African Journal of Advanced Pure and Applied Sciences (AJAPAS)
Online ISSN: 2957-644X
Volume 3, Issue 1, January-March 2024, Page No: 282-291
Website: https://aaasjournals.com/index.php/ajapas/index
معامل التأثير العربي 2023: (1.55) SJIFactor 2023: 5.689 ISI 2022-2023: 0.557

Sexual dimorphism and regional variations in size and reproduction of Cuttlefish, *Sepia officinalis* L. 1758 (Sepiida, Coleoidea) in Libyan waters

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Received: January 19, 2024	Accepted: March 24, 2024	Published: March 26, 2024

Abstract:
This study presents a comprehensive investigation of various population dynamics and reproductive parameters in Sepia officinalis in the coastal waters of Libya, Utilized a dataset of 1171 individuals. This study focuses on potential differences in sex-based and regional variations, between Tripoli and Benghazi subpopulations. The result confirmed sexual dimorphism, in smaller males. The sex ratio is nearly balanced. Most individuals exhibit hypo-allometric growth, their body weight increases at a slower rate than their mantel length. The spawning season for S. officinalis in Libyan waters spans from March to September, reaching its peak during the warmer months of July and August. Females displayed a continuous cycle of ovarian development, with peak ripeness observed throughout the year. Males exhibited a more seasonal pattern, with ripe testes dominating during the warmer months of May to September. The estimated size at first maturity (L50) differed slightly between sexes; females matured earlier and at a slightly smaller 8.42 cm compared to males overall. The study revealed regional variations in size at maturity; The pattern in Tripoli subpopulation deviated from this trend, females matured earlier, reaching a size of 9.06 cm compared to males, who matured at 10.12 cm. Conversely, Benghazi females take their time to reach maturity at a smaller size of 8.32 cm, while Benghazi males matured earlier and were smaller at 6.78 cm.

Keywords: Sepia officinalis, dimorphism, Libyan waters, Benghazi, Tripoli.

Cite this article as: H. M. Ghmati, H. A. Ensair, A. F. Kara, M. M. Elhejaji, A. S. Abid, H. A. Al-Maghbon, N. S. Altrabulsi, and W. K. Saadawi, "Sexual dimorphism and regional variations in size and reproduction of Cuttlefish, *Sepia officinalis* L. 1758 (Sepiida, Coleoidea) in Libyan waters," *African Journal of Advanced Pure and Applied Sciences (AJAPAS)*, vol. 3, no. 1, pp. 282–291, January-March 2024.

إزدواج الشكل الجنسي والاختلافات الإقليمية في الحجم والتكاثر في حبار السيبيا في المياه الليبية

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الملخص
هذه الدراسة تقدم تحقيقًا شاملاً للديناميكيات السكانية المختلفة و معايير التكاثر لـ *Sepia officinalis* في المياه الساحلية الليبية، باستخدام مجموعة بيانات تتكون من 1171 فردًا. تركز هذه الدراسة على الاختلافات المحتملة حسب الجنس والمنطقة، بين التجمعات السكنية في طرابلس وبنغازي. أكدت النتائج ازدواج الشكل الجنسي، مع وجود ذكور أصغر حجماً. النسبة بين الجنسين متوازنة تقريبًا. تظهر معظم الافراد نموًا اقل في القياس، حيث يزداد وزن الجسم بمعدل أبطا من طول الرداء. يمتد موسم التكاثر لـ *S.officinalis* في المياه الليبية من مارس إلي سبتمبر ويصل ذروته خلال الاشهر الاكثر دفئاً في يوليو و اغسطس. أظهرت الاناث دورة مستمرة من تطور المبايض، مع ملاحظة ذروة النضج على مدار العام. أظهرت الذكور نمطاً موسمياً أكثر، حيث تهيمن الخصي الناضجة طيلت الاشهر الاكثر دفئا من مايو وحتى سبتمبر. يختلف الحجم المقدر للنضج عند (L50) قليلاً بين الجنسين؛ تصل الاناث لمرحلة النضج في وقت مبكر، وبحجم أصغر قليلا يبلغ 8.42 سم مقارنة بالذكور بشكل عام. كشفت الدراسة عن اختلافات إقليمية في حجم النضج؛ اختلف النمط في طرابلس عن النمط العام حيث نضجت الاناث في وقت مبكر ووصلن لحجم 9.06 سم مقارنة بالذكور الذين نضجوا عند 10.12 سم وعلى العكس من ذلك تأخذ أناث بنغازي وقت للنضج بحجم أصغر يبلغ 8.32 سم، بينما ينضج ذكور بنغازي في وقت مبكر وكانوا اصغرا حجماً عند 6.78 سم.

الكلمات المفتاحية: حبار السيبيا، ازدواجية الشكل، بنغازي، طرابلس.

