International Journal Of Biology and Biological Sciences Vol. 3(5), pp. 053-066, December 2014
Available online at http://academeresearchjournals.org/journal/ijbbs
ISSN 2327-3062 ©2014 Academe Research Journals

Full Length Research Paper

Energy storage and allocation of pearl oyster *Pinctada radiata* (Leach, 1814) in relation to timing of pearl seeding

Moussa R. M.[1]*, ElSalhia M.[2] and Khalifa A.[2]

[1]Aquaculture Division, Invertebrate Aquaculture Laboratory, National Institute of Oceanography and Fisheries, Alexandria, Egypt.
[2]Fisheries Division, Fish Physiology Laboratory, National Institute of Oceanography and Fisheries, Alexandria, Egypt.

Accepted 4 December, 2014

Seasonal and monthly changes in biochemical components and condition indices of the gonadal-visceral mass (GVM), gill, mantle, and adductor muscle of *pearl* oyster *Pinctada radiata* were analyzed from May 2012 to May 2013 in Eastern Egyptian Mediterranean coast in relation to reproductive cycle to provide information about the suitable time for implantation surgery. Protein was the major structural part of GVM and adductor muscle which was utilized to supply energy for late gonad development and spawning. Glycogen in adductor muscle might be for meeting the energy demands for spawning and gonad rebuilding, while accumulated gill glycogen was considered a major energy source for spawning and maintenance. Energy requirements were met by proteins and glycogen to a greater extent as compared to lipid. Year could be divided into three phases: somatic growth and maintenance, gametogenesis, and spawning phase. High condition index (CI) and fatness values were observed in May and June, whereas the lowest were observed after spawning (August). Contrarily, the shell component index minimum value was recorded in May then it increased till it reached the maximum value in August. The suitable time for pearl implantation surgery was October as oysters would be more energetic.

Key words: Protein, lipid, glycogen, condition index, shell component index, fatness, pearl oyster *Pinctada radiata*.

INTRODUCTION

Pearl oyster *Pinctada radiata* was reported as lessepsian immigrants for the first time by Monterosato (1878) in 1874 as *Meleagrina sp* in Egyptian Mediterranean coast (Dogan and Nerlovic, 2008). It is continually colonizing new habitats in the eastern Mediterranean Sea (Tlig-Zouari and Zaouali, 1994, 1998; Galil and Zenetos, 2002; Gofas and Zenetos, 2003; Zenetos et al., 2005) which has led to spectacular increase of its spatial distribution (Zenetos et al., 2004, 2005, 2007). It has ecological and also economic importance as it was the greatest source of natural pearls in the world, since ancient times (Almatar, 1992). Nonetheless, *P. radiata* studies are scarce, limited among Mediterranean countries, and are confined to Tunisia (Tlig-Zouari, 1993; Tlig-Zouari and Zaouali, 1994, 1998; Tlig-Zouari et al., 2009, 2010) and recently to Egypt (Abdul aziz and Ali, 2009; Moussa, 2013).

In Egypt, *P. radiata* is a candidate species for mariculture and pearl production. The growth pattern affecting the pearl formation in *P. radiata* was comparatively studied from different stations along Alexandria coast, Egypt. The maximum reported *P. radiata* size until now was recorded from Abu-Qir,

*Corresponding author. E-mail: ragiamoussa@yahoo.com.au. Tel: +201120020346, +2012 08 40 40 47.

Alexandria. It was 113 mm DVL and 144.71 g T.wt. The recommended size range for seed implantation in *P. radiata* was 65-89 mm (Moussa, 2013). As yet, there is no information about the suitable time for pearl formation surgery in *P. radiata*. According to Haws (2002) and Taylor and Strack (2008), the method for inducing pearl formation is related to their reproductive status which is strongly committed to the energy storage-utilization cycles (Gabbott, 1983; Berthelin et al., 2000). Nutrient reserves are accumulated prior to gametogenesis in the form of glycogen, lipid and protein substrates for utilization in gamete production (Barber and Blake, 1981; Brokordt and Guderley, 2004; Mathieu and Lubet, 1993). Consequently, oysters become highly vulnerable to manipulation and grafting due to very large loss of input energy in gametogenesis (Ca´ ceres-Martı´nez et al., 2006). Although knowledge of energy storage is necessary to determine the suitable moment of pearl formation operations, there is an obvious lack of information about the strategies for energy allocation during reproduction in the genus *Pinctada*.

The condition index (CI) is a rapid measure of ecophysicological state in commercially exploited bivalve and other molluscan species (Lucas and Beninger, 1985; Yildiz and Lök, 2005). It is considered a very practical method used for following gametogenetic activities of bivalves through tracing the monthly values (Okumuš and Stirling, 1998). Verily, CI has been utilized by a number of authors to clarify the spawning season of bivalves (Clemente and Ingole, 2009; Park et al., 2011; Yang et al., 2011, Celik et al., 2012) and measure the reproductive output (Al-Barwani, 2007; Li et al., 2011, Celik et al., 2012). Furthermore, CI can be used for monitoring the commercial quality and time for harvest (Manley and Walker, 2011).

The aim of this study was to describe the seasonal cycles of energy storage-utilization and seasonal condition index in different organs in relation to the reproductive cycle to provide information about: (i) preliminary recommendations to the fishing community targeting *P. radiata* to protect the spawning stock and the collective spawns; (ii) biochemical composition as an aspect of quality of seafood; (iii) the optimum harvest period for *P. radiata* in the Eestern Egyptian Mediterranean coast, and (iv) the suitable time for performing surgical grafting. Ultimately, the results will provide valuable information for *P. radiata* population management, culture and pearl production.

MATERIALS AND METHODS

Oyster collection and sample preparation

Four hundred and eighty pearl oyster *P. radiata* in commercial size with dorsoventral length between 50 and 73 mm, total weights between 19 and 44 gm were obtained from Mamurah on the eastern Alexandria, Egypt (Figure 1) (N 31 18 0297; E 030 01 6760) by Scuba diving at 7-15 m from May 2012 to May 2013. Samples of 40 oysters were collected at monthly intervals and transported to the National Institute of Oceanography and Fisheries, Alexandria, Egypt for biochemical, and weight determinations. Fouling organisms were carefully removed from the shell surface using a scalpel. Each specimen was measured to record the total weight (TWT) and dorsoventral length (DVL) (umbo to lip) with Vernier calipers to the nearest 0.5 mm. Oysters were opened and shucked. After removing the valves, oyster meat was excised and drained on blotting paper prior to wet weight measurement. The soft parts were separated into four groups; gonad-visceral mass (GVM), gill, mantle, and adductor muscle tissues. The GVM was analyzed as a unit because of the physical difficulty in separating the organs.

Biochemical analysis

Ten oysters were shucked monthly; four tissue groups (GVM, gill, mantle, and adductor muscle tissues) were separated from each oyster for further protein, lipid and glycogen analysis. Protein concentration was determined using Bradford (1976) assay with blue brilliant of coomassie as reagent and bovine serum albumin as standard. Total lipid was quantified according to CPFAC (2001) assay by using Chloroform / methanol / water extraction. Carbohydrate and glycogen were determined using the phenol-sulphuric acid (Dubois et al., 1956). The results are expressed in mg of protein, glycogen and lipid using a standard one gram tissue weight. A chemical analysis was done without distinction between sexes.

Condition indices

A total of 30 oysters were shucked monthly. The separated GVM, gill, mantle, adductor muscle tissues and shell valves of each oyster sample were dried on aluminum foil at 80°C for 48 h to constant weight. The condition index, shell component index and fatness were calculated as follows:

- The condition index % (CI) = [dry meat weight/dry shell weight] × 100 (Walne, 1976, Crosby and Gale, 1990).
- The shell component index % = [Shell wet weight/ total wet weight)] × 100.
- Fatness (Meat Yield) % = [Meat wet weight/total wet weight] × 100 (Freeman, 1974).

Statistical analysis

Means, standard deviations, standard errors of the mean

Figure 1. Map of the study area in Mamurah, Alexandria, Egypt.

