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Invasion dynamics of a non-indigenous bivalve clam; *Venerupis aurea* (Gmelin, 1791), in Lake Qarun, Egypt.

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ABSTRACT

The invasive clam *Venerupis aurea* is native to the Mediterranean Sea. The species has now become established in Lake Qarun, Egypt. This is the first record of the establishment of this clam in the Lake. The histograms of the length-frequency distribution indicated multiple consecutive cohorts and the species is able to establish self-sustaining population. Total weight increased relatively slower than shell length in summer. Isometric growth pattern was recorded in other seasons. Negative allometric growth pattern during summer may explain spawning. The female to male ratio is 1: 0.87. Sexual maturity is attained at 11.8 mm shell length in summer and 16.6 mm shell length in winter. Histological studies revealed two different stages in both male and female clams namely, immature and developing. The results from these preliminary studies have indicated that *V. aurea* is able to reproduce and survive in Lake Qarun and has an important impact in the local community of the Lake.

KEYWORDS

Bioinvasion, Veneridae, Lake Qarun; Egypt.

1. INTRODUCTION

Biological invasions consist of human induced species translocation such as global shipping and aquaculture or of natural range expansions (Schmidt *et al*., 2008). *Venerupis aurea* (Gmelin, 1791) is endemic to the Mediterranean Sea and have penetrated through the Suez Canal and successfully colonized Lake Timsah (Fouda and Abou Zied, 1990). This clam was first recorded from the Canal by Tillier and Bavay (1905). The presence of this clam in Lake Qarun has been recorded for the first time since 1994 (El-Shabrawy, 2001).The standing crop of the clam in the Lake showed an increase from 12 org. m^2 in 1994 to 51 org. m^2 in 1995 (El-Shabrawy, 2001). The clam has been brought in from the Mediterranean with mullet fry of *Mugil cephalus* and *Liza ramada* (General Authority for Development of Fish Resources, 2000; El-Ghobashy, *et al*, 2011).

Although the biological and ecological studies on bivalves that have economic damage to local communities are relatively large (Bedir *et al*., 2008; Bownes and McQuaid, 2006; Branch and Stefani, 2004; Schmidt *et al.,* 2008; Tan and Morton, 2006; Waangkulangkul and Lheknim; 2008), the studies on invasive edible species is very rare (El-Ghobashy, *et al*, 2011. Kandeel, 2006; 2008; Schmidt *et al*., 2008)

The aims of the present study are therefore to estimate the population structure, length-weight relationship, sex ratio, and onset of sexual maturity of the invasive clam *V. aurea* to assess the stock position of the species in Lake Qarun, Egypt. An attempt was also made to compare the population of *V. aurea* in Lake Qarun with populations established in other areas.

2. MATERIALS AND METHODS

2.1. Study area

Lake Qarun (Fig. 1), one of the largest lakes of Egypt, is a closed saline lake in the northern part of El-Fayoum Depression at the Western Desert (approx. 85 km to the southwest of Cairo). The Lake lies between 30° 34\` and 30° 49\` E longitude and 29° 25\` and 29° 34\` N latitude at 44 m below the sea level and spread in an area of about 226 km^2. Lake Qarun receives about 470 million cubic meters of drainage water through 12 drains, annually, of which "Bats" and "Wadi" drains carry most of the water brought to the lake (Mansour *et al.,* 2000). The Lake is almost entirely sustained by inflow from the Nile River and salinity of the lake has increased gradually over time (Ball, 1939), that ranged between 26.4 and 43.9 gL^{-1} during 2001 (Fathi and Flower, 2005). Increased salinity caused a profound effect on the faunal composition of the Lake and reduced their standing crop (El-Shabrawy, 2001; Fishar, 2000). Average water temperature reached a maximum in summer (28.6 ^oC) and a minimum value (15.4 ^oC) in winter (Shadrin *et al*., 2016).

