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## ASPECTS OF REPRODUCTIVE BIOLOGY OF THE MUSSEL, *BRACHIDONTES PHARAONIS* (FISCHER, 1870) (MYTILIDAE: BIVALVIA: MOLLUSCA) FROM THE NORTHWESTERN COAST OF SUEZ GULF, EGYPT

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### ABSTRACT

Reproductive cycle of the two populations of *Brachidontes pharaonis* (Mytilidae: Bivalvia: Mollusca) at Adabia and Ain Sokhna (northern western coast of the Suez Gulf), was studied during the period from January to December 2014. The present results showed that sex is separated in this species, but the microscopic examinations are necessary for determination of gonads in both sexes. The first appearance for gonads was observed in males and females at 6.3 and 6.5 mm shell length, respectively. However, the largest immature individuals reach 8.6 mm shell length. The overall sex ratio indicated over dominant females at both sites, with 1:1.57 (males: females) at Adabia, increased slightly to 1:1.81 (males: females) at Ain Sokhna, with statistical significant differences ( $X^2 = 8.33$  and  $5.14$  significant at  $P < 0.001$  and  $P < 0.05$ ), respectively. Gonads of both sexes appeared scattered within mantle tissues at both left and right sides of the animal. Mature male testes have whitish or creamy color, while females ovaries are more conspicuous, appeared lobulated and composed of a large numbers of oogenic follicles occupying most of the mantle depending on stages of egg maturation. In early stages, ovaries appear small in size, translucent, and have small oocytes. The sexually matured females have dark yellow to orange ovaries due to occurrence of full ripe ova full of yolk granules. Based on oocyte maturation, five developmental stages were recognized, comprised: stage I (immature), stage II (early stage of development), stage III (maturing stage), stage IV: late developing stage (ripe and spawning egg) and stage V (spent stage). Each developmental stage was characterized and identified by its specific features. The average values of mantle index and developmental stages showed that, the populations of *B. pharaonis* at Adabia and Ain Sokhna have lengthy breeding seasons extends from September to the end of the following April, with two peaks of spawning in early spring and late autumn at Adabia, extends through early and late spring, and through autumn at Ain Sokhna. The oocyte diameter varied from 30.60 to 112.20  $\mu\text{m}$ , and averaged  $49.41 \pm 8.64$ ,  $59.16 \pm 13.23$  and  $71.95 \pm 12.25$   $\mu\text{m}$ , at stages, II, III and IV (ripe eggs) before spawning, respectively.

**Keywords:** Bivalve, Mollusca, Gulf of Suez, reproduction, oocytes, maturation.

### INTRODUCTION

Bivalves play an important role in the ecosystem equilibrium and constitute an important member of the near shore biota, contributing significant part in the food chain and in the modification of sea bottom nature at their occurrence (Desouky, 2009). *B. pharaonis* (Fischer, 1870) is a small intertidal bivalve, belongs to family Mytilidae, Order Mytilida, within class Bivalvia, Phylum Mollusca (Vine, 1986; Rusmore-Villaume, 2008). This species is a euryhaline, eurythermal, diet generalist, anchors itself to hard substrates in the mid-littoral zone with abyssal threads, forming mytilid beds, and reaches high

densities and completely covers rocky shores when wave exposure and sedimentation conditions are optimal (Safriel *et al.*, 1980).

This species has high fecundity, early maturity, with planktonic larvae, and its tolerance of high salinity and high temperature enable this species to occupy diverse habitats across a wide geographic range. This species had been reported in high-salinity lagoons, on open coasts, and in polluted waters (Radwan, 2014). Consequently, based on this tolerance *B. pharaonis* was reported across wide geographical range including the western Pacific Ocean, the Indian Ocean, the Red Sea (Taylor, 1971; Sasekumar, 1974; Barash and

Danin, 1986; Morton, 1988), and had migrated to the Mediterranean Sea after opening Suez Canal (Sara *et al.*, 2000, 2008; Zenetos *et al.*, 2005; Radwan, 2014), but its occurrence in some areas is controversial (Sara *et al.*, 2008).

According to Barash and Danin (1986), *B. pharaonis* occurs along eastern African coasts, from the Red Sea to southern Africa, in the Indian Ocean except for the Persian sub region and Malaysia, and in the western Pacific Ocean. Sasekumar (1974) reported *B. pharaonis* in Malaysia, while Arcidiacono and Di Geronimo (1976) supported the occurrence of *B. pharaonis* along the western African coasts. This species was reported from the Red Sea including Gulfs of Suez and Aqaba by several authors particularly Sharabati (1984), Vine (1986), Head (1987), Mastaller (1987), Mohamed (1992), Rusmore-Villaume (2008), and Radwan (2014). It was also treated among Suez Gulf fauna reported by El Mekawy (2016), and El-Sayed *et al.* (2017) and its biometric relationships was also studied by El-Sayed, *et al.* (2016).

In spite of scattered studies on distributions, ecology, interaction between adults and recruitments on substrates, age structure, reproduction, and length weight relationships for *Brachidonte spharaonis* under its specific name or its synonyms were treated in few articles in the Red Sea, Gulf of Suez as well as at Suez Canal and at eastern Mediterranean Egyptian coasts by Mohamed (1992, 1997) and Radwan (2014), there are no intensive studies were done on the reproduction of different populations of this species at the north-western coasts of the Gulf of Suez.

Therefore, this study aims to investigate the reproductive cycle and gonad maturation of *Brachidonte spharaonis* collected from the intertidal and shallow subtidal zone at the north-western portion of the Suez Gulf.

