8.51 cm for ♂. My study found that size when puffer fish first become sexually mature varies slightly from other research, but both sexes reach maturity in their first year. These findings are similar to earlier studies on puffer fish [24]and [23]. [7] noted that in Iskenderun Bay, in the North-eastern Mediterranean, females reached maturity at 7.8 cm and males at 8.3 cm, which happens in their first or second year of life. [23] reported that in Suez, Egypt, female *Torquigener flavimaculosus* reached maturity at 8.2 cm, while males reached maturity at 9.5 cm.

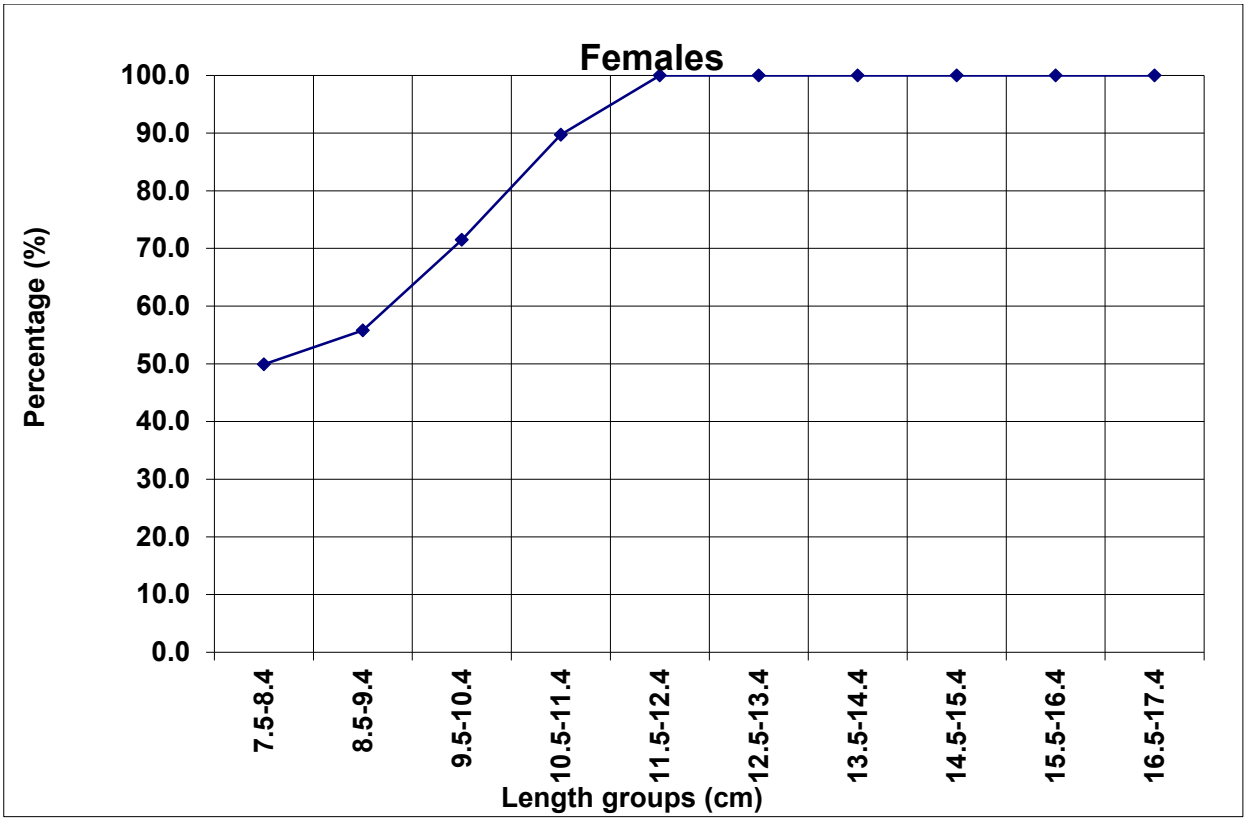


Figure 2. shows the length of female *Torquigener flavimaculosus* from the Tobruk shore in eastern Libya at the time of first sexual maturity between January and December 2022.