(mean ± SE) of biochemical compositions were calculated and tested for normal distribution with MS-Excel software. ANOVA was employed to compare the biochemical composition monthly values monthly between different examined tissues and for each tissue. In addition, ANOVA was applied to monthly values of condition index, shell component index and fatness. The assumptions of normality of the data and homogeneity of variances were tested, and the appropriate transformations were applied when necessary. Post Hoc Test (Tukey HSD) was made when significant differences were found (Zar,1984, Sokal and Rohlf, 1995). Statistical analyses were performed using the software package SPSS "Statistical package for social sciences" (version 20). The statistical level of significance was set at P <0.05 for all analyses. The Kendall's rank correlation coefficients were calculated to test the strength of association among oyster weight relationships, and biochemical components.

RESULTS

Biochemical composition

The biochemical composition significantly varied seasonally and monthly between GVM, gill, mantle, and adductor muscle in addition to monthly significant difference in the energy content of each organ. Protein, lipid and glycogen proportions were within the range of 58-84 mg g^{-1}, 11-23 mg g^{-1}, and 7-21 mg g^{-1}, respectively.

Protein

The protein content was significantly different monthly between the four examined organs examined (ANOVA: F range: 45-741, $P < 0.05$). The GVM showed monthly different protein contents (ANOVA: F =101.025, $P < 0.05$) with two peaks in the months of March and October as 23.55 ± 2.09 and 31.70 ± 1.89 mg g^{-1}, respectively. Relatively constant GVM protein content at around 14 mg g^{-1} was noticed between peaks. There were two protein accumulation periods (January to March and September to October) followed by a sharp decrease (Figure 2A). Significant monthly differences were also detected in the protein content of gill tissue (ANOVA: F = 9.209, $P <$ 0.05), with a marked increase in February and March, then decreased to reach the lowest value of 6.90 ± 0.80 mg g^{-1} in June (Figure 3). A pattern of accumulation of gill protein was re-observed thereafter to reach a maximum value of 11.69 ± 0.86 mg g^{-1} in October then slightly decreased in November and December (Figure 2B).

The mantel protein content was significantly different

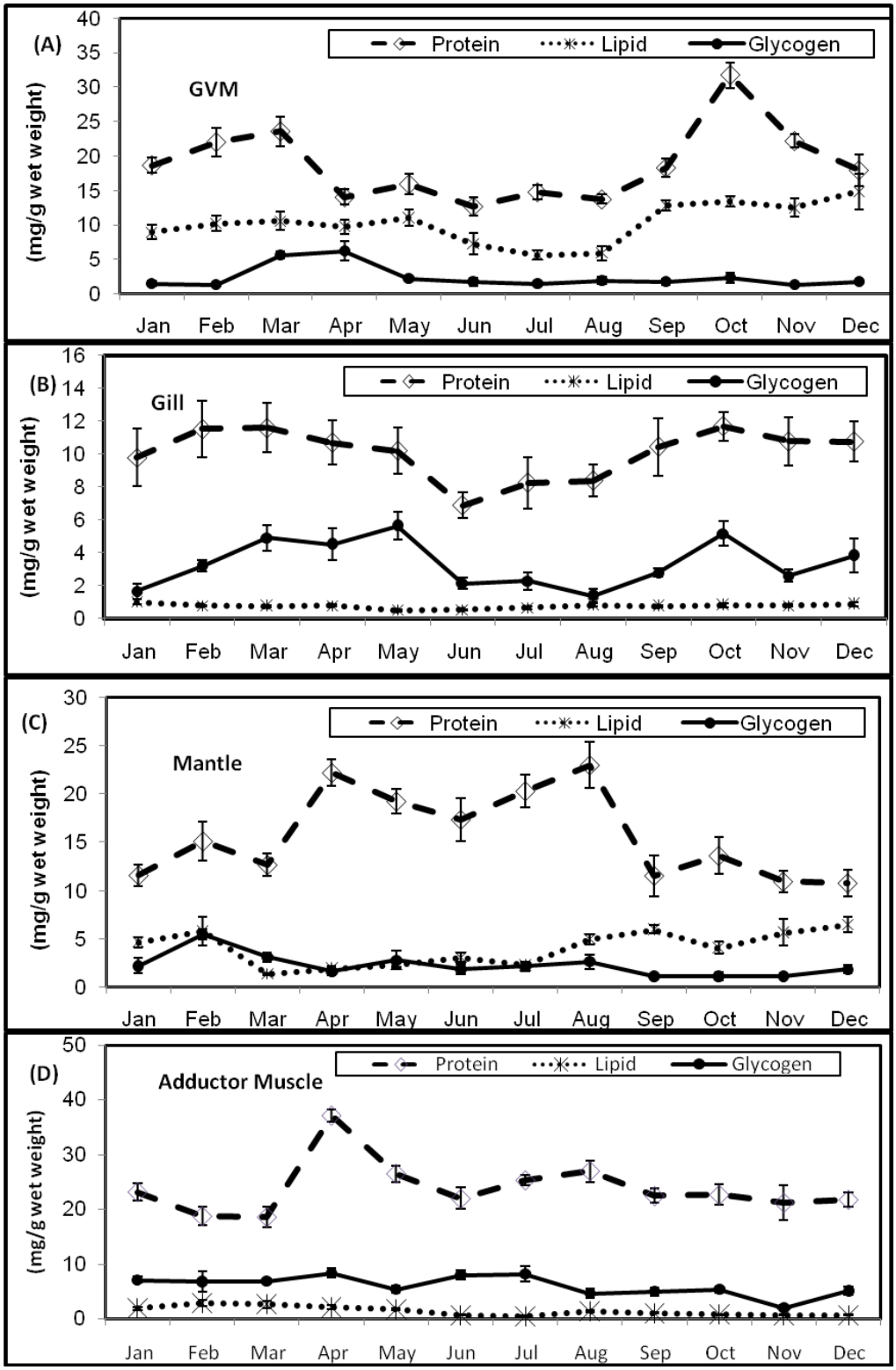

Figure 2. Monthly variation in the biochemical composition of *Pinctada radiata* in protein, lipid and glycogen in: (A) GVM, (B) Gill, (C) Mantle, and (D) Adductor Muscle. Data are presented as percentages of the biochemical component on wet weight. Error bars: mean ± SD.