## MATERIALS AND METHODS

### 1- Study area and sample collection:

Samples of *Brachidonte spharaonis* were collected monthly during the period from

January to December 2014 from the intertidal and shallow subtidal rocky zones from Adabia (32°30'3.84"E- 29°50'46.41"N) and Ain Sokhna (32°21'39.30"E-29°33'29.40"N). These sites lie at 18 km and 65 km south Suez City, respectively, and extend along the north-western portion of Suez Gulf. The habitats at these sites are characterized by rocky, sandy, sand-rock and coral reefs at particularly Ain Sokhna, but has sandy bottom with scattered rock boulders at Adabia.

The individuals of this species were collected by hand and detached from its substrates using forcipies of different sizes and sharp knife. The collected specimens were kept in 10% formalin solution and others in 70 % ethanol, and then transferred into the Laboratory of Marine Invertebrates at Faculty of Science, Al Azhar University for further examination. The specimens were cleaned, sorted and shell length, shell breadth, and shell width were measured by Caliper vernier, and weighted to the nearest 0.01mg using an electric balance.

### 2-Gonad examinations:

The monthly specimens of different size classes of *B. pharaonis* were dissected and gonads were examined, then removed and weighted to the nearest mg using an electrical balance. The sex and gonads conditions were determined by microscopic examination of smears of the gonads according to Gab- Alla (2007). The maturity stages of examined ovaries were estimated and classified into 5 stages according to Seed and Brown (1977), comprising: stage I (immature), stage II (early stage of development), stage III (maturing stage), stage IV (late developing stage or ripe and spawning egg) and stage V (spent stage after spawning).

The sex ratios of *B. pharaonis* were calculated monthly and presented as ratios of males to females. Gonad somatic index was calculated monthly by cutting all mantle tissues associated with gonads in each mature males and females and presented as percentage of

total the body weight according to Ansel *et al.* (1963) as following:

$$\text{Gonadosomatic index} = \frac{\text{Total mantle weight}}{\text{Total body weight}} \times 100$$

Egg size was measured for each developmental stages using binuclear microscope using eye piece ocular micrometer. The longest and shortest axes of each egg were measured to the nearest micrometer.

## RESULTS

### 1- General morphology:

The collected specimens of *B. pharaonis* varied from 2.8 to 37.3 mm in shell length, 1.6 to 22.3 mm in shell breadth, and from 1.2 to 15.9 mm in shell width. Shell is equivalent, in equilateral, attached to substrate by stout byssus. Sculptures are numerous, with fine radial bifurcating ribs, which become coarser posteriorly. The shell margins are crenulated. The hinge has dysodont teeth. Most shells had dark brown-black color and internally tinged violet-black.

### 2- Gonad morphology:

The sex in *B. pharaonis* is separated and cannot be differentiating externally but it can determine through examination of the internal viscera. The macro- and microscopic examinations showed that gonads of both sex appear scattered within mantle tissues at both left and right sides of the animal. Male testes appear whitish or creamy in color; while sexually matured females have dark yellow to orange ovaries due to occurrence of full ripe ova fill of yolk granules. Both gonads of males and females have main duct branched to smaller ducts attached to several acinus or lobules forming egg nests or oogonic follicles in females or testes in males. During egg maturation these eggs increase in size and shapes based on yolk accumulation.

In very small individuals, gonads are hardly differentiated into testes or ovaries, but appear as transparent tissues have very small follicles without yolk granules in immature individuals.

While in mature individuals, testes are enormous, being branched and lobulated.

In females, ovaries are more conspicuous, lobulated, and composed of large numbers of oogenic follicles occupying most of the mantle depending on stages of egg maturation. These follicles have irregular size and shape and they are connected together by a connective tissue. Each follicle has different groups of eggs at variable stages of development with spherical or semispherical ova.

### 3- Maturity stages in female:

The results of the present work revealed that, there are 5 stages of gonad maturation in females of this species, these are:

#### - Stage I: Immature

During this stage no signs for occurrence of gonads are detected, therefore, this stage comprises very small individual of this species, ranged in shell length between 6.3 and 6.5 mm (Figure, 1 a).

#### - Stage II: Early stage of development

During this stage gonads of both males and females can be differentiate. Examined ovaries showed that there were several oogenic follicles have transparent very small oocytes without yolk granules. This stage comprise both of early stages of maturation and resting stages, but ovaries and testes of individuals of the last stage are characterized by high ratios of connective tissues and widely separated follicles (Figure, 1c).

#### - Stage III: Maturing stage

This stage is characterized by beginning of yolk deposition in the ova. Ova are spherical, with yolk granules occupying from one to three quarters of the ovum volume (Figure, 1d). Color of ovaries varied from faint yellow to yellowish or yellow.

#### - Stage IV: Late developing stage (ripe and spawning egg)

This stage is characterized by complete deposition of yolk in the ova and yolk granules

fill all spaces of ova. Free oocytes are released and gradually fill the lumen of follicles leading to broken of follicle wall. Mature oocytes at this stage have polygonal shape due crowding (Figure 1 e). Color beings deep yellow.

- Stage V: Spent stage

Ovaries at this stage being almost empty, characterize with shrinking oogenic follicles containing few remains of unspawning opaque oocytes or egg remains (Figure, 1 f). The unspawned ova have irregular shapes, surrounded by fibrous connective tissues in addition to few numbers of free transparent oocytes, beings very similar to early maturing stage. The sex can still be identified by the presence of the un resorbed oocytes.