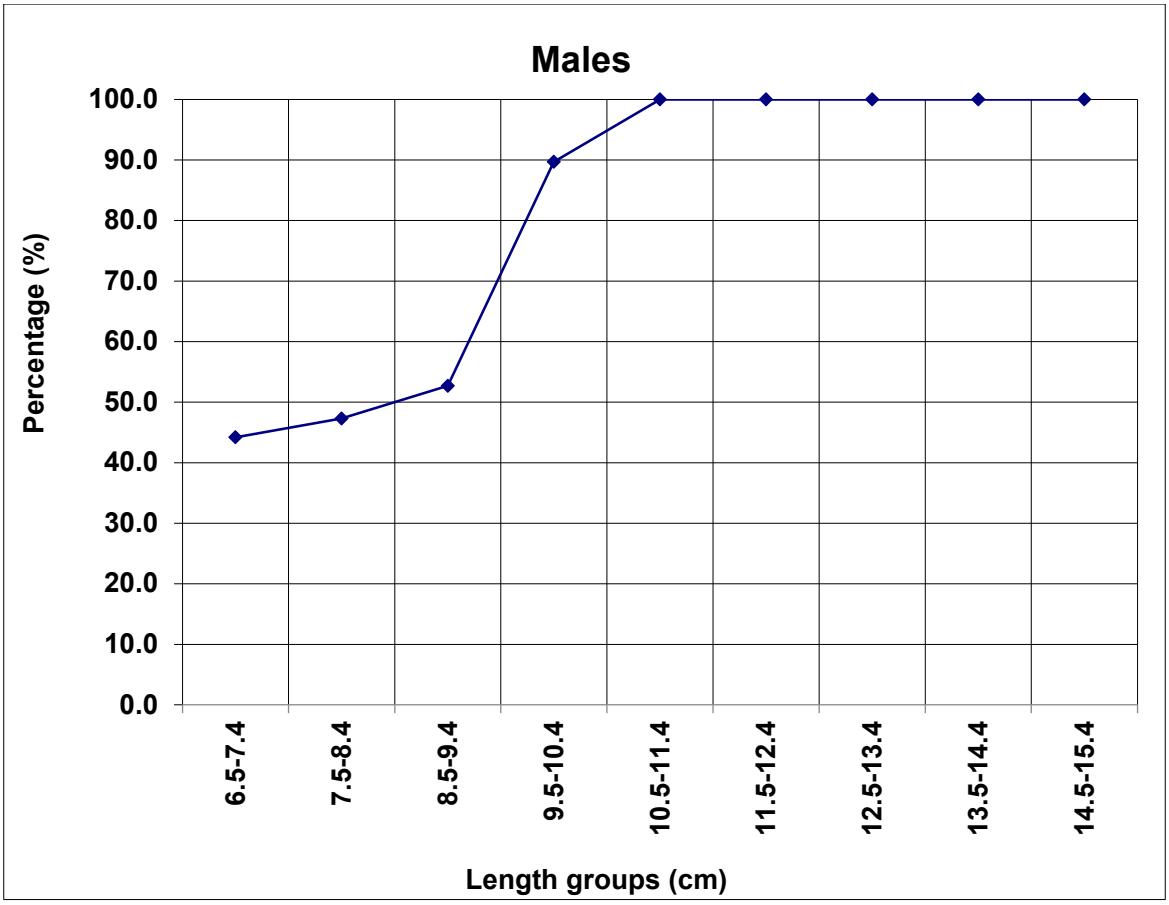
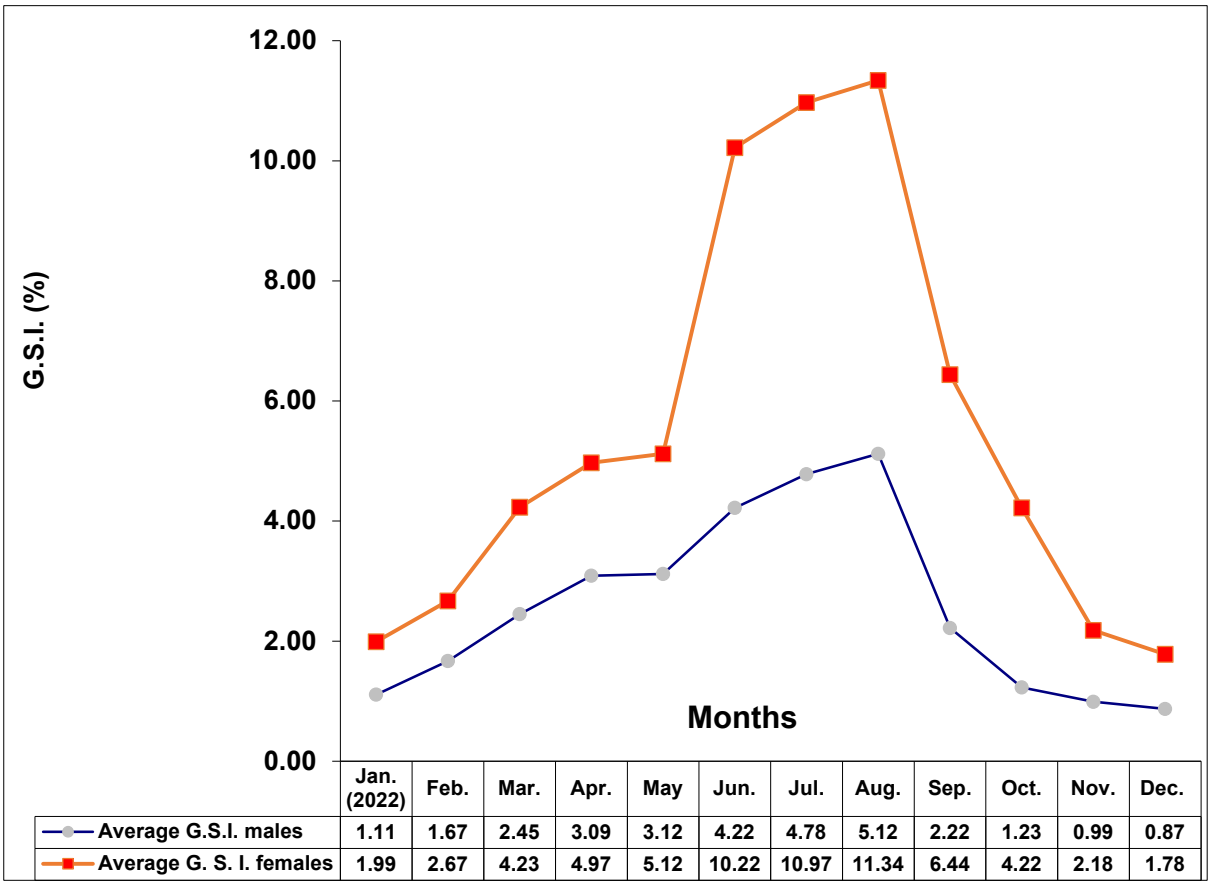


