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Lipids and Glycogen Utilization in the Intertidal Gastropod Mollusc Species *Turbo Lunella coronatus* (Gmelin 1791)

إستخدامات الدهون والجليكوجين في الحلزون البحري *Turbo coronatus* (Gmelin 1791)

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Abstract: This study examines the turban shell *Turbo coronatus* strategies for storing lipids and glycogen for reproductive investment. The monthly variations of total lipids and glycogen contents were studied over a period of 12 months from March 2003 to February 2004. Samples of 40-50 individuals were handpicked from the surface of the intertidal rocks at Shaikh Ebrahim Island every month. Two spawning events occurred during the year, the first one in April-May, while the second peak took place from July to November. The onset of male spawning was earlier than the onset of female spawning by an average of 4 weeks. Spawning was associated with a decrease in total lipids content, whereas gametogenesis was associated with an increase in total lipids content. The highest concentration of total lipids content was recorded during June and July for males and females respectively. Glycogen contents fluctuated throughout the period of this study with a significant increase ($p< 0.05$) in May for both sexes. In contrast to lipids, glycogen concentrations were very low, and did not contribute to the gametogenetic effort. Our results suggest that energy for reproduction is primarily derived from stored lipids.

Keywords: *gametogenesis, glycogen, lipid, snail, spawning, Turbo coronatus*.

المستخلص: استهدف هذا البحث دراسة إستراتيجية تخزين الدهون والجليكوجين لغرض التكاثر في الحلزون البحري *Turbo coronatus* وذلك من خلال متابعة التغيرات الموسمية في تركيز الدهون والجليكوجين على مدى 12 شهراً بدأ من مارس 2003 إلى فبراير 2004. توصلت الدراسة إلى أن الحلزون يتكاثر على فترتين في السنة، الأولى خلال الربيع (أبريل ومايو) وفترة ثانية مستمرة بين شهري يوليو ونوفمبر. كما لوحظ بدأ التكاثر لدى الذكور حوالي 4 أسابيع قبل الإناث. ولقد تزامن تكوين الأمشاج مع الزيادة في تركيز الدهون الكلية بينما دلت نتائج البحث على حدوث نقص حاد في تركيز الدهون اثناء عملية التكاثر. كما بلغ أعلى تركيز للدهون الكلية في شهر يونيو للذكور ويوليو للإناث وشهد الجليكوجين تذبذباً خلال فترة الدراسة بحيث سجل زيادة ملحوظة خلال شهر مايو لكلا الجنسين وكان تركيزه قليلاً جداً بشكل عام مقارنةً بتركيز الدهون. ولقد خلصت الدراسة إلى أن الطاقة اللازمة للتكاثر مستمدة أساساً من الدهون المخزنة.

كلمات مدخلية : *تكوين الأمشاج، جليكوجين، دهون، حلزون، تكاثر، توربوكوروناتس.*

INTRODUCTION

The gastropod mollusc *Turbo coronatus* Gmelin 1791 is a species which belongs to the family Turbinidae. *T. coronatus* is widely distributed in many areas in Bahrain such as Budaiya, Meridien, Asry, Marina club and Jaww (Green, 1994). The species *T. coronatus* has a shell size that ranges from 2024- mm in length and 1417- mm in width (Green, 1994). They are intertidal animals widely distributed on rocks with algae browsing from them (Jones, 1986). Despite their abundance in Bahrain, relatively little is known about their main energy source for gametogenesis.

Biochemical changes are known to accompany gametogenesis in marine organisms. Stored glycogen is reported to play a major role as energy source during gametogenesis, whereas lipids are the nutritive storage product of the gonads (Wenne and Polak, 1989). Several studies on marine mollusc have shown that the lipid level is inversely related to changes in glycogen level (Waldock, 1979). On the other hand, lipids are reported to play a major role in gamete development in bivalves. Several investigators have shown that the proportion of lipid at the time of gamete liberation affects the viability and proportion of larvae that reach the first-feeding stage (Gallager and Mann, 1986). In some bivalve species, the bulk of lipids in the diet accumulate in the female gametes. Thus the total energy intake (up to 94%) is transferred to gametes during gametogenesis (Martinez, *et al*. 2000). During this period, carbohydrates are converted into lipids and stored in the ripening gametes (De Gaulejac, *et al*. 1995; Galap, *et al*. 1997). Thus sexual maturation can be reflected by the total lipid and triacylglycerol content in the gonad of *Pectin maximus* (Besnard, 1988).