**4-Size at first maturity:**

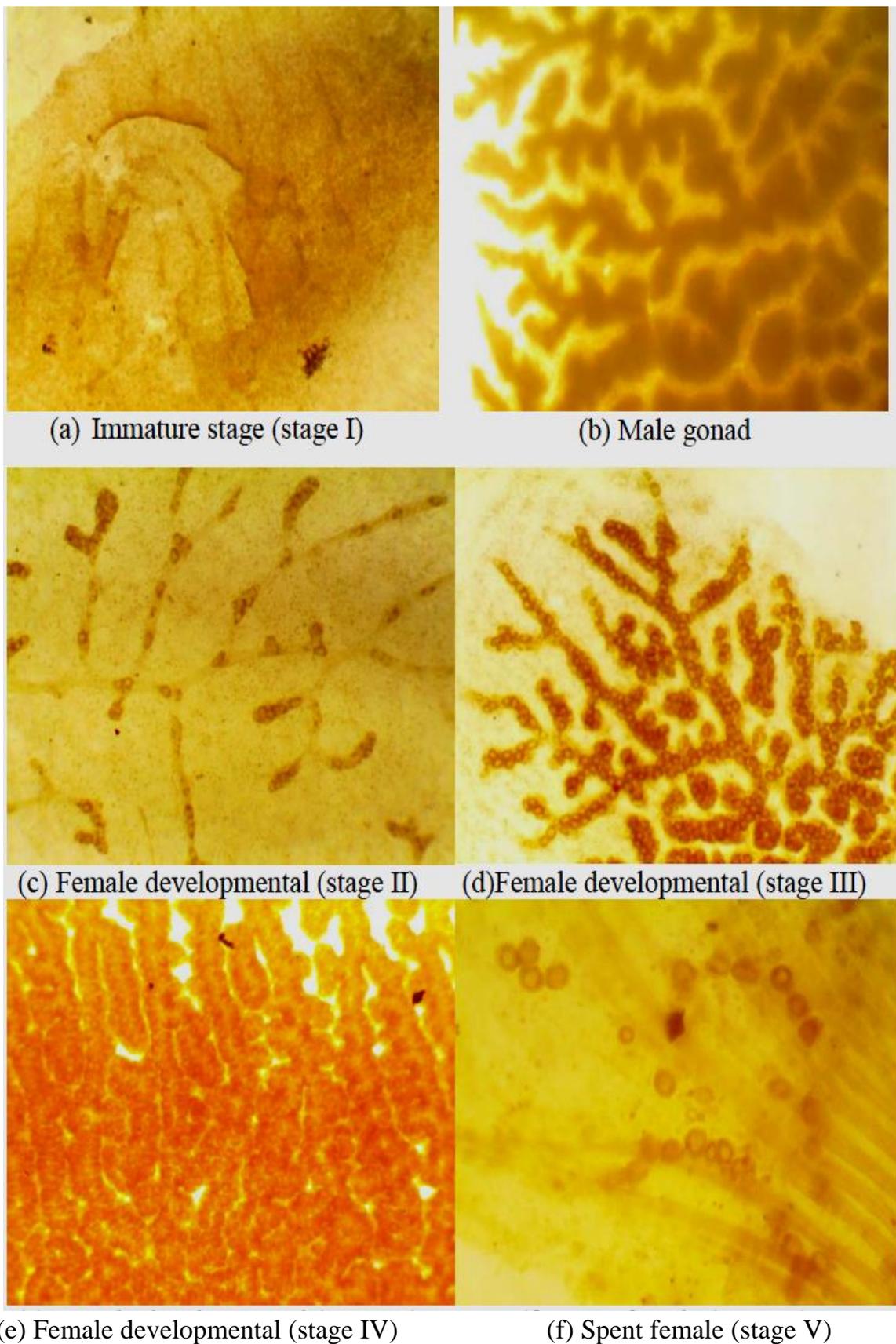
The size of *B. pharaonis* ranges between 3.6 and 33.2 mm at Adabia and between 2.8

and 37.3 mm at Ain Sokhna. The microscopic examinations exhibited that the first appearance for gonads in both males and females was recorded between 6.3 mm and 6.5 mm shell length for both sexes respectively at Adabia and at 5.7 mm shell length in males and 5.6 mm shell length in females at Ain Sokhna. Therefore, no signs for occurrence testes or ovaries were detected less than these sizes at both sites. However, the largest size for immature reached 8.6 and 8.0 mm shell length at both sites, respectively. Hence, the size classes between 6.3-8.6 mm and 5.7- 8.0 mm considered an overlapping between immature and maturing individuals, while all individuals larger than these sizes were maturing and have gonads at different stages of maturity at Adabia and Ain Sokhna, respectively (Table, 1).

**Table (1): Monthly variations in maturity stages in female's ovaries of *B. pharaonis* from Adabia and Ain Sokhna Gulf of Suez (Data as %).**

Sites & stages Months	Adabia					Ain Sokhna				
	I	II	III	IV	V	I	II	III	IV	V
January	40.00		20.00	20.00	20.00	20.00		20.00	40.00	20.00
February		14.00	57.00		29.00	29.00		14.00	43.00	14.00
March		10.00	20.00	30.00	40.00	13.00	13.00	38.00		38.00
April			60.00	20.00	20.00	13.00	13.00	25.00	50.00	
May	25.00	25.00	50.00				14.00	14.00		71.00
June			100.00				43.00	43.00		14.00
July	Did not visit									
August	29.00		57.00	14.00		17.00	33.00	50.00		
September	20.00		20.00	20.00	40.00				17.00	83.00
October			40.00	40.00	20.00		50.00	13.00	25.00	13.00
November		12.50			87.50	17.00	33.00	17.00	17.00	17.00
December			43.00	57.00		33.00	33.00		17.00	17.00

I= Stage 1 (immature); II= Stage 2; III=Stage3; IV= Stage4 and V= Stage5 (Spent ovary).