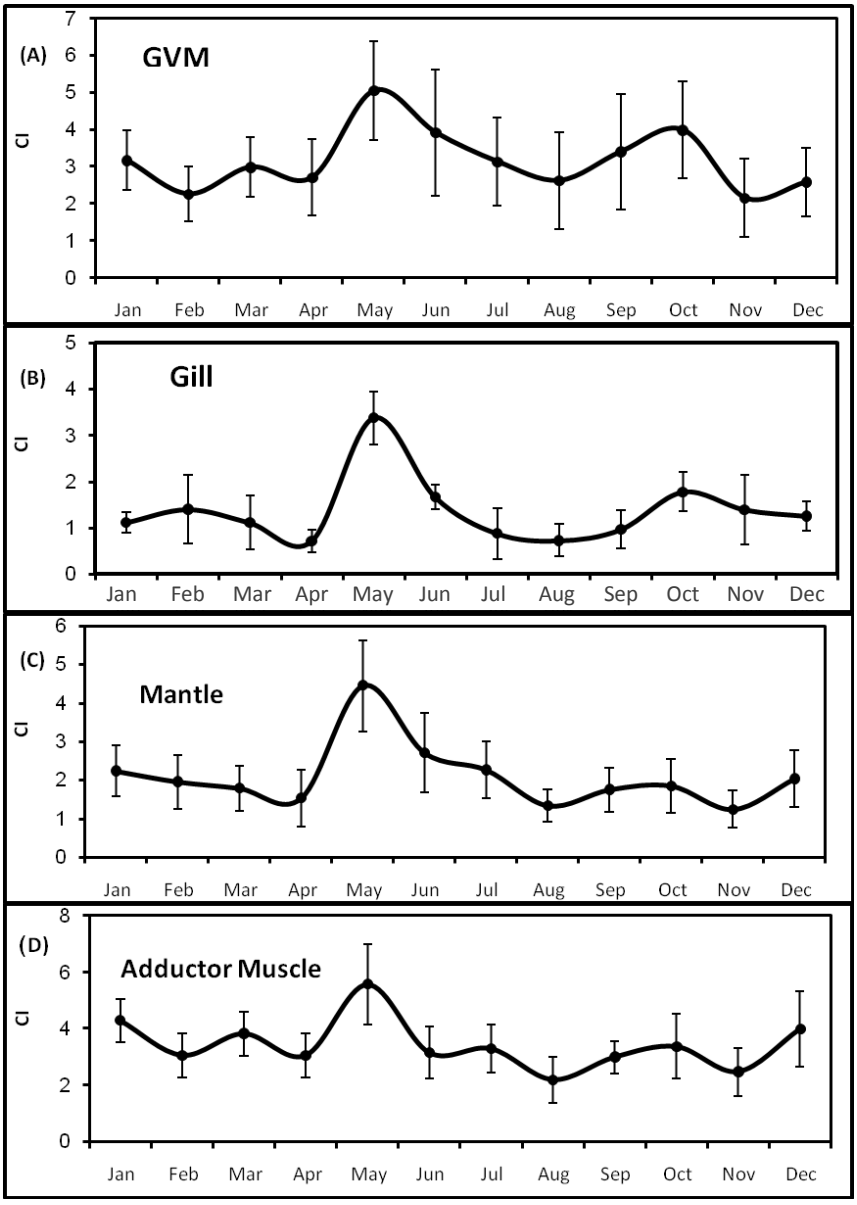

Figure 3. Monthly variation in condition index (CI) of *Pinctada radiata* in: (A) GVM, (B) Gill, (C) Mantle, and (D) Adductor Muscle. Error bars: mean ± SD.

among months (ANOVA: $F = 57.125$, $P < 0.05$), with two peaks in April (22.18 ± 1.35 mg g^{-1}) and August (22.97 ± 2.37 mg g^{-1}), whereas mantle tissue showed the minimum protein content in November and December as 10.93 ± 2.12 and 12.65 ± 1.16 mg g^{-1}, respectively (Figure 2C).

Protein content of adductor muscle was significantly different monthly (ANOVA: F =61.315, $P < 0.05$). It was minimum in March (18.62 ± 1.86 mg g^{-1}) and then increased to a maximum of 37.12 ± 1.11 mg g^{-1} in April, whereas the secondary peak was observed in August (27.01 ± 1.97 mg g^{-1}). Afterward, protein content declined and remained around 21 mg g^{-1} until December (Figure 2D).

Lipid

The lipid content was also significantly different monthly

among the four examined organs (ANOVA: *F* range: 137-1299, p < 0.05) showing no consistent pattern, and values were higher in the GVM than the somatic tissues. The lipid content of GVM was significantly different (ANOVA: *F*=41.769, *P* < 0.05) with relatively constant values from January to May at around 9 mg g^{-1} followed by sharp decrease to a minimum of 5.84 ± 0.98 mg g^{-1} in August and a further increase to reach the maximum value of 14.81 ± 2.55 mg g^{-1} in December (Figure 2A).

Despite the significant difference in the gill lipid content (ANOVA: *F* = 18.001, *P* < 0.05), it did not show a particular pattern of storage or mobilization of lipid. As such, it remained within a range of 0.5-1 mg g^{-1} throughout the year experimental period with maximum peak in January (1.03 ± 0.18 mg g^{-1}) (Figure 2B).

The mantle lipid showed monthly significant difference (ANOVA: *F* = 47.002, *P* < 0.05) with accumulation period from July (2.27 ± 0.29 mg g^{-1}) to December (6.45 ± 0.81 mg g^{-1}) followed by reduction with a minimum value of 1.38 ± 0.10 mg g^{-1} in March (Figure 2C). In addition, the monthly significant adductor muscle lipid content (ANOVA: *F* = 60.074, *P* < 0.05) remained within a relatively range from 0.7 to 2 mg g^{-1} throughout the study period with maximum peak in February (2.86 ± 0.54mg g^{-1}) (Figure 2D).

Glycogen

The glycogen level showed a close relationship with the reproductive cycle. Glycogen content varied between 1 and 8% wet weight of tissue and was significantly different between the examined tissues (ANOVA: *F* range: 33-203, *P* < 0.05). The monthly significant glycogen content of GVM (ANOVA: *F* = 70.406, *P* < 0.05) was around 1 mg g^{-1} with maximum peaks in March (5.64 ± 0.50 mg g^{-1}) and April (6.16 ± 1.39 mg g^{-1}) (Figure 2A).

The monthly significant difference of gill glycogen (ANOVA: *F* = 39.371, *P* < 0.05) was observed through its accumulation from January (1.66 ± 0.48 mg g^{-1}) to May (5.64 ± 0.83 mg g^{-1}) then sharp decrease of glycogen content was noticed to reach the lowest value in August (1.39 ± 0.41 mg g^{-1}). Afterward, further glycogen accumulation period was observed until reaching the value of 5.17 ± 0.74 mg g^{-1} in October then glycogen content declined until being re-accumulated in January (Figure 2B).

The monthly significant mantle glycogen content (ANOVA: *F* = 38.241, *P* < 0.05) was around 2 mg g^{-1} throughout the study period except for February glycogen content that reached the maximum value of 5.48 ± 0.57 mg g^{-1}. The mantle glycogen content remained constant at 1.1 mg g^{-1} from September to November representing the lowest values (Figure 2C).

The monthly significant adductor muscle glycogen content (ANOVA: *F* = 31.695, *P* < 0.05) from December to March was around 6 mg g^{-1}, and then increased to reach the maximum value of 8.36 ± 0.88 mg g^{-1} in April. Decreasing of glycogen content was attaining the lowest value in November (1.99 ± 0.47 mg g^{-1}) except for June and July that kept the glycogen content values around 8 mg g^{-1} (Figure 2D).

Correlation between oyster weight relationship and biochemical composition were summarized in Table 1. GVM weight was strongly correlated positively to total weight and somatic (gill, mantle and adductor muscle) (0.05 < *P* < 0.001). Both GVM lipid and protein contents were negatively correlated (*P* ≤ 0.05) to GVM weight, somatic weight and somatic protein. In addition, GVM protein was negatively correlated (*P* ≤ 0.05) to total weight.

Condition indices

Significant differences in monthly CI values were observed in GVM (ANOVA: *F* = 13.949, *P* < 0.001), gill (ANOVA: *F* = 108.814, *P* < 0.001), mantle (ANOVA: *F* = 38.541, *P* < 0.001), and adductor muscle (ANOVA: *F* = 26.269, *P* < 0.001). The maxima GVM, gill, mantle and adductor muscle CI were in May, then decreased gradually until it reached its lowest value in August which further increased in September reaching the second peak in October that would be followed by a decline in November values.

The fatness was statistically significant (ANOVA: *F* = 23.680, *P* < 0.001) among months. The trend of fatness was closely similar to that of CI with peak in May and October (Figure 4).

The monthly shell component index values were significantly different from May to September (ANOVA: *F* = 80.91, *P* < 0.001). Contrary to CI and fatness, the shell component index minimum value was recorded in May then increased till it reached the maximum in August (Figure 5).

DISCUSSION

Considering the obvious paucity of information on the *Pinctada radiata*'s biology, the present work makes a useful contribution to the current knowledge on the reproduction of *P. radiata* in Egyptian Mediterranean coasts which enormously reflects on performance of oyster culture and pearl production. This paper is the first to report on biochemical compositions of *P. radiata* to provide valid information of seasonal variation in energy allocation with special regard to reproductive cycle.

Biochemical composition of few *Pinctada* species has been studied previously such as in *P. margaritifera* (Gangnery, 1997), *P. fucatamartensii* (Hiroaki Saito,

Table 1. *Pinctada radiata* correlation coefficients relating weight relationships and biochemical components.

