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Water Content as a New Tool for Discrimination between some Shellfishes

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ABSTRACT

The quality requisites of shellfishes are primarily dependent on the water quality, assuring a healthy product and a safe consumption. Water (moisture) content of five species from two different environments was conducted for a period of one year (July, 2010-June, 2011) for this purpose. Among the most exploitable and economically important Egyptian species, three brackish (the bivalves *Cerastoderma glaucum*, *Fulvia fragilis* and the gastropod *Thais carinifera*) and two salt water (the bivalves *Donax semistriatus* and *Donax trunculus*) shellfishes were chosen. Estimation on monthly basis revealed the incompatibility of moisture content with spawning period. Present results proved that water content varied according to the environmental factors rather than spawning seasons. Marine species had lower demand of water than brackish species. The differences in the average of the body water content between genera [*Donax* sp. (0.13), *C. glaucum* (0.75), *F. fragilis* (2.35) and *T. carinifera* (3.68)] was greater than that within the same genus [*D. semistriatus* (0.10) and *D. trunculus* (0.16)]. The gastropod *T. carinifera* had higher water content than the bivalves. Hierarchical clusters analysis was a good tool to differentiate moisture content between genera, as well as between species from different environments. The present study put in the perception of the probability of use water content in discrimination and classification between species.

Key words: Water content, Suez Canal, Mediterranean sea, bivalve, gastropod

INTRODUCTION

Moisture content is one of the most important biochemical constituents of the shellfishes. So, it is of great importance to know variations in the moisture content of them, as it may be correlated with spawning or other physiological activities or may reflect the impact of the surrounding environment on them. Most molluscs have open circulatory systems, which mean that their organs are surrounded by watery blood that contains nutrients and oxygen. Changes in watery environment represent a challenge for the tissues of these molluscs and alternatively on their water contents (Ruiz and Souza, 2008). So, we assume that moisture content may be used as a tool to differentiate between genera as well as between species from different environments (brackish and sea water). For this purpose, we select five shellfishes (the bivalves; *C. glaucum*, *F. fragilis*, *D. semistriatus*, *D. trunculus* and the gastropod *T. carinifera*) among the most exploitable species in Egypt and also worldwide.

The cockles are among the most familiar marine invertebrates, which include the largest and fastest growing living members of the Bivalvia class (Yonge, 1982). *C. glaucum*, commonly known as the lagoon cockle, is a species of Egyptian brackish water bivalve and is also quite common in Danish water, Baltic and in the Atlantic region: in the Netherlands and British Isles (Mohammad, 2002).

F. fragilis another cockle species among the brackish water bivalve and common in several other countries, such as; Turkey (Ozturk and Poutiers, 2005), Tunisia (Rifi *et al*., 2011), Italy, Spain (Zenetos *et al*., 2003), Malta (Goud and Mifsud, 2009) and Egypt (Mohammad *et al*., 2006).

The wedge clams, *D. trunculus* and *D. semistriatus* are one of the most economically important marine bivalves in Egypt. These saltwater species are widespread Mediterranean species. They occur usually on the coasts of Western Europe and north-western Africa. They inhabit the shallowest two meters of coastline and are commercially harvested for food (Alkaradawe *et al*., 2014).

Gastropods, on the other hand, represent ~2% of the marine molluscs harvested worldwide (Leiva and Castilla, 2001). Muricidae is a large family comprising 8 subfamilies represented by ~1300 species that are distributed worldwide (Houart, 2001). Despite that remarkable number of species, only a few muricids have commercial value. Among those, *T. carinifera* is widely distributed in the Indian Ocean, in the Red Sea, in Suez Canal in the Mediterranean Sea and in the Pacific Ocean (Ine, 1986; Gofas *et al*., 2001; Streftaris *et al*., 2005; Radwan *et al*., 2009), is also harvested for human consumption and has large commercial value due its cheapness and to the large amount of meat that containing.

These five species have a considerable economic importance, both in terms of aquaculture and harvested aquatic resource. Accordingly, the main purpose of this research was: (1) To evaluate water content and its percentage in these shellfishes and (2) To assess the possibility of discrimination and classification on the basis of water content.