**Figure (1):** Photomicrographs showing gonadal maturity stages of *Brachidontes pharaonis* from Ain Sokhna, Gulf of Suez (X 100).

### 5- Sex ratio:

A total of 1103 specimens were collected monthly from the study areas, Adabia, (532), and Sokhna (571). Subsamples (10 specimens of all sizes) were dissected monthly from each site to investigate the occurrence and determine maturity stages. Data in Table (2) and Figure (2) demonstrate the monthly changes and overall sex ratios for *B. pharaonis*.

The present results showed that, out of the examined 110 individuals at each site 63 females (57.27%), 40 males (36.36%) and 7 immature individuals (6.36%) were recorded at Adabia, compared with 65 females (59.09%), 36 males (32.73%) and 9 immature individuals (8.18%) at Ain Sokhna. These results indicated that the general sex ratio (males to females) for the two population of this species is 1: 1.575 at Adabia and 1:1.81 at Ain Sokhna. These values indicate to higher ratios for females than males at the study areas, being remarkable at Ain

Sokhna than Adabia, with high statistical significant differences of  $X^2 = 8.33$ , and 5.14 ( $P < 0.01$  and  $P < 0.05$ ) at the two sites, respectively. There were also monthly and seasonal fluctuations, but had no significant differences using t-test ( $P > 0.05$ ), except during March ( $X^2=10.00$ ,  $P < 0.01$ ) at Adabia, where all specimens were females (Table, 2).

The present results showed that, these ratios were not constant throughout the year for the two sites. Females were recorded with low considerable values during January (1:0.8) and June (1: 0.43) at Ain Sokhna and Adabia, respectively, but they over-numbered males during most months of the year, reached to 1: 4.00 in November and October at both sites, respectively. In contrast, equal ratios of males and females were recorded during April and October for Adabia and December for Ain Sokhna. But all males disappeared completely during March at Adabia (Table, 2).

**Table (2): Monthly variations in sex ratios of *B. pharaonis* collected from Adabia and Ain Sokhna, Gulf of Suez.**

Sites	Adabia					Ain Sokhna				
	Males	Females	Immature	Sex ratio	$X^2$	Males	Females	Immature	Sex ratio	$X^2$
				♂♂ : ♀♀					♂♂ : ♀♀	
Months										
January	5	3	2	1 : 0.60	0.50	5	4	1	1 : 0.80	0.11
February	3	7		1 : 2.33	1.60	3	5	2	1 : 1.67	0.50
March	0	10		All are females	10.00**	2	7	1	1 : 3.50	2.78
April	5	5		1 : 1.00	0.00	2	7	1	1 : 3.50	2.78
May	2	6	2	1 : 3.00	2.00	3	7		1 : 2.33	1.60
June	7	3		1 : 0.43	1.60	3	7		1 : 2.33	1.60
July	Did not visit									
August	3	5	2	1 : 1.67	0.50	4	5	1	1 : 1.25	0.11
September	5	4	1	1 : 0.80	0.11	4	6		1 : 1.50	0.40
October	5	5		1 : 1.00	0.00	2	8		1 : 4.00	3.60
November	2	8		1 : 4.00	3.60	4	5	1	1 : 1.25	0.11
December	3	7		1 : 2.33	1.60	4	4	2	1 : 1.00	0.00
Overall ratio	40	63	7	1 : 1.575	5.14*	36	65	9	1 : 1.81	8.33**

Note that: \* =  $P < 0.05$ , and \*\* =  $P < 0.01$ .

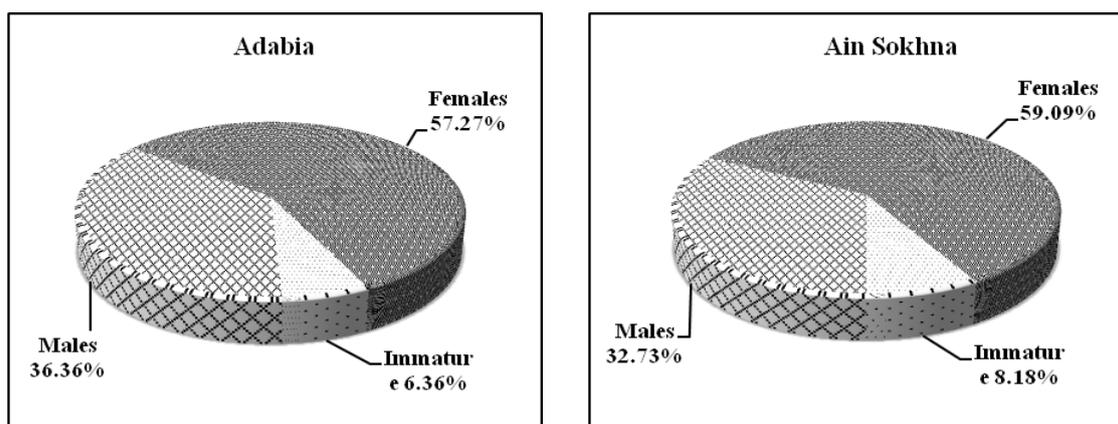


Figure (2): Percentage of sex composition of *B. pharaonis* at Adabia and Ain Sokhna.

