

## Validated age and growth estimates of two clam species in a saltwater lake on the Suez Canal in Egypt

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### ABSTRACT

This is the first time to document the validated age and growth estimates for two clam species; *Ruditapes decussatus*, and *Gafrarium pectinatum* in Lake Timsah, which is a salt water lake on the Suez Canal in Egypt. Age validation for the two species was done to confirm the annual deposition of each growth increments on the shell surface and was conducted using the edge analysis method. Age validation of these two species showed that the period of the major growth was summer, while the slower growth was documented in winter. The age of the two species was determined by counting the growth increments on the shell surface. Maximum age for *R. decussatus* (4 y) and *G. pectinatum* (3 y) were recorded in a 37.2 mm and 42.1 mm in the two species, respectively. Also, length frequency analysis using FiSAT was used to confirm the number of year classes (age) in the two species. The von Bertalanffy Growth Parameters;  $L_{\infty}$ ,  $k$  and  $t_0$ , were 40.4 mm, 0.268 and -1.11y for *R. decussatus* and 36.3 mm, 0.81 and 0.05 y for *G. pectinatum*. Besides age, the morphometric characters of the two species were discussed. The results of this study will help in better fisheries and ecosystem management.

**Keywords:** age determination, validation, Lake Timash, *Ruditapes decussatus*, *Gafrarium pectinatum*.

### INTRODUCTION

Lake Timsah is one of three lakes that are connected to the Suez Canal in Egypt. The lake bottom is mainly muddy or sandy and is the habitat of a wide range of ecological and economical important taxa such as the clams of family Veneridae (Gabr & Gab-Alla, 2008). Among these clams are *Ruditapes decussatus* (Linnaeus 1758) and *Gafrarium pectinatum* (Linnaeus 1758) that are been collected on a commercial scale. Few studies were published to address the commercial importance of clam species in the area. For example, Yassin *et al.* (2009) documented the fisheries statistics in the region and mentioned that *R. decussatus* and *G. pectinatum* make up to 25 % of the clam fisheries in Lake Timash. The authors also showed that there was an increase in the total clam catch from 499 t in 1999 to 2088 t in 2006 and then declined in 2008 to 1793 t. There are no available literature about the age and growth of the carpet shell

clam, *Ruditapes decussatus* in Lake Timsah. Nevertheless, the population dynamics of the same species was investigated in other locations, for example, in UK (Urrutia *et al.*, 1999), Greece (Chryssanthakopoulou & Kaspiris, 2005) and Turkey (Serdar *et al.*, 2007). On the other hand, few studies are available for *Gafrarium pectinatum* in Egyptian waters. Gab-Alla *et al.* (2007) for example, studied its reproductive biology besides analyzing its length frequency over one year in northern Red Sea.

Knowledge of growth parameters is essential for understanding the biology and productivity of bivalves. Studying vital rates (namely growth) is necessary for modeling population dynamics, which, in turn, is crucial to support exploitation and management (Peharda *et al.*, 2007) and to propose effective measures for the protection of the species (Katsanevakis, 2007). Growth rates also indicate how much time is required to reach a certain marketable size (Urban, 2000), and the relationship between size and age is essential to implement appropriate management strategies (Keller *et al.*, 2002). Several methods are used to estimate age and growth of bivalves, but there are practical problems with most of them. Size-frequency distribution analysis is a subjective method, inappropriate for species with a relatively long annual recruitment period and/or highly variable individual growth rates within age groups (Cerrato, 1980). Mark and recapture experiments demand a lot of time to gather data and should only be carried out in areas where fishing activities are prevented (Gaspar, *et al.*, 2004). Analyses of oxygen and carbon isotopic composition are also used for determining the age of bivalves, although the determination of equilibrium or disequilibrium conditions between carbonate and seawater is often difficult, chiefly when large changes in both temperature and seawater isotopic composition take place throughout time (Keller *et al.*, 2002). Besides these methods, counting growth increments on the shell surface or on thin sections of whole valve or of the chondrophore region have proven to be among the most efficient techniques to determine the age of various bivalve species (Ropes and Jearld, 1987).