2. 2. Sampling procedure

Samples were collected using 20 x 20 cm^2 stainless steel grab sampler randomly placed in the substratum about 0.5 m in depth. Samples were collected in four seasons only; winter (February), spring (April), summer, (July), and autumn (October) 2016. All the clams from the grabs were separated and kept in labeled containers filled with 6% formaldehyde-seawater solution. Some specimens were fixed in Bouin’s solution for histological preparation.

2. 3. Laboratory procedure and data analysis

In the laboratory, shell lengths (maximum distance on the anterior-posterior axis) of all the specimens were measured to the nearest 0.1 mm by using a Vernier caliper. Other size parameter, such a total weight (precision of 0.0001 g) was measured using top-loading digital balance.

Length measurements were used to construct length-frequency distribution for each sample collected from the Lake using class intervals of 1 mm size. Cohort analysis was made by means of Bhattacharya's methods, available in FISAT II (FAO, 2005), and used to distinguish exiting cohorts in sampling seasons.

The relationship between total weight (TW; in grams) and shell length (SL; in mm) was described by the following allometric equation:

$$\log TW = \log a + b \log SL \quad \text{or} \quad TW = aSL^b \quad \text{(Ricker, 1975)}$$

Where TW is a dependent variable; SL is the independent variable*;* *a* and *b* are intercept (initial growth coefficient) and slope (relative growth rate of variables) values, respectively. Weight and length were converted to natural logs and a linear regression equation was determined: The association degree between size parameters was calculated by the determination coefficient (r^2). Additionally, data were submitted to an analysis of variance (ANOVA) to estimate variance ratio (*F*) and the significance level of r^2.

The deviation of the b value of the regression function from the isometric value (b = 3) was tested using Student's *t*-test, as expressed by the following equation (Monti *et al*., 1991):

$$t_s = (b - \beta)/s_b$$

where t_s = *t*-test value; b = slope; β = isometric value of the slope; s_b = standard deviation of the slope (b). A comparison between the obtained values of *t*-test and the correspondent tabled critical values allowed for determination of the statistical significance of the b values. A significance deviation indicates a negative ($b < 3$) or positive ($b > 3$) allometric relationship.

The shell length at which the gonad begins to develop from the rudimentary virgin state (sexually undifferentiated or juvenile gonad) to the state of sexually differentiated was taken to be the size at onset of sexual maturity. Sexual products (mature oocytes/spermatozoa) were examined microscopically. Statistically significant deviations from the expected sex ratio of 1:1 were assessed by Chi-square (x^2) analysis with one degree of freedom (Bailey, 1995).

Statistical analysis was carried out using MINITAB software (version 13, 2000). Statistical significance was considered at P = 0.05.

The percentages of sexually mature individuals in 1-mm class intervals were calculated. A logistic curve was fitted to the data in order to estimate the length, at which 50% of individuals were sexually mature (SM_{50}) during winter (February) and summer (July) 2016.

Gonads were dissected from fixed specimens, dehydrated in graded ethanol, cleared in xylene, embedded in paraffin wax and sectioned at 6-8 µm. Sections were mounted and stained with Ehrlich's hematoxylin and Eosin Y following Howard and Smith (1983).

3. RESULTS

3.1. Population structure

Length-frequency distribution of *V. aurea* in Lake Qarun exhibits considerable seasonal changes (Tab. 1 and Fig. 2). The shell length range varied between 4.0 and 30.9 mm. The earlier mode (X = 6.37, S.D. = 2.86) was recorded in summer season where the length class represented 6.3 % of the whole population. The latter mode (X = 26.10, S.D. = 3.25) was represented by 4.4 % of the whole population during autumn at a length class of 28 mm. The largest animal ever found, in spring, was 30.9 mm long and represented only 1.0 % of the total population.