### 6- Spawning season:

During this study, the occurrence of spent females (females released their eggs) of *B. pharaonis* was investigated. At Adabia, the appearance of spent females was recorded in January, increased slightly in February, reached the first peak in March but decreased again in the following April, and disappeared completely from May to August, then arises in September and October to reach the second peak in November, but disappeared again in December (Table, 1).

On contrast at Ain Sokhna, spent females of this species were recorded all the year except in April, July and August only. However, they have high frequency during May and September, showed the three peaks of spawning in March, May and September (Table 1).

These data show that, spawning season of *B. pharaonis* at Adabia appeared to be in early spring and late autumn. While at Ain Sokhna the spawning season has two peaks in early and late spring and third one in autumn (Table 1).

### 7- Oocyte diameter:

The present results showed that no evidence for oocytes or ovaries was detected at stage I, but the first appearance was detected at stage II. The examined ovaries showed that oocytes being pass through 4 stages to reach

full maturing egg (ripe egg) which become ready for spawning. During these stages, the developing oocytes showed great changes in sizes and colour at each stage. At stage II or the early developed egg, the size of oocytes varied from 30.60-51.00  $\mu\text{m}$ , and averaged  $49.41 \pm 8.64 \mu\text{m}$ , increased to vary between 40.80 and 91.80  $\mu\text{m}$  and averaged  $59.16 \pm 13.23 \mu\text{m}$  at stage III (maturing eggs) and reached the maximum size before spawning and ranged between 51.00 and 112.20  $\mu\text{m}$  and averaged  $71.95 \pm 12.25$  at stage IV.

### 8- Gonadosomatic index:

The values of gonadosomatic index (represented by mantle included gonad weight) for *B. pharaonis* from Adabia and Ain Sokhna during this study are given in Table (3) and Figure (3). These data showed that there is an obvious gradual increase in gonad weight for females at Ain Sokhna started from September, reaching the maximum value ( $7.45 \pm 1.81$ ) in the following January, but exhibited gradual decline during the following months reaching the minimum average ( $2.43 \pm 0.86$ ) in August.

At Adabia, an increase in female's ovaries weight was characterised by the highest average, beings  $7.58 \pm 2.58$ , at February and showed also high values in April- May and October but declined in March, September and reached the lowest average ( $3.77 \pm 0.95$ ) in November.

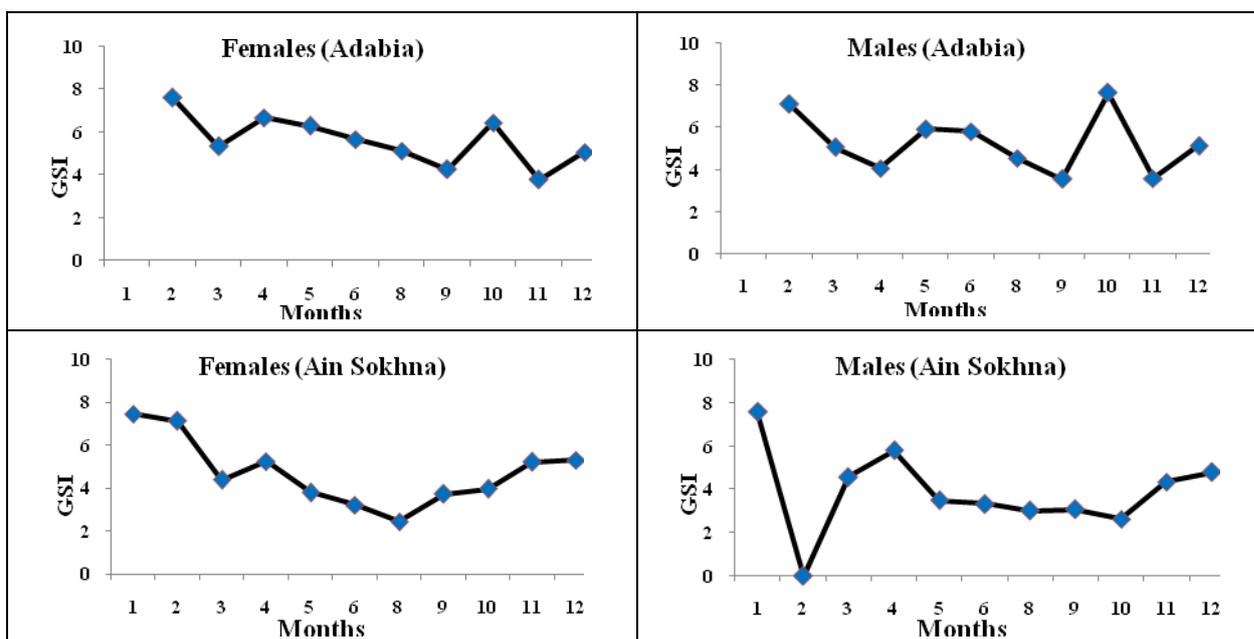
With exception values of male gonadosomatic index during February at Ain Sokhna, the average values of male gonadosomatic index at the two sites had the same females pattern, and reached the maximum averaged of  $7.65 \pm 1.07$  and  $7.56 \pm 2.9$  in February and January, but declined to the lowest values, being  $3.56 \pm 1.16$  and  $2.63 \pm 0.42$

in September and October at Adabia and Ain Sokhna, respectively.