Component		GVM W	SW	TW	SG	GVM G	SL	GM L	SP	GVM P
GVM W	r	-	0.946*	0.968*	-0.056	0.057	-0.573*	-0.263	0.326	-0.720*
	p		<0.001	<0.001	0.862	0.861	0.052	0.410	0.302	0.008
SW	r		-	0.961	0.016	0.222	-0.503	-0.013	0.269	-0.676*
	p			<0.001	0.960	0.487	0.095	0.968	0.398	0.016
TW	r			-	0.009	0.153	-0.554	-0.196	0.374	-0.740*
	p				0.977	0.636	0.062	0.541	0.232	0.006
SG	r				-	0.153	0.156	0.079	-0.014	0.367
	p					0.636	0.629	0.807	0.967	0.241
GVM G	r					-	-0.404	0.038	0.322	0.034
	p						0.192	0.908	0.307	0.918
SL	r						-	0.443	-0.550	0.414
	p							0.149	0.064	0.181
GVM L	r							-	-0.413	0.251
	p								0.183	0.431
SP	r								-	-0.644*
	p									0.024
GVM P	r									-
	p									-

GVM W, dry weight of gonad; SW, somatic tissues dry weight; TW, total dry weight; SG, somatic tissue glycogen; GVM G, GVM glycogen; SL, somatic tissue lipid; GVM L, GVM lipid; SP, somatic tissue protein; GVM P, GVM protein. (r: Kendall's test; *: Statistically significant at $P \leq 0.05$).

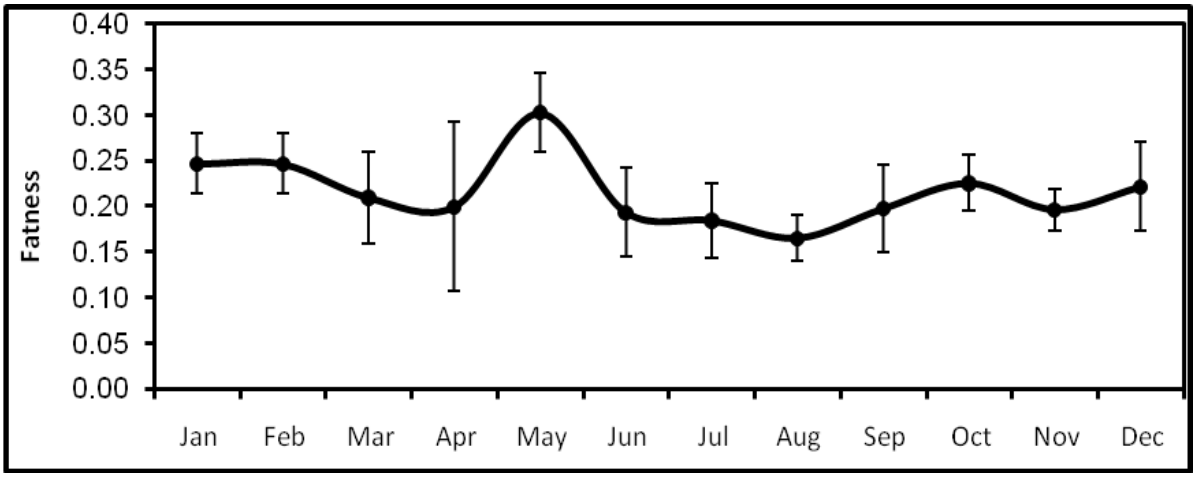

Figure 4. Monthly variation in fatness of *Pinctada radiata*. Error bars: mean ± SD.

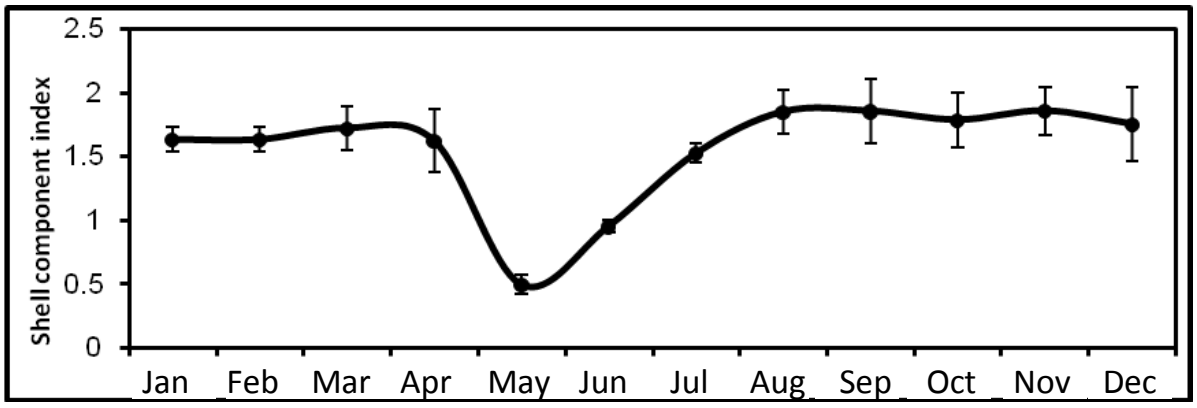

Figure 5. Monthly variation in shell component index of *Pinctada radiata*. Error bars: mean ± SD.

2004), and *P. mazatlanica* (Robles and Saucedo, 2009). In studying biochemical composition in relation to reproductive cycles, the analysis of various body components will be more informative than estimation in the entire body to elucidate the mobilization of the tissue reserves to the gonad during gametogenesis (Giese et al., 1967; Giese, 1969; Liu et al., 2008). The present results of biochemical components analysis of GVM, gill, mantle and adductor muscle showed significant monthly variation in accordance with the reproductive cycle but did not experience the same variation trends. Similar findings have been recorded in many bivalves such as *Ruditapes philippinarum* (Robert et al., 1993); *Glycymeris glycymeris* (Galap et al., 1997), and *Laternula elliptica* (Ahn et al., 2003).

Biochemical synthesis was intensively involved in gametogenesis. The present findings about *P. radiata* revealed that the major constituents of GVM were protein followed by lipid then glycogen. The presence of biochemical components within the GVM may be as a result of mobilization of preformed reserves within the body tissue of an animal as mentioned by Blackmore (1969) and their fluctuations might be attributed to relative contribution of gonad growth and spawning activity.

Protein

Protein is the most abundant biochemical component in tissues and it may be an alternative energy reserve in some bivalve species during gametogenesis (Beninger and Lucas, 1984; Galap et al., 1997). In *P. radiata*, protein was the major structural part of GVM and adductor muscle which was utilized to supply energy for gametogenesis particularly in gonad development and spawning as previously pointed out by Beninger and Lucas (1984), Galap et al. (1997), and Yan et al. (2010). Protein was accumulated in the entire *P. radiata* tissues before gametogenesis then gradually declined as far as June. Similar findings were reported for the contribution of gonadal protein in gametogenesis in wedge clam *Donax cuneatus* (Nagabhushanam and Talikhedkar, 1977) and Pacific oyster *Crassostrea gigas* (Berthelin et al., 2000), whereas the utilization of adductor muscle protein was mentioned by Barber and Blake (1981), Martinez and Mettifogo (1998) and Acosta-Salmon (2004).

Re-accumulation of protein patterns were noticed in mantle and adductor muscle between July and August, while storage was from September to October in GVM and gill tissues. The phytoplankton richness might be the reason for the accumulated protein and might be further utilized as energy source for *P. radiata* gonad rebuilding, growth and maintenance. Noteworthy, the gradual gonadal protein's declination till spawning ended may be attributed to intervals of *P. radiata* gonads releasing rather than at once.

The present study revealed monthly protein dynamics with a substantial increase before spawning where energy requirements were met by proteins and glycogen to a greater extent as compared to lipid. Similarly, Wolowicz et al. (2006) mentioned that protein and carbohydrates content increases during gametogenesis, whereas it declines after spawning.

Lipid

Lipids play a crucial role in maturing gonadal tissues and constitute a major component of *P. radiata* GVM as found in marine bivalves (Zandee et al., 1980). Generally, lipid content was observed to increase before mass spawning occurrence, and then markedly decreased (Racotta et al.,

2003; Dridi et al., 2007; Liu et al., 2008; Colaco et al., 2009), whereas in P*. radiata*, the lipid concentrations accumulated prior to somatic growth in GVM and mantle. Lipid storage before gametogenesis was in GVM and adductor muscle then fell during spawning similarly to *Crassostrea gigas* (Ren et al., 2003). Moreover, accumulated GVM and mantle lipid in September might be mobilized to provide energy during cold months, while in *Crassostrea gigas*, the gonadal lipid was responsible for meeting the energy demand in cold months (Bayne, 1976; Barber and Blake, 1981; Ren et al., 2003). Before now, utilization of lipid as energy source was mentioned by Holland (1978), Gabbott (1983) and Fraser (1989), and observed in *Scapharca broughtonii* and *Ruditapes philippinarum* (Park et al., 2001; Robert et al., 1993).