Meanwhile, age validation is essential to confirm that the increments used in counting are deposited annually and reflect the absolute age of the species (Campana, 2001). Methods that are used in the age validation include the release of known age and marked individual where the animals can be marked either externally (Quinn *et al.*, 1991), or immersion mass-marked using temperature fluctuations (Volk *et al.*, 1999) or chemicals (Campana, 1999). A different technique that is used successfully to validate the annual deposition of growth increments in aquatic animals is the bomb radiocarbon. This technique is recommended for long living species and was used with fish (Campana & Jones, 1998) and with bivalves (Kilada *et al.*, 2007a; Kilada *et al.*, 2009). A third validation method is the otolith or shell marginal increment analysis (MIA) which is the most commonly used for fish (Carlson *et al.*, 1999) and was applied successfully in bivalves (Kilada *et al.*, 2007b). Moreover, the edge analysis is a

different validation method which has similar properties to MIA and is used to record the presence of the marginal increments as opaque or translucent zones (Labropoulou & Papaconstantinou 2000). The absence of age and growth information initiated the importance of the present study which presents the first report of the validated age structure and growth of two clam species living in Timsah Lake. The clam age is determined by counting the growth increments on the shell surface of the two species in addition to the use of length frequency analysis. Age validation is conducted to confirm the annual deposition of each increment and is done using the edge analysis. Besides age and growth, the study will investigate the morphometric characters of the two clam species in Lake Timash. These characters should indicate the growth trend in the shell relatively to the flesh of each species.

### MATERIALS AND METHODS

The study was conducted in the salt-water Lake Timsah which is located on the Suez Canal at 30°34'N and 32°18'E. The average water depth in the lake is about 6 m although some areas are 16 m deep. The salinity in the lake varies between 36 ‰ and 44 ‰ and temperature varies between 15°C in December and 28°C in August.

#### Morphometric Measurements:

Sampling of *Ruditapes decussatus* and *Gafrarium pectinatum* were carried out at monthly intervals between November 2008 and October 2009. In each month, a sample of 50 -60 individuals of each species was randomly collected by hand from depths ranging less than 1 m. Total number for *R. decussatus* and *G. pectinatum* was 626 and 699, respectively. The shell length (SL, maximum distance along the anterior-posterior axis), width (SW, maximum distance along the 2 valves-lateral axes) and height (SH, maximum distance along the dorsal-ventral axis) were measured to the nearest 0.1 mm. The total weight of each clam was determined and then the shell was separated from the soft tissues, which were weighed after being drained from excess water. The dry weight of soft tissue was recorded after keeping the tissue in an oven at 100 °C for 48 h. The mean and range of all measurements are recorded in Table 1.

The length–height and width relationships were described by a linear regression:

$$Y = a + bX$$

where  $Y$  is the clam height (mm) and  $X$  is the length (mm). Length–weight relationships were calculated using the equation:

$$Y = a * X^b$$

where  $Y$  is a weight variable (total dry tissue weight, g) and  $X$  is the shell length (mm);  $a$  and  $b$  are model parameters. In order to assess if the  $b$  value obtained in the linear and non-linear regressions was significantly different from the isometric value of  $b = 1$  and 3, respectively, a t-test ( $H_0: b = 1$ ) with a confidence level of 95% ( $\alpha = 0.05$ ) was applied. Calculated  $b$  values of 1 (length–height) or 3 (length–weight) were defined as being in the isometric range, while  $b < 1$  (3)

was defined as negative allometry and  $b > 1$  (3) as positive allometry. The relationships between the wet and dry flesh weight was done to examine the flesh water content.

### Age determination

The age was determined in 90 and 94 individuals of *R. decussatus* and *G. pectinatum*, respectively by counting the growth rings observed on the shell surface of each individual after cleaning. The increments were also investigated in a thin section of the chondrophore region. Thin section was prepared by sectioning the shell along the anterior-posterior axis with a low-speed Isomet saw mounted with two diamond blades, 2 mm apart. The thin section was mounted on a glass slide and ground down to 140  $\mu\text{m}$  thickness with a Buehler PETRO-THIN thin sectioning system. The section surface was hand ground with silicon carbide grinding powder of grit size 200 and then examined using an Olympus binocular microscope using reflected light at 40 x magnification. The growth increments were counted in the chondrophore region.

The von Bertalanffy growth curves for all age-length data of the two species were fitted by non-linear regression using the statistical package SYSTAT (2009):

$$L_t = L_\infty(1 - e^{-k(t-t_0)})$$

where  $L_t$  is the length-at-age  $t$ ;  $k$  is a growth coefficient;  $L_\infty$  is the asymptotic length and  $t_0$  is the theoretical age at 0 length.