These results indicated that, this species has lengthy breeding season extends from September to the following January at Ain Sokhna and from October to the following February at Adabia. The fluctuation in these

**Table (3): Gonads index of *B.pharaonis* at Adabia and Ain Sokhna (using mantle weight with gonads).**

Months	Average $\pm$ SD	Adabia			Ain Sokhna		
		No.	Males	Females	No.	Males	Females
January	X $\pm$ S.D		-	-	15	$7.56 \pm 2.9$	$7.45 \pm 1.81$
	Range		-	-		4.28-12.87	5.55-10.58
February	X $\pm$ S.D	20	$7.11 \pm 2.09$	$7.58 \pm 2.58$	13	All female	$7.12 \pm 2.04$
	Range		4.45-9.57	2.50-12.69			3.17-10.26
March	X $\pm$ S.D	20	$5.06 \pm 0.85$	$5.30 \pm 2.27$	20	$4.57 \pm 0.41$	$4.39 \pm 1.45$
	Range		4.50-6.72	3.16-8.48		4.28-4.86	2.16-7.49
April	X $\pm$ S.D	20	$4.06 \pm 0.89$	$6.66 \pm 2.55$	17	$5.80 \pm 2.04$	$5.26 \pm 2.84$
	Range		3.04-5.22	3.54-8.99		3.21-10.21	1.72-11.33
May	X $\pm$ S.D	20	$5.92 \pm 1.97$	$6.28 \pm 1.67$	18	$3.48 \pm 0.44$	$3.79 \pm 1.38$
	Range		3.22-8.57	3.77-8.69		2.76-3.83	1.97-5.88
Jun	X $\pm$ S.D	20	$5.82 \pm 2.06$	$5.65 \pm 2.18$	20	$3.32 \pm 1.11$	$3.23 \pm 0.76$
	Range		2.75-8.59	3.14-8.89		2.11-5.42	2.18-4.21
July			Area did not visit				
August	X $\pm$ S.D	20	$4.54 \pm 1.25$	$5.11 \pm 1.32$	18	$2.99 \pm 0.96$	$2.43 \pm 0.86$
	Range		3.05-6.14	3.01-8.02		2.15-4.59	1.59-4.42
September	X $\pm$ S.D	20	$3.56 \pm 1.16$	$4.25 \pm 0.60$	20	$3.07 \pm 0.91$	$3.74 \pm 1.24$
	Range		2.43-4.74	3.40-5.67		1.99-4.41	2.52-7.33
October	X $\pm$ S.D	16	$7.65 \pm 1.07$	$6.41 \pm 2.42$	10	$2.63 \pm 0.42$	$3.97 \pm 1.56$
	Range		6.52-8.73	3.72-11.37		2.01-2.92	2.56-6.45
November	X $\pm$ S.D	18	$3.58 \pm 1.32$	$3.77 \pm 0.95$	15	$4.35 \pm 1.37$	$5.21 \pm 1.46$
	Range		2.31-5.14	2.54-5.78		2.76-6.77	3.06-7.17
December	X $\pm$ S.D	19	$5.14 \pm 0.81$	$5.06 \pm 0.93$	15	$4.77 \pm 2.2$	$5.32 \pm 1.52$
	Range		4.04-6.31	4.15-6.93		2.81-8.87	2.80-7.32



**Figure (3), Gonads index of *B. pharaonis* at Adabia and Ain Sokhna (using mantle weight included gonads).**

data may be correlated with partially spawning behaviour in females at two study sites.

## DISCUSSION

Detailed studies on reproduction of bivalves were carried out at several localities around the world comprised equatorial, tropical, temperate and cold water regions. For family Mytilidae and very close families, the articles treated reproductive activity were considered, particularly those either carried out along the Egyptian coastal waters of the Red Sea, Mediterranean Sea and Suez Canal or those carried out in the very near regions along European, African and Asian coasts of the previous mentioned seas or those on the Atlantic and Indo-Pacific regions. Other works studied the relationships between the effects of environmental conditions and reproductive activities were also considered (Nayar, 1977; Seed and Brown, 1977; Abou Zied, 1991; Galinou-Mitsoudi and Sinis, 1994; Gab-Alla *et al.*, 2007; Kandeel *et al.*, 2013; Abdel Razek *et al.*, 2014 and Radwan, 2014).

In the present study, microscopic examinations were used to determine gonadal stages in both sexes of *Brachidontes pharaonis*. The gonads in this species appear scattered within mantle tissues at both left and right sides of the animal, showing color changes associated with gonad maturation, particularly those accompanied with developmental stages in ovaries. All changes in gonadal morphology in both sexes and changes in oocytes color and sizes to reaching ripe eggs in females are in well agreement with that mentioned by Nayar (1977).

During ovarian maturation, eggs are increasing in size and shapes based on yolk accumulation according to Seed and Brown (1977). The oocytes of examined mature females were passed through 4 stages to reach full mature ova. During these stages, oocytes increased from 30.60 to 51.0  $\mu\text{m}$  at stage II reaching 51.0 to 112.2 and averaged 71.95  $\mu\text{m}$  at the final stage of maturation and become ready for ovulation at stage IV. While those

unspawned ova were resorbed and have irregular shapes and size. The obtained results are in full agreement with those described by Nayar (1977) on general bivalves, Galinou-Mitsoudi and Sinis (1994) on *Lithophaga lithophaga* from Aegean Sea (eastern Mediterranean), Gab-Alla *et al.* (2007) on *Gafrarium pectinatum* and *Macraolorina* from northern parts of Suez Gulf, and Abdel Razek *et al.* (2014) on *Modiolus auriculatus* from the Egyptian coasts of the Red Sea.