Stored energy in most animals is used during nutritional stress and reproductive period (Barber and Blake, 1981). In fishes, lipid is stored in the somatic tissues of the liver and then transported to the ovary during egg production (Henderson, 1996; Adams and Huntington, 1997). Bivalves, in contrast, store glycogen mainly in somatic tissues, and in a few cases in the digestive gland. Glycogen is then converted to lipid and stored in the digestive gland, or delivered directly to the ovary (Robert, *et al*. 1993; Pazos, *et al*. 1996; Galap, *et al*. 1997). In adult bivalves, carbohydrates, especially glycogen, is considered the main energy source. In contrast lipids are the main energy source for larvae, which is related to the initial lipid content of the eggs (Whyte, *et al*. 1992). In bivalves, eggs generally contain large amounts of lipid; however lipid content varies between species. Eggs of the intertidal bivalve *Macome balthica* contain about 30% lipids of their ash free dry weight (AFDW), whereas eggs of the cockle *Cerastoderma edule* and the mussel *Mytilus edulis* contain only about 11% and 20% lipids respectively (Honkoop, *et al*. 1999). Several studies on marine invertebrates have also revealed that the male gonads are comparatively richer in polysaccharide and protein nitrogen than the female gonads due to the high nucleic acid content of male gamete (Morais, *et al*. 2003).

The aim of this study on the seasonal changes in glycogen and total lipids contents in the snail *T. coronatus* is to describe the utilization and involvement of glycogen and total lipids in the process of gametogenesis and spawning in both sexes. The purpose of choosing *T. coronatus* was for its importance as a source of food for humans, however, relatively little is known about seasonal changes in their biochemical composition, calorific content and their reproductive cycle. This study is part of a comprehensive study on the edible snail *T. coronatus* which include the following researches:

1. Seasonal variations in total biochemical composition and the nutritional value of the marine edible snail *T. coronatus* Gmelin.
2. The fatty acids of the marine edible snail *T. coronatus* Gmelin 1791.
3. Reproductive cycle in the edible marine snail *T. coronatus* (Gmelin1791) in Bahrain water.

MATERIALS AND METHODS

Sampling and Preparation of Materials

T. coronatus were obtained from the rocky shores of Shaikh Ebrahim Island which is located between Askar and Jaww (Figure 1). Shaikh Ebrahim Island is an Island with an area of 1233 m^2. This Island is characterized by abundant

marine algae, plankton and sea grass which makes it a perfect habitat for the breeding, incubation and feeding of many organisms (Khalaf, 1995). A sample of 40-50 individuals were hand-picked from the surface of the intertidal rocks every month. Snails with the mean shell size of 22.120.25±mm in length, and 17.60.10±mm in width were used over the experimental period; snails were measured with vernier calipers (± 0.1 mm). Water temperature and salinity were measured on the collection day.

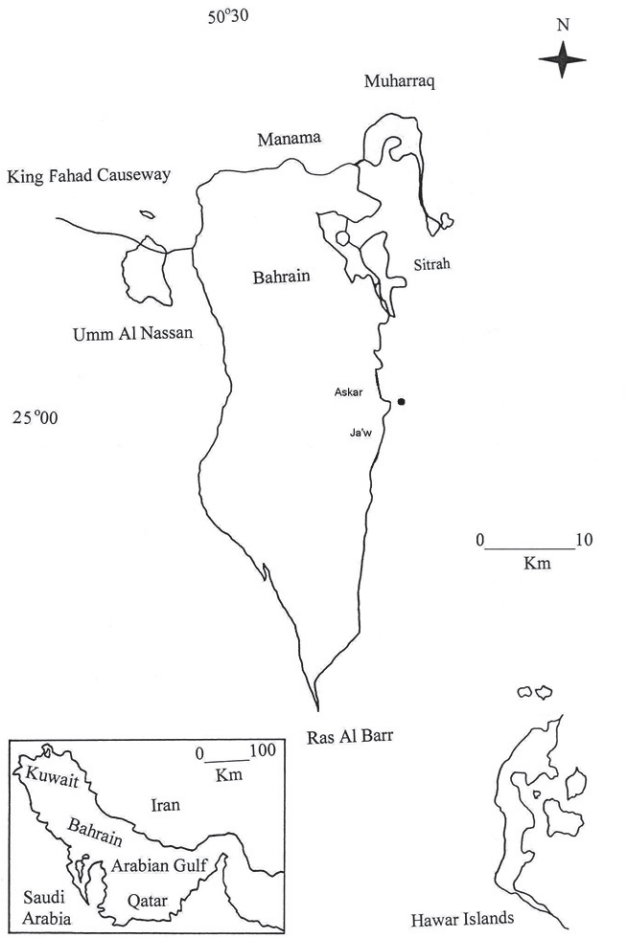

Fig. 1. Map of the sampling area, Shaikh Ebrahim Island (Bahrain), located between Askar and Jaww.