The gill lipid content did not show a particular pattern of storage or mobilization. It remained within a range of 0.5-1 mg g^{-1} throughout the year of experimental period. Likewise, the role of gill tissue for the storage of nutrients during reproduction of *P. sterna* was quite secondary or negligible which was shown by significantly lower biochemical constituents (García and Saucedo, 2008).

Glycogen

Glycogen is the main carbohydrate constituent, representing about 50% of total carbohydrates (Robert et al., 1993). Glycogen is a preferred form of energy reserve particularly in oysters because of fast glycogen catabolism that provide instant energy under hypoxic or anoxic conditions (Hummel et al., 1989; Whyte et al., 1990).

GVM with mature *P. radiata* gonad was found to have high glycogen content similarly to *Crassostera* gigas (Berthelin et al., 2000), whereas *Crassostrea cucullata* was found to store more glycogen in immature oysters (Nagabhushanam and Bidarkar, 1978). In this context, Berthelin et al. (2000) suggested that the presence of numerous mantel storage cells have led to maximal glycogen metabolism and storage capacity which would result in glycogen mobilization to gonad and consequently developing mature gonad with highly glycogen content. In adductor muscles, the storage glycogen pattern was observed twice; in April (prior to late spawning) and June-July (prior to gonad rebuilding) which may be for meeting the energy demands for spawning and gonad rebuilding. In addition, accumulated gill glycogen in May and October was considered a major energy source for spawning and maintenance, respectively.

Catabolism process converting carbohydrates into lipids in *P. radiata* GVM was observed during gametogenesis (February) leading to increase of the lipid content due to initiation of gonad lipid storage. Additionally, glycogen catabolism was suggested to occur also in spawning (May) and after gamete release (September) as a result of lipid depletion.

The knowledge of the biochemical composition of any organisms as an aspect of food quality is extremely important to demonstrate the nutritive value. Generally, biochemical assays and nutrients of Mollusca play a vital role in physical growth, development, maintenance of normal body function of physical activity and health (Periyasamy et al., 2011). In particular, oysters are one of the most nutritionally well-balanced foods, containing protein, carbohydrates and lipids. The present study revealed that adult *P. radiata* was found to have high protein and low lipid content.

Energy

A substantial increase in the protein, glycogen and lipid contents reflected a conservative strategy for storing and mobilizing these energy reserves at the time of reproduction while the increase of biochemical constitutes after spawning indicates energy anabolism in the recovery process.

In terms of seasonality, it was demonstrated that there were two complimentary phases for administering available energy; one for storage and the other for mobilization of reserves to meet biological processes of maintenance of the cellular machinery, growth of somatic tissues, and reproduction (Ansell, 1974; Comely, 1974; Gabbott, 1975; Sastry, 1979; Bayne, 1976; Taylor and Venn, 1979; Bayne et al., 1982; Barber and Blake, 2006).

In *P. radiata,* a summary of the biochemical findings was demonstrated in Figure 6 in order to draw a chart for energy allocation for different energy reserves of oyster tissues over twelve months (that is, a year). The year could be divided into three phases: somatic growth and maintenance phase from September to December; gametogenesis phase from January to April; and spawning phase from May to August. Dots squares were referred to storage periods, while the others were for utilization times that were through providing energy for oyster biological activities or by reserves mobilization from tissue to others. The difference between number storage months and utilization months could be considered as indication of oyster biochemical status whether being energetic or exhausted. The highest difference values (4) were attained in October and February where oysters were more energetic, while June and November represented the months of exhaustion due to spawning peak and either growth or maintenance, respectively. These findings are in general agreement with those found in other bivalves where gonad ripening was concomitant with reduction of somatic growth, as in *C. opercularis* (Taylor and Venn, 1979), *P. maximus*

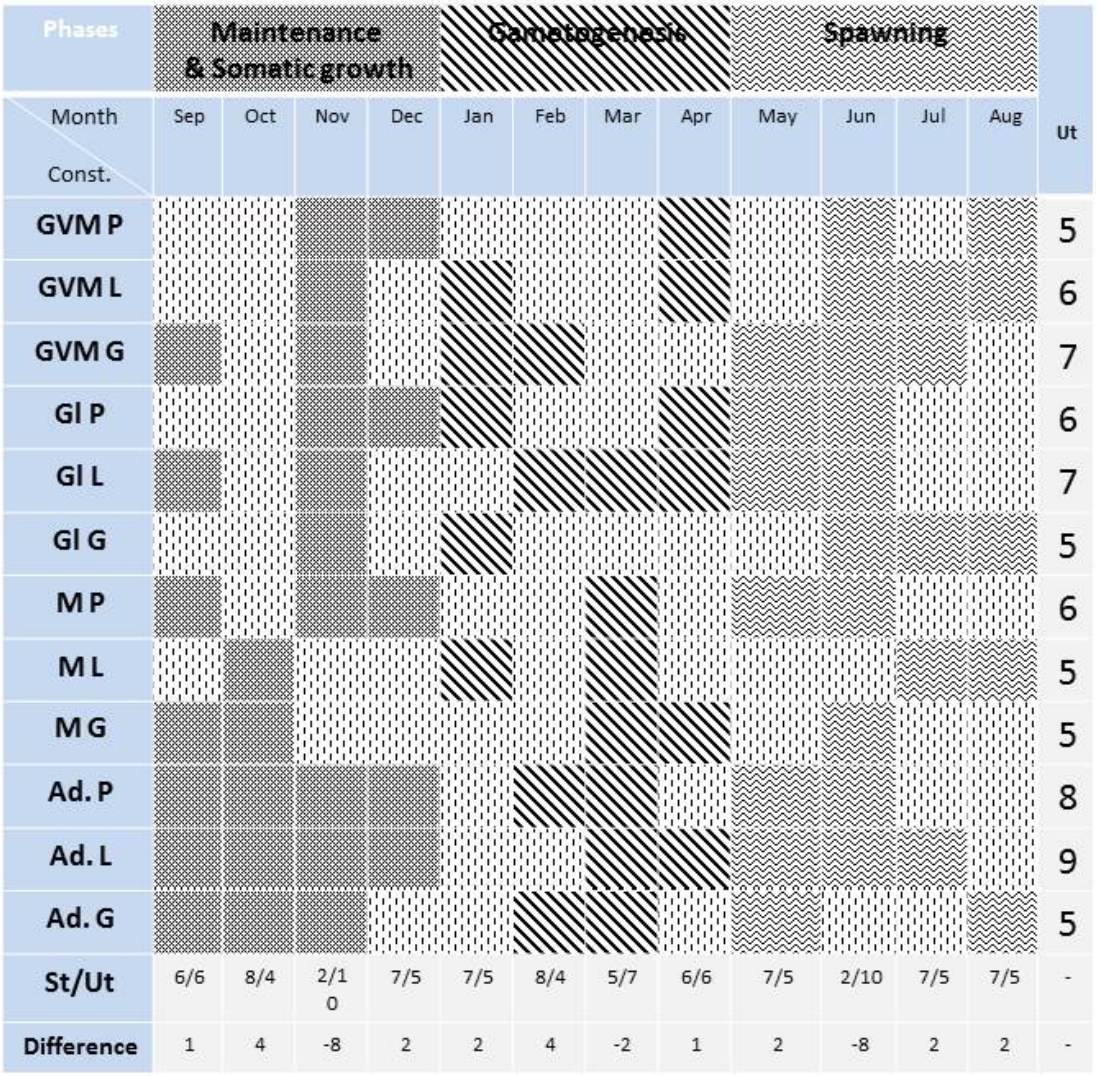

Figure 6. Energy chart illustrating the storage phases (St) and utilization phases (Ut) of the biochemical constitutes (Const.) over twelve months in GVM P: Gonad-Visceral Mass Protein; GVM L: Gonad-Visceral Mass Lipid; GVM G: Gonad-Visceral Mass Glycogen; Gl P: Gill Protein; Gl L: Gill Lipid; Gl G: Gill Glycogen; M P: Mantle Protein; M L: Mantle Lipid; M G: Mantle Glycogen; Ad. P: Adductor muscle Protein; Ad. L: Adductor muscle Lipid; Ad. G: Adductor muscle Glycogen.

