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Research Article
Reproductive Biology of the Horned Octopus *Eledone cirrhosa* (Lamarck, 1798) from the Mediterranean Sea off Alexandria, Egypt

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Abstract

Background and Objective: The fishery resources play a leading role in the economy of coastal countries. Abundant pelagic and valuable demersal resources represent the country's main source of employment. This work selected the horned octopus *Eledone cirrhosa* from the Mediterranean Sea off Alexandria, Egypt to study sexual maturity indices, the histological study of the gonads of both sexes and measurement of six heavy metals in the seawater and the gonads. **Materials and Methods:** Sex ratio, GSI (Gonado-Somatic Index), RGS (Record Gonado-Somatic), VNI (Vas deferens Needham's sac Index), OGI (Oviduct Gland Index) and HI (Hayashi index) are measured monthly and their fluctuations are commented through UNIANOVA (ANCOVA) analysis; (METHOD=SSTYPE(3) and COVARIANCE P. **Results:** Males mature earlier than females. Seminiferous tubules show a centripetal sequence of spermatogonia, spermatocytes I, spermatocytes II, spermatids and spermatozoa. The histological picture of ovarian lobules, oocytes and accessory glands are commented. Both morphometric parameters and histological overviews highlight two periods of spawning: spring to summer spawning and fall spawning which is less intense. Males reached maturity in June to January, while spermiogenesis was intense until the moment of copulation. Vitellogenesis begins in November and extends to the next laying period. Gonadal development in females is non-synchronous and later than in males, about eight months for females and four months for males. Six metal elements were measured by spectrometry of Atomic Emission: Manganese, Cadmium, Iron, copper, zinc and lead and Measurements. **Conclusion:** Metal bioaccumulation in gonads affect the process of gametogenesis in Abu Qir bay where samples are less viable and fertile than those of Ras el Tin beach.

Key words: Sexual maturity indices, ANCOVA, seminiferous tubules, ovarian lobules, spawning, metal bioaccumulation

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Octopus of the genus *Eledone* encompasses small to medium-sized benthic species which inhabit sandy and rocky substrates up to 300 m deep. Among the most economically important species are *E. moschata, E. massyae* and *E. cirrhosa*. The *E. cirrhosa* is an exclusively benthic species, which lives in the depths of 10 to 50 m[1,2]. It is an abundant species in the Mediterranean Sea of Egypt, Arabian Gulf, Red Sea, the Aegean Sea, Northern Chilean waters, the Atlantic Ocean, the Liguria Sea, Indian Ocean, English Channel, Southern Celtic Sea-Bay of Biscay, Galician waters, Portuguese waters, Central and Southern Tyrrhenian Sea, Sicilian Channel, Eastern Ionian Sea, Northern and Central Adriatic Sea, Northeastern Atlantic basins, Morocco to Norway, British Isles, the North Sea off Aberdeen, Iceland, the Gulf of Cadiz (SW Spain), the Gulf of Lions and the northwest of the Iberian Peninsula[3-5]. This entire biodiversity range of *E. cirrhosa* is fundamental for the balance and stability of the ecosystem in nature, boosting and diversifying the most different habitats for commercial use, such as agricultural, fishing, livestock and biotechnology. Octopuses of the same trophic level are functionally equivalent regardless of the identity of the species[6]. In the Catalonian Sea of the Western Mediterranean *E. cirrhosa* reproduces during spring and summertime with some animals that may reproduce during April. In Egypt, this species has great economic importance because of its commercial value, which gives its a special place among the species exploited in the region. During sampling which is carried out from April, 2016 to February, 2017, an important bias was noticed in the mutual prosperity between sexes and sampling site. Landings of *E. cirrhosa* show a periodic motif related to its biological cycle and its commercial curiosity which are sufficiently variable with its habitat assortment. It shows a long scale bathymetric occurrence in the Mediterranean Sea. This species shares *Octopus vulgaris* in the commissary landings in the Mediterranean fisheries. The biology of *E. cirrhosa* has been mentioned in several works. However, few authors have insisted on gonadal biology and gametogenesis. This species is gonochoric, short-lived and its life cycle ends with reproduction[7-10]. Reproductive activity in cephalopods is usually a cyclical and seasonal phenomenon whose manifestation affects the gonad, the genital tract and reproductive behaviour. Studies of the reproductive activity of octopods and especially *Octopus* spp. showed a very marked seasonal periodicity and irregularity in the spawning periods in different marine ecosystems[11]. All the more, *Octopus vulgaris* males reach their sexual maturity at an early age to females[12]. In Galician waters, mature females of *Octopus vulgaris* emerge from September to December and during May to June. Among the most important octopod species interesting is the *E. cirrhosa* which is the second target species by the national fleet after *Octopus vulgaris*. Many works have been dedicated to the study of its life cycle in other areas. In the Arabian Gulf, the spawning season of most cephalopods was restricted. In the Mediterranean Sea, Belcari *et al*.[13] showed that in *E. cirrhosa* the number of mature females was never zero during the year and the laying period was spread over several months. This finding was similar to that in the Aegean Sea[14] and Iquique in Northern Chilean waters[15] on *Octopus mimus*.

Akyol *et al*.[16] highlighted the similarities and differences that exist in the development phases of the gonad in females of, *Octopus vulgaris*; *E. cirrhosa* and *E. moschata* which coexist in the same areas and undergo the same measures of the cephalopod fishery[17,18].

In Egypt, as a result of the continued environmental crimes involving mining companies has increased, concern about the danger to human and animal health due to exposure to toxic metals. The bibliographic survey on the reproductive changes in marine cephalopods caused by toxic metals contaminating the environment, including possible permanent damage to the fauna genetic bank in the affected regions is still unknown. Toxic metals, mistakenly called "heavy metals", refer to a group of elements that do not have known physiological functions in living organisms. Therefore, they can produce harmful effects on metabolic functions, even in very small amounts. Bioaccumulation and the high reactivity of metals such as mercury, lead and cadmium, among others, make intoxications public health problems. The impact of toxic metal poisoning on the marine production system is still difficult to measure, due to the lack of statistical information. Still, they also contribute to the destruction of the animal genetic bank, mainly of marine fauna, since these animals are exposed to toxic metals due to the proximity to the areas contaminated with their natural habitat.

This study aimed to measure sexual maturity indices: sex ratio, GSI (Gonado-Somatic Index), RGS (Record Gonado-Somatic), VNI (Vas deferens Needham's sac Index), OGI (Oviduct Gland Index) and HI (Hayashi index); the histological picture of the gonads of both sexes and measurement of six heavy metals in seawater and gonads. Finally, test whether these heavy metals exert a negative impact on the reproductive cycle. These results can help specify the periods adequate for the biological rest of *E. cirrhosa* in the Mediterranean Sea of Alexandria, Egypt.

MATERIALS AND METHODS

Sampling and morphometric parameters: The study was conducted on the horned octopod *E. cirrhosa.* Animals were collected from Ras el Tin beach; 296 females and 282 males and 368 animals collected from Abu Qir; 194 females and 174 males). This collection was taken place monthly at the level of trawler landings coastal waters operating Ras el Tin beach and Abu Qir bay from April, 2016 to February, 2017. Samples were fished at depths ranging from 10-20 m, on sandy to rocky bottoms. Measures of morphometric samples taken were.