After collection, the organisms were transported to the laboratory and kept in seawater for 24 hours in order to allow gut clearance. The snails used in this study were collected over a 12 month period, between March 2003 and February 2004 at four weeks intervals. The shells of the organisms collected were broken and the soft tissues were dissected and separated into two sex groups based on the appearance of their gonads. The females had a distinct olive-green ovary that was uniform in color, whereas males had cream-colored gonads and light-brown splotches at times. Gonad materials were quite distinct from the dark-brown digestive gland (Jones, 1986). The soft tissues of 20 females and 20 males were removed, weighed and homogenized separately (Table 1). Homogenates were analyzed independently, and monthly results were recorded.

Table 1. Number of sample analyzed during the period of study.

Variables	Females	Males
No. of Turbo snails analyzed/month	20	20
No. of individuals analyzed/year	240	240
No. of homogenates analyzed/ month	4	4
No. of homogenates analyzed/year	48	48

Biochemical Analysis

The homogenates of 20 organisms (approximately 20 grams) from each sex group were divided into eight samples of two grams each. Four samples of each were used for the lipids and glycogen analysis.

Total lipids

Extraction of lipids was carried out within 24 hours of collection. For the lipids assay, four samples of separate male and female tissue homogenates each weighing two grams were used. The homogenates were extracted with a mixture of chloroform and methanol (2:1v/v) by the procedure of Folch, *et al*. (1957).

Glycogen

Glycogen extraction was performed immediately after collection in order to reduce the effect of rapid hydrolysis of glycogen (Zandee, *et al*. 1980). Four replicate samples of separate male and female homogenates were used. Two grams of each homogenized tissue were weighed and used for glycogen extraction. Glycogen was extracted by the procedure of Robinson (1992).

Statistical Analysis

Monthly data of total lipids and glycogen concentrations were analyzed using ONE WAY ANOVA to test for seasonal variations. However, the monthly mean values of each measured parameters were statistically compared using Duncan's Multiple Range Test, which is designed to specifically pin point comparisons between months. All statistical analysis was performed using STATGRAPHIC software version 5. Further statistical analysis involved calculation of correlation matrix and regression analysis for all the parameters.

RESULTS

Temperature and Salinity

A significant fluctuation in water temperature and salinity was recorded throughout the study, with a maximum water temperature value of 36.900.00°±C in August, and a minimal value of 19.760.03%± in December. The salinity showed a significant fluctuation ranging between 40 and 45‰.

Shell Size and Tissue Weight

The monthly changes in shell size, whole and tissue weight are summarized in Table (2). A minimal drop in the whole weight was registered in September (3.42±0.12g), whereas whole weight increased to reach a maximum value in February (5.58±0.12g). A sharp decrease in shell length (16.06±0.30) and in shell width (15.40±0.28-16.77±0.29) was registered in October and from September to November respectively. The wet weight decreased to reach a minimum value in June.

None of the above parameters were significantly correlated with monthly variations in water temperature over the sampling period except for the whole weight which negatively correlated with water temperature ($r = -0.60, p \leq 0.05$) as shown in Table 4. Similarly, whole and wet tissue weight, and one biochemical parameter (glycogen) showed no correlation with salinity with the exception of total lipids which negatively correlated with salinity ($r = -0.63, p \leq 0.05$) Table (3). Shell lengths on the other hand, have shown a significantly positive correlation with both whole weight (($r = 0.52, p \leq 0.05$) and wet weight ($r = 0.3, p \leq 0.05$) (Table 3).

Table 2. Monthly variations in shell length (mm), width (mm), whole weight (g), and tissue wet weight (g) of Turbo coronatus (mean ± SE).

Month / Year	Length (n=20)	Width (n=20)	Whole weight (n=20)	Tissue Wet weight (n=20)
March 03	22.90±0.43^{c}	17.92±0.17^{c,d}	4.71±0.16^{d,e}	1.14±0.10^{g}
April 03	21.42±0.50^{c}	17.35±0.21^{b,c,d}	4.77±0.15^{d,e}	0.83±0.04^{c,d}
May 03	22.17±0.37^{c}	18.40±0.32^{d}	4.85±0.21^{e}	0.64±0.03^{b}
June 03	22.50±0.51^{c}	17.15±0.33^{b,c}	3.89±0.15^{a,b}	0.38±0.02^{a}
July 03	22.82±0.38^{c}	17.77±0.26^{b,c,d}	5.00±0.22^{e,f}	1.07±0.03^{f,g}
August 03	21.87±0.37^{c}	17.55±0.30^{b,c,d}	4.05±0.14^{a,b,c}	0.90±0.04^{d,e}
September 03	20.12±0.28^{b}	15.40±0.28^{a}	3.42±0.12^{a}	0.65±0.02^{b}
October 03	16.06±0.30^{a}	15.62±0.49^{a}	3.68±0.14^{a,b}	0.71±0.03^{b,c}
November 03	21.92±0.37^{c}	16.77±0.29^{b}	4.15±0.19^{b,c,d}	0.69±0.03^{b,c}
December 03	22.35±0.55^{c}	18.40±0.24^{d}	5.74±0.19^{g}	1.18±0.03^{g}
January 04	22.85±0.44^{c}	17.92±0.32^{c,d}	5.09±0.19^{e,f}	1.03±0.03^{e,f,g}
February 04	24.47±0.54^{d}	17.52±0.55^{b,c,d}	5.58±0.37^{f,g}	1.18±0.06^{g}
ANOVA F-Ratio	12.145*	07.19*	11.48*	26.04*

* Statistically significant at P=0.05.
n = no. of individuals.