To examine precision, number of growth increments on the shell surface of both species was made by two readers for each specimen independently. The bias graph displays the number of growth increments obtained by one reader against a second reader in reference to an equivalence line where the age 1 has the same results of age 2. Specifically, for all animals assigned a given age by age 1, the mean age and 95% confidence intervals of the ages assigned by age 2 are plotted against the age 1 results (Campana *et al.*, 1995; Campana, 2001; Kilada *et al.*, 2007). Precision estimates were calculated by using the coefficient of variation (CV) as described by Chang (1982) and Morales-Nin and Panfili (2002):

$$CV_j = 100 * \frac{\sqrt{\frac{\sum_{i=1}^R (X_{ij} - \bar{x}_j)^2}{R-1}}}{\bar{x}_j}$$

where  $X_{ij}$  is the  $i^{\text{th}}$  age estimate of the  $j^{\text{th}}$  clam,  $\bar{x}_j$  is the mean age of the  $j^{\text{th}}$  clam, and  $R$  is the number of times each clam is aged. CV is averaged across clams to produce a mean. CV is more flexible and statistically more robust than other measures of precision, such as percent agreement or average percent error (Kimura & Lyons, 1991).

### Age Validation:

The validation procedure was used to confirm the annual deposition of single growth increment on the shell surface and to determine the time of

deposition. Age validation for *R. decussatus* and *G. pectinatum* was done by applying the edge analysis which records the presence of the marginal increment as either an opaque or translucent zone (Gaspar *et al.*, 1995; van der Walt & Beckley, 1997; Labropoulou & Papaconstantinou, 2000; Campana, 2001; Gaspar *et al.*, 2004). The edge analysis method investigates the change in relative frequency of each edge zone which is plotted across seasons where the cycle frequency should equal one year in true annuli. The marginal increment checked by Olympus SZX16 at x 10 magnification.

#### **Length-Frequency Analysis:**

Total number of clams used in the length-frequency was 626 and 699 individuals of *R. decussatus* and *G. pectinatum*, respectively. Length-frequency distributions were calculated per 1 mm size-classes and the modal length of the cohorts was identified applying the Bhattacharya method (1967) using the FISAT software package (Gayanilo *et al.*, 2005).

## **RESULTS**

#### **Morphometric Measurements:**

Morphometric relationships between several size parameters in the two clam species are presented in Tables 1 and 2. The allometric coefficient ( $b$ , slope) of these relationships is related allometrically in all cases and reflects ontogenetic changes in the shell and flesh. The shell of *R. decussatus* and *G. pectinatum* tend to become proportionally higher and wider (positive allometric,  $b > 1$ ,  $P > 0.01$ ) possibly providing better anchorage in the mobile sediment for larger animals.

Table 1: Mean, range and Standard Error (SE) of morphometric measurements in the two species collected from Lake Timsah.

Species	Variable	n	Minimum	Maximum	Mean	Standard Error
<i>Ruditapes decussatus</i>	Shell Length (mm)	627	16.40	38.00	26.16	0.15
	Shell Width (mm)	625	10.00	29.00	18.58	0.12
	Shell Height (mm)	625	7.30	17.50	11.12	0.07
	Total Weight (g)	625	0.61	9.56	3.04	0.05
	Dry Flesh Weight (g)	624	0.05	0.88	0.21	0.01
<i>Gafrarium pectinatum</i>	Shell Length (mm)	519	19.20	52.40	29.39	0.211
	Shell Width (mm)	519	14.70	40.20	21.90	0.15
	Shell Height (mm)	519	6.60	20.10	10.67	0.09
	Total Weight (g)	518	1.32	24.87	5.03	0.12
	Dry Flesh Weight (g)	515	0.03	1.40	0.24	0.01

Table 2: Morphometric relationship parameters for *R. decussatus* and *G. pectinatum* from Lake Timsah. Values between parentheses are the upper and lower 95 % confidence levels.