Ventral mantle length (LVM) in millimetres:

$$\text{Gonado-Somatic index (GSI)} = \frac{\text{Pg}}{\text{P-Pg}} \times 100$$

$$\text{Record gonado-somatic (RGS)} = \frac{\text{Pg}}{\text{P}} \times 100$$

where, P is the eviscerated body weight, Pg is the weight of the gonad:

$$\text{Vas deferens Needham's sac index (VNI)} = \frac{\text{VD}}{\text{VML}}$$

where, VD is the diameter of the Vas deferens in the male:

$$\text{Oviduct gland index (OGI)} = \frac{\text{OGD}}{\text{VML}}$$

where, OGD the diameter of the oviduct gland:

$$\text{Hayashi index (HI)} = \frac{\text{SCW}}{\text{SCW+TEW}}$$

or:

$$\text{HI} = \frac{\text{OCW}}{\text{(OCW+OVW) for both sexes}}$$

where, SCW is the weight of spermatophoric complex, TEW is the weight of the testis, OCW is the weight of oviduct gland and OVW is the weight of the ovary.

Ethical clearance for this study was obtained from the Alexandria University ethics committee.

Measurement of heavy metals

Total metals in the seawater: For the development of this study, some monitoring parameters were adopted to characterize certain water quality standards and the degree of contamination by Manganese, Cadmium, Iron, copper, zinc and lead in the water body of the two studies sites. The quality of the water can be represented by several parameters, which reflect its main biological characteristics. Water samples (1 L) to determine the concentration of total metals were collected using a "van Dorn" type bottle. In each site, seawater was collected at 30 and 70% depth corresponding to the site of *E. cirrhosa* sampling. The water sampled at each collection site was preserved in the field with 1.5 mL of concentrated nitric acid. In the laboratory, the 1 L sample was concentrated to 100 mL. During the concentration process, a total of 5 mL of nitric acid was added to each sample. The concentrated material was filtered through glass fibre filters, GFC, with 0.45 µm porosity and placed in 100 mL volumetric flasks. The volume was then diluted to 100 mL and the reading of the total metals was performed on an Atomic Absorption Spectrophotometer (model SpectrAA 220, Varian). The results were expressed in mg L^{-1} of water.

Total metals in the gonads: The thawed samples were dried in an oven at 70°C for 48 hrs. The dry samples have weighed and crushed. An amount of 0.1-0.3 g of powder of each dry sample weight was put in vials and measured in the presence of nitric acid (65%) for 1 hrs at room temperature 25°C. The vials were then heated in a sand bath for 12 hrs to a temperature of about 140°C. The materials obtained were filtered, adjusted to 100 mL with distilled water and kept at 4°C until analysis. Six metal elements were measured: Manganese, Cadmium, Iron, copper, zinc and lead. Measurements of metal contents were made by spectrometry of Atomic Emission Coupled to Induced Plasma (ICP Atom. Scan 16). Two types of standard samples were used for the stability of the system as well as for its inter-calibration. These were the ICP quality mono-elements (D1-H) and quality control standards (D1-QC). The results were expressed in µg g^{-1} dry weight. The levels of different metals in the seawater were analyzed in triplicate by Atomic absorption spectrophotometer and expressed in µg g^{-1}.

Microscopic test: To study gametogenesis, the fragments of gonads were directly incubated for 3-4 days in Bouin`s fixative before being dehydrated and included in paraffin wax. The methodology was as follows: 3 ethyl alcoholic baths at 95°C

for one and half hours; 3 ethyl alcoholic baths at 100°C for one and half hours; 3 baths of toluene during 3 hrs; 2 paraffin baths for 4 hrs; 1 paraffin bath for 1 night; embedding in paraffin wax. The staining technique was hematoxylin, eosin and triple stains[19]. The 3 baths of 10 mm each in toluene; 3 baths of 10 min each in alcohol at 100°C; Hematoxylin staining for 1 min; washing with running water; staining with eosin mixture 2 min; or triple stains; washing with running water; dehydration in an oven; mounting in Canada Balsam[20]. Histological analysis of these gonads was made by light microscopy on sections of 5-8 µm thickness. Stages of oocyte maturation were described. Microscopic images were made under a microscope photonic camera with a camera (Leica). Regarding the reproductive periodicity of *E. cirrhosa* if it reproduces during all months of the year (continual motif) or only during the months with more favourable environmental conditions (intermittent or recurrent motif), stages of oocyte maturation from previtellogenesis to spent phases were described in the two study locations. The frequency of monthly occurrence of these stages was commented throughout the year, to determine the reproductive period of the species.

Statistical analysis: The morphometric parameters: sex ratio, GSI (Gonado-Somatic Index), RGS (Record Gonado-Somatic), OGI (Oviduct Gland Index), VNI (Vas deferens Needham's sac Index) and HI (Hayashi index) are measured monthly and their fluctuations are commented through IBM SPSS Statistics 19. The particular analyses were: UNIANOVA (ANCOVA) analysis, (Method = SSTYPE (3); Intercept = Include; Print = ETASQ Homogeneity descriptive and Criteria = Alpha, 0.05) and Covariance P. Analysis of covariance is an analysis that tests the main effects and interactions of categorical variables, of a continuous dependent variable, to control the effects of other selected continuous variables, which would covert with the dependent. The concentrations of metals in samples were expressed on average (Mean±SEM water and gonad, means Difference, 95% confidence interval, R square and p-value). The monthly average was obtained by the sum of the measurements of four samples. The differences among concentrations were depending on; the locality and the month of sampling. Two-tailed analyses of variance (ANOVA) are applied. The averages of the concentrations are compared with each other using unpaired t-test and F-test to compare variances.

RESULTS

Sexual maturity indices: This study recorded the collection data and sex ratios of *E. cirrhosa* from Ras El-Tin beach and Abu Qir bay. In this study 578 and 368 animals were collected from Ras El-Tin beach and Abu Qir bay respectively. Sexes were separated and counted from both collections each month. Sex ratios were calculated and tabulated in Table 1 and 2. The sexual maturity indices were measured monthly from April, 2016 till March, 2017 to test the equality of fecundity between males and females among seasons in the two study sites. In Ras el Tin beach males were dominated during August-March with the minimal number found during December while in Abu Qir bay more males were collected from April-July, December and March. In Ras el Tin beach females were dominated in May-September and February-March while in Abu Qir bay more females were collected from July-September and November-December. The ventral mantle length (LVM) showed variability in the maturation phases among males and females. In males and females of Ras el-Tin

Table 1: Records of samples collection and sex ratio of specimens collected from Ras el-Tin

					Sex ratio	
Months	Total	Male (M)	Female (F)	M/F	♀	♂
April, 2016	37	21	16	1.31	43.24	56.75
May, 2016	37	15	22	0.68	59.45	40.54
June, 2016	37	19	18	1.05	48.64	51.35
July, 2016	35	13	22	0.59	62.85	37.14
August, 2016	51	24	27	0.88	52.94	47.05
September, 2016	56	27	29	0.93	51.78	48.21
October, 2016	38	19	19	1.0	50.0	50.0
November, 2016	51	24	27	0.88	52.94	47.05
December, 2016	44	15	29	0.51	65.90	34.09
January, 2017	47	31	16	1.93	34.04	65.95
February, 2017	73	40	33	1.21	45.20	54.79
March, 2017	72	34	38	0.89	52.77	47.22
Total	578	282	296	0.95	51.21	48.78