Table 3. Correlation analysis between some environmental factors, total lipids and glycogen and different weight and shell size parameters.

Correlation parameter	Coefficient (r)	Sample size (n)	Significant level (p)	Correlation
Salinity and length	-0.07	13	0.82	No correlation
Salinity and width	0.14	13	0.66	No correlation
Salinity and whole weight	0.35	13	0.23	No correlation
Salinity and wet weight	0.22	13	0.46	No correlation
Salinity and glycogen	-0.02	13	0.93	No correlation
Salinity and total lipid	-0.63	13	0.01	Negative
Temp. and length	-0.36	13	0.22	No correlation
Temp. and width	-0.33	13	0.26	No correlation
Temp. and whole weight	-0.60	13	0.02	Negative
Temp. and wet weight	-0.46	13	0.10	No correlation
Temp. and glycogen	0.38	13	0.19	No correlation
Temp. and total lipid	0.23	13	0.43	No correlation
Shell length and whole weight	0.52	13	<0.05	Positive
Shell length and wet weight	0.30	13	<0.05	Positive

Table 4. Monthly variations in total lipids and glycogen concentration (%) (mean± SE). Figures in the same column having same superscript are not significantly different at p ≤0.05..

Month/Year	Total lipids concentration (n=4)		Glycogen concentration (n=4)	
	Male	Female	Male	Female
March 2003	1.60±0.00^{d}	1.61±0.02^{b}	0.08±0.00^{b}	0.09±0.01^{b}
April 2003	1.38±0.01^{b}	2.10±0.10^{c}	0.15±0.00^{c}	0.08±0.00^{b}
May 2003	1.93±0.16^{e}	1.65±0.00^{b}	0.29±0.04^{d}	0.16±0.00^{c}
June 2003	3.40±0.21^{f}	2.10±0.00^{c}	0.13±0.01^{c}	0.04±0.01^{a}
July 2003	2.18±0.01^{e}	2.50±0.13^{d}	0.15±0.01^{c}	0.09±0.01^{b}
August 2003	1.33±0.07^{b}	1.49±0.05^{b}	0.08±0.01^{b}	0.06±0.00^{b}
September 2003	1.50±0.00^{c}	1.50±0.00^{b}	0.08±0.01^{b}	0.03±0.01^{a}
October 2003	1.30±0.14^{b}	1.50±0.08^{b}	0.08±0.01^{b}	0.08±0.01^{b}
November 2003	1.18±0.03^{a}	1.13±0.03^{a}	0.08±0.01^{b}	0.08±0.01^{b}
December 2003	1.65±0.00^{d}	1.65±0.00^{b}	0.08±0.01^{b}	0.08±0.01^{b}
January 2004	1.61±0.02^{d}	1.58±0.00^{b}	0.05±0.00^{a}	0.09±0.01^{b}
February 2004	1.60±0.00^{d}	1.50±0.00^{b}	0.05±0.00^{a}	0.09±0.01^{b}
ANOVAF-Ratio	43.66*	41.63*	22.10*	9.84*

* Statistically significant at P=0.05.
n = no. of homogenates.

Biochemical Composition

The monthly variations in the total lipids and glycogen contents of the soft tissues over the sampling period are shown in Table (4) and Figures (2-5).

There was a distinct seasonal cycle of changes in total lipids content. The results indicated a significant decrease in April (1.38±0.01%), July (2.18±0.01%), and October (1.3±0.14%) for males. In contrast, a significant decrease was registered