MATERIALS AND METHODS

The study was conducted in the brackish (Timsah Lake, which is located on the Suez Canal at 30°34'N and 32°18'E) and salt-water (Idku that is located on the Mediterranean coast of Egypt, Latitude: 31°31'N, Longitude: 30°3'E). Average of salinity varied from 36-43 psu, for the brackish and saltwater, respectively. Specimens were collected at monthly intervals from July, 2010 -June, 2011. Shell measurements were made to the nearest 0.01 mm using a vernier caliper. The wet tissues were blotted and their weights (wet weights) were measured to the nearest 0.001 g with an electrical balance. Sexes were separated. The sexual dimorphism of *C. glaucum* and *D. semistriatus* is largely gonadal. Ovaries are green and dark red in females' *C. glaucum* and *D. semistriatus*, respectively. Testes are creamy white-pale orange in males of both species. The dry weights were recorded after drying the tissue to a constant weight for 48 h at 80°C. The water (moisture) content was determined by weight difference before and after drying the samples. Percentage water (moisture) content was estimated, as percentage of dry weight to the wet weight.

Statistical analysis: Statistical analysis was evaluated by Microsoft Excel and SPSS 18 statistical packages. Mean of the different parameters were analyzed graphically and submitted to analysis of the variance (ANOVA) followed by multiple range LSD test to find any significant ($p<0.05$) fluctuations during the year or between the studied species. Primers for Windows (version 5.2.0) were used to determine the degree of similarity between the different species.

RESULTS

Figure 1-3 elucidate monthly significant variations ($p<0.01$) of water content and its percentage for both sexes of *C. glaucum* and *D. semistriatus* but the variation was not