Sex in *Brachidontes pharaonis* is separate, but no external sexual dimorphism can be determined. The present results exhibited that, no gonads were detected in small males less than 6.3 mm shell length or females smaller than 6.5 mm at Adabia and 5.7 mm and 6.0 mm at Ain Sokhna. Hence, the smaller individuals beyond these sizes are called immature or juveniles, but some of those individuals at these sizes may have gonads (testes or ovaries) and the largest immature may reach 8.0 mm shell length. The obtained results are in agreement with that mentioned by Radwan (2014) on the same species at the same localities which ranged between 5- 10 mm shell length, but being slightly lower than that reported by Kandeel *et al.* (2013) on *Cerastoderma gluacum* from Lake Qarun, Egypt which attained maturity between 8.5 and 9.6 mm for males and females, respectively. These findings are also in agreement with those reported by Galinou-Mitsoudi and Sinis (1994), and Abdel Razek *et al.* (2014) on other bivalves. The onset of first maturation at this size class reflects effects of favorable conditions and stability of populations of this species at the studied areas.

The obtained results showed that, the overall sex ratio for *B. pharaonis* was 1:1.56 and 1:1.81 (males to females) at Adabia and Ain Sokhna, respectively. These ratios indicated over dominance of females at both sites, with statistical significant difference between the two sexes at the studied areas. However, these ratios are not stable and showed monthly variations, revealing increasing male numbers in January at both

sites and only through June and September at Adabia. On the other hand, female numbers were higher in most months during the course of this study, as well as are higher than those reported by Abou Zied (1991), Gab-Allah, *et al.* (2007), Park *et al.* (2012) on *Crassostrea gigas* during 2007, Kandeel *et al.* (2013), Abdel Razek *et al.* (2014), but lower than that reported by Park *et al.* (2012) on *C. gigas* collected in 2008. These authors calculated sex ratio very close to 1:1 (males to females) in their studies on different species of bivalves.

The annual changes in sex ratio were reported by Park *et al.* (2012). They found that, sex ratio had been changed from 1:1 (males to females) during 2007 to 1:2.8 in 2008. On the other hand, Galinou- Mitsoudi and Sinis (1994) found that sex ratio was changed associated with changes in shell size. They reported that the ratio was changed from 3:1 (males to females) in individuals up to 7 cm to 1:1 for individuals greater than 7 cm shell length. However, no data on sex ratio depending on size were considered during this study.

The results of the present work revealed that, there are 5 stages of ovarian maturation in females of this species. These stages were classified microscopically based on gradual increase in egg size and accumulation of yolk granules in developed eggs, which in accordance to that described by Mladineo *et al.* (2007), for *Modiolus barbatus* but Vaschenko *et al.* (2013) recognized 6 stages for maturation of gonads in both males and females of *M.modiolus*.

The obtained results exhibited occurrence of these stages with various frequencies all the year round, showing high frequencies of late spawning eggs or ripe eggs during March, September and December at Adabia and in March, May and September at Ain Sokhna, associated with remarkable increase in spent stages at the same or the following months.

These data exhibited lengthy breeding season based on gonadosomatic index and

appearance and disappearance of ripening eggs for *B. pharaonis* collected from the northern limit of the Suez Gulf which starts in late autumn and passed through winter to the following spring, showing at least two peaks of spawning. The same results were obtained at Ain Sokhna, showing disappearance of ripe eggs and spent stages during June- August, with high values in March and September. After spawning the examined mantle tissues associated with ovaries were characterized at spent stages with high ratio of fibrous tissues, comprised few of irregular ripe eggs accompanied with very clear atresia or semi resorbed egg of irregular shapes.

These results are similar with that recorded by Abou-Zied (1991) on *Venerupis aurea* from Lake Timsah (Suez Canal) which had monthly variations in stages of maturity proved that the population of lake Timsah contains more than one breeding groups, separated based on shell length into two groups. The first group was varied from 20 – 25 mm, while the larger one ranged between 30 - 35 mm, and the whole population was spawned from August till December for the rest of the year. But the present results are in contrast to that carried out on other bivalves obtained by Gab-Allah *et al.* (2007) on *Gafrarium pectinatum* and *Maetra orolina* from the same localities which spawned during spring from March to June and had only three maturity stages. However, these data are in agreement with that mentioned by Galinou-Mitsoudi and Sinis (1994) on *Lithophaga lithophaga* which are breeding almost simultaneously correlated with a decline in the highest water temperature (27°C), an increase in salinity (>31‰) and a decrease in the dissolved oxygen (6.5 ppm). Small percentages of mature individuals appear during the first winter months thus lengthening the reproductive period. This phenomenon is attributed to the temperature difference in deeper waters, the delay in gamete release by young individuals, tide, wave action and changes in salinity (Galinou-Mitsoudi and Sinis, 1994). Also these data agree with that mentioned by Machreki-Ajmi (2013)

on *Cerastoderma glaucum* (Mediterranean Sea) which are spawning most of the year, without prolonged periods of reproductive inactivity; Crnčević *et al.* (2013) on *Glycymeris nummaria* from Mali Ston Bay, Adriatic Sea, Croatia.

The disappearance of late stages of maturity particularly stages IV (ripe eggs) and V (spent stage) was greatly correlated with prevailing environmental factors, and coincides with those reported by Seed and Brown (1977) on *Modiolus smodiolus* (L.), *Cerastoderma edule* (L.) and *Mytilus edulis* L. in Strangford Lough, Northern Ireland and to population stability. They suggested that localized environmental factors are exceedingly important in controlling the annual reproductive cycle of this species. They also added that, variations in the duration of the spawning periods in these bivalves can perhaps be explained in terms of both environmental stability and the immediate physical conditions experienced by the separate populations. They also added that, the reproductive strategies exhibited by the species are considered in relation to population stability and to the different patterns of mortality which characterize these species in their respective local habitats.