(Paulet and Boucher, 1991; Duinker, 2002), and *P. magellanicus* (Roma´n et al., 2002; Thompson and MacDonald, 2006). It is worth noting that *P. radiata* energy loss during spawning was higher than that lost during the gametogenesis.

Apart from *P. radiata* commercial value for use as human foodstuff, pearl production is a more attractive business venture because of the high value, lightweight and nonperishable nature of the final product (Haws, 2002) in addition to providing increased economic opportunities to remote marine communities. This study provides a reference point for knowing the suitable time for pearl implantation surgery in *P. radiata* that is recommended to be carried out in October because of having less gonadal development to facilitate seeding of the saibo (mantle) allograft and reduce stress besides being more energetic.

Condition indices

The condition index (CI) of bivalves is an economic indicator of market products (Orban et al., 2002),

generating information that may be used as a benchmark for adaptive management (Polasky et al., 2011). This could be useful and simple, representative and responsive technique in the selection of broodstock for future hatchery operations as it is less expensive and time consuming compared to histological methods state (Gribben et al., 2004, Mercado-Silva, 2005). In addition, it also has advantage of being easier to teach and implement in basic shellfish enterprises (Adjei-Boateng and Wilson, 2011).

The seasonal and monthly variations of the relative CI were correlated with gametogenesis cycle. *P. radiata* started to shunt energy from growth to gamete production in January which was reflected in the high CI values in May and June (Figure 3). The drop in the CI values between June and August was likely correlated to releasing gametes. After spawning, allocating most of energy into storage was translated to higher CI. Based on biochemical analysis and CI, the spawning season can be defined as the warm summer months. In accordance, a commercial ban on the harvesting of *P. radiata* along Egyptian Mediterranean coast is recommended to be from May to August for population management.

As a result of gonad discharge formed in oyster mantle cavities in spawning period, significant decreases occur in fatness resulting in unfeasibility of commercial harvest in such a period as reported in *Ostrea edulis* (Yildiz et al., 2011). Our results revealed that the recommended harvesting time for *P. radiata* is before spawning as previously mentioned for *Crassostrea gigas* (King, 1977); blood cockle *Anadara inaequivalvis* (Sahin et al., 2006), and bearded horse muscle *Modiolus barbatus* (Peharda et al., 2007). Kendall′s rank correlation matrices showed that before spawning, gonad was less in protein and lipid contents, whereas the somatic tissues were rich in protein content. This correlation illustrated that during the recommended time for consumption, where both fatness and CI were maximum, abstaining from gonad consumption due to its bitter taste would be balanced by using the somatic tissues which would be highly proteinous. For acquiring sweet oyster taste, the suitable time for consuming was April where high glycogen content was attained in the entire tissues.

Conclusion

The findings of this study suggested that energy requirements of *P. radiata* for gametogenesis were met by proteins and glycogen to a greater extent as compared to lipid. Gametogenesis took place in winter-spring at the expense of reserves accumulated previously during the autumn period. On biochemical basis, the year could be divided into three phases: somatic growth and maintenance phase from September to December; gametogenesis phase from January to April; and spawning phase from May to August. A single spawning occurred in summer, subsequently, a commercial ban on the harvesting of *P. radiata* along Egyptian Mediterranean coast was recommended to be from May to August. However, the suitable consumption time was before spawning where the CI and fatness values were the maximum. Meanwhile, the bitter gonad taste could be averted by consuming somatic tissues which were highly proteinous. Moreover, the best time for consuming tasteful oysters was April where glycogen content is high. For pearl formation surgery in *P. radiata*, the suitable period was October where oysters would be less gonad developed and more energetic.

REFERENCES

Abdul-Aziz KK, Ali AE (2009). The Health Status and Genetic Variations of the Bivalve, *Pinctada radiata* Affected by Environmental Pollution. The Egypt. Sci. Mag., 6 (3): 22-30.

Acosta-Salmon H (2004). Broodstock Management and Egg Quality of the Pearl Oysters *Pinctada margaritifera* and *Pinctada fucata*. Ph.D. Thesis. James Cook University, North Queensland.

Adjei-Boateng D, Wilson JG (2011). Body condition and gametogenic cycle of *Galatea paradoxa* (Mollusca: Bivalvia) in the Volta River estuary, Ghana. Estu. Coast. Shelf Sci.1-5.

Ahn I Y, Surh J, Park YG, Kwon H, Choi KS, Kang SH, Choi HJ, Kim KW, Chung H (2003). Growth and seasonal energetics of the Antarctic bivalve *Laternula ellipticafrom* King George Island, Antarctica. Mar. Ecol. Prog. Ser., 257:99–110.

Al-Barwani SM (2007). Seasonal variations in reproduction, growth, sex ratio and condition index in *Perna viridis* (Linnaeus 1758) (Mollusca: Mytilidae). Ph.D. Thesis, Universiti Putra Malaysia, Malaysia..

Almatar S (1992). Status of the pearl oyster fishery of Kuwait. SPC Pearl Oyster Info. Bull. No.18.

Ansell AD (1974). Seasonal changes in biochemical composition of the bivalve *Chlamy radiata* from the Clyde Sea area. Mar. Biol., 25:85–99.

Barber BJ, Blake NJ (2006). Reproductive physiology. In: Shumway SE, Parsons GJ, editors. Scallops: biology, ecology, andaquaculture, 2nd ed. Amsterdam, The Netherlands: Elsevier, pp. 357–416.

Barber BJ, Blake NJ (1981). Energy storage and utilization in relation to gametogenesis in *Argopecten irradians concentricus* (Say). J. Exp. Mar. Biol. Ecol., 52:121-134.

Bayne B L, Bubel A, Gabbott PA, Livingstone DR, Lowe DM, Moore MN (1982). Glycogen utilization and gametogenesis in *Mytilus edulis*. Mar. Biol. Lett. , 3:89–