Introduction

Coleoidea is a subclass of Cephalopoda that includes all of the major groups of soft-bodied Cephalopoda such as octopus, squid and cuttlefish. The common Cuttlefish, Sepia officinalis are found across the eastern Atlantic Ocean from the North Sea all the way to the Cape of Hope. It is also widely distributed in the Mediterranean Sea [1]. *S. officinalis* is a nectobenthic animal that typically inhabits the upper slopes of continental shelf waters up to a maximum depth of 1000 meters [2][3]. The species lives primarily on sandy and muddy bottoms [4]. *S. officinalis* is characterized by rapid growth and a short lifespan, they live for about two years during their cycle [5], and their population management depends on spawning and recruitment [6] *S. officinalis* is gonochoristic with one or two annual cycles of reproductive, the time of breeding season varies depending on location [7]. Worm water such as the Mediterranean Sea exhibit year-round spawning in *S. officinalis*, with pecks in early spring and late summer [5]. Also S. officinalis spawning behavior demonstrates a link between body size-related patterns, with individuals migrating inshore for breeding first [4]. A large number of adults died all at once after spawning season [8]. *S. officinalis* does not provide any parental care for their young, after mating, the female lays her eggs in the nest on the seabed and leaves them to fend for themselves, *S. officinalis* are predators, and predatory behavior is driven by visual cues [9]. In addition to being an important fishery resource, they also serve an ecological role both as predators and prey [10], A wide variety of fishing gear can be used to catch *S. officinalis* [11]. However, its short lifespan and variable growth rates make its populations volatile and vulnerable to overfishing. This study sheds light on the reproductive variations of *S. officinalis* off east and west coasts of Libya. It highlights the complex interplay of environmental factors, sex, and other influences that shape the lives of these creatures.

Material and methods

S. officinalis was sampled from commercial landings in two regions of the Libyan coast: Tripoli (Western regions) and Benghazi (Eastern regions) (Figure.1). Sampling was conducted monthly for a period one year, between October 2009 and October 2010.

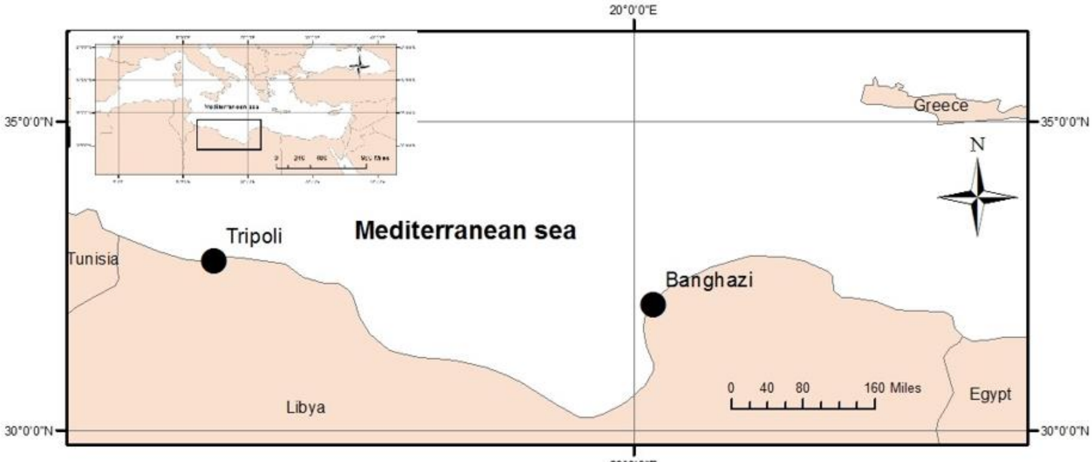

Figure 1: Map of Libya cost showing area monitoring in the text.

The collected *S. officinalis* was maintained in a fresh state until their arrival at the laboratories of the Marine Biology Research Centre (MBRC). The body weights (BW) and dorsal mantle lengths (DML) for all were measured. After dissection, the wet weight of the gonad (GW) for all individuals and the wet weight of the nidamental glands for females were measured. The Kolmogorov-Smirnov test (KS) was employed to statistical compare the mean lengths between male and female *S. officinalis*, with the combined data from both sexes being analyzed. Chi-square tests (0.05) were conducted to assess where the observed sex ratios deviated significantly from a 1:1. A scale established by [12] was utilized in an attempt to ascertain the maturity stage of each individual *S. officinalis* (Table 1). All measurements were within 0.1 mm, and weights determined to within 0.01 g.