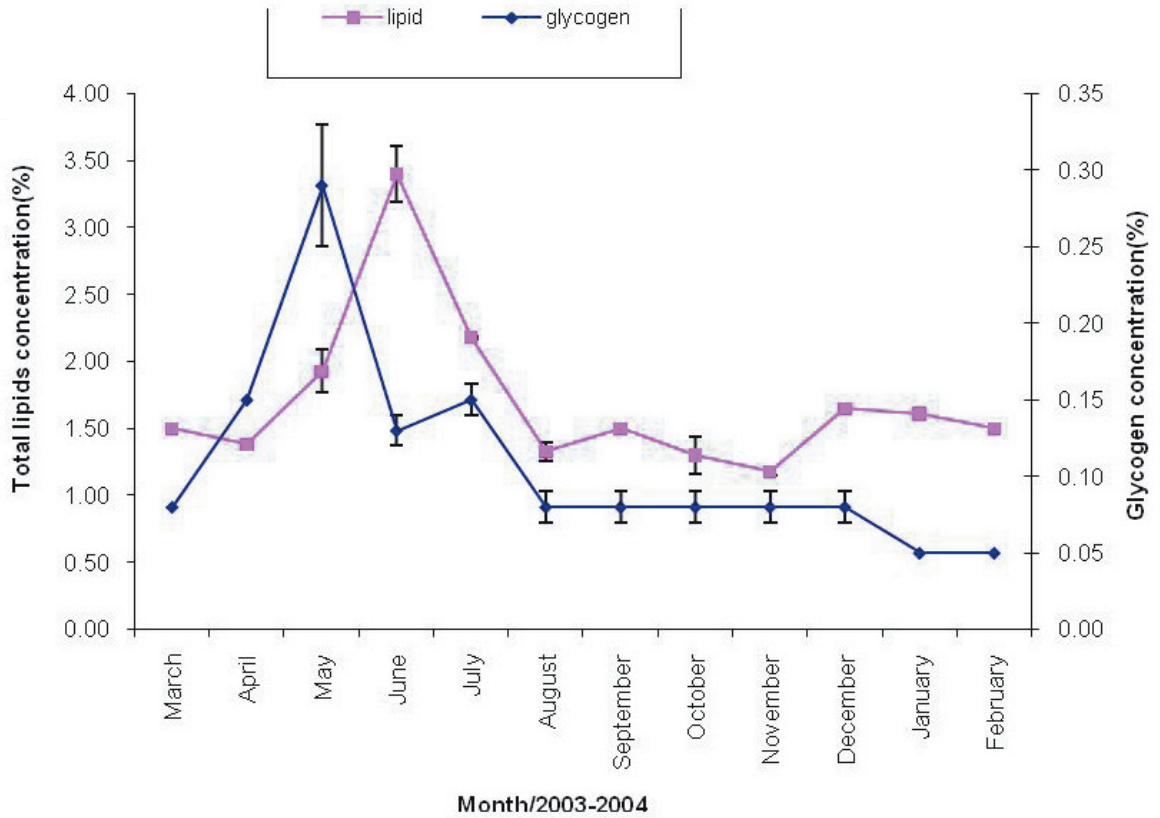

Fig. 2. Monthly changes in total lipids and glycogen concentration (%) in males of *Turbo coronatus* (mean±SE).