Sex ratio: F/(F+M)×100 OR M/(M+F)×100. F: Number of females, M: Number of males, F+M: Total number of sexed samples

Table 2: Records of samples collection and sex ratio of specimens collected from Abu Qir Bay

Months	Total	Male (M)	Female (F)	M/F	Sex ratio ♀	Sex ratio ♂
April, 2016	38	21	17	1.23	44.73	55.26
May, 2016	37	19	18	1.05	48.64	51.35
June, 2016	21	12	9	1.30	42.85	57.14
July, 2016	47	25	22	1.13	46.88	53.19
August, 2016	38	12	26	0.46	68.42	31.57
September, 2016	40	9	31	0.29	77.50	22.50
October, 2016	20	6	14	0.42	70.0	30.0
November, 2016	33	14	19	0.73	57.57	42.42
December, 2016	33	18	15	1.20	45.45	54.54
January, 2017	11	7	4	1.75	36.36	63.63
February, 2017	21	13	8	1.62	38.09	61.90
March, 2017	29	18	11	1.63	37.93	62.06
Total	368	174	194	0.89	52.71	47.28

Table 3: Analysis of morphometric parameters of specimens collected from Ras El-Tin

	N°		LVM (mm)	P (g)	Pg (g)	GSI (%)	RGS (%)	OGI	HI
Immature	♂	30	11.7-140.3	14.7-.87.2	0.32-16.57	2.22-23.4	2.17-19.0	2.03-19.7	0.42-0.12
Stage 1			22.20±10.50	17.50±1.200	1.20±0.250	64.840±2.760	6.765±4.735	6.765±4.735	0.2030±0.0130
	♀	6	120.6-180.4	530.2-970.1	19.2-42.1	3.75-4.5	3.62-4.33	0.22-0.04	37-0.51
			205.5±25.10	2146±1132	64.25±12.15	7.250±2.950	0.0300±0.0100	0.0300±0.0100	0.3320±0.02100
Developing	♂	25	45.3-81.6	36.2-115.2	14-21.2	63.06-22.5	38.67-18.40	5.3-11.1	0.33-0.27
Stage 2			60.35±15.05	95.2±96.7	7.15±4.210	6.650±1.050	6.200±0.9000	6.200±0.9000	0.3100±0.00001
	♀	12	75.4-140.1	135.7-934.3	10.2-64.2	8.90-7.3	7.51-6.87	7.1-7.7	0.33-0.58
			110.9±29.25	452.4±314.2	34.10±16.80	10.40±2.100	9.400±1.700	9.400±1.700	0.6200±0.00003
Maturing	♂	24	60.5-110.6	115.2-461.5	22.4-32.7	24.13-7.6	19.44-7.08	17.4-6.05	0.23-0.42
Stage 3			85.35±24.85	112.6±41.10	27.25±1.230	17.0±4.10	14.40±3.000	14.40±3.000	0.3200±0.00010
	♀	11	110.2-139.1	210.3-2120.1	26.3-89.8	14.29-4.43	12.50-4.23	11.4-3.01	0.46-0.30
			124.9±14.25	1201±7.10	73.10±34.20	18.70±12.30	4.530±1.520	4.530±1.520	0.4700±0.01000
Mature	♂	27	42.5-180.5	80.2-1110.2	23.7-72.5	41.94-6.98	29.55-6.53	24.1-2.6	0.42-0.18
Stage 4			79.05±36.55	110.1±72.1	32.70±1.500	23.00±8.900	18.25±5.850	18.25±5.850	0.22±14.26
	♀	14	32.7-370.2	12.6-130.1	1.6-37.4	14.54-40.34	12.69-28.74	12.7-11.5	0.32-0.62
			255.3±115.0	135.2±912.2	27.20±7.50	19.50±5.000	16.20±3.500	10.10±1.400	0.4230±0.00300
Spawning	♂	31	70.5-230.6	130.4-3200.2	31.1-96.2	31.31-3.09	23.84-3.00	22.9-0.02	0.29-0.45
Stage 5			95.55±25.05	232.4±143.1	29.15±1.820	1.79±1.18	11.56±11.34	11.56±11.34	0.3400±0.02000
	♀	17	115.6-170.6	202.4-1320.2	37.2-53.2	3.19-4.19	18.37-4.02	12.4-3.02	0.41-0.2
			175.6±4.950	1252±132.4	58.20±1.200	1.365±1.335	2.810±0.2100	2.810±0.2100	0.3520±0.02000
Spent	♂	10	115.7-140.9	970.1-1310.6	94.8-172.8	10.83-15.18	9.77-13.18	5.7-13.1	0.27.3-.0.35
Stage 6			103.2±12.55	655.5±314.7	48.65±46.15	0.2350±0.1350	6.500±0.8000	6.500±0.8000	0.430±0.02400
	♀	15	90.6-130.3	340.8-920.3	2.5-36.8	0.62-416	0.73-3.99	7.3-3.9	0.37-0.1
			135.6±5.300	1115±195.2	104.8±68.00	0.600±5.500	8.500±4.600	8.500±4.600	0.640±0.27100

it was longer than those of Abu Qir Bay. The eviscerated body weight and the weight of the gonads of both sexes in Ras el-Tin were heavier than those of Abu Qir during the maturity phases. The Gonado-Somatic Index (GSI) increased from immature to mature phases to spawning phase in both sexes then diminishes at the spent phase. The Record Gonado-Somatic (RGS), the Oviduct Gland Index (OGI) and the Hayashi index (HI) showed that both males and females of Ras el Tin beach are more vital and healthy than those of Abu Qir bay in Table 3 and 4. The number of males and females was always higher in Ras el Tin beach than those in Abu Qir bay. Females were dominated during August-December in both study sites. Males dominated during April-May, July and December in both study sites in Fig. 1(a-b). The Gonado-Somatic Index of males and females of Ras el Tin beach was higher than those specimens of Abu Qir bay in Fig. 1(c-d). The Record Gonado-Somatic of Ras el Tin beach was higher than those specimens of Abu Qir bay in Fig. 1(e-f). The vas deferens Needham`s index of mature males of Ras el Tin beach was higher than that of specimens of Abu Qir bay in Fig. 1g. The Oviduct Gland Index of mature females of Ras el Tin beach was higher than those specimens of Abu Qir bay except the weight of 430 g of Abu Qir bay in Fig. 1h. The Hayashi index of both sexes of Ras el Tin beach was higher than specimens of Abu Qir bay in Fig. 1(i-j).