Table 1 Gonad maturity scale used for *S. officinalis*

Stage	Males	Females
I	Gonad is very small and difficult to find	Gonad is very small and difficult to find
II	Gonad translucent or whitish	Gonad is translucent or whitish, with no structure visible. Nidamental glands enlarged. Accessory nidamental glands appear white
III	The gonad appears enlarged. No particles visible in Needham's sac	Gonad is no longer translucent, and some structures are visible. Nidamental glands further enlarged. Accessory nidamental glands appear beige-yellow
IV	Gonad large. Spermatophores visible in Needham's sac	The gonad is large and many eggs are visible but may be compressed together in the proximal part of the oviduct. There may be different egg stages in the distal part of the oviduct. Accessory nidamental glands appear orange
V	Spermatophores in conduct. Few or no spermatophores visible in Needham's sac	As above but almost all eggs are large (4±8 mm), most in the proximal part of the oviduct. Accessory nidamental glands may appear red
VI	Spent	Spent

Based on the whole weight (W) in grams and the DML in cm, a least squares regression analysis [13] was used to estimate constants a and b.

$W = aDML^b$

An index of sexual development was calculated using the gonadosomatic index (GSI), which is defined as

$$GSI(\%) = \frac{gonad\ mass(g)}{\text{cuttlefish}\ mass(g) - gonad\ mass\ (g)} \times 100$$

Females and males' length at 50% of cuttlefish were mature was determined by using a two-parameter logistic ogive, with gonad stages III and IV considered to be mature, cuttlefish per 5 cm size class.

$$Propotion\ mature = \frac{1}{1 + e^{\frac{-F_i - L_{50}}{\delta}}}$$

Where F_i the size-class and L_{50} is the DML (mm) at which 50% of females are mature and δ is the width of ogive. by minimizing the sum of squares of discrepancies between the observed and estimated values to estimate L_{50} and with δ created for use with Microsoft Excel's solver routine, the ogive was fitted using an iterative technique (Microsoft Corporation 2000).

Results and discussion

Length distribution

Analyses of 1171 *S. officinalis* revealed a significant size difference between males and females (Figure. 2). Mantle length values ranged from 6 to 21 cm in females (n = 481) and 4 to 23cm in males (n = 690) The largest males reached 22.1cm and 1029.4 g, while the largest females were 20.4 cm and 948.6 g (KS =0.108, p = 0.003). This pattern held true within both the Tripoli (n = 570) and Benghazi (n = 601) subpopulations. In Tripoli, males had a wider size range (4.9 to 22.9 cm) compared to females (6 to 21 cm), with the largest individual again exceeding females (males 22.1 cm, 1029.4g; females: 20.4 cm, 948.6 g; KS =0.217, p<0.0001). Benghazi also displayed a significant male size advantage, though with a smaller effect size (males: 6 to 20 cm, 22.1, 1029.4g; females: 6 to 21 cm, 20.2, 672.3g; KS =0.078, p = 0.033).

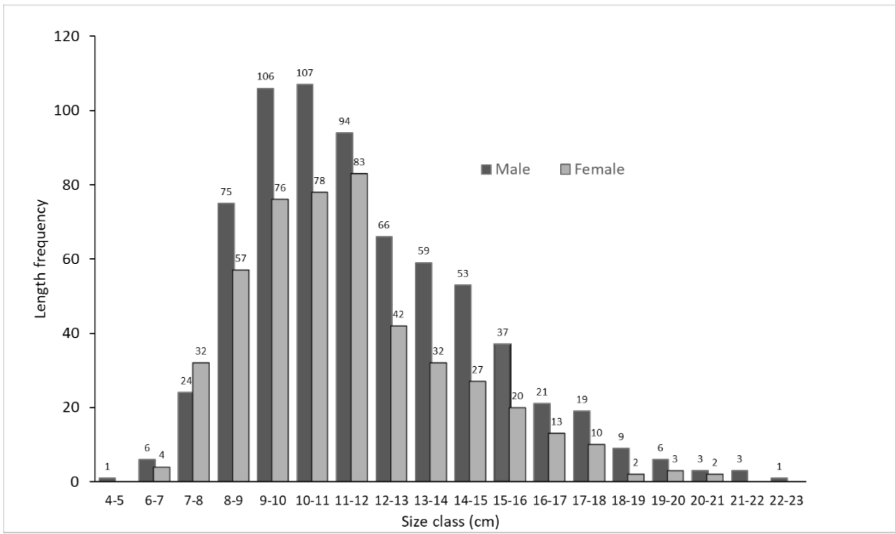

Figure 2: The number of males and females of Sepia officinalis sampled in the various size classes.

Our study reveals a clear pattern of sexual size dimorphism in *S. officinalis*, with females attaining significantly larger sizes than males. Notably, the effect size of this difference is greater in the Tripoli subpopulation compared with Benghazi. This pattern of female's size advantage aligns with reports from most cuttlefish, like *S. elegans* and *S. orbignyana* among others [14]. However, this pattern is universal within the Sepiidae family [15].