On the other hand, these results are in contrast to that mentioned by Rajagopal *et al.* (1998) on *P. viridis* from Indian waters which exhibits two spawning periods and 50% of the population spawns during May–June and September–December, with some annual variations. Interestingly, these periods were associated with higher annual temperatures in the backwaters and showed increased spawning activity during the above periods, with larval density in the backwaters increased during the summer and northeastern (NE) monsoon periods. They indicated that, temperature appears to regulate the onset of reproductive events.

The relationships between environmental factors and spawning were treated with several authors. Barnes (1957) showed that synchronization of spawning in marine

epibenthic communities particularly turned to the main phytoplankton outburst and only indirectly to temperature. He also emphasized that, under tropical marine conditions, factors such as temperature and food availability were maintained at near constant levels, and therefore, seasonal breeding cycles were often superimposed. However, in spite of this intensive study on *B. pharonis* in the northern portion of the Gulf of Suez, further detailed studies on other common and frequent species are still necessary to give clear picture on biological activities of bivalves and their ecological and biological role at this area of sensitive ecological importance.

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## مظاهر التكاثر فينوع براكيديونتس فاراونيس (الرخويات- المصرايعات) المجمعة من الجزء الشمالي الغربي لخليج السويس

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تشير نتائج الدراسة الحالية أن نوع براكيديونتس فاراونيس *Brachidontes pharaonis* وهو أحد أنواع الرخويات ذوات المصراعين منفصل الجنس إلا أنه لا يمكن تمييزه من الشكل الخارجي، حيث أوضح الفحص المجري للعينات وجود مناسل متفرعة داخل الأحشاء تقع على الجهتين اليمنى واليسرى للبرنس (mantle). وتتميز المناسل الذكرية (الخصى) باللون الأبيض الكريمي بينما توجد المبايض على شكل فصوص متفرعة تحتوى على أعداد كبيرة من حويصلات البيض التي تتفرع في جزء كبير من البرنس، كما تمر مبايض الإناث بعدة مراحل وصولاً إلى مرحلة التبويض وتتلون المبايض الناضجة باللون الأصفر الداكن أو البرتقالي بسبب تراكم وكثافة حبيبات المح وتطور مراحل النضج، وتتباين البويضات في أحجامها وألوانها وتصنف في خمس مراحل واضحة هي: (١) المرحلة الأولى: وتضم الأفراد غير الناضجة، (٢) المرحلة الثانية: وهالمرحلة التي يمكن فيها تحديد البويضات، (٣) المرحلة الثالثة: أو مرحلة النضج، حيث تظهر فيها البويضات ويبدأ تكوين حبيبات المح بدرجات متفاوتة، (٤) المرحلة الرابعة: وهي المرحلة المتأخرة للنضج، وتتميز بظهور بويضات كاملة وجاهزة للتبويض، (٥) المرحلة الخامسة: وهي مرحلة التبويض وخروج البويضات من المبيض، مع وجود بقايا من البويضات المتأكلة.

وتشير نتائج فحص المبيض إلى اختلاف أحجام البويضات خلال مراحل النضج المختلفة، حيث تراوح حجم البويضات ما بين 30.60-51.00 ميكرون بمتوسط  $49.41 \pm 8.64$  ميكرون في المرحلة الثانية، وازداد فيما بين 40.80-91.80 ميكرون بمتوسط  $59.16 \pm 13.33$  ميكرون في المرحلة الثالثة، ثم تراوح الحجم فيما بين 51.00-112.20 بمتوسط  $71.95 \pm 12.25$  ميكرون في المرحلة الرابعة. ولقد أوضحت الفحوصات المجهرية ارتباط ظهور المناسل بطول الصدفة حيث بدأ ظهورها في الذكور بداية من طول 6.3 مم وفي الإناث عند الطول 6.5 مم، بينما سجلت أكبر عينة غير ناضجة عند طول 8.6 مم ومن ثم تمثل الأطوال فيما بين 6.3-8.6 مم منطقة تداخل بين الأفراد غير الناضجة والأفراد الناضجة في الشقين بمنطقتي الدراسة.

ولقد سجلت النسبة الشقية بين الذكور والإناث 1:1.07 و 1:1.81 في الأدبية والعين السخنة على التوالي طوال العام، وتشير التحاليل الإحصائية باستخدام مربع كاي إلى فروق معنوية ( $P < 0.05$  و  $0.01 < P < 8.33$ ) في الأدبية والعين السخنة على التوالي. مع وجود اختلافات إحصائية واضحة بين النسب الشقية خلال الشهور والمواسم المختلفة.

وتشير نتائج الدراسة باستخدام مراحل نضج البويضات ومؤشر العلاقة بين وزن البرنس شاملاً وزن المناسل أن موسم التكاثر لمجتمع براكيديونتس فاراونيس بمنطقتي الأدبية والعين السخنة موسم ممتد يبدأ في شهر سبتمبر ويمتد إلى شهر أبريل من العام التالي، وتصل ذروة موسم تكاثر العينات المجمعة من الأدبية في بداية الربيع ونهاية الخريف، بخلاف عينات العين السخنة التي تتميز بوجود ذروتين إحداها في بداية فصل الشتاء والربيع مع ظهور ذروة ثالثة أقل نسبياً في فصل الخريف.