Table 4: Analysis of morphometric parameters of specimens collected from Abu Qir

	N°		LVM (mm)	P (g)	Pg (g)	GSI (%)	RGS (%)	OGI	HI
Immature	♂	40	8.2-90.3	25.1-230.7	0.51-15.8	2.07-0.74	2.03-6.84	0.42-7.4	0.73-0.84
Stage 1			17.10±7.20	28.60±3.500	2.105±1.595	4.840±2.760	6.765±4.735	1.11±1.100	0.6050±0.1250
	♀	10	75.2-80.8	720.9-1680.4	41.6-69.4	0.06-4.30	5.77-4.12	0.01-0.001	79-0.73
			150.2±17.20	3746±2065	99.75±30.35	7.250±2.950	0.7100±0.1500	0.0010±0.0001	0.7350±0.05500
Developing	♂	40	36.1-62.4	60.1-230.7	13.2-25.7	0.62-12.53	21.96-10.84	1.6-4.1	0.68-0.71
Stage 2			42.15±9.1	170.3±110.2	11.65±8.450	6.650±1.050	6.200±0.9000	2.200±0.1000	0.7200±0.01000
	♀	20	55.1-75.8	280.5-1250.6	20.1-96.3	7.71-6.34	7.16-0.76	2.1-3.4	0.73-0.88
			80.1±17.5	740.7±510.0	61.00±35.30	10.40±2.100	0.5600±0.3200	3.0100±0.100	0.8300±0.05000
Maturing	♂	40	45.2-92.7	180.3-790.6	31.5-47.9	21.16-6.44	17.47-6.05	5.1-1.00	0.73-0.62
Stage 3			75.15±18.15	255.6±75.25	34.65±3.150	17.0±4.10	14.40±3.000	4.40±0.1000	0.6800±0.05000
	♀	20	90.1-102.3	330.8-4650.6	37.8-140.1	12.90-2.58	11.423.01	3.4-0.021	0.82-0.70
			94.7±8.9	2721±1930	94.00±46.10	18.70±12.30	0.8900±0.1000	1.110±0.110	0.7400±0.04000
Mature	♂	40	37.1-100.2	190.6-3420.6	26.1-90.6	31.90-2.72	13.69-0.02	7.1-0.1	0.78-0.72
Stage 4			58.3±23.7	303.2±112.6	48.90±2.800	23.00±8.900	18.25±5.850	5.00±1.200	0.39±27.61
	♀	20	17.3-262.1	132.1-360.4	3.7-45.8	13.02-14.55	2.60-12.70	9.2-7.1	0.77-0.78
			168.1±95.8	245.6±114.9	35.80±10.00	19.50±5.000	0.5550±0.1050	16.20±3.500	0.7050±0.02500
Spawning	♂	40	48.2-140.5	210.4-5810.7	28.3-130.1	29.7-2.29	13.45-2.23	8.2-0.001	0.63-0.72
Stage 5			63.2±17.6	465.7±255.3	44.95±3.350	1.79±1.18	11.56±11.34	4.15±4.11	0.6700±0.05000
	♀	20	94.7-110.5	415.8-2860.4	51.7-86.4	14.19-3.11	12.43-3.02	4.1-0.001	0.65-0.7
			120.3±2.30	3141±280.1	88.50±2.100	1.365±1.335	1.075±0.1750	0.710±0.01000	0.6900±0.04000
Spent	♂	10	84.3-90.5	970.1-1310.6	44.8-72.8	0.10-15.1	4.61-5.55	2.9-9.7	0.48-0.69
Stage 6			83.1±7.12	655.5±314.7	48.65±46.15	0.2350±0.1350	6.500±0.8000	4.200±0.2000	0.7650±0.07500
	♀	15	69.5-95.8	310.8-800.1	1.5-26.8	0.37-4.1	0.44-2.19	1.2-2.8	0.56-0.8
			111.2±3.100	1115±195.2	104.8±68.00	0.600±5.500	0.715±0.02500	5.100±2.200	0.950±0.7500

LVM: Ventral mantle length (to the nearest mm). P: Eviscerated body weight. Pg: Weight of the gonad

Table 5: Analysis of heavy metals in the sea water and gonads

	Mn (μg g^{-1})		Cd (μg g^{-1})		Fe (μg g^{-1})		Cu (μg g^{-1})		Zn (μg g^{-1})		Pb (μg g^{-1})	
	Water	Gonad	Water	Gonad	Water	Gonad	Water	Gonad	Water	Gonad	Water	Gonad
Ras El Tin												
January	1.0±0.5	1.7±0.8	1.7±0.7	3.1±0.6	0.5±0.2	1.2±0.9	3.3±0.1	3.8±0.1	7.4±0.1	6.5±0.1	1.7±0.7	1.2±0.6
February	1.2±0.1	1.6±0.5	1.9±0.1	3.0±0.2	0.7±0.1	1.3±0.7	4.1±0.7	2.7±0.9	6.9±0.1	5.9±0.6	1.8±0.6	1.4±0.1
March	0.9±0.6	1.5±0.4	1.6±0.4	2.9±0.6	0.6±0.4	1.1±0.6	3.7±0.5	3.6±0.6	6.1±0.8	5.8±0.9	2.5±0.5	1.7±0.8
April	0.7±0.4	1.3±0.1	1.4±0.2	2.7±0.1	0.7±0.8	1.2±0.5	3.9±0.8	3.7±0.5	5.7±0.7	4.7±0.4	1.6±0.4	1.3±0.5
May	1.3±0.4	1.9±0.4	1.8±0.6	3.3±0.2	0.9±0.5	1.7±0.8	3.7±0.2	3.7±0.3	7.7±0.5	6.9±0.3	1.9±0.1	1.8±0.1
June	1.4±0.7	1.5±0.6	1.7±0.8	3.1±0.2	0.8±0.7	1.4±0.3	4.3±0.2	2.9±0.1	6.8±0.8	5.7±0.7	1.7±0.5	1.9±0.4
July	1.1±0.9	1.7±0.8	1.9±0.2	2.8±0.1	0.9±0.1	1.6±0.4	3.9±0.2	3.8±0.1	6.4±0.3	5.9±0.4	2.3±0.1	1.5±0.7
August	0.9±0.8	1.2±0.9	1.3±0.1	2.9±0.7	0.6±0.3	1.7±0.1	3.8±0.4	3.9±0.7	5.9±0.9	4.9±0.6	1.6±0.5	1.7±0.2
September	1.2±0.5	1.6±0.3	1.6±0.1	3.3±0.1	0.9±0.1	1.4±0.2	3.6±0.3	3.6±0.6	7.9±0.4	6.8±0.1	1.9±0.3	1.9±0.3
October	1.5±0.9	1.8±0.7	1.8±0.2	3.2±0.3	0.6±0.4	1.5±0.2	4.4±0.3	2.4±0.6	6.8±0.7	5.7±0.4	1.7±0.2	1.6±0.4
November	0.8±0.3	1.4±0.1	1.5±0.5	3.7±0.4	0.9±0.7	1.3±0.2	3.9±0.1	3.9±0.8	6.5±0.8	5.5±0.3	2.6±0.2	1.8±0.6
December	0.9±0.1	1.6±0.6	1.7±0.1	2.9±0.1	0.8±0.1	1.9±0.3	3.8±0.4	3.6±0.1	5.9±0.9	4.9±0.5	1.9±0.5	1.5±0.7
Abu Qir												
January	1.5±0.6	1.2±0.8	2.1±0.8	3.8±0.7	1.2±0.4	1.7±0.7	3.9±0.6	5.2±0.6	7.7±0.4	10.0±0.9	2.1±0.9	2.6±0.5
February	1.5±0.3	2.4±0.3	2.3±0.6	4.1±0.5	1.1±0.5	1.8±0.9	2.8±0.8	4.3±0.3	7.1±0.8	8.8±0.9	1.9±0.8	1.9±0.8
March	1.9±0.1	1.7±0.4	2.2±0.4	3.6±0.7	1.3±0.1	1.6±0.6	3.1±0.7	3.9±0.9	7.9±0.8	7.9±0.7	2.2±0.6	2.8±0.5
April	2.6±0.4	2.1±0.8	2.8±0.2	3.7±0.8	1.5±0.2	1.8±0.1	3.9±0.3	4.5±0.7	8.4±0.5	9.2±0.6	2.4±0.6	2.7±0.8
May	1.8±0.3	2.1±0.7	2.3±0.9	3.9±0.4	1.5±0.2	1.6±0.2	1.8±0.2	5.7±0.5	7.3±0.1	9.3±0.1	2.7±0.4	2.6±0.6
June	2.2±0.2	1.9±0.3	2.4±0.2	4.2±0.3	1.3±0.6	1.9±0.2	1.9±0.2	4.4±0.5	7.7±0.5	8.9±0.6	2.9±0.1	2.9±0.1
July	1.9±0.5	2.9±0.1	2.5±0.1	3.9±0.1	1.8±0.1	1.5±0.5	1.5±0.7	3.7±0.5	7.9±0.7	7.8±0.4	2.8±0.1	2.6±0.8
August	1.7±0.8	2.2±0.4	2.9±0.5	3.7±0.4	1.9±0.9	1.9±0.3	1.6±0.4	4.2±0.7	8.6±0.8	9.7±0.5	2.7±0.5	2.9±0.6
September	1.8±0.9	2.4±0.7	2.3±0.5	3.9±0.6	1.5±0.4	1.6±0.4	1.9±0.4	5.1±0.7	7.5±0.5	10.3±0.7	2.6±0.4	2.5±0.7
October	2.7±0.7	2.6±0.3	2.4±0.l6	4.2±0.7	1.4±0.1	1.9±0.6	1.3±0.3	4.7±0.5	7.9±0.8	8.9±0.6	2.8±0.7	2.9±0.1
November	2.2±0.5	2.8±0.1	2.8±0.2	3.8±0.1	1.8±0.5	1.7±0.7	1.7±0.8	3.8±0.9	7.8±0..1	7.8±0.9	2.7±0.5	3.8±0.3
December	2.8±0.8	2.4±0.8	2.7±0.4	3.9±0.1	1.9±0.1	1.9±0.1	1.9±0.1	4.9±0.4	8.7±0.9	9.9±0.8	2.6±0.4	2.9±0.9