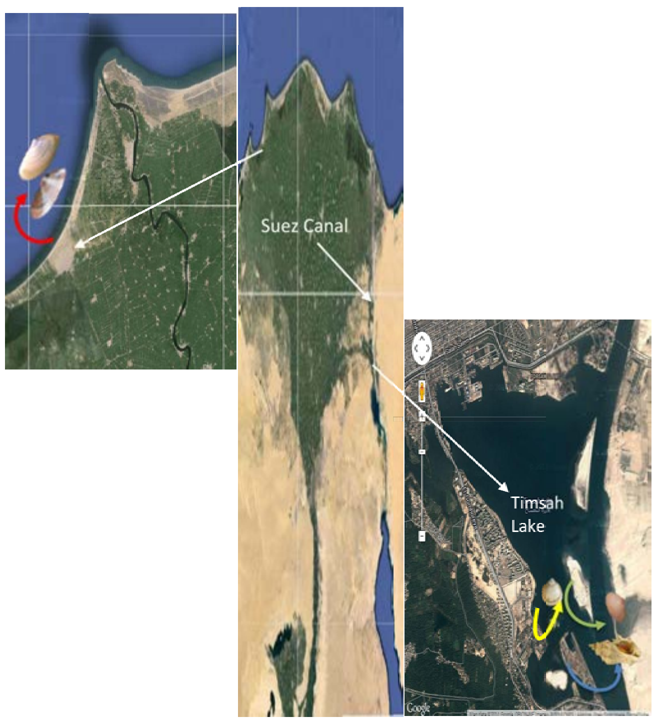

Fig. 1: Map of the investigated area and monitoring sites in this study

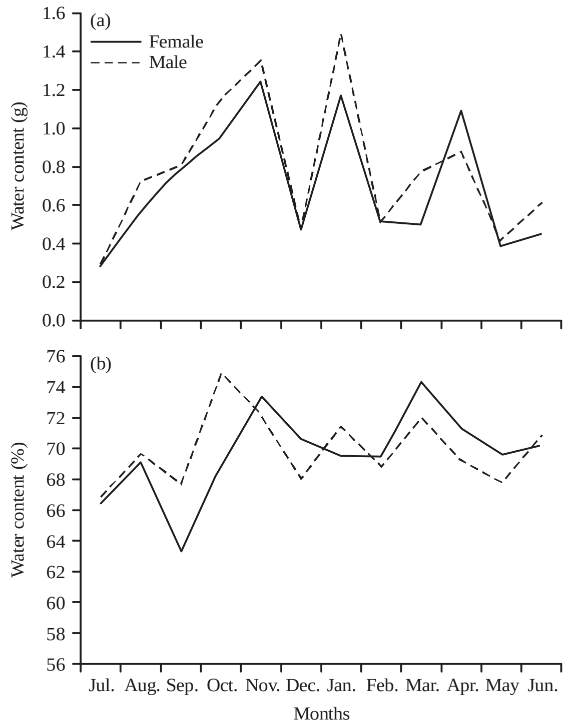

Fig. 2(a-b): Monthly variation in (a) Water content and (b) Its percentage in both sexes of *Cerastoderma glaucum*

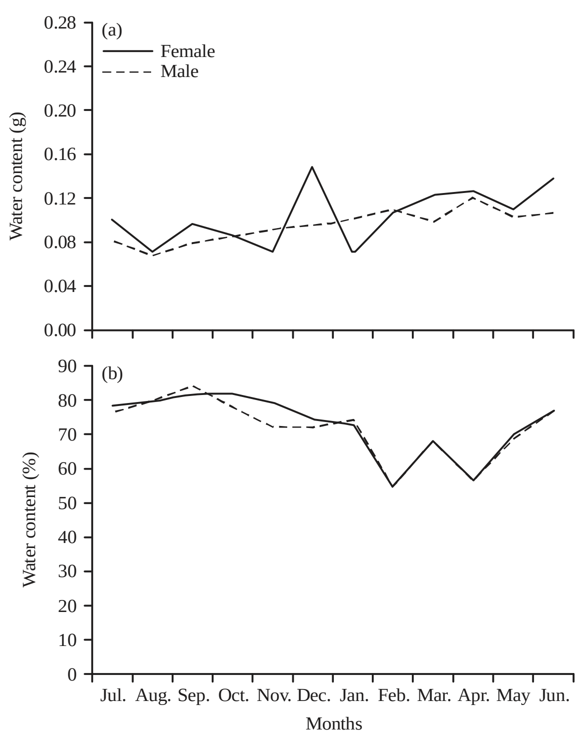

Fig. 3(a-b): Monthly variation in (a) Water content and (b) Its percentage in *Donax semistriatus*

significant between sexes (p = 0.85 and 0.24, for them, respectively). This was ascertained by the high similarity between sexes as shown in Fig. 4. However, moisture of males was slightly higher than females in *C. glaucum* and the reverse was true in *D. semistriatus*.

Water content of both sexes of *C. glaucum* and *D. semistriatus* increased with the increase in shell length, as shown in Fig. 5a and 6a. The increasing in water content of male *C. glaucum* was slightly higher than that of females (b = 1.87 and 1.49 for them, respectively). *D. semistriatus* showed also the reverse trend (b = 1.23 and 1.41 for male and

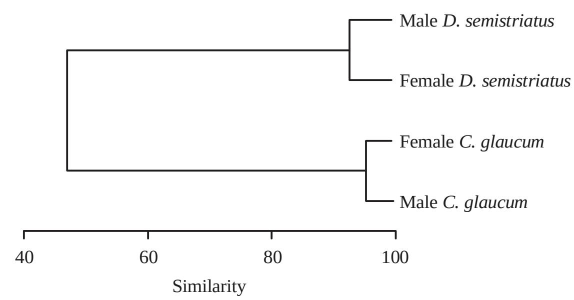

Fig. 4: Dendrogram for hierarchical clusters analysis shows the similarity in water content between the studied species based on monthly variation during the year