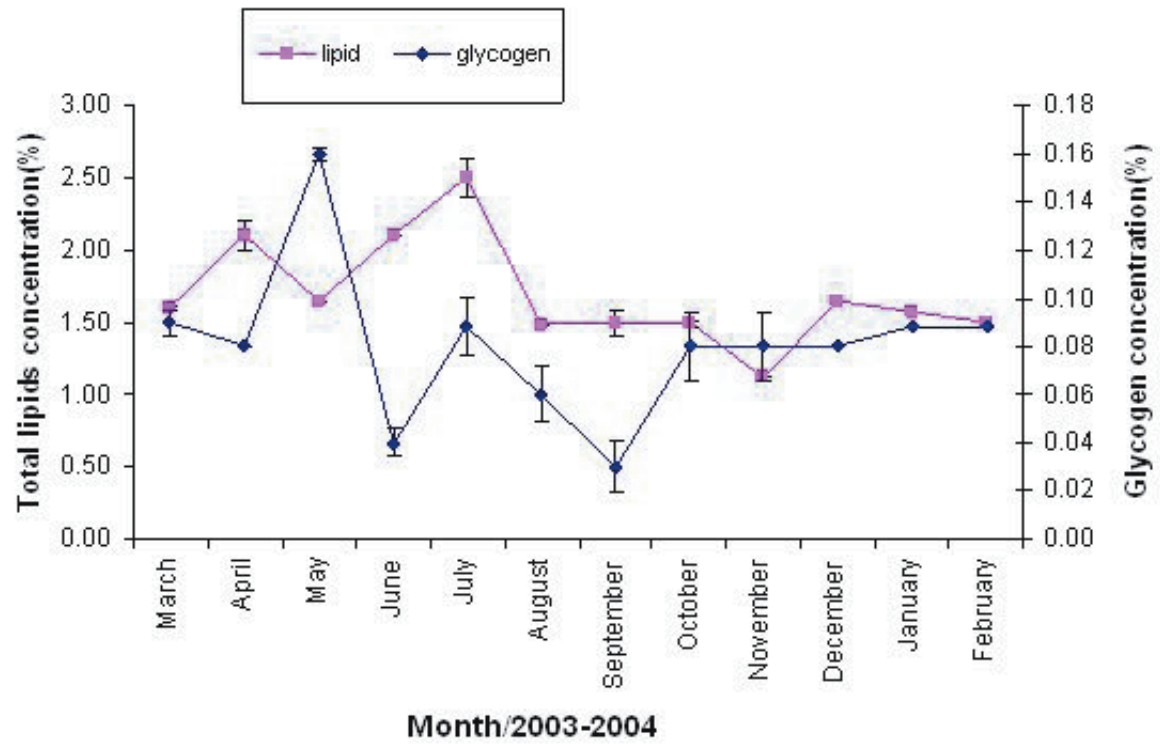

Fig. 3. Monthly changes in total lipids and glycogen concentration (%) in females of *Turbo coronatus* (mean±SE).

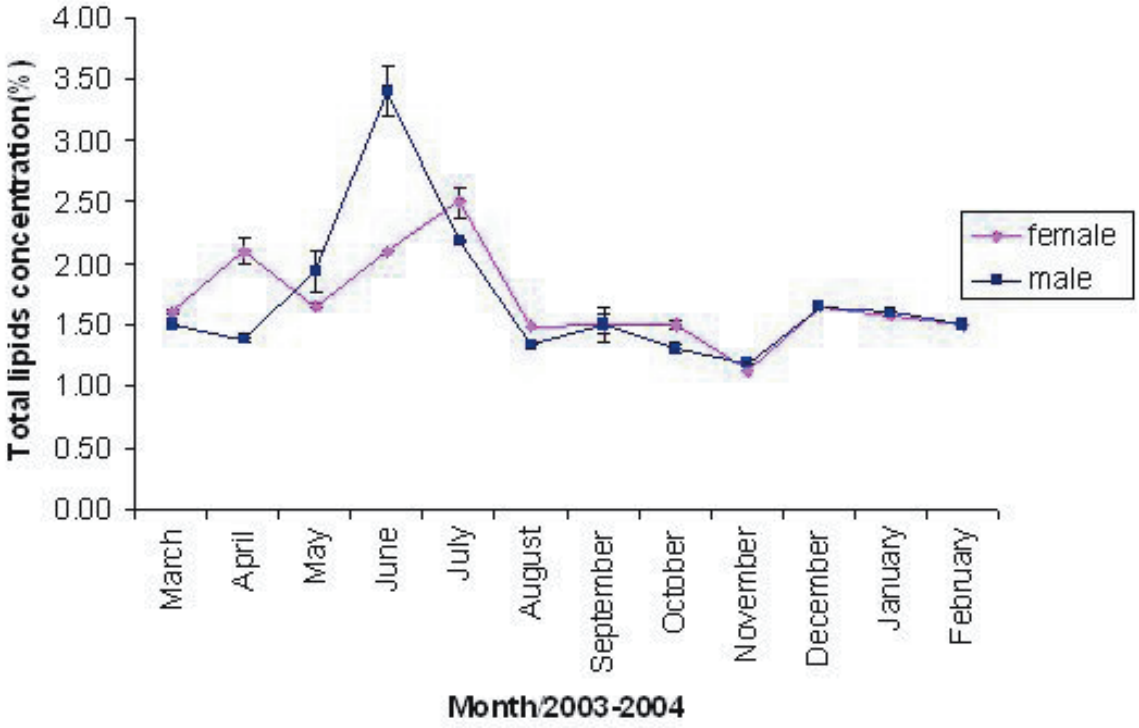

Fig. 4. Monthly changes in total lipids concentration (%) in males and females of *Turbo coronatus* (mean±SE).