3. 2. Length-weight relationship

Coefficient of determination (r^2) varied between 0.888 and 0.980 (Tab. 2) when seasonal linear regressions between SL and TW of *V. aurea* was established. Slope values (*b*) fluctuated between 2.49 and 2.96. Student's *t*-test revealed that TW increased relatively slower than SL (negative allometric growth) during summer. Slope values did not significantly deviated from 3 value ($P > 0.05$) indicating isometric growth pattern in winter, spring and autumn.

3.3. Sex ratio

V. aurea is found to be a gonochoric species and sex reversals were not observed. Microscopic examination of gonadal materials revealed, 53.5% females and 46.5% as males. The ratio of females to males (1 F: 0.87 M) was statistically non-different from the theoretical sex ratio of 1:1 (x^2 = 0.7806, $P > 0.05$)

3.4. Onset of sexual maturity

The relationship between shell length (SL) and the percentage of mature *V. aurea* for winter and summer samples revealed size at which 50% of the population reached maturity (SM_{50}) as 11.8 mm and 16.6 and mm SL for both seasons, respectively. The smallest mature clams were 15 mm and 12 mm SL and the largest immature ones were 18 mm and 11 mm SL for the two seasons, respectively (Fig 3).

3.5. Histological studies

Sexually undifferentiated (juvenile) gonads (Fig. 4) showed very few follicles embedded in the connective tissue. The follicles were separate and smaller in diameter. The gonadal cells (GC) were embedded in the follicle walls and contain large conspicuous and dense basophilic nuclei. Follicle lumen (L) was very large with no trace of gametes

Sexually differentiated or developing gonads were compose of numerous and irregular follicles. Different stages of oogenesis could be recognized in female clams (Fig. 5 A). Fully developed oocytes (ripe ova, RO) were often without attachment to the follicle wall and moved into the lumen of the follicles. Testicular follicles also, contained germ cells at different stages of spermatogenesis depending upon the reproductive condition of the clam (Fig. 5 B).

4. DISCUSSION

The successful introduction of an invasive species is divided into four successive phases: arrival, settlement, expansion and persistence (Mollison, 1986; Reise *et al*., 2006). For the invasion of the venus clam *Venerupis aurea* in Lake Qarun, we could detect the four phases of introduction. The four successive phases of bioinvasion were recorded for comb-jellies, *Mnemiopsis leidyi* A. Agassiz, 1865, at Lake Qarun (El-shabrawy and Dumont, 2016). This species may ultimately prove fatal to predators that, in nature, depend heavily on various diets of plankton, planktonic fish eggs, and fish larvae. (Purcell *et al*., 2001)

The clam *V. aurea* belonging to the family Veneridae is one of the most commercially important species of bivalves in Egyptian waters. The success of spawning of this clam in Lake Qarun was confirmed by the presence of different cohorts of juveniles in collected samples and the presence of mature gametes (ripe ova and morphologically ripe spermatozoa) in the lumen of the gonadal follicles (Fig.5 A and B). These results may refer to the availability of clam's foods in the Lake throughout the year. Also, it seems that the relatively moderate water temperature in winter (average value = 15.4) and warm in summer (average value = 28.6) (Shadrin *et al*., 2016).) are within range of clam's normal metabolism.

The relationship between body weight and shell length allow life span and morphological comparisons between related species or between populations of the species from different habitats and/or regions (Gaspar *et al*., 2001; Mohammad *et al*., 2014). Parameters of total weight-shell length relationships of the different species of Venerupis are shown in Table (3). All species presented higher determination coefficients (r^2). Slope values (*b*) were variable among species and also between different geographical areas. The prevalence of isometries and negative allomtries over positive allometries is common in the different species. . For the invasive clam*; V. aurea*, negative allometric growth pattern during summer may reflect spawning stress (Johannessen, 1973; Kandeel, 2008). The results of this study agree with Kandeel (2008) and Mohammad *et al*. (2014). However, the prevalence of isometric and positive allometric growth over negative allometric growth for 25 bivalve species including 6 venerids from the Algarve coast in southern Portugal was reported by Gaspar *et al*. (2001)

Although the presence *of Venerupis aurea* has been recorded in Lake Qarun since 1994 (El-Shabrawy, 2001). This is the first report of its distribution and relatively abundance in a habitat (Lake Qarun) differs from its native. Further researches to estimate vital parameters of the population dynamics (growth, recruitment and mortality) are required. The potential impact of this clam on Lake Qarun ecosystem is required because the unique importance of Lake Qarun ecosystem and also because the economic importance of the clam in coastal cities of Egypt.