105.
Bayne BL (1976). Marine mussels: their ecology and physiology. Bayne BL eds. Cambridge University Press Publisher, Australia, p506 .
Beninger PG, Lucas A (1984). Seasonal variations in condition, reproductive activity, and gross biochemical composition of two species of adult clam reared in a common habitat: *Tapes decussates L.* (Jeffreys) and *Tapes philippinarum* (Adams and Reeve). J. Exp. Mar. Biol. Ecol., 79: 19–37.
Berthelin C, Kellner K, Mathieu M (2000). Storage metabolism in the Pacific oyster (*Crassostrea gigas*) in relation to summer mortalities and reproductive cycle (West coast of France). Comp. Biochem. Physiol. B., 125: 359-369.
Blackmore DT (1969). Studies on *Patella vulgata.*I. Growth, reproduction and zonal distribution. J. expt. mar. Biol. EcoL., 3: 200-213.
Bradford MM (1976).A rapid and sensitive method for the quantitation of microgram quantities of protein utilizingthe principle of protein-dye binding. Anal.Biochem., 72:248–254.
Brokordt KB, Guderley HE (2004). Energetic requirements during gonad maturation and spawning in scallops: sex differences in *Chlamy sislandica* (Muller, 1776). J. Shellfish Res., 23: 25–32.
Ca´ceres-Martı´nez C, Barrios-Ruı´z D, Benı´tez-Torres A (2006). Comparative stereological and histological study of the gonad,digestive gland and muscle of *Atrina maur a* from San Ignacio BCS, Mexico. In: Book of abstracts, 1st Inter. Workshop Phys.,Repro. Nut. in Moll., La Paz, B.C.S., Mexico, p 33.
Celik MY, Karayucel S, Karayucel Y, Ozturk R, Eyuboolu B (2012). Meat yield, condition index and biochemical composition of mussels (*Mytilus gallo* (Lamarck, 1819) in Sinop, Sout of the Black sea. J. Aquat. Food Prod. Tech., 21: 198-205.
Clemente S, Ingole B (2009). Gametogenic development and spawning of the mud clam, *Polymesoda erosa* (Solander, 1876) at Chorao Island, Goa. Mar. Biol. Res., 5: 109-121.
Colaco A, Prieto C, Martins A, Figueiredo M, Lafon V*et al* (2009). Seasonal variations in lipid composition of the hydrothermal vent mussel *Bathymodiolus azoricus* from the Menez Gwen vent field. Mar. Enviro. Res., 67: 146-152.
Comely C A (1974). Seasonal variation in flesh weights and biochemical
content of the scallop *Pecten maximus*in the Clyde Sea Area. J. Cons. Int. Explor. Mer., 35:281–295.
Crosby MP, Gale LD (1990). A review and evaluation of bivalve condition index methodologies with a suggested standard method. J. Shellfish Res., 9: 233-237.
Dogan A, Nerlović V (2008). On the occurrence of *Pinctada radiata* (Mollusca: Bivalvia: Pteriidae), an alien species in Croatian waters. Acta Adri., 49: 155-158.
Dridi S, Romdhane MS, Elcafsi M (2007). Seasonal variation in weight and biochemical composition of the Pacific oyster, *Crassostrea gigas* in relation to the gametogenic cycle and environmental conditions of the Bizert lagoon, Tunisia. Aqua., 263: 238–248.
Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smith F (1956).Colorimetric method for determination of sugars and related substances. Analy. Chem., 28: 350-356.
Duinker A (2002). Processes related to reproduction in great scallops (*Pecten maximus L*.) from Western Norway. Doctoral Thesis, Bergen University, Norway.
Fraser AJ (1989). Triacylglycerol content as a condition index for fish, bivalve, and crustacean larvae. Can. J. Fish. Aquat. Sci., 46:1868–1873.
Freeman KR (1974). Growth, mortality and seasonal cycle of *Mytilus edulis* in two Nova Scotianem bayments. Bedford Inst. of Oceanography Publ., Dartmouth,Canada.
Gabbot PA(1975). Storage cycles in marine bivalve molluscs: A hypothesis concerning the relationship between glycogen metabolism and gametogenesis. In: Scotland Aberdeen University Press (Eds.), Proceedings of the 9thEurop. Mar. Biol. Symposium, Aberdeen, Uk, pp 191-211.
Gabbott PA (1983). Developmental and seasonal metabolic activities in marine molluscs. In: Hochachka, P.W., (Eds.), The Mollusca, Vol. 2, Enviro. Biochem. Physiol., pp 165-217.
Galap C, Leboulenger F, Grillot JP (1997). Seasonal variations in biochemical constituents during the reproductive cycle of the female dog cockle *Glycymeris glycymeris*. Mar.Biol., 129:625-634.
Galil BS, Zenetos A (2002). A sea change -exotics in the Eastern Mediterranean Sea. In: Invasive Aquatic Species in Europe. Distribution, Impacts and Management. Leppakoski E, Gollasch S, Olenin S (Eds.), Dordrecht, Boston, London. Kluwer Academic Publishers. pp. 325-336.
Gangnery P (1997). E´ tude in situ etexpe´rimentale de le´ ffort dereproductio´ n chez l'huitreperlie´re *Pinctada margaritifera*. Me´mE´cole Sup d'Inget de Tech pour l'Agric. French Polynesia, France.
Garcia NV, Saucedo PE (2008). Energy storage and allocation during reproduction of Pacific winged pearl
oyster *Pteria sterna* at Bahía de la Paz, Baja California Sur, Mexico. J. Shellfish Res., 27(2):375-383.
Giese AC, Hart MA, Smith AM, Gheung MA (1967).Seasonal changes in body component indices and chemical composition in the Pismo clam *Tivela stu / ltorum*. Comp. Biochem. Physiol., 22: 549-561.
Giese AC (1967). A new approach to the biochemical composition of the mollusk body.Oceanogr. Mar. Biol.

Annu. Rev., 7:175-229.
Gofas S, Zenetos A (2003). Exotic molluscs in the Mediterranean basin: current status and perspectives. Oceanography and Marine Biology: An Annual Review. 41: 237-277.
Gómez-Robles E, Saucedo P E (2009). Evaluation of quality indices of the gonad and somatic tissues involved in reproduction of the pearl oyster *Pinctada mazatlanica* with Histochemistry and Digital Image Analysis. J. Shellfish Res., 28(2):329-335.
Gribben PE, Helson J, Jeffs AG (2004). Reproductive cycle of the New Zealand geoduck, *Panopea zelandica*, in two North Island populations. Vel., 47:59-71.
Haws M (2002). The basics of pearl farming: ALayma's Manual. Center of Tropical and Subtropical Aquaculture. Hawaii, U.S.A. Spec Pub. p127.
Hiroaki S (2004). Lipid and FA Composition of the Pearl Oyster *Pinctada fucata martensii*: Influence of Season and Maturation Lipids. 39(10):997-1005.
Holland DL (1978). Lipid reserves and energy metabolism in the larvae of benthic marine invertebrates. In: *Biochemical and* Biophysical Perspectives in Marine Biology, Malins DC, Sargent JR (Eds). Academic Press, New York. (4):85-123.
Hummel H, De Wolf L, Zurburg W, Apon L, Bogaards RH, Van Ruitenburg M (1989).The glycogen content in stressed marine bivalves: the initial absence of a decrease .Comp. Biochem. Physiol. B., (4):729–733.
King MG (1977).Cultivation of the Pacific oyster, *Crassostrea gigas*, in a non-tidal pond. Aqua., 11(2):123-36.
Li Q, Yang L, Ke Q, Kong L (2011). Gametogenic cycle and biochemical composition of the clam *Mactra chinensis* (Mollusca: Bivalvia): Implications for aquaculture and wild stock management. Mar. Biol. Res., 7: 407-415.
Liu WG, Li Q, Yuan YD, Zhang SH (2008). Seasonal variations in reproductive activity and biochemical composition of the cockle *Fulvia mutica* (Reeve) from eastern coast of China. J. Shellfish Res., 27: 405-411.
Lucas A, Beninger PG (1985). The use ofphysiological CI in marine bivalve aquaculture. Aqua., 44:187-200.
Manley J, Walker R (2011). Condition index for Georgia oysters. Occasional papers of the marine extension service, University of Georgia Vol. 12, p 9.
Mathieu M, Lubet P (1993). Storage tissue metabolism
and reproduction in marine bivalves -a brief review. Inv. Rep. Dev., 23:123-129.
Mercado-Silva N (2005). Condition index of the Eastern oyster, *Crassostrea virginica* (Gmelin, 1791) in Sapelo Island Georgia: Effects of site, position on bed and pea crab Parasitism. J. Shellfish Res., 24(1): 121-126.
Monterosato, TAD (1878) Note sur quelques coquilles draguees dans les eaux de Paleme. J. de Conchyliologie., 26:143-160.
Morriconi E, Lomovasky BJ, Calvo J, Brey T (2002). The reproductive cycle of *Euromalea exalbida* (Chemnitz, 1795) (Bivalvia: Veneridae) in Ushuaia bay, Beagle Channel (Argentina). Inv. Rep. Dev., 42:61-68.
Moussa RM (2013). Biometric studies of growth pattern affecting pearl production in pearl oyster *Pinctada radiata.* Egypt. J. Zool., 60:397-414. Nagabhushanam R, Bidarkar DS (1978). Studies on seasonal changes in the biochemical constituents of the oyster, *Crassostrea cucullata*. Ind. J. Fish., 25(1-2):156–164.
Nagabhushanam R, Talikhedkar PM (1977). Seasonal variations in protein, fat and glycogen of the wedge clam *Donax cuneatus*. Ind. J. Mar. Sci., 6:85–87.
Okumus I, Stirling HP (1998). Seasonal Variations in the meat weight condition index and biochemical composition of mussels (*Mytilus edulis* L.) in suspended culture in two Scottish sea lochs. Aqua., 159:249-261.
Orban E, di Lena G, Nevigato T, Casini I, Marzetti A, Caproni R (2002). Seasonal changes in meat content, condition index and chemical composition of mussels (*Mytilus galloprovincialis*) cultured in two different Italian sites. Food chem., 77:57-65.
Park HJ, Lee WC, Choy EJ, Choi KS, Kang CK (2011). Reproductive cycle and gross biochemical composition of the ark shell *Scapharca sub-crenata* (Lischke, 1869) reared on subtidal mudflats in temperate bay of Korea. Aqua., (322-323): 149-157.
Paulet YM, Boucher J (1991). Is reproduction mainly regulated by temperature or photoperiod in *Pecten maximus*. J. Inv. Rep. Dev., 19:61–70.
Peharda M, Richardson CA, Mladineo I, Šestanović S, Popović Z, Bolotin J, Vrgoč N (2007). Age, growth and population structure of *Modiolus barbatus* from the Adriatic. Mar. Biol., 151(2): 629-638.
Periyasamy N, Srinivasan M, Devanathan K, Balakrishnan S (2011). Nutritional value of gastropod *Babylonia spirata* (Linnaeus, 1758) from Thazhanguda, Southeast coast of India*.* Asian Pacific. J. Trop. Biomed., 12: 249-252.
Polasky S, Nelson E, Pennington D, Johnson L (2011). The impact of land-use change on ecosystem services, biodiversity and returns to landowners: a case study in the state of Minnesota. Enviro. Reso. Eco., 48:219-242.
Racotta IS, Ramirez JL, Ibarra AM, Rodriguez-Jaramillo MC, Carreno D *et al* (2003). Growth and gametogenesis in the lion-paw scallop *Nodipecten subnodosus*. Aqua., 217:335-349.
Ren JS, Marsden ID, Ross AH, Schiel DR (2003). Seasonal variation in the reproductive activity and biochemical composition of the Pacific oyster (*Crassostrea gigas*) from the Marlborough Sounds,