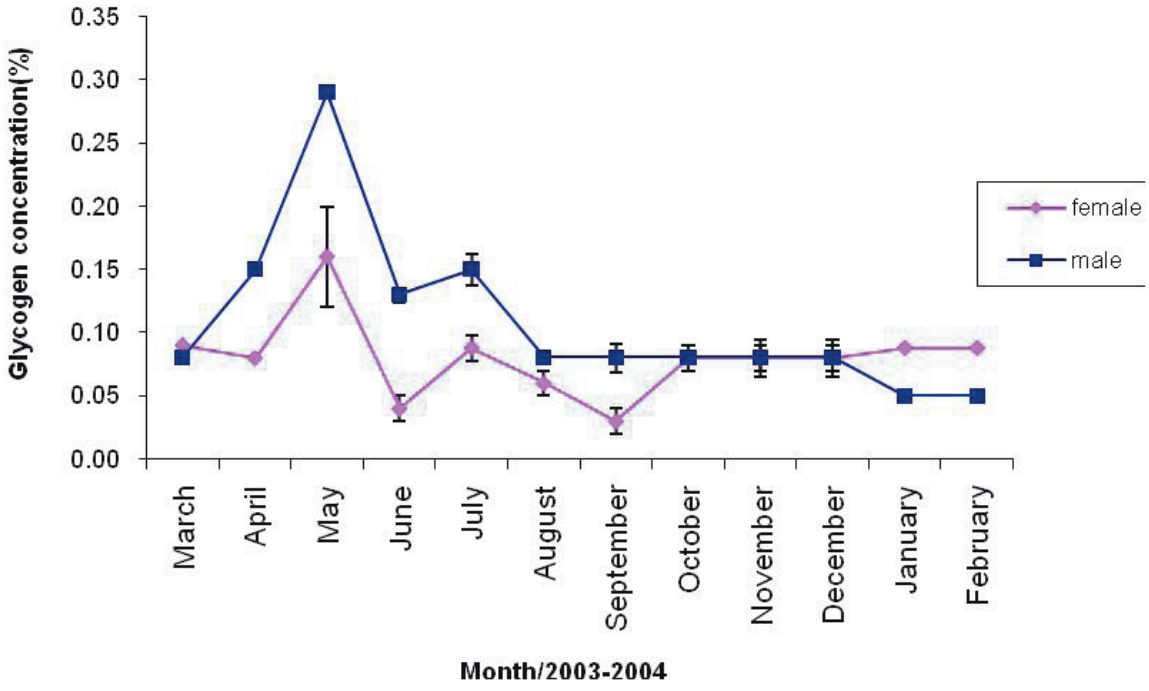

Fig. 5. Monthly changes in total glycogen concentration (%) in males and of *Turbo coconatus* (mean±SE).

in May (1.65±0.00%), August (1.49±0.05%), and November (1.13±0.03%) for females. The total lipids content increased to its greatest level in June (3.4±0.21%) for males and in July (2.5±0.13%) for females. In males, the glycogen content showed a slight fluctuation. Glycogen content increased from March (0.08±0.00%), reaching a maximum level in May (0.29±0.04%), which was then followed by a steady decrease till August. Thereafter, glycogen content was constant till December (0.08±0.01%), followed by a slight decrease till February (0.05±0.00%). In females, glycogen content varied between (0.03±0.01-0.16±0.00%). The lowest value was recorded in September (0.03±0.01%) and the highest in May (0.16±0.00%). No relationship existed between total lipids and glycogen in both sexes, however, a positive correlation was observed in total lipids and glycogen contents ($p < 0.005$) between male and female.

DISCUSSION

Gametogenesis is an energy-demanding process which mobilizes nutrients from ingested or stored nutrients and subsequently utilizes reserve from the body tissue is required (Pipe, 1987). The present investigations have revealed that adult male *T. coronatus* spawns during the months of April, and between the months of July to November, whereas adult female spawns in May, and between August and November. The present study also indicates that the main spawning season for *T. coronatus* occurred during the spring-summer season with a peake in April for males and in May for females. A second but continuous spawning event occur between July and November for males and between August and November for females. The results of the present study confirm the findings of our previous study which reported two spawning events in *T. coronatus*; the first major event occurred in May, and a second continuous spawning was recorded between September and December (Freije and Al-Sayed, 2008).

This study also revealed that the onset of male spawning was earlier than the onset of female spawning by an average of four weeks. This indicates a significantly longer period is needed to stimulate female spawning. This result from the study on *T. coronatus* largely agree with the study of Siah, *et al*. (2002) on the soft-shell clam *Mya arenaria* in which the males were found to be in the spawning stage one month earlier than the females. No correlation was found between spawning and temperature during the research period. However, a negative correlation was found between total lipids and salinity ($r = -0.63$, $p \leq 0.05$) during the research period. The present investigations have revealed that *T. coronatus* are capable of spawning twice a year; (spring, and summer) with the main spawning event occurring in spring. In marine organisms such as bivalves, gametes maturation and spawning is mainly affected by two factors, water temperature and food availability, and secondarily, by salinity and photoperiod (Pouvreau, *et al*. 2000). In tropical temperatures (temperature always around 28°C), however, the reproductive cycle is less distinct, and continuous spawning become predominant (Wada, *et al*. 1995; Pouvreau, *et al*. 2000). On the other hand, Martinez, *et al*. (2000) have postulated that low temperatures are probably associated with normal gametes development and accumulation of energetic reserves in *Argopecten purpuratus*; however, the last stage of gametogenesis (spawning) could then be accelerated by increasing temperature.