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Table (1): Percentage-size frequency distributions of *V. aurea* in Lake Qarun during four occasions. N = number of clams measured.

Season	Shell length (mm)													
	4.0	6.0	8.0	10.0	12.0	14.0	16.0	18.0	20.0	22.0	24.0	26.0	28.0	30.0	N
Winter 2016	2.2	2.2	2.2	11.8	12.7	9.7	15.7	10.8	10.8	4.9	4.9	5.9	5.9	0.9	102
Spring	5.9	10.2	11.7	11.7	6.6	15.9	5.9	4.0	5.9	2.4	7.0	3.4	5.9	3.5	88
Summer	4.5	6.3	5.4	4.5	17.0	16.1	12.5	9.6	6.3	3.6	4.5	3.6	4.5	1.6	112
Autumn	-	1.1	2.2	4.4	8.9	15.7	14.4	14.4	10	6.7	5.6	8.9	4.4	3.3	90

Table (2): Seasonal variation in regression parameters (log *a* and *b*) of shell length (SL, mm) and total weight (TW, g.) relationships of *V. aurea* collected from Lake Qarun. Values of Student's *t*- test (*t*), level of significance from isometric value of the slope; *β* = 3 (*p*), coefficient of determination (r^2), variance ratio (*F*), and number of animals examined (N) are also given.

Length-Weight relationship							
Season	Log *a* ± S.D.	*b* ± S.D.	*t*	*p*	r^2	*F*	N
Winter 2016	-3.64±0.09	2.76±0.05	4.80	N.S.	0.972	2761.3	102
Spring	- 4.00±0.14	2.96±0.07	0.57	N.S.	0.967	1892.4	88
Summer	-3.34±0.10	2.49±0.03	17.00	<0.025	0.980	5994.2	112
Autumn	-3.35±0.06	2.53±0.08	5.88	N.S.	0.888	1108.8	90

All relationships were highly significant ($p < 0.0001$), N.S. = non-significant ($p > 0.05$), S.D. = standard deviation.

Table (3): Values of equation parameters relating total body weight (TW) with shell length (SL) for the different species of *Venerupies* according to the expression log TW = log *a* + *b* log SL.

Species	Location	log *a*	*b*	Relationship (*t*-test)	r^2	*P*	References
Venus fasciata	Algarve coast, southern Portugal.	-3.00	2.63	- allometry	0.859	<0.05	Gaspar *et al*. (2001)
Venus verrucosa	The Bizerte Lagoon, Tunisia.	4.00	3.24	-allometry in summer	0.953	ns	El-menif et al. (2008)
Venerupis rhomboides	Algarve coast, southern Portugal.	-4.70	3.61	+ allometry	0.902	<0.05	Gaspar *et al*. (2001)
Venerupis pullastra	Seljehölen, western Norway.	-4.48	3.48	ns	ns	ns	Johannessen (1973)
Venerupis pullastra	Etap, Lake Timsah, Suez Canal, Egypt.	-3.61	2.73	ns	0.922	=0.0001	Gabr (1991)
Venerupis pullastra	Taawen, Lake Timsah, Suez Canal, Egypt.	-3.95	3.00	ns	0.941	=0.0001	Gabr (1991)
Venerupis aurea	Lake Timsah, Suez Canal, Egypt.	-1.70	2.33	isometric	0.980	ns	Abou-Zied (1991)
Venerupis aurea	Bridge, Lake Timsah, Suez Canal, Egypt	-3.22	2.57	-allometry in summer	0.93	ns	Mohammad *et al* (2014)
Venerupis aurea	Taawen, Lake Timsah, Suez Canal, Egypt	-3.39	2.68	-allometry in summer	0.88	ns	Mohammad *et al* (2014)
Venerupis aurea	Etap, Lake Timsah, Suez Canal, Egypt.	-2.85	2.26	-allometry in summer	0.68	ns	Mohammad *et al* (2014)
Venerupis aurea	Lake Timsah, Suez Canal, Egypt.	-3.59	2.69	isometric in most months	0.967	<0.0005	Kandeel (20008) •
Venerupis aurea	Lake Qarun, Egypt	-3.58	2.68	isometric in most seasons	0.952	<0.0001	Present study