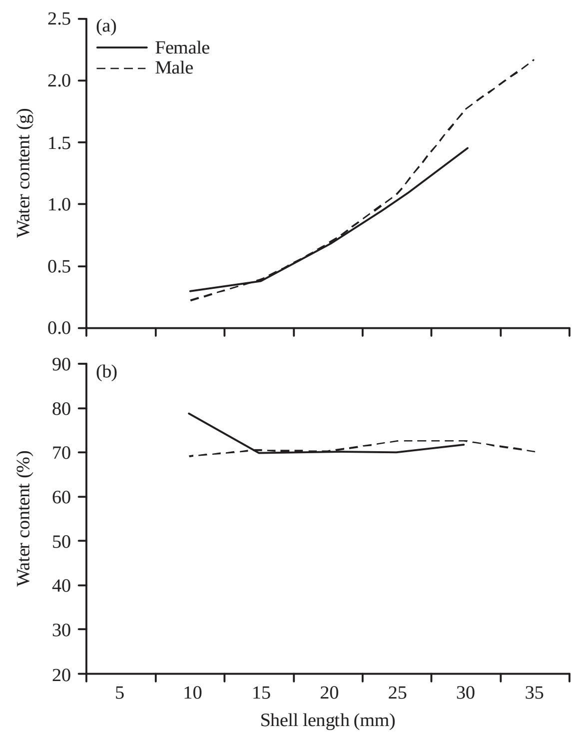

Fig. 5(a-b): Variation in (a) Water content and (b) Its percentage with shell length in *Cerastoderma glaucum*

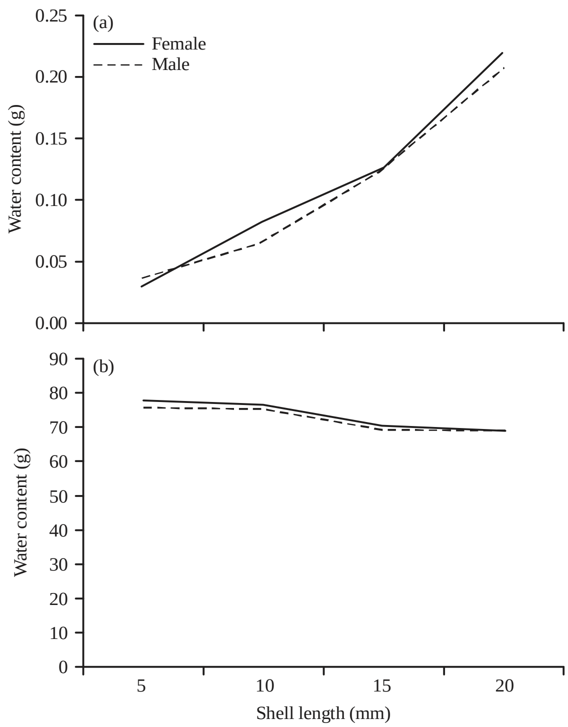

Fig. 6(a-b): Variation in (a) Water content and (b) Its percentage with shell length in *Donax semistriatus*

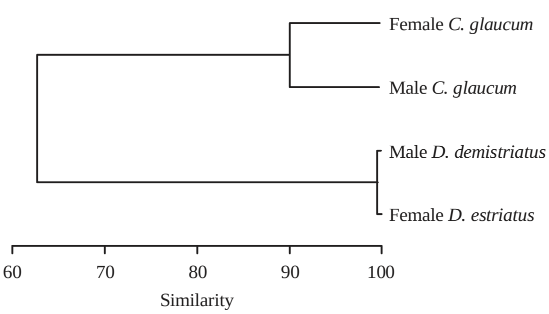

Fig. 7: Dendrogram for hierarchical clusters analysis shows the similarity in water content between the studied species according to the shell length

female, respectively). However, there was no difference between sexes (p = 0.93 and 0.99 for *C. glaucum* and *D. semistriatus*, respectively). Generally, similarity between sexes in *D. semistriatus* was higher than that in *C. glaucum* as shown in Fig. 7. Reversely, the moisture percentage declined as the shell length of female *C. glaucum*, male and female *D. semistriatus* increased (b = -1.34, -2.58 and -3.23 for them, respectively) as shown in Fig. 5b and 6b. However, variation of moisture percentage with shell length was not noticeable in *C. glaucum* due to weak correlation (R^2 = 0.3 for both sexes).