Figure 3. shows the length of male *Torquigener flavimaculosus* from the Tobruk shore in eastern Libya at the time of first sexual maturity between January and December 2022.

Gonado-somatic index (GSI):

For the fish species **Torquigener flavimaculosus**, the (G.S.I.) reached its highest point in June (4.22 ♂ and 10.22 for ♀), July (4.78 for males and 10.97 for females), and August (5.12 for males and 11.34 for females). This shows that the spawning season happens during the summer. After this period, the GSI dropped to its lowest values in December, with males at 0.87 and females at 1.78 (see Fig. 4). The study's **T. flavimaculosus** reproductive cycle is similar to that of other tetraodontidae species, the majority of which also reproduce in the summer months [17] and [23]. The GSI values for females in this study were greater than

those for males. Therefore, females typically have a greater gonado-somatic index (GSI) than ♂, in summer reproduction season. According [23], there is a strong inverse link between GSI and the HSI (hepato-somatic index), with the former having very high values in the summer. This implies that gonad development and spawning depend on the fat that is stored in the liver.



	Jan. (2022)	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.
Average G.S.I. males	1.11	1.67	2.45	3.09	3.12	4.22	4.78	5.12	2.22	1.23	0.99	0.87
Average G. S. I. females	1.99	2.67	4.23	4.97	5.12	10.22	10.97	11.34	6.44	4.22	2.18	1.78

Figure 4. From January to December 2022, the gonado-somatic index of male and female Torquigener flavimaculosus from the Tobruk coast in eastern Libya varied monthly.

Fecundity:

Fisheries, population studies, and the food supply all depend on knowing how many eggs a fish can produce. The most developed eggs from mature fish were counted in order to estimate the quantity of eggs (fecundity) in this study. Samples were collected during the

spawning season to measure fecundity. To estimate the absolute fecundity for the species **Torquigener flavimaculosus**, we looked at 25 adult female fish that ranged in length from 7.5 to 17.4 cm. These females were divided into groups based on their size, with each group covering a 0.9 cm range. The results showed that as the fish grew longer, they produced more eggs (see Table 2). The average AF per fish from 844 to 3,214 for fish measuring 7.5 to 17.4 cm, with an overall average of 1,771 ± 279.4 eggs per fish. This result is very close to what [7] found. They reported that for Yellow Spotted Puffers (*Torquigener flavimaculosus*) in Turkish coast the average fecundity was 1967±324 for fish ranging in length from 8.1 to 16.4 cm.

Table (2). From January to December 2022, female *Torquigener flavimaculosus* from the Tobruk beach in eastern Libya were measured for fecundity and total length of body (cm).

Total length (cm)		Absolute Fecundity			
Range	**Average**	**No.**	**Minimum**	**Maximum**	**Average**
7.5-8.4	**7.9**	**2**	**844**	**911**	**878±47.4**
8.5-9.4	**8.1**	**2**	**1078**	**1089**	**1085±7.8**
9.5-10.4	**9.9**	**3**	**1197**	**1234**	**1216±26.2**
10.5-11.4	**11.1**	**2**	**1221**	**1267**	**1248±32.5**
11.5-12.4	**12.1**	**2**	**1458**	**1489**	**1474±21.9**
12.5-13.4	**12.9**	**3**	**1488**	**1564**	**1529±53.7**
13.5-14.4	**13.9**	**4**	**1612**	**2389**	**2100±549.4**
14.5-15.4	15.1	**3**	**1978**	**2879**	**2546±637.1**
15.5-16.4	15.9	**2**	**1999**	**2923**	**2661±653.4**
16.5-17.4	**16.9**	**2**	**2135**	**3214**	**2974±762.9**
Average		**25**			**1771±279.4**

Conclusion

The gonado-somatic index, which gauges reproductive activity, the fish size reach Mature sexuality, the ratio of males to females, and the fecundity (number of eggs produced) are among the important data it contains. This information is essential for determining the species' population size and comprehending how it reproduces. When and how many new fish enter the population can be predicted with the use of fecundity.

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Conflicts of Interest

Declare conflicts of interest or state the "Authors declare no conflicts of interest". Authors must identify and declare any personal circumstances or interest that may perceived as in appropriately influencing the representation or interpretation of reported research results.

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