Key: ns, not specified; r^2, determination coefficient; *P*, significance level of r^2. •, the values are the mean at the sampling sites or throughout the study period.

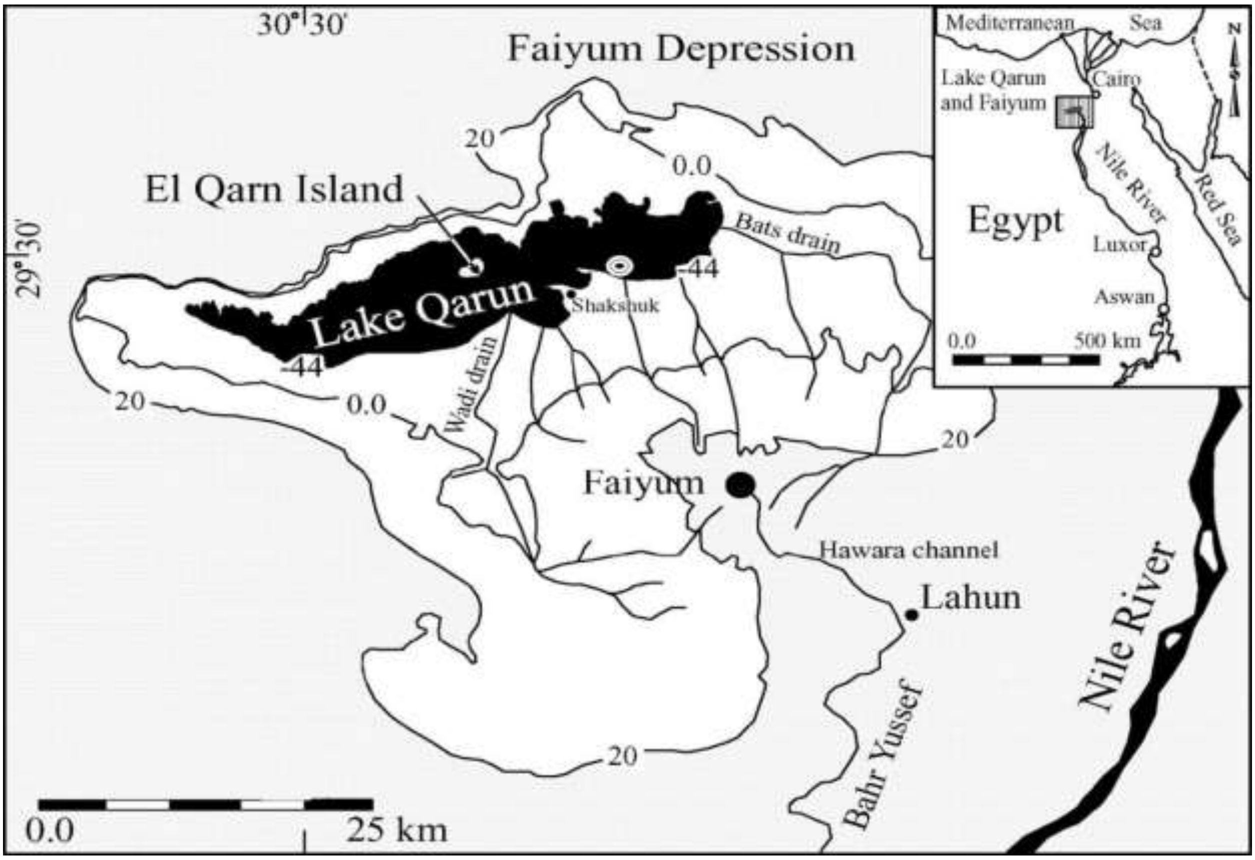


Figure 1: Map of Lake Qarun showing sampling site ( ).

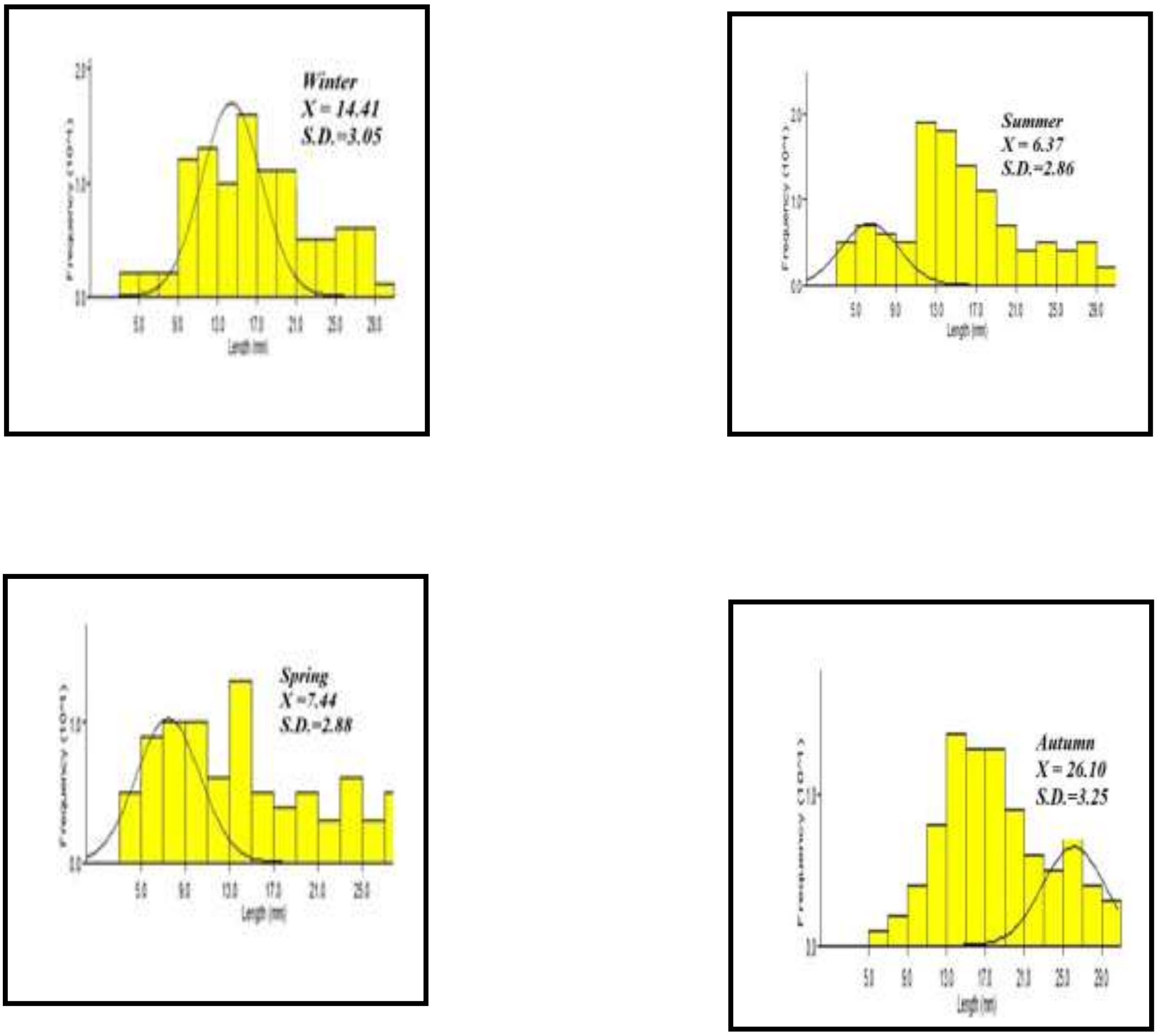
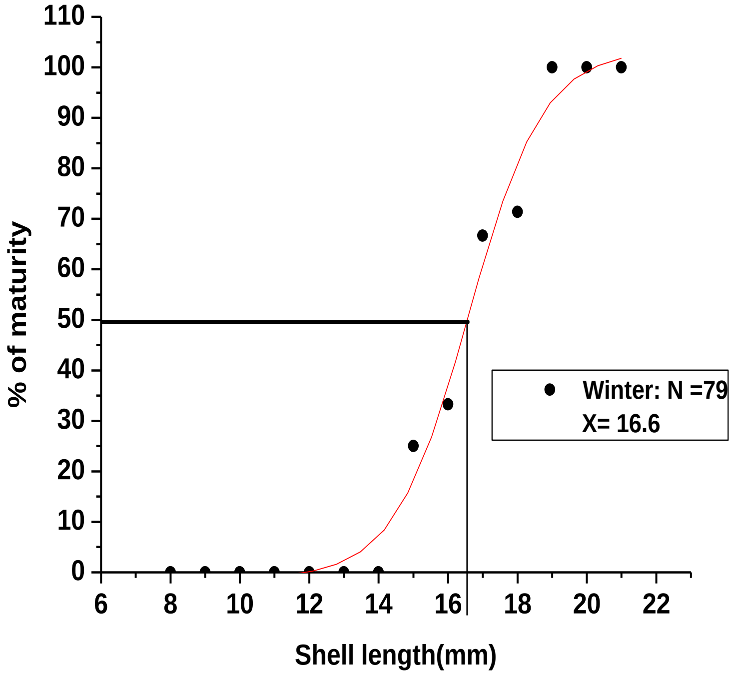