Sex Ratio

Analysis of the data revealed that the overall ratio of male to female *S. officinalis* (1.43:1), (1:1) (X^2=37.3, df = 1, p >1.01). Closely matched the expected sex ratio of 1:1. Further investigation in both Tripoli (1:0.68) and Benghazi (1:0.71) confirmed this observation, finding no significant deviation from the expected ratio in either location. (1:1) (X^2= 20.4, df = 1, p >6.08) and (X^2= 16.9, df = 1, p >3.7) respectively. Our findings indicate a near-balanced sex distribution within the studied *S. officinalis* populations. This result contrasts with the observation of [16], who reported a female-biased sex ratio (M:F) of 0.5:1 in Benghazi and a male-biased ratio of 1.4:1 in the Sousa area, both on the Libyan eastern coast. Globally, on the western coast of Algeri, a female-biased ratio (0.7:1) was observed [17]. In Turkey, [4] documented females' dominances, while [18] observed males' dominances in Izmir. Similarly, on Mallorca Island in the western Mediterranean, [19] reported a male-biased sex ratio. Our study detected a slightly higher number of males in Benghazi and Tripoli, possibly hinting at reproductive migration patterns; males may travel farther in search of mates, impacting the observed sex ratio in specific areas and seasons. [20] found males to be highly mobile during spawning, traversing various spawning areas, in contrast to territorial females who stayed within specific breeding gonads. These contrasting movement patterns, coupled with the mesh size differences of the two fishing gears employed, likely explain the observed disparities in size, maturity stage, and sex ratio of captured individuals [21].

The mantle length-body weight relationship for Sepia officinalis

S. officinalis females exhibited a substantial range in body mass, spanning from 41.4 to 984.3 g, offering a glimpse into their diverse size; in contrast, males displayed the potential for even greater heft, reaching weights between 19.8 and 1084.9 g. To further explore size variation, length-weigh relationships were established for the entire dataset and for each population separately. For overall population, weights were estimated by:

$W= 0.2727xDML^{2.6847}$; n=1171, r^2=0.970.

Similar relationships were formulated for the Tripoli (W_T) and Benghazi (W_B)

$W_T = 0.24xDML^{2.7478;}$ n=570, r^2= 0.978

$W_B = 0.28x\ DML^{2.666;}$ n=601, r^2= 0.969

The slopes of these formulas, representing growth patterns, differed significantly between regions ($p<0.05$). Figure 3 (a. b) showed fitted mantle length-mass relationships for *S. officinalis* in different regions broken by sex.

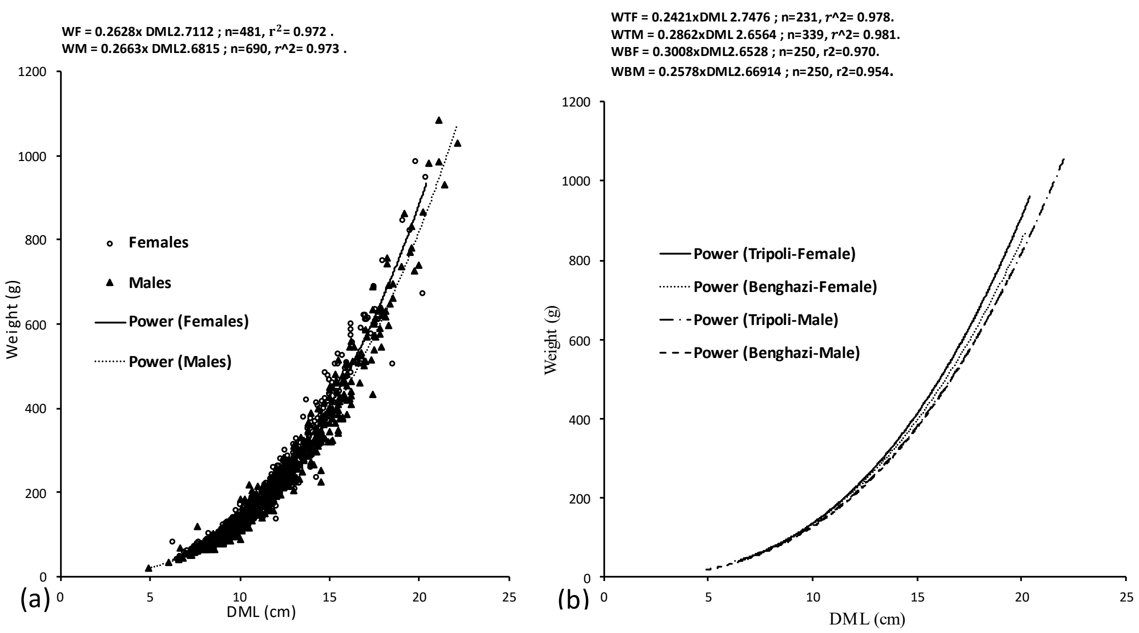

Figure 3: The regression and plot of weight vs mantle length of Sepia officinalis broken down by sex (a) and regions (b).