Species	Morphometric Relationship	n	r <sup>2</sup>	a	b	Allometry
<i>R. decussatus</i>	SW - SL	625	0.83	4.53 (3.75 – 5.30)	1.16 (1.12 – 1.20)	Positive
	SH - SL	623	0.86	5.04 (4.35 – 5.72)	1.90 (1.834 – 1.956)	Positive
	TW - SL	624	0.89	0.0001 (0.00 – 0.00)	2.83 (2.75 -2.90)	Isometric
	FDW - SL	623	0.72	0.0001 (0.00009 – 0.000011)	3.13 (2.98 – 3.28)	Isometric
	FWW - FDW	624	0.86	-0.022 (-0.031 - - 0.014)	0.20 (0.19 – 0.20)	Negative
<i>G. pectinatum</i>	SW - SL	699	0.94	-1.47 (-2.08 - -0.87)	1.42 (1.39 – 1.443)	Positive
	SH - SL	699	0.87	5.64 (4.90 – 6.38)	2.25 (2.19 – 2.32)	Positive
	TW - SL	697	0.90	0.001 (0.001 – 0.001)	2.530 (2.473 – 2.587)	Negative
	FDW - SL	694	0.68	0.0001 (0.00009 – 0.000011)	2.70 (2.57 – 2.83)	Negative
	FWW - FDW	515	0.94	-0.04 (-0.05 - -0.03)	0.23 (0.22 – 0.23)	Negative

### Age determination:

Examination of shell sections showed distinct growth patterns within the shell of the two species and identified a wide light-colored band followed by a narrow dark-colored growth band (Fig. 1A). These bands were found to correspond to the wide opaque and the narrow light areas noticed on the external surface of the shells (Figs 1B – C). The same number of increments was visible in the thin sections of the chondrophore region as well as on the shell surface of both species and therefore, the external shell was used for the age determination in all clams. In *R. decussatus* and *G. pectinatum*, the maximum number of increments was 4 and 3, respectively. The maximum age in the former was found in a 37.20 mm SL individual, while in *G. pectinatum*, the maximum age was recorded in 42.10 mm SL clam. The von Bertalanffy growth parameters (VBGP) of *R. decussatus* and *G. pectinatum* was estimated from the length-at-age relationship (Fig. 2; Table 3) and the mean length at each year class were estimated (Table 4).

Table 3: Von Bertalanffy parameters calculated from the age-length relationship for *R. decussatus* and *G. pectinatum*. Values between parentheses are the upper and lower 95 % confidence levels.

	<i>R. decussatus</i>	<i>G. pectinatum</i>
$L_{\infty}$	40.41 (35.50 – 45.33)	36.31 (32.94 – 39.67)
$K$	0.268 (0.20 – 0.33)	0.81 (0.47 – 1.16)
$t_0$	-1.106 (-1.42 – -0.79)	0.053 (0.03 – 0.06)

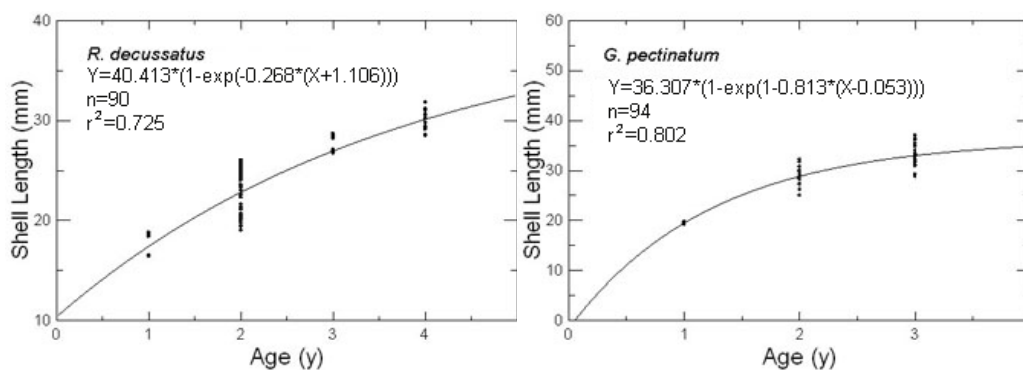


Fig. 2: Growth curve for *R. decussatus* and *G. pectinatum* collected from Lake Timsah.

The Bias plot comparing the results of the two readers indicated no bias in the ageing process (Fig. 3).