Figure 2: Bhattacharya's Length frequency distributions of *V. aurea* at Lake Qarun during winter (n= 102), spring (n =88), summer (n =112) and autumn (n = 90).



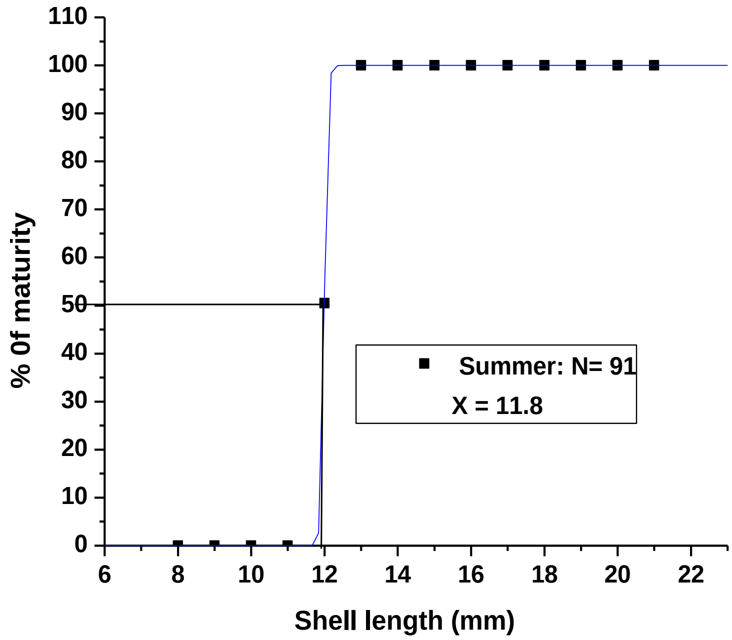


Figure 3 : Relationship between shell length (mm) and percentage of maturity for *V. aurea* collected from Lake Qarun during winter and summer seasons. The size of 50 % maturity is demonstrated. N = number of individual examined.