Measurement of heavy metals in the seawater and the gonad: The purpose of this study was to determine the concentrations of the metals: Mn, Cd, Fe, Cu, Zn and Pb in the seawater and gonads of *E. cirrhosa* in the two study sites during the study months. The values obtained for the average levels of these heavy metals are listed in Table 5. In the seawater of Ras el Tin beach, manganese contamination was higher during January-February, May-July and September-

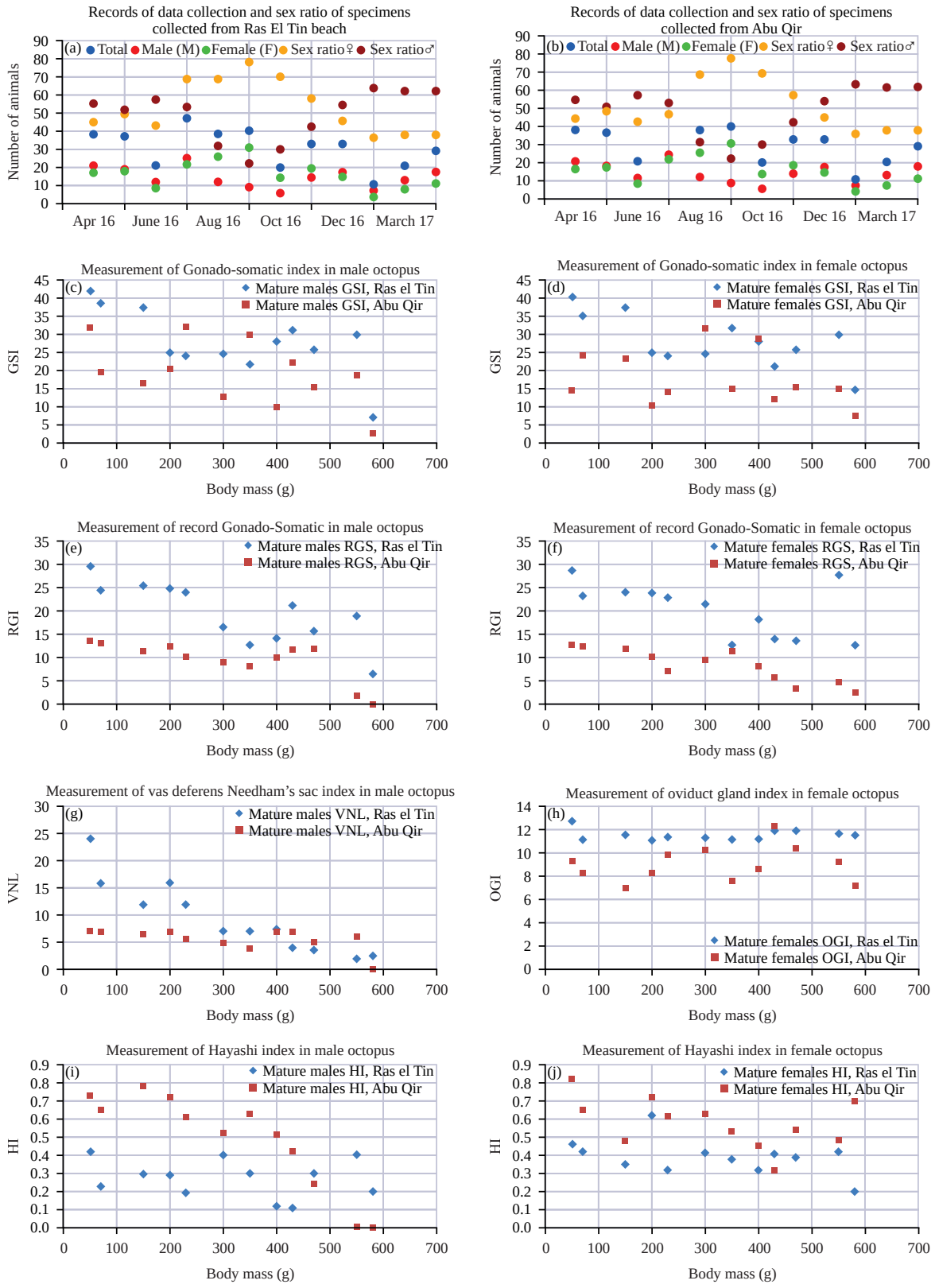

Fig. 1(a-j): (a-b) Sex ratio of *E. cirrhosa*, (c-d) GSI in males and females, (e-f) RGI in males and females, (g-h) VNL in male and OGI in female and (i-j) HI values in males and females

October. Its value in the gonads was higher than in the seawater in all months of the year. In the seawater Abu Qir bay it was higher during April and October-December. Its concentration in the gonads was higher than those in Ras el Tin specimens, especially during February, April-May and July-December. Cadmium contamination in the seawater of Abu Qir bay was higher than that in Ras el Tin beach. More or less, there was no difference in the cadmium concentration in the gonads of both study sites. Iron contamination showed a higher concentration in the seawater of Abu Qir Bay. There was no difference in the iron concentration in the gonads of both study sites. Copper contamination was higher in the seawater of Ras el Tin beach than in Abu Qir Bay. Gonads of Abu Qir bay contained excessive copper pollution than those of Ras el Tin beach. Zinc and lead contaminations were slightly higher in the seawater of Abu Qir Bay. Gonads of Ras el Tin beach contained a smaller amount of Zinc than those of Abu Qir Bay. From these analyses together with the maturity indices this study concludes that heavy metals affect the reproductive cycle of *E. cirrhosa* sampled from Abu Qir Bay.