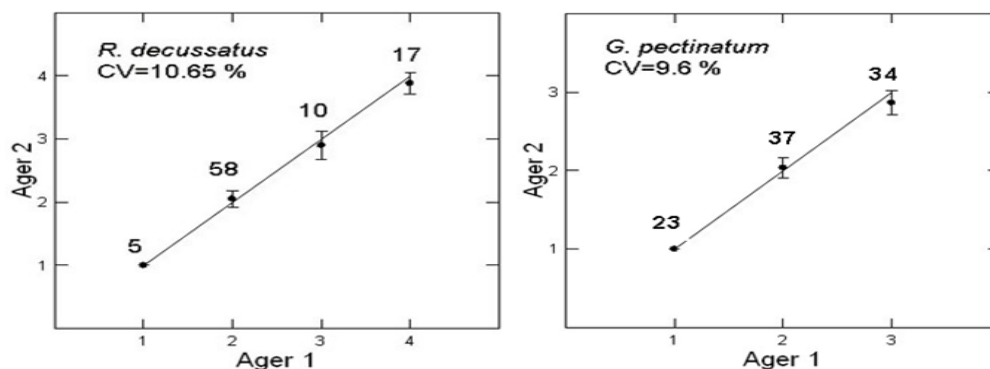


Fig. 3: Age bias graph for *R. decussatus* and *G. pectinatum* for age reader 1 and 2 aging the clam by counting the growth increments on the shell surface. Each error bar represents the 95 % confidence interval about the mean age assigned by ager 2 to all clams assigned a given age by ager 1. The values indicate the number of clams aged at each age group and the solid line represents 1:1 equivalence. (Cv = Coefficient of variation).

Also the mean coefficient of variation (CV) that measures the precision was found to be 10.65 % and 9.6 % for *R. decussatus* and *G. pectinatum*, respectively. There is no absolute rule for an acceptable CV for ageing studies as precision is affected by longevity, the structure used for ageing and the difficulty in reading the growth increments. In fish, a CV of 5 % is considered as limit for acceptable age readings for short lived species (Laine *et al.*, 1991) although Campana, (2001) states that CV of 5 % is common in fish. The result in the present study was thus considered precise and unbiased, and therefore the counts generated by one reader for the entire set of shells were used for the analysis.

#### Length-Frequency Analysis:

The analysis revealed that there are 4 and 3 modes in the length-frequency data for *R. decussatus* and *G. pectinatum*, respectively (Fig. 5). The mean length of each mode was calculated and was found to be comparable to mean of length at age that was calculated from the von Bertalanffy equation for each species (Table 4).

Table 4: Mean length (mm) at each year class for *R. decussatus* and *G. pectinatum*. The values are provided from von Bertalanffy equation and from the length-frequency analysis using FiSAT package.

Species	Year 1		Year 2		Year3		Year 4	
	Increments	FiSAT	Increments	FiSAT	Increments	FiSAT	Increments	FiSAT
<i>R. decussatus</i>	17.43	19.69	22.83	23.84	26.97	26.24	30.13	29.79
<i>G. pectinatum</i>	19.50	23.79	28.50	28.82	33.00	36.20		

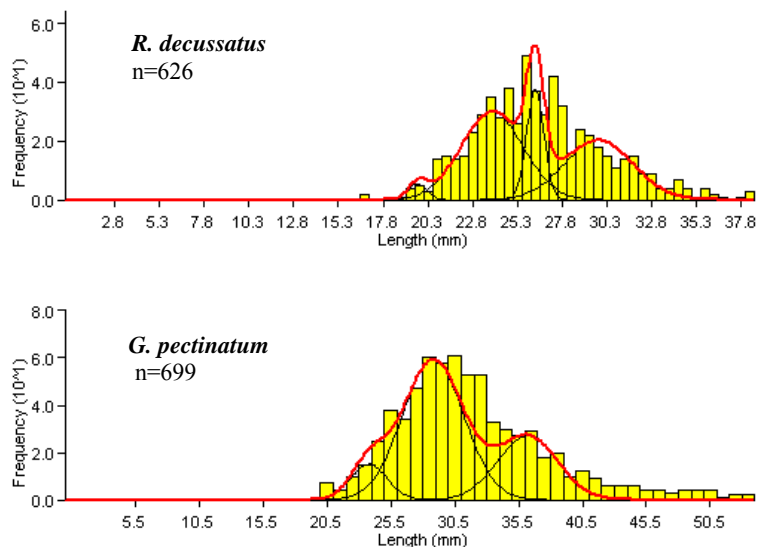


Fig. 4: Composite distribution of the pooled length frequency sample of *R. decussatus* and *G. pectinatum* collected from Lake Timsah determined by Bhattacharya method in FiSAT software.