New Zealand. New Zealand J. Mar. Fresh. Res., 37:171-182.
Robert R, Trut G, Borel M, Maurer D (1993). Growth,fatness and gross biochemical composition of the Japanese oyster *Crassostrea gigas*, in stan way cylinders in the Bay of Arcachon, France. Aqua., 110: 249–261.
Roma´ n G, Martı´nez G, Garcı´a O,Freites L (2002).Reproduccio´ n. In: Maeda-Martı´nez AN, editor. Los moluscos pectinidos deIberoame´ rica: Ciencia y Acuicultura. Limusa, Mexico City.pp27–60.
Sahin C, Duzgunes E, Okumus I (2006). Seasonal variations in condition index and gonadal development of the introduced blood cockle *Anadara inaequivalvis* (bruguiere, 1789) in the southeastern black sea coast. Turk. J. Fish. Aquatic Sci., 6: 155-163.
Sastry AN (1979). Pelecypoda (excluding ostreidae). In: Giese AC, Pearse JS, editors. Reproduction of Marine Invertebrates. New York: Academic Press. Vol. 5.pp: 113–292.
Sokal RR, Rohlf FJ (1995). Biometry: the principles and practice of statistics in biological research. New York: W.H. Freeman. pp 887.
Taylor A C, Venn TJ (1979). Seasonal variation in weight and biochemical composition of the tissues of the queen scallop *Chlamy sopercularis* from the Clyde Sea area. J. Mar. Biol. Assoc.,59:605–621.
Taylor J, Strack E (2008). Pearl Production. In: Southgate PC, Lucas JS editors. The pearl oyster: biology and culture. The Netherlands: Elsevier. pp 273–302.
Thompson RJ, MacDonald BA (2006). Physiological integrations and energy partitioning. In: Shumway SE, Parsons GJ editors. Scallops: biology, ecology, and aquaculture, 2nd ed. Amsterdam, The Netherlands: Elsevier. pp 493–520.
Tlig-Zouari S (1993). Contribution `al'étudeéco biologique dedeuxesp`eces de mollusques lamellibranches *Pinctada radiata* (Leach, 1814) et Pinna nobilis, Linné 1758 des ˆılesKerkennah.Th`ese en Sciences, Eco-biologie marine, Universitéde Tunis, Faculté des Sciences de Tunis, pp215.
Tlig-Zouari S, Rabaoui L, Irathni I, Ben Hassine OK (2009).Distribution, habitat and population densities of the invasive species *Pinctada radiata* (Molluca: Bivalvia) along the Northern and Eastern coasts of
Tunisia. Cah. Biol. Mar., 50:131–142.
Tlig-Zouari S, Rabougi L, Irathni I, Diwara M, Ben Hassine OK (2010).Comparative morphometric study of the invasive pearl oyster *Pinctada radiata* along the Tunisian coastline. Biolo., 65: 294-300.
Tlig-Zouari S, Zaouali J (1994). Reproduction de *Pinctada radiata* (Leach, 1814, Mollusque: Bivalve) dans les ˆılesKerkennah(Tunisie). Mar. Life., 4: 41–45.
Tlig-Zouari S, Zaouali J (1998). Etude des quelques caract`eres biométriques de *Pinctada radiata* des iles Kerkennah (Tunisie Méridionale). Ann. Inst. Océanogr., 74: 217–224.
Walne PR (1976). Experiments on the culture in the sea of the butter fish *Venerupis decussata* L. Aqua., 8:371–381.
Whyte JNC, Englar JR, and Carswell BL (1990).Biochemical composition and energy reserves in *Crassostrea gigas* exposed to different levels of nutrition. Aqua., 90, 157-172.
Wołowicz M, Sokołowski A, Bawazir AS, Lasota R (2006). Effect of eutrophication on the distribution and ecophysiology of the mussel *Mytilus trossulus* (Bivalvia) in southern Baltic Sea (the Gulf ofGdan´sk). Limnol.Oceanogr., 51:580-590.
Yan H, Li Q, Yu R, Kong L (2010). Seasonal variations in biochemical composition and reproductive activity of Venus clam *Cyclina sinensis* (Gmelin) from the yellow river delta in northern China in relation to environmental factors. J. Shellfish Res., 29 (1), 91-99.
Yang HS, Kang DH, Park HS, Choi KS (2011). Seasonal changes in reproduction and biochemical composition of the cockle, *Fulvia mutica* (Reeve, 1884), in Cheonsu Bay off the west coast of Korea. J. Shellfish Res., 30: 95-101.
Yildiz H, Berber S, Acarlı S, Vural P (2011). Seasonal variation in the condition index, meat yield and biochemical composition of the flat oyster *Ostrea edulis* (Linnaeus, 1758) from the Dardanelles, Turkey. Italian J. Ani. Sci. 17 (2): 203-210.
Yıldız H, Lök A (2005). Meat yield of mussels (*Mytilus galloprovincialis* (Lamarck, 1819) in different size groups in Kilya Bay -Dardanelles. J. Fish. Aquat. Sci., 22:75-78.
Zandee DI, Kluytmans JH, Zurburg W (1980) Seasonal variations in biochemical composition of *Mytilus edulis* with reference to energy metabolism and gametogenesis. Neth. J. Sea Res., 14, 1-29.
Zar JH (1984). Biostatistical analysis. Englewood Cliffs, NJ: Prentice-Hall. 718pp.
Zenetos A, Cinar ME, Pancucci-Papadopoulou *MA*, *Harmelin JG, Furnari G*, Andaloro F, Bellou N, Streftaris N, Zibrowius H (2005). Annotated list of marine alien species in the Mediterranean with records of the worst invasive species. Med. Mar. Sci. 2: 63-118.
Zenetos A, Gofas S, Ruso G, Templado J (2004). CIESM atlas of exotic species in the Mediterranean. Vol. 3.Molluscs. In: Briand F(ed). CIESM Publishers,Monaco,pp 376.
Zenetos A, Vassilopoulo V, Salomisi M, Poursanidis D (2007). Additions to the marine alien fauna of Greek waters (2007 update). JMBA2 – Biod. Reco.,5928: 1-8.