HISTOLOGY

Microscopic study of the male gonad: The male reproductive tract consists of a testis, a spermatophoric duct connecting the spermatic organ to the spermatophoric sac and a penis. This set forms the complex spermatophores. Formed spermatophores pass through a sperm duct where the packaging of the spermatophores takes place, before being put in the pocket of Needham. The beginning of genital maturation is marked by the activity of seminiferous tubules. The testis consists of several elongated seminiferous tubules whose outline is semicircular in Fig. 2a. They are delimited by thin connective tissue membranes. At the intersection of these seminiferous tubules, blood sinuses and interstitial cell assembles are observed in Fig. 2b. Spermatogonia are less differentiated and lie close to the external limiting membrane of the tubule. Observation of the seminiferous tubule from the periphery to the central core shows spermatogonia, spermatocytes I, spermatocytes II, spermatids and spermatozoa, which lie in the central lumen of the seminiferous tubules in Fig. 2c-d. In each segment of the seminiferous tubules, the process of spermatogenesis occurs in a centripetal sequence and regular. So, every moment, almost every spermatocyte II of the seminiferous tubules is in the process of division. Spermatocytes I have large nuclei and exhibit progressive condensation of chromosomes (growth phenomenon); they are followed by spermatocytes II, which have smaller nuclei. Spermatids are, at first, spherical and lengthen thereafter (spermiogenesis) in Fig. 2e. Once the elongation of spermatids completes, sperm have undergone a process of maturation and they are ready to fertilize in Fig. 2f. Indeed, the phenomenon of transition from spermatids to mature spermatozoa is accompanied by changes in size, shape and metabolic properties in Fig. 2g. After mating, the testicular seminiferous tubules shrink and the central zone becomes empty in Fig. 2h. It is possible to observe all stages of male sex cells development, from spermatogonia to spermatozoa: Primary spermatogonia at the periphery of the seminiferous tubules have dark-coloured nuclei. Secondary spermatogonia are larger with more apparent nucleoli in the form of large black dots. Spermatocytes have smaller nuclei than spermatogonia. They appear circular and denser. The distinction between primary and secondary spermatocytes is not possible under light microscopy; Spermatids are easily identifiable by the elongated form of their nucleus which is very dense; Spermatozoa are cells with flagella and are located in the seminiferous tubules lumen. Though following up the histological preparations of the testis in each month per year, maturity of the testis can be assumed to last about four months. Males reached maturity in June-January, while spermiogenesis is intense until the moment of copulation which begins in April-August and fall spawning.

Histological study of the female gonad: The female reproductive tract is composed of an ovary, a pair of oviducts and accessory glands. The ovary is rounded in shape, it occupies the lower part of the mantle in the mature females and its weight varies concerning maturity phases. In immature females, the ovary and accessory glands are small, round and translucent. Sometimes there is a small white border at the level of the insertion of the oviduct into the glands. Oocytes are not visible in the ovary and the gonad weight is less than 25 g. In females with the growth phase, the ovary and accessory glands are larger but remain round. They gradually become creamy white, with still translucent parts. A grey disc, which may become dark, surrounds the white edging, report in the accessory glands. The gonad weighs are on average between 20 and 96 g. The oocytes become apparent but do not individualize. In females at the end of their maturation; the ovary is bigger and gains a yellow colour. The accessory glands are round at first but flatten towards the end of this stage. The grey disc grows, but still do not take the form of a crown yet (without striation). Oocytes begin to be individually discernible. In mature females, the ovary is bulky and turns bright yellow. The oocytes are distinctly visible with granulations. The accessory glands are flat and the grey crown covers them partially. This crown is marked by a deep striation.

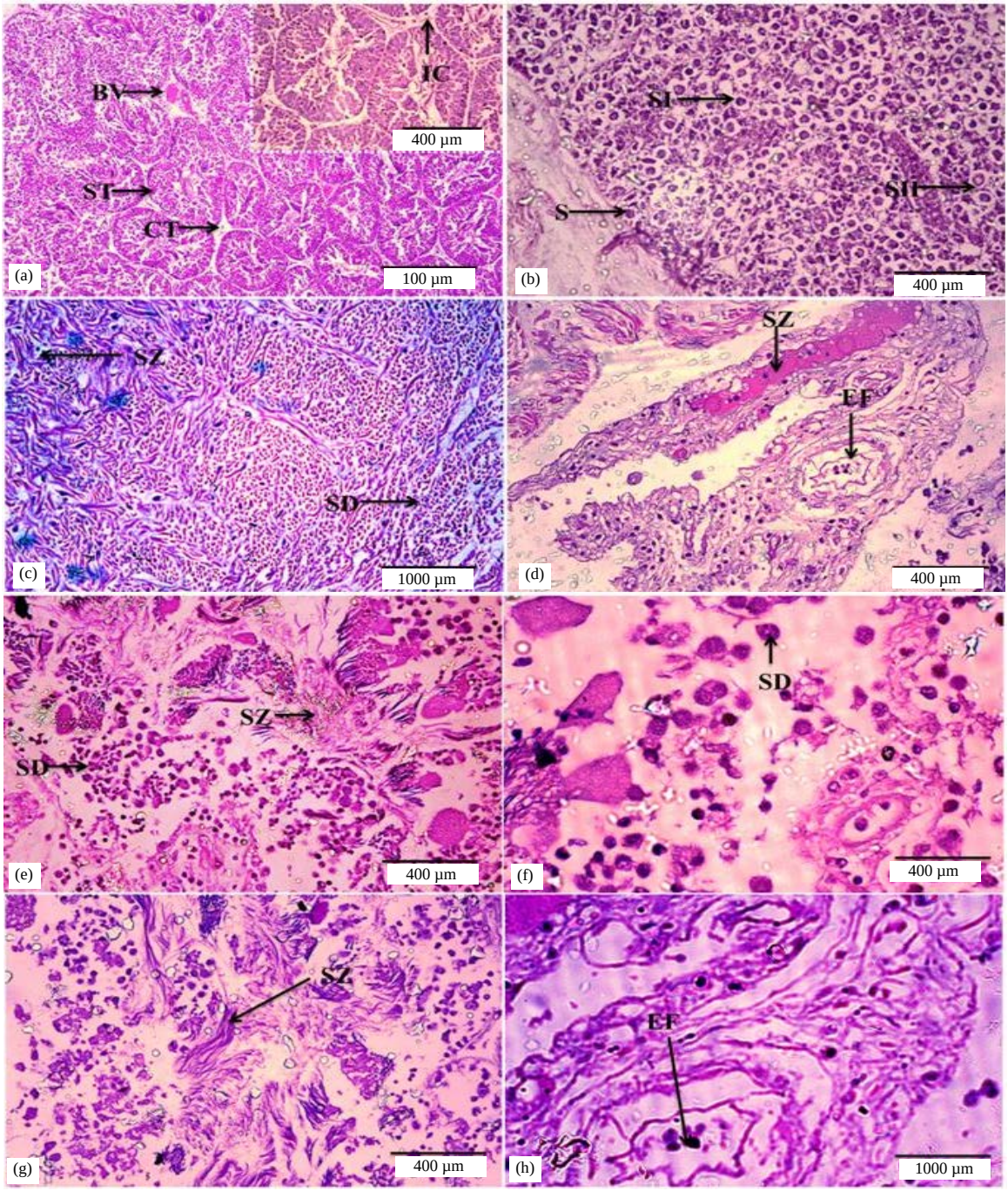

Fig. 2(a-h): (a-b) CS in the testis showing seminiferous tubules, spermatogonia, connective tissue, blood vessels and assemblage of interstitial cells, (c-d) CS in the testis showing the seminiferous tubule from the periphery to the central core, spermatogonia, spermatocytes I, spermatocytes II, spermatids and differentiation of spermatids to spermatozoa, which was in the central lumen, (e) CS in the testis showing spermatids were, at first, spherical and lengthened, (f) CS in the testis showing the elongation of spermatids completed, the sperms have undergone a process of maturation, (g) CS the testis shows the transition from spermatids to mature spermatozoa and (h) CS in the testis showing shrinking of seminiferous tubules and the central zone became empty of spermatozoa