### Age Validation:

Percentage of the dark zones in the marginal increment of the shells of *R. decussatus* and *G. pectinatum* are shown in Fig. 5. The number of the dark zones in the marginal increments of the shells of the two species reached its maximum and minimum in summer and winter, respectively. There was a significant difference in the number of dark zones between different seasons (Kruskal-Wallis  $P=0.012$ ). Based on these results, the increments analysis shows that a single increment is formed in the two clam species annually starting in summer months in Lake Timsah.

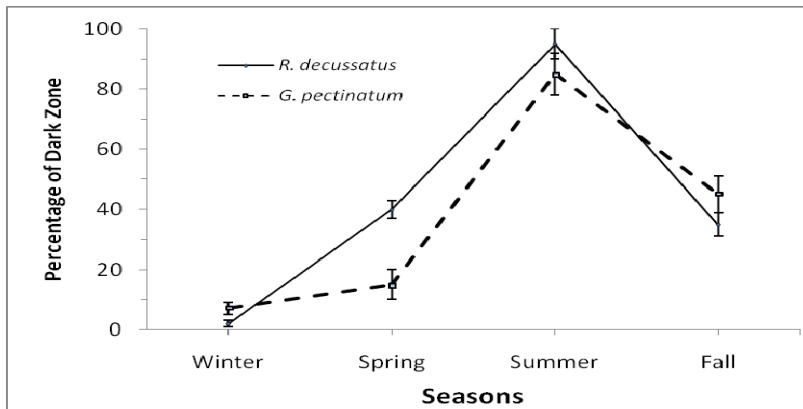


Fig. 5: Seasonal variation in the number of the dark zones associated with the shell margin of *R. decussatus* and *G. pectinatum* (Error bars =  $\pm$  SE).

## DISCUSSION

The validation of annual deposition of growth bands is essential to support the use of the number of these bands as age indicator. In the present study, the method of edge analysis was applied in order to confirm this hypothesis and to allow understanding of when they are deposited. The drop in the percentage of dark zones between summer and winter supports the hypothesis of annual band formation of the two species. The method also showed that the period of major growth was summer while slower growth was documented in winter as shown in the change in the frequency of dark zone of the marginal region. This is similar to that observed for the Greenland smoothcockle in Eastern Canada (Kilada, *et al.*, 2007), in which a single annual band is proven to be deposited during summer months. In Lake Timsah, the average water temperature reaches its peak during August and continues to decline till it reaches its minimum in December (El-Shenawy, *et al.*, 2009). Seasonal variation in temperature may be the main reason of the variation in the percentage of dark zone observed on the clam shell. This was documented for other bivalves by Anwar *et al.* (1990).

The growth patterns were visible on the external shell surface of the two species where a prominent band separates 2 successive growth increments (Fig. 1). The growth in *R. decussatus* is rapid during the first year until it reaches about 17 mm and slows down thereafter (Table 4). The estimated growth parameters for *R. decussatus* were compared to those estimated in other places (Table 5). In the present study, the asymptotic length ( $L_{\infty}$ =40.41 mm) was smaller than that documented in Spain ( $L_{\infty}$ =45.05 mm, Urrutia *et al.*, 1999). In this study, the authors demonstrated the age-at-length relationship by back calculating the length to each growth band. Maximum age in this study was 6 years as compared to 4 in the present study and this may be the reason of the difference in  $L_{\infty}$  values. In contrast, in Greece the asymptotic size was very dissimilar and was larger ( $L_{\infty}$ =69.00 mm, Chryssanthakopoulou & Kaspiris, 2005). Those authors documented that the maximum age was in a 49 mm-clam and was 5 years. These discrepancies could possibly be a consequence of the error in estimating the asymptotic size. When estimating the growth parameter, the authors assumed that the maximum age can reach 19 years, which is not realistic, and also assumed that the clam would be 69 mm in size. Therefore, the results are age-biased since the life span of *R. decussatus* is less than 6 years.

On the other hand, *G. pectinatum* showed similar growth pattern. The growth was faster during the first year and then decreased afterwards. The clam was about 20 mm, 29 mm and 33 mm in the first, second and third years. The length at sexual maturity for *G. pectinatum* was estimated in the Suez Bay that is located at about 100 km south of Timsah Lake and was found to be 18 mm (Gab-Alla *et al.*, 2007). This size corresponds to approximately 1 y in age which means that the species can be collected commercially after one year from spawning.