The present study corroborates the findings of previous Mediterranean investigations by demonstrating hypo-allometric growth in *S. officinalis* (Table 2). This implies that as *S. officinalis* individuals increase in size, their weight accrues at a progressively slower rate relative to their DML. The intercept parameter (a) tends towards higher values in the Western Mediterranean compared to the Eastern region. This discrepancy may be attributable to environmental factors such as food availability or temperature regimes. Furthermore, Table 2 highlights the presence of sexual dimorphism in the DML-weight relationship. Females exhibit higher slope values (b) compared to males, suggesting that they accumulate more weight relative to their size as they mature.

Table 2 Parameters of the relationship between DML and weight for *Sepia officinalis* off the East and West Libyan coasts and in other locations, according to different authors in the Mediterranean.

Author	Sex	a	B	N	Locality
[22]	Comb	0.22	2.77		Adriatic Sea
[23]	F M Comb	0.232 0.244 0.237	2.73 2.69 2.72	286 457 743	East-Adriatic Sea
[24]	Comb	0.343	2.55		North-Adriatic Sea
[25]	F M	0.31 0.51	2.81 2.69	505 444	Western- Mediterranean
[26]	F M Comb	0.35 0.46 0.39	2.61 2.49 2.55	597 461 1058	Algiers coast
[17]	F M Comb	0.44 0.46 0.43	2.50 2.45 2.49	253 353 581	western coast of Algeri

[16]	F M	0.39 0.33	2.50 2.54	66 32	Benghazi-Libya
[16]	F M	1.32 0.58	1.98 2.34	25 35	Sousa area-Libya
[27]	Comb	1.01	2.10 3	104	Ain El-ghazala Lagoon-Eastren Libya
This study	F M Comb	0.24 0.28 0.24	2.74 2.65 2.74	231 339 570	East- Libya coast (Tripoli)
This study	F M Comb	0.30 0.25 0.28	2.65 2.66 2.66	250 351 601	West- Libya coast (Benghazi)

Spawning season

S. officinalis Enjoys an extended spawning period, stretching from Marsh to September, However, the real party starts in the warmer months of July and Augus when the reproductive activity reaches its peck (2.39±0.20, 2.40±1.17) respectively. Individual GSIs ranged from 0.0034to 9.59. During the year, the majority of average GSI values for females tended to be higher than the males, GSI values for females ranged from 0.16±0.04 to 4.93±0.44 with peak in July, while the male's ranged between 0.48± 0.06 to 1.64± 0.08 with peak in August (Figure 4).

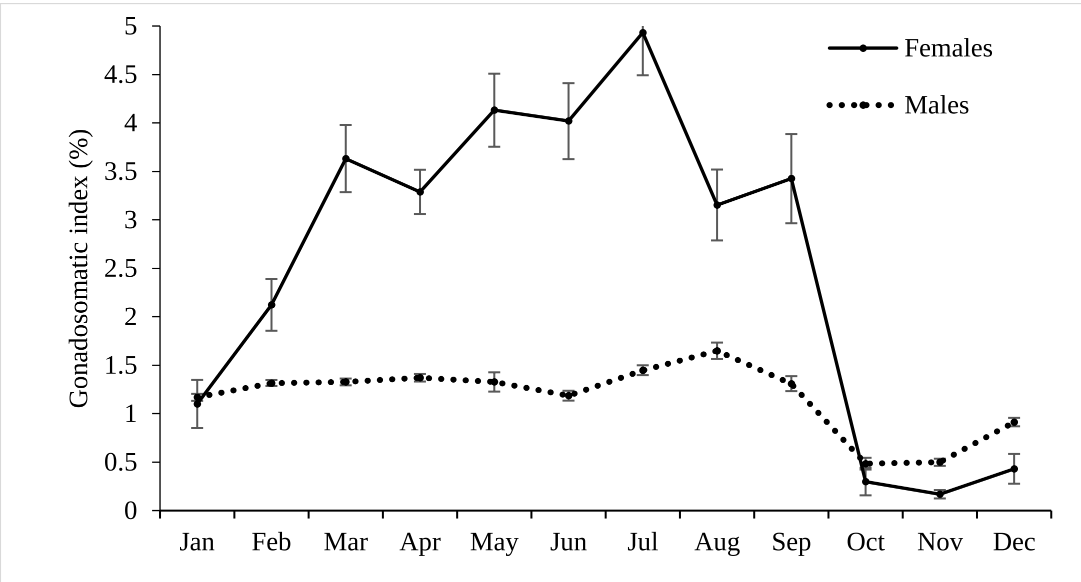

Figure 4: Temporal variation of gonadosomatic index of male and female *S. officinalis* from the Liban Coast. Error bars indicate one standard error.