The bivalve water contents increased also with weight (Fig. 8-9). Females were more watery than males especially in

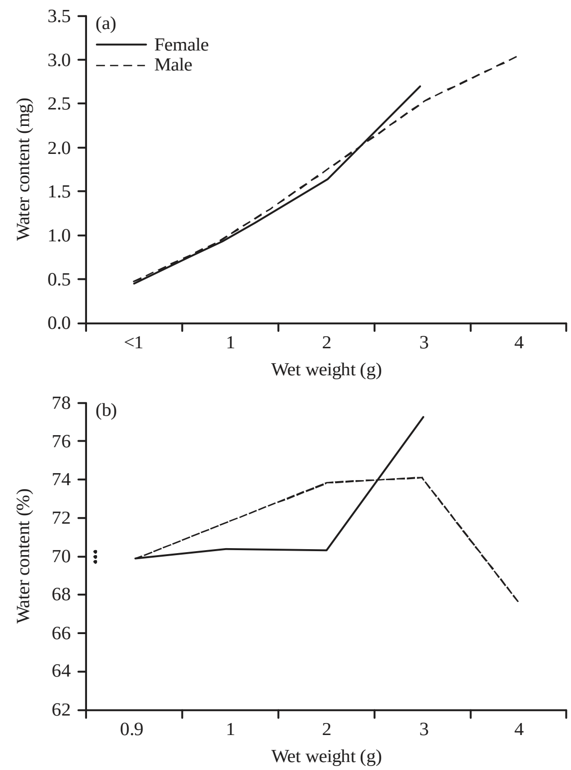

Fig. 8(a-b): Variation in (a) Water content and (b) Its percentage with wet weight in *Cerastoderma glaucum*

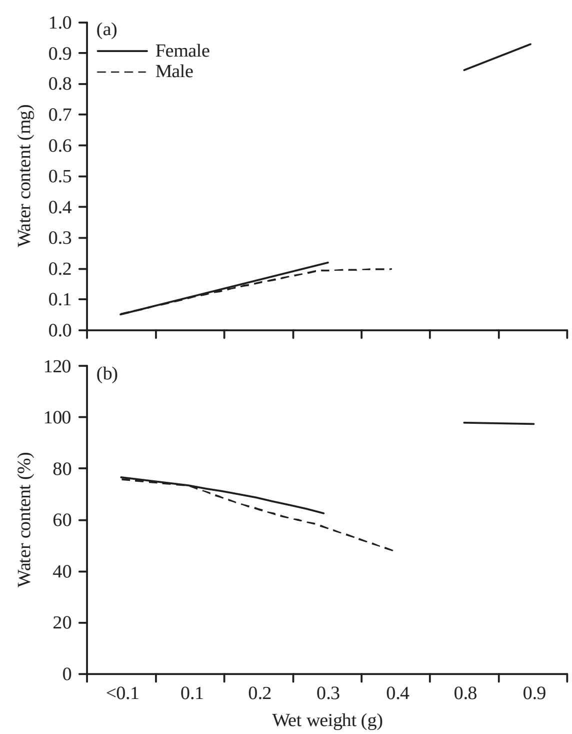

Fig. 9(a-b): Variation in (a) Water content and (b) Its percentage with wet weight in *Donax semistriatus*

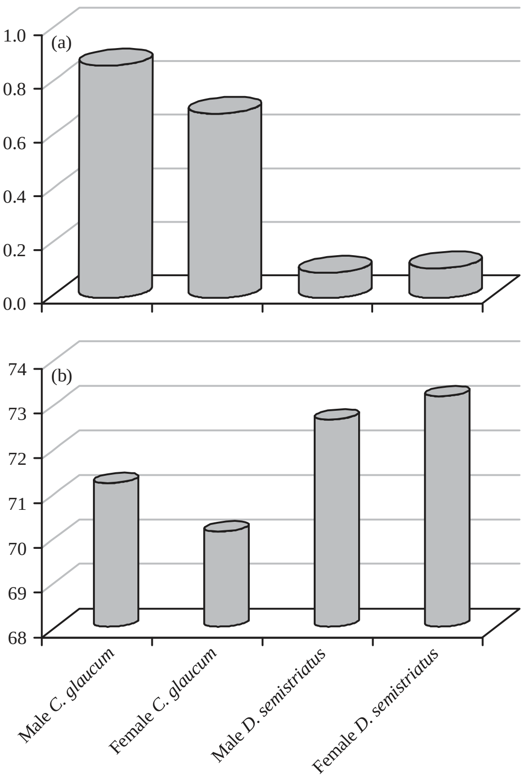

Fig. 10(a-b): Total average of (a) Water content and (b) Its percentage in *Cerastoderma glaucum* and *Donax semistriatus*