In the present study, major spawning coincided with the relatively moderate water temperature in April (29.70±0.85) and May (27.835±0.38°C). Most probably energy reserves would have been built up during the cold winter season in Bahrain (January-February), and the beginning of the spring season (March-April), where water temperature usually ranges between 18°C and 22°C (Madany and Al-Sayed, 2001). However, in the present study energy reserves were built up during the hot summer season (June-July), and spawning also occurred during the hot season between July and November. Therefore, water temperature appears to play no role in *T. coronatus* ability to build reserve energy and spawn as well. Gametogenesis could most probably be attributed to food availability, a parameter which was not determined during this study. These findings are consistent with several other studies on the pacific oyster *Crassotrea*

gigas (Sang-Gyun, *et al*. 2003), the mollusc *Perna picta* (Shafee, 1989) and the intertidal bivalve *Macoma balthica* (Honkoop, *et al*. 1999) in which the building of energy reserves was independent of temperature fluctuation.

On the other hand, most studies on turbinids in the world have shown that turbinids undergo an annual spawning cycle with a single spawning event each year (Toshiaki, 1993; Hideki, *et al*. 1995; Foster, *et al*. 1999). In accordance *T. coronatus* Gmelin 1791 on the Transkei coast in South Africa has a distinct spawning peak between December and February; however multiple spawnings have also been suggested due to the low levels of breeding throughout the year (Lasiak, 1986). Nevertheless, the turban shell *T. torquatus* Gmelin 1791 in New South Wales, Australia as well as several trochid species from Japan and Mexico are capable of spawning twice a year (Belmar-Perez, *et al*. 1993; Noda, *et al*. 1995; Ward and Davis, 2002). Partial or incomplete spawning was also recorded in several gastropod species (Belmar-Perez, *et al*. 1993; Ward and Davis, 2002). The green snail *T. marmoratus* L. in Indonesia also has one distinct spawning period during the warmer months from June to September in subtropical areas in the Northern Hemisphere; however, spawning occurs several times throughout the year in tropical waters (Dwiono, *et al*. 2001).

In the present study, spawning was associated with a decrease in the total lipids content in both sexes, probably as a result of loss of lipids in the spawned gametes, whereas it increase immediately after spawning. Several authors have shown that in marine invertebrates, lipids increased before spawning and decrease immediately after spawning, probably as a result of loss of lipid in spawned oocyte (Whyte, *et al*. 1990; Robinson, 1992; Ruiz, *et al*. 1992; Berthelin, *et al*. 2000).

Averaging the pre-spawning total lipids level and subtracting the post-spawning level gives a loss of 0.22% in April, 2.07% in July-August, and 0.32 % in October-November for males. The loss of total lipids content in male was slightly different than that in females (0.45% in May, 1.01% in August, and 0.37% in November), most probably because of the different biochemical composition of the gamete. Several studies on marine invertebrates have found a difference in biochemical content between male and female gonads (Morais, *et al*. 2003). The higher June-July total lipids values are probably caused by better feeding conditions during the spring-summer growing season which contributed consequently strongly to the relatively high total lipids values in both sexes.

The level of glycogen measured in the whole body of *Turbo coronatus* (less than 0.3%) was much lower than many marine invertebrates such as *Mytilus edulis* L. (2%) (Bayne, *et al*. 1982), *Crassostrea gigas kumamoto* (less than 5%) (Robinson, 1992) and *Crassostrea gigas* (less than 6%) (Berthelin, *et al*. 2000), two explanations are presented for this fact. Firstly, glycogen is not the main energy reserve in *Turbo coronatus*. Secondly, glycogen is not one of the main constituents of *Turbo coronatus* gametes. Furthermore, partial quantitative differences were found in glycogen concentration in males and females. Both sexes showed much higher glycogen levels during the spring season (mainly in April) comparative to other seasons, most probably due to food availability. However, males were found to have a higher glycogen content than females during this season. This could be attributed to the possible difference in biochemical contents between male and female gonads. No appreciable difference was found between the sexes in the proportion of glycogen level. In general the relative amount of glycogen varied little through the year. Therefore, the possible explanation may be the availability of food supply.

No correlation was found between total lipids and glycogen concentration in both sexes. Therefore, lipids would probably play the major role in gamete development in the edible snail *T. coronatus*.

CONCLUSION

In conclusion, the results obtained in this study show that *T. coronatus* is capable of spawning twice a year with a peak during the spring season (April-May).

The onset of male spawning was significantly

earlier in contrast to onset of female spawning by an average of four weeks. Spawning was associated with a decrease in total lipids content, whereas gametogenesis was associated with an increase in total lipids content. However, the Pattern of the loss of total lipids content in males was slightly different to females. Glycogen content gradually increased to reach its maximum level in May for both sexes; thereafter, a relatively constant very low glycogen levels persisted throughout the year. Therefore, *T. coronatus* would most probably yield gamete which contains a comparatively high amount of lipids in relation to glycogen.

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