From January until May, the females in Tripoli held the upper hand. Their GSI values consistently surpassed those of their Benghazi counterparts, this suggests a head start in eggs production for Tripoli females during the first half of the spawning season. While throughout the middle reproductive period of June to September GSI values for Benghazi females were dominant. On the other hand, at the end of the year, female GSI values were low in both areas mimicking the same pattern (Figure 5).

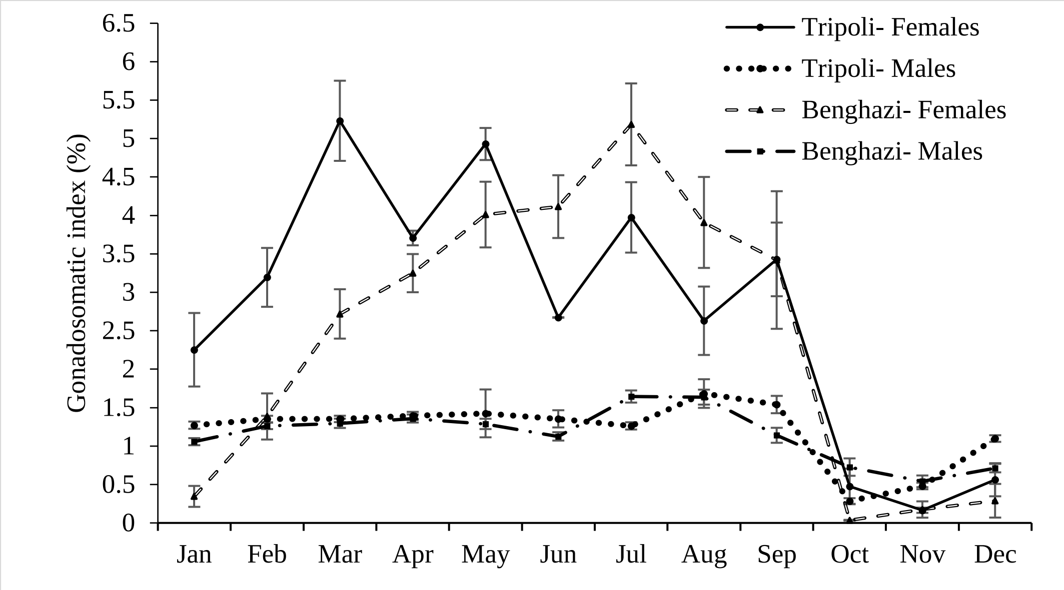

Figure 5: Monthly variation in the gonadosomatic index for females and males in Tripoli and Benghazi of *S. officinalis* Error bars indicate one standard error

The reproductive cycle of the common *S. officinalis* has been the subject of numerous studies due to its commercial significance and behavior. However, discrepancies exist regarding the timing and duration of its spawning season, particularly across geographical regions. The protracted spawning season aligns with the observed gonado-somatic index pattern in the current study, where GSI reaches its minimum from October to December, indicating a prolonged reproductive period with fluctuations in gonadal throughout the year. [26] reported a long period of spawning in the Algiers region from March to July, with a peak in June. Similarly, [19] identified a peak breeding period from March to June. Water temperature plays a crucial role in cephalopod reproduction [28] Tripoli experiences warmer temperatures earlier in the year compared to Benghazi. This could lead to earlier maturation and egg production in Tripoli females, explaining their higher GSI values in the first half of the season. The fish's mature ovaries are found throughout the year. In the months from March to July more than 50% of sampled females had ripe gonads. While the spent ovaries appear only in May at 8.69% indicating a brief period of recovery before the cycle starts again. The active stages dominated from December to February suggesting ongoing development eggs. The highest occurrence of immature or early maturing ovary in October and November at 76.6% and 67.4% respectively. (Figure 6).