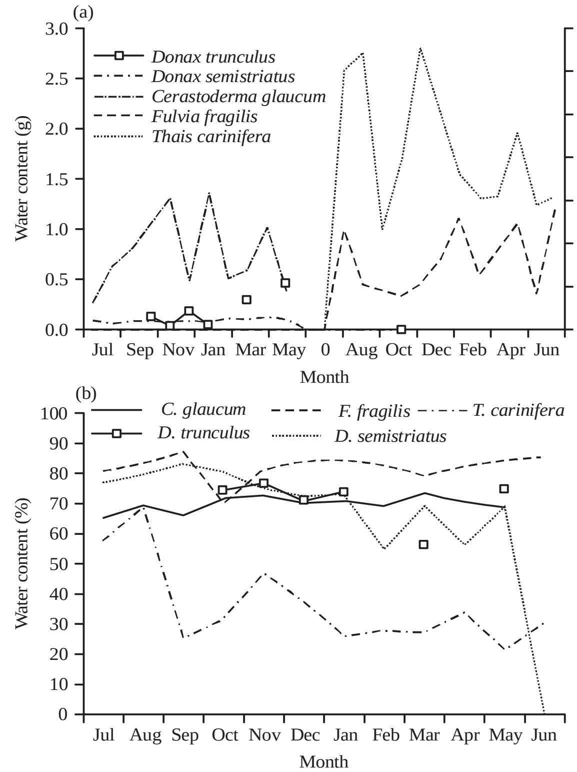

Fig. 11(a-b): Monthly variation in (a) Water content and (b) Its percentage in the studied species

the largest size. This was clear from the negative relationship of its percentage with size that appeared in male *C. glaucum* and male *D. semistriatus* (b = -0.22 and -7.33 for them, respectively).

Generally, the average water content and its percentage in both sexes of the two bivalves were graphed in Fig. 10. The difference between sexes was little and non significant (p>0.05). Hence, the data was pooled and compared with the other molluscs. The gastropod *T. carinifera* had the highest water content as seen in Fig. 11a. The maximum and minimum water contents of the studied species were 6.56 (Nov.) and 2.33 (Sep.), 2.58 (Jan.) and 0.84 (May), 1.36 (Jan.) and 0.27 (Jul), 0.46 (May) and 0.04 (Nov.) and 0.12 (Apr.) and 0.06 (Aug.) for *T. carinifera*, *F. fragilis*, *C. glaucum*, *D. trunculus* and *D. semistriatus*, respectively. Significant variations (p<0.01) were detected between all species except that between *D. trunculus* and each of *C. glaucum* (p = 0.17) and *D. semistriatus* (0.86). Water content percentage of *F. fragilis* exhibited monthly little variation with a distinct drop (70.18%) in Oct. (Fig. 11b). The highest percent of water content of *D. semistriatus* (83.31) appeared in Sep. while the lowest (54.94 and 56.49) in Feb. and Apr. Moisture percentage ranged between 56.20 and 76.78, 65.42 and 73.51 and 21.61 and 68.85 in *D. trunculus*, *C. glaucum* and *T. carinifera*, respectively. No significant variations were detected between the bivalves *C. glaucum* and *D. trunculus* (p = 0.57), *C. glaucum* and *D. semistriatus* (p = 0.17) and *D. trunculus* and *D. semistriatus* (p = 0.63), but the others exhibited significant variations (p<0.05).