ST: Seminiferous tubules, S: Spermatogonia, CT: Connective tissue, BV: Blood vessels, IC: Interstitial cells, SI: Spermatocytes I, SII: Spermatocytes II, SD: Spermatids, SZ: Spermatozoa

In post-laying females, the ovary appears as an empty bag. It narrows and turns brown or even black. Observation of ovary samples shows connective tissue, blood sinuses, oocytes and nerve cells in Fig. 3a. The connective tissue is richly vascularized and contains oocytes at varying stages of development in Fig. 3b. The maturation of genital cells is accompanied by the development of follicular cells. During oogenesis, these initially follicular cells lengthen, multiply and surround the oocytes. The maturation of these gametes can be subdivided into 3 phases: previtellogenesis (or follicular phase), Vitellogenesis and post-laying. The phenomenon of oogenesis is continuous; the boundaries between phases are arbitrary and only conventional benchmarks help better understand the process.

Previtellogenesis or follicular phase

- **Stage 1:** During this phase, the follicle is formed. Oocytes are spherical with circular nuclei of large diameter. The chromatin of these cells is not dense; one or two large nucleoli are seen. Lipid inclusions are absent in these cells. This stage contains both the oocytes that lack follicular cells and those surrounded by a layer of follicular cells in Fig. 3c
- **Stage 2:** Subsequently, the oocytes grow in lengthening. They surround themselves with a second layer of follicular cells. These cells, now organize in two layers, form the folds inside the oocyte. These folds penetrate deep into the cell as they invade its cytoplasm. These folds are occupied by a blood sinus and some connective tissue the nucleoli increase in size and the chromatin is always loose. Lipid inclusions are observed in the cytoplasm. Follicular cells increase in volume and become cubic with large nuclei, occupying most of the volume in Fig. 3d-f

Vitellogenesis

- **Stage 3:** During this phase, the synthesis of vitellus was terminated. The cytoplasm of the oocyte is invaded by follicular folds; Lipid inclusions multiply and become larger. The first vitelline plaques, stains bright red by the mixture hematoxylin-eosin, appears in the cytoplasm, it is the beginning of the Vitellogenesis. A zona pellucida is formed between the follicular cells and the oocyte in Fig. 3g
- **Stage 4:** At this stage, the interior of the cell is completely coloured red by the presence of yolk which invades the cell. The follicular folds are pushed back to the outside. The oocytes elongate and become very large. Lipid inclusions are now in the form of large globules in the middle of the yolk. The chromatin is denser and the nuclei have a more homogeneous internal structure in Fig. 3h

Post-spawning

- **Stage 5:** This phase marks the end of oogenesis. The oocyte is emitted into the ovarian cavity. The empty follicle regresses and it is no longer made of a cluster of cells and begin to disorganize in Fig. 3i. The current combined study of the average GSI; RGS; VNI, OGI, HI and the different gametic stages histology in both sexes shows that the octopod can breed throughout the year with two peaks of maximum lying in spring to summer and fall

DISCUSSION

The present work aims to study the gonadal biology of the horned octopus *E. cirrhosa* in Ras el Tin beach and Abu Qir Bay of the Mediterranean Sea of Alexandria. Analysis of biological parameters revealed the presence of a broad difference in gonadal maturity in Ras el Tin beach samples from those of Abu Qir. The morphometric parameters estimated by this study, GSI (Gonado-Somatic Index); RGS (Record Gonado-Somatic); OGI (Oviduct Gland Index); HI (Hayashi index), were commented in previous studies on the same genus or/species in the Atlantic, Northern and Central Adriatic Sea, the North Sea off Aberdeen, English Channel and the Mediterranean Sea[21-23]. The findings of these authors were consistent with current results in term GSI and RGS increase during maturation and spawning phases whereas HI increased during immature and spent phases. Current results concluded that the sex ratio showed a dominance of females during the spawning season. This study tried to test the effect of heavy metal bioaccumulation in the gonads of the horned octopus *E. cirrhosa*. Tissue analysis of the gonad in Ras el Tin beach (reference station) and Abu Qir gonad samples reveals the bioaccumulation of six metal elements: manganese, cadmium, iron, copper, zinc and lead. This study shows a clear variation in bioaccumulation depending on the time of sampling and the locality. Given the average annual concentrations, the degree of gonad contamination is higher in Abu Qir for the six metals studied and it affects the gonadal maturation vigorously. Trace metals are needed by humans and other animals in small amounts, but they can cause damage, in higher concentrations. They affect the blood, kidneys,