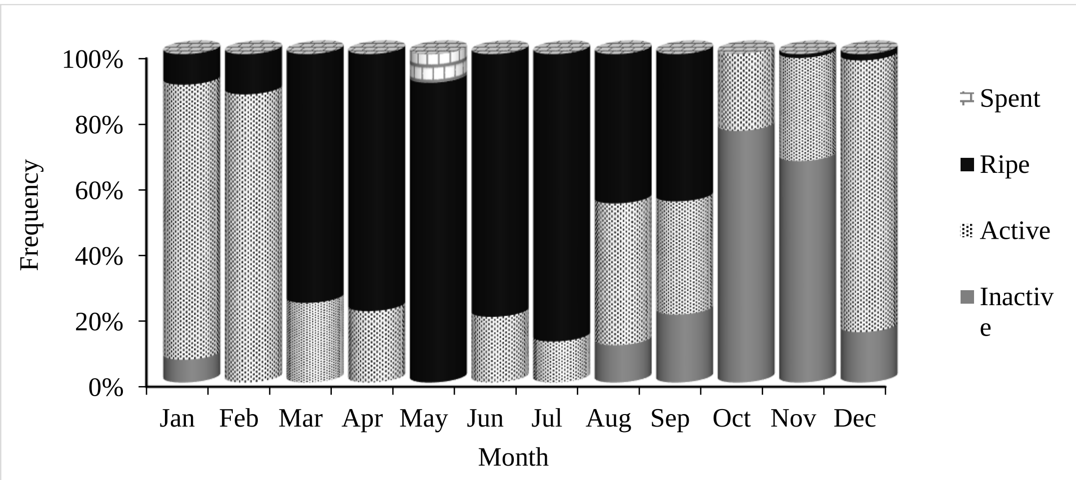

Figure 6: The monthly changes in the frequency of the different maturity stages of ovary of *S. officinalis*

Similer to females, males mature are also found throughout the year. No males with free sperm spent were encountered . The wormer months, May to September experience an increase in ripe testes, with July boasting the highest percentage at 82.8%. While inactive testes were present during August to December. Dominated in October at 53.7%. The majority of the active stages dominated in Marsh, April, November and December.(Figure 7)

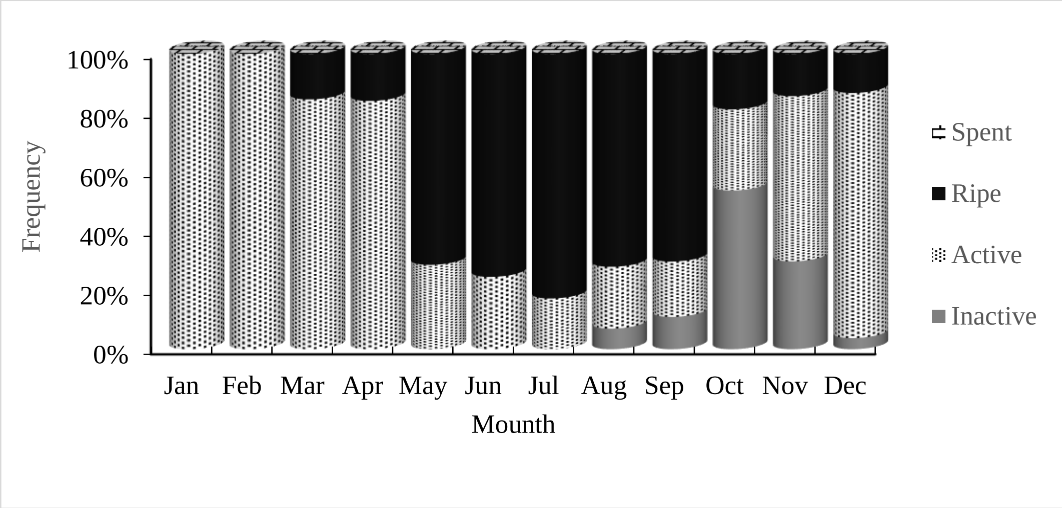

Figure 7: The monthly changes in the frequency of the different maturity stages of testis of *S. officinalis*

Size at maturity

The smallest mature females were 6.7cm DML (46.1g), while their males counterparts started maturing a bit later at 7.6cm DML (61.4g). But as they grow the females reach maturity at a large size of 9.07 cm DML (δ=0.89 cm) compared to 8.11 cm DML (δ=0.58cm) for males. Regardless of sex, the size-at-50% maturity was estimated for *S. officinalis* is around 8.42 cm DML (δ=1.00 cm) (Figure 8).

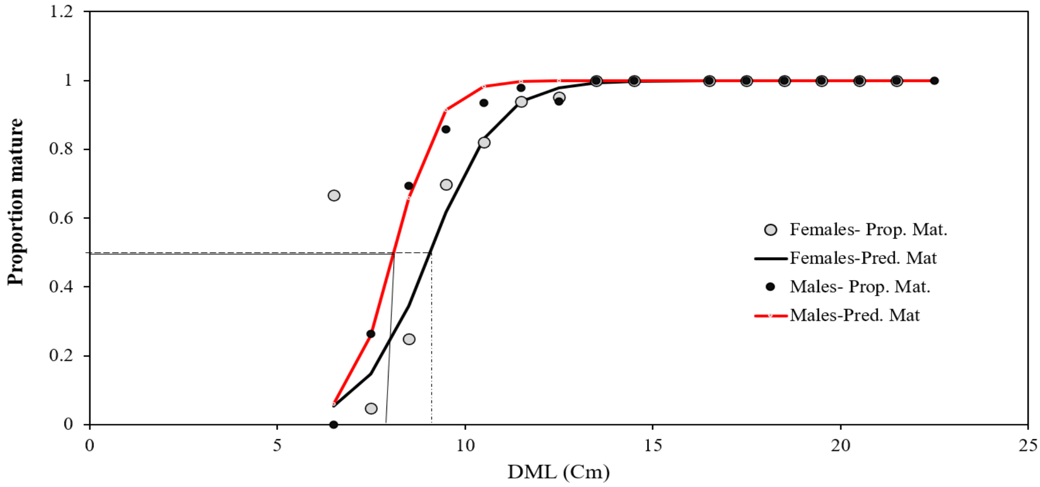

Figure 8: The proportion of *S. officinalis* that were mature in each 5Cm DML category and the fitted logistic curve for each sex.