The water content increased as the shell length increased (Fig. 12a). *T. carinifera* showed a different trend, water content increased till shell length 60 mm, after which it declined up to 70 mm, thereafter, it began to increase again. However, *F. fragilis* showed the maximum increase in water content (b = 2.59), as the shell length increased by 1 mm. It was followed by *C. glaucum* (b = 1.77) and the lowest was *D. semistriatus* (b = 1.36). No obvious variation was detected in the moisture percentage with the increase of length in *C. glaucum* and *F. fragilis* (Fig. 12b). *T. carinifera* exhibited negative correlation with the shell size, the maximum water percentage was recorded in the smallest snail (62.24%), it declined to 28.95% in the largest size. Negative correlation with size was also recorded for the clams *D. trunculus* and *D. semistriatus*. These results are well represented in Fig. 13. It separated the investigated species into three groups. The first group was the brackish bivalves (*F. fragilis* and *C. glaucum*) that had a positive correlation with shell length, the second group was the marine bivalves (*D. trunculus* and *D. semistriatus*) that had a negative correlation and isolated the gastropod *T. carinifera* in the third group.

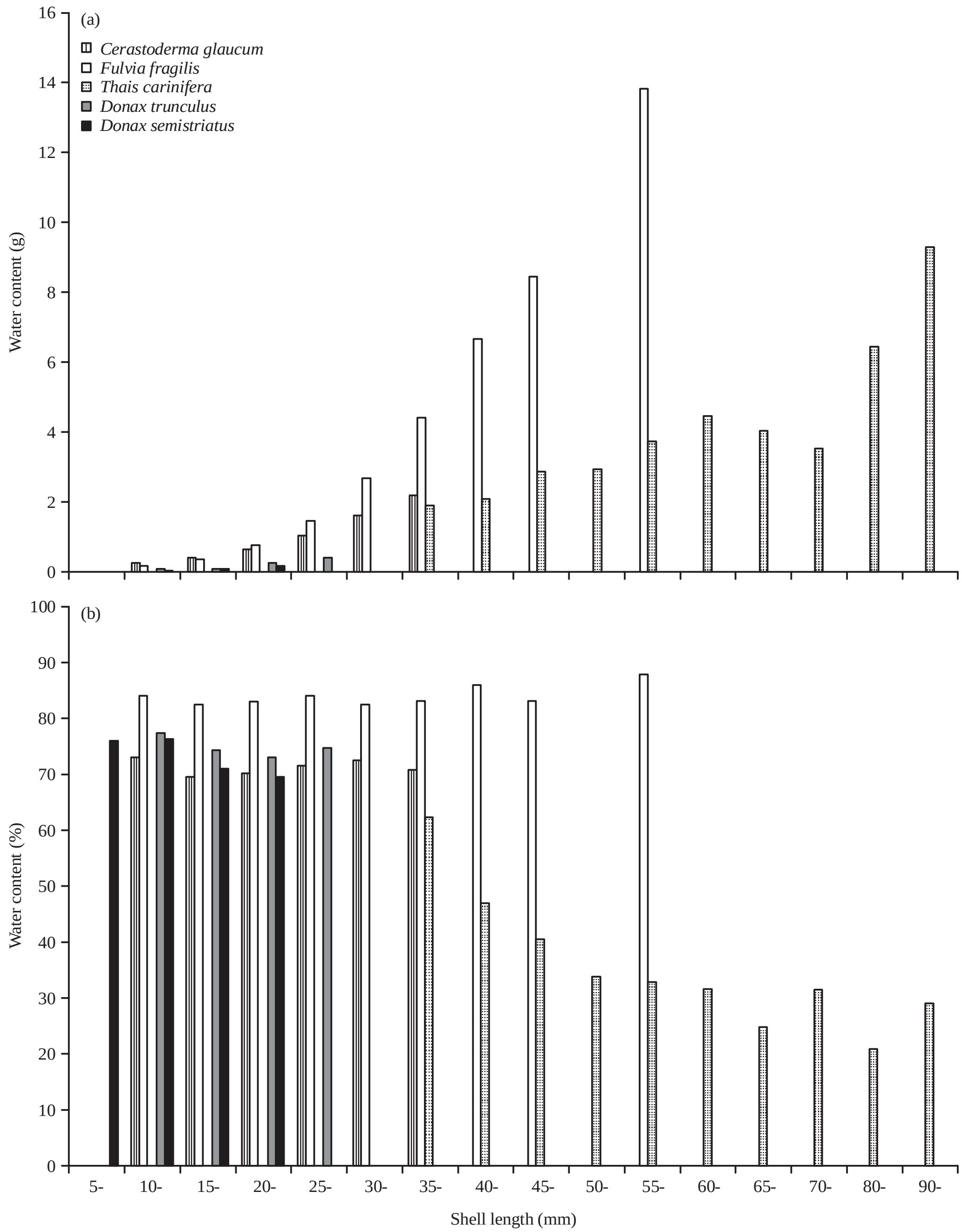

Fig. 12(a-b): Variation in (a) Water content and (b) Its percentage with shell length in the studied species