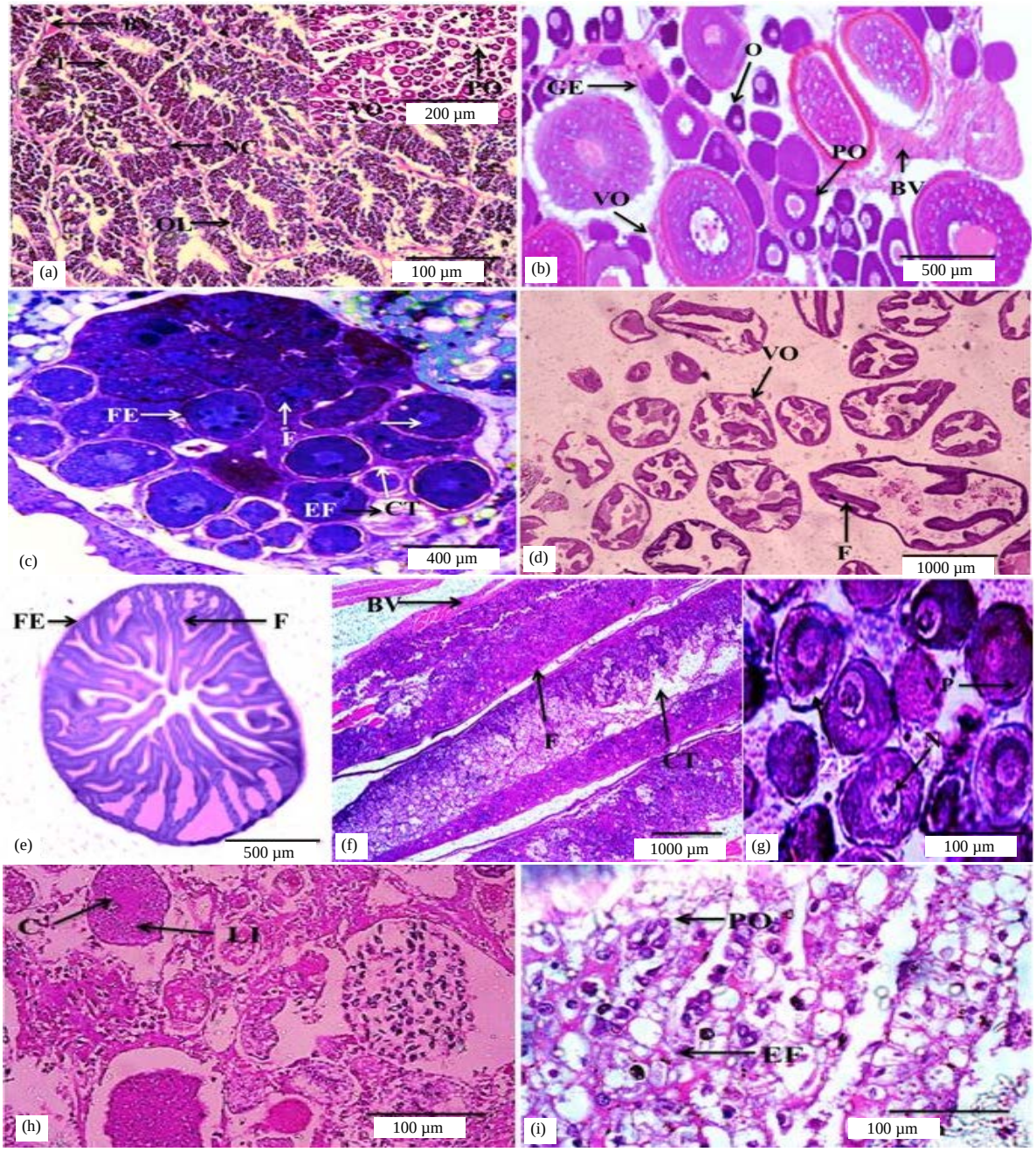

Fig. 3(a-i): (a) CS in the ovary showing of ovarian lobules, oocytes, connective tissue, blood vessels and nerve cells, (b) CS in the ovary showing connective tissue was richly vascularized and contained oocytes at varying stages of development, (c) CS in the ovary showing two types of previtellogenic oocytes, one type was not surrounded by follicular cells and the other type was surrounded by a layer of follicular cells, (d-f) CS in the ovary the second layer of follicular cells formed the folds which invaded its ooplasm, (g) CS in the ovary showing the first vitelline plaques appeared in the ooplasm and a zona pellucida was formed between the follicular cells and the oocyte, (h) CS in the ovary showing the oocytes became very elongated and very large. Lipid inclusions were now in the form of large globules in the middle of the yolk. The chromatin was denser and the nuclei have a more homogeneous internal structure and (i) CS in the ovary showing the empty follicle regressed and was no longer made of a cluster of cells that began to disorganize

OL: Ovarian lobules, NC: Nerve cells, O: Oogonium, PO: Previtellogenic oocytes, VO: Vitellogenic oocytes, FC: Follicular cells, F: Folds, O: Ooplasm, VP: Vitelline plaques, ZP: Zona pellucida, LI: Lipid inclusions, C: Chromatin, N: Nucleus, EF: Empty follicle, GE: Germinal epithelium, C: Chromatin

digestive and reproductive systems. They are teratogenic and are causative agents of hypertension. Lead for example affects the nervous, vascular, immune, pulmonary, urinary and reproductive systems and is possibly carcinogenic. The octopus collects and ingests particles containing metal elements from surrounding sediments and associated micro-fauna. Several authors were interested to investigate the problem of heavy metals bioaccumulation in marine invertebrate. This study investigates the effect of heavy metals on the maturation of *E. cirrhosa*. Samples of Abu Qir show a delay in maturation and a decrease in the number of ripe gametes during the year. Cd is a non-essential metal of high toxicity; even at low levels, as found in species of this study may cause physiological dysfunction in octopus making it impossible to survive in the ecosystem. Cu and Zn concentrations found in gonadal tissue do not pose a risk of contamination to the local population as they are below the standards. Cu and Zn metals are elements essential and are easily regulated by the metabolism that is why the accumulation of these metals is scarce. Pb has no beneficial or nutritional effects on organisms, being extremely toxic. This result is evidenced through the average GSI; RGS; VNI, OGI, HI and the different gamete stages histology. The result of this work provides further details to gonadal indices, gametogenesis and the effect of heavy metals accumulation on sexual maturation and spawning. This result help provides clarification of the spawning time, larval survival, impact on recruitment and best management of *E. cirrhosa* stocks in the Mediterranean Sea of Alexandria. In comparison with other results, Donnaloia *et al*.[24] revealed that the sex ratio was dominated in the Mediterranean Sea by males. The same result has been reported in different regions for *E. massyae* and *Octopus vulgaris*[22-24]. The VNI (Vas deferens Needham's sac Index) is first measured in this study using the same equation of OGI with one modification; the diameter of the oviduct gland in females is replaced by the diameter of the Vas deferens in males. Lourenço *et al*.[25] concluded that the sex ratio is slightly in favour of males and showed significant inter-annual fluctuations according to the bathymetry. Moreover, these authors concluded that the reproductive activity of the octopod *E. cirrhosa* seems to be continuous because mature females are encountered throughout the year. According to Pascual *et al*.[26] and Gonçalves *et al*.[27], the deviation to the 1:1 ratio could be due to ecological or morphological reasons.

The same observation makes an effect in *Octopus maya*[26]. The current combined study of the average GSI; RGS; VNI, OGI, HI and the different gametic stages histology shows that the octopod can breed throughout the year with two peaks of maximum lying in spring to summer and fall. This situation is very different from that found in the Atlantic Iberian waters. In the French and Spanish coasts, the spawning season occurs between mid-April and July[28-30]. In the European waters[31-33] concluded that the spawning season is spread over a short period of the year (mid-March and end of June). This breeding season is carried out by two types of broodstock which can be distinguished by their mantle size. Males reach maturity in June to January, while spermiogenesis is intense until the moment of copulation[34-37].

CONCLUSION

The number of males and females in Ras el Tin beach is always higher than those of Abu Qir concerning body mass as a fixed factor except for 230, 300 and 350 body mass. Significant differences ($p<0.05$) in the RGS values are remarkable at all maturity phases. The mean values of OGI in female or VNI in male increases from the immature to the spawning phases and a decrease in the spent phase. Significant differences ($p<0.05$) in the OGI and VNI values are noticed at all maturity phases except between mature and spawning phases. HI, the value differs between both sexes. Gonadal development in females is non-synchronous and later than in males, about eight months for females and four months for males. The average concentrations of heavy metals are compared with each other using an unpaired t-test and F test to compare variances.

SIGNIFICANCE STATEMENTS

This study concludes that the sexual maturity indices and histological overview of the gonads of specimens in Ras el Tin beach seem hygienic than those in Abu Qir Bay. The six heavy metals studied exert negative feedback on the biology of *E. cirrhosa* in Abu Qir bay.

This study will help the researcher to uncover the critical areas of reproductive biology cephalopods that many researchers were not able to explore. Thus a new theory on the improvement of cephalopods productivity in the marine ecosystem may be arrived at.

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