Figure 4: Transverse section through sexually undifferentiated (juvenile) gonad of *V. aurea* in Lake Qarun, gonial cell (GC), lumen (L). Scale bar = 50 *u*m.

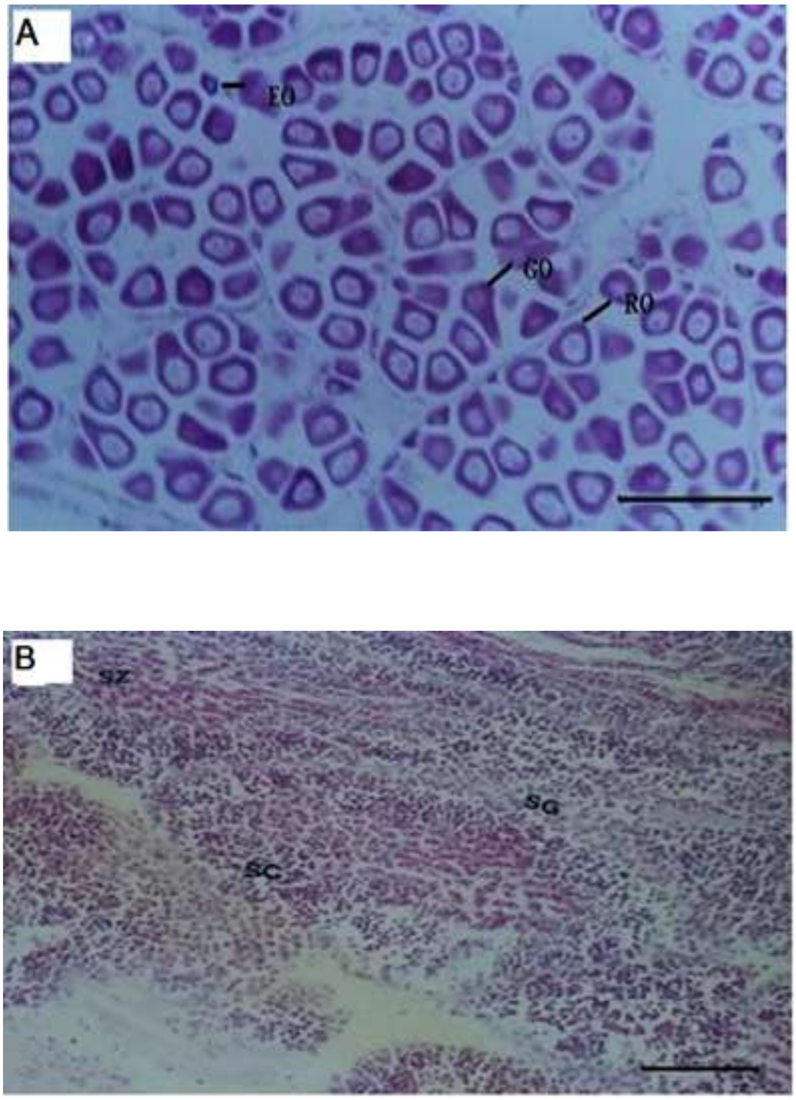


Figure 5: Transverse sections through developing gonads of female (A) and male (B) *V. aurea* in Lake Qarun, early oocytes (EO), growing oocytes (GO), ripe ova (RO), spermatogonia (SG), spermatocytes (SG) and spermatozoa (SZ).Scale bar = 200 µm.