There is no available literature documenting the growth of this species, nevertheless in India, Jagadis and Rajagopal (2007 a; b) showed that *G. tumidum* had a different growth rate than *G. pectinatum* (Table 5) which may be explained by the occurrence of different temperature regimes.

Table 5: Von Bertalanffy Growth parameters estimated for different clam species in different locations

Species	$L_{\infty}$	k	$t_0$	Location	Reference
<i>Ruditapes decussatus</i>	69.00	0.211	-1.173	Greece	Chryssanthakopoulou & Kaspiris, 2005
<i>R. decussatus</i>	45.05	0.026	4.052	Spain	Urrutia <i>et al.</i> , 1999
<i>R. decussatus</i>	40.41	0.268	-1.106	Egypt	Present Study
<i>R. philippinarum</i>	43.32	0.697	-0.267	UK	Humphreys <i>et al.</i> , 2007
<i>Gafrarium tumidum</i>	50.67	0.288	-1.05	India	Jagadis & Rajagopal, 2007a; b
<i>G. pectinatum</i>	36.31	0.81	0.053	Egypt	Present Study

The mean length at each age class that was estimated from the length-frequency data was similar to the results estimated from age-at-length relationship (Table 4). However, the values obtained from length-frequency

analysis were slightly larger than those provided from counting the growth increments on the shell surface.

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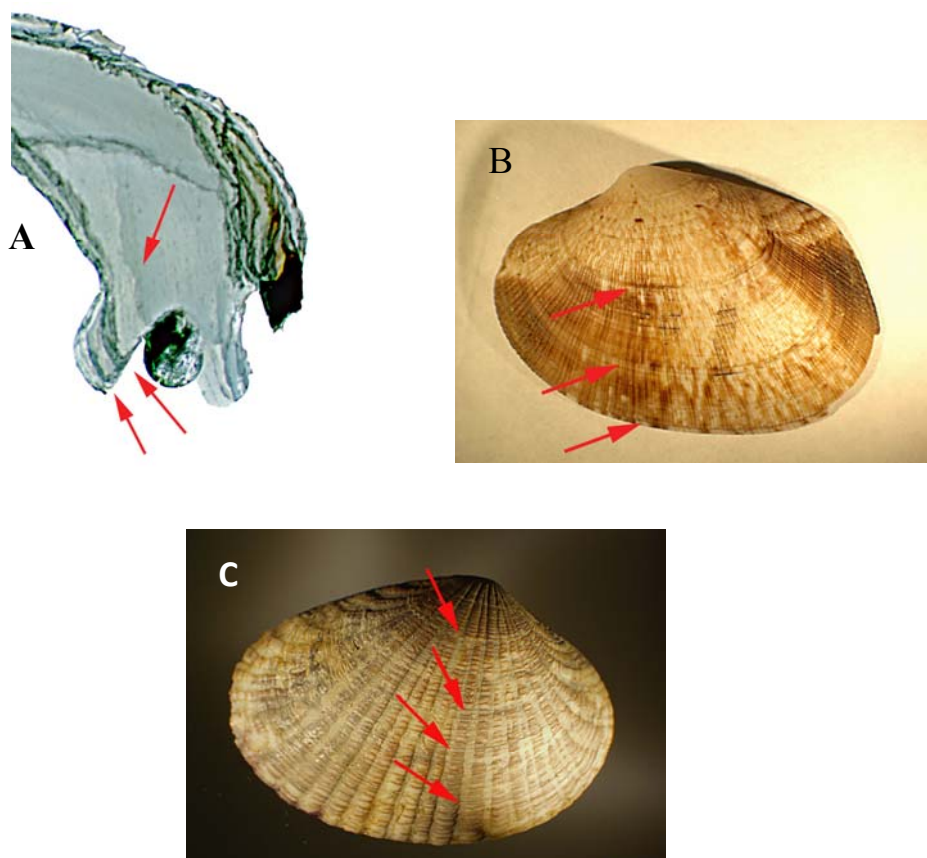


Fig. 1: Growth increments (arrows) visible in various clam species. A. transverse thin section (140  $\mu\text{m}$ ) of *Ruditapes decussatus*. B. Shell of *Ruditapes decussatus*. C. Shell of *Gafrarium pectinatum*.