In Tripoli, females reach maturity at a size of 9.06 cm DML (δ=1.13 cm), compared to their male counterparts, who hit the milestone at 10.12 cm DML (δ=0.83cm). This might seem counterintuitive, but it suggests that females prioritize early maturity and egg production, even at a slightly smaller size. In Benghazi, the females take their time, reaching maturity at a larger 8.32 cm DML (δ=1.45 cm), while the males mature earlier and smaller, at just 6.78 cm DML (δ=1.12cm). Tripoli females might be able to reproduce earlier and more frequently through their lifespan, even if their initial clutch size is similar to that of Benghazi female's. This hints at a different strategy, with males prioritizing early competition for mates, even at a smaller size. (Figure 9)

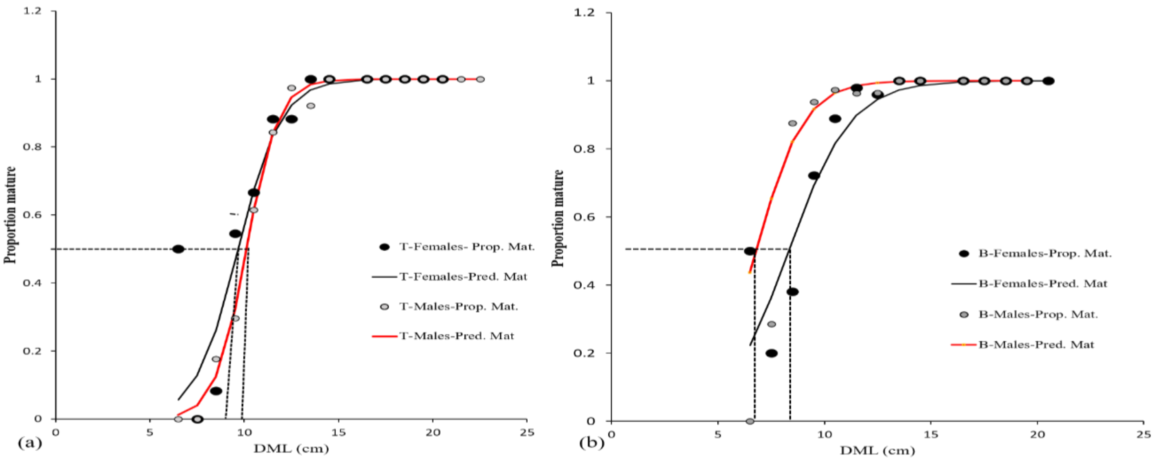

Figure 9:(a,b) The proportion of *S. officinalis* that were mature in each 5Cm DML category and the fitted logistic curve for each sex per area, a:Tripoli, b: Benghazi.

Our results in Libyan waters indicate that males mature slightly later than females with smaller minimum size, which could be explained by different reproduction strategies; in contrast, females reach a large maximum size at maturity compared to males, which is intriguing this could be explained by energy constraints during egg development. Males in both regions mature at a smaller size than females. This aligns with their role as sperm producers, where early maturation can increase mating opportunities even with limited energy investment in body size, our observation agrees with the concept of sexual size dimorphism in cephalopods, where resources allocation during reproduction leads to divergent growth patterns [29], the observed smaller size of males at maturity, compared to females, is consistent with the emphasis on early maturation in many cephalopods' species [30]. Also [31] reported that egg development in cephalopods is energetically demanding, potentially limiting females' size and growth. [32] shows the trade-off between the clutch size, egg size and female's size. In our study this, the trade-off may be manifested in larger size of females, which allows them to invest more resources in egg production while maintaining sufficient body size for survival and parental care.

Conclusion

This study investigated key biological parameters of *S. officinalis* in Libyan waters. Significant sexual size dimorphism was observed, with females attaining larger sizes. The overall sex ratio suggested near- balanced distribution, contrasting with previous reports. Hypo-allometric growth was observed, with regional differences in the length- weight relationship between sexes. The spawning season extended from Marsh to September, with females generally exhibiting higher GSI values. Females matured slightly earlier and at smaller size than males overall, but this trend reversed in the Tripoli subpopulation. This study contributes to understanding *S. officinalis* population dynamic and reproductive strategies, highlighting potential regional variations and differences from previous reports.

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