Depending on weight, there was obvious positive correlation with the wet weight (Fig. 14a). *F. fragilis* exhibited the highest moisture content specially in the heaviest animals while *D. trunculus* and *D. semistriatus* contained the lowest. Hierarchical clusters analysis (Fig. 15) also isolate *F. fragilis* and *C. glaucum* in a group dissimilar with *Donax* sp. (similarity = 0). The percentage of water content (Fig. 14b) shows little variations with the increasing in weight except *T. carinifera* that exhibited a negative relationship (b = -1.24) with weight.

The least values of water content were estimated in the marine bivalves (0.10 and 0.16 for *D. semistriatus* and *D. trunculus*, respectively) as shown in Fig. 16a. It gradually increased to 0.75 and 2.35 g in the brackish bivalves *C. glaucum* and *F. fragilis*, then it reached to the highest (3.68 g) in the gastropod *T. carinifera*. This variation between genera was highly significant ($p<0.01$) except that between *D. semistriatus* and *D. trunculus* ($p = 0.75$). Meanwhile, percentage of water content of *T. carinifera* represented the minimum value (32.89 g) and the maximum value was

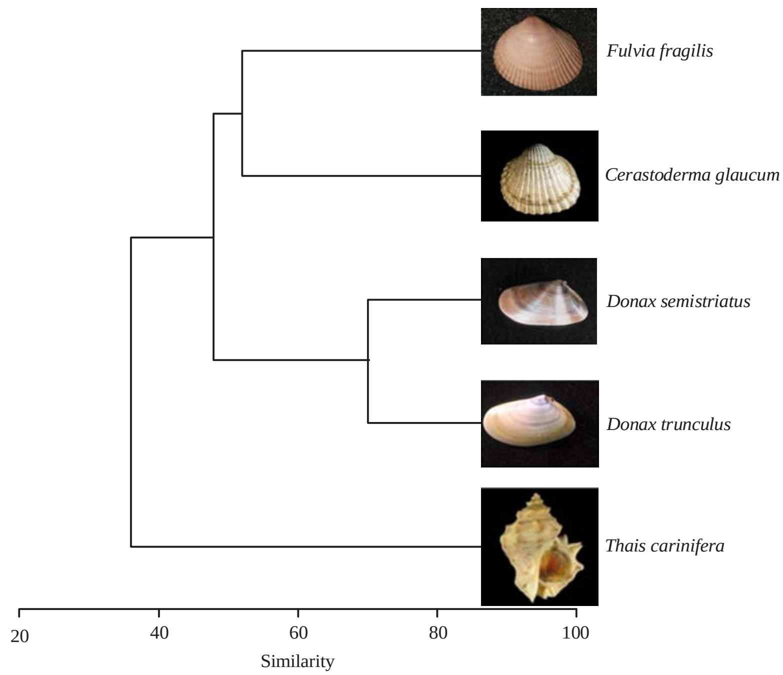

Fig. 13: Dendrogram for hierarchical clusters analysis shows the similarity in water content between the studied species based on shell length

represented by *F. fragilis* (Fig. 16b). Also, the variation between species was highly significant except that between *D. semistriatus* and *D. trunculus* (p = 0.50).

DISCUSSION

The present study revealed that monthly variation in water content of male *C. glaucum* was slightly higher than female, while the reverse was the case in *D. semistriatus*. This means that the monthly water demand differs between sexes of brackish water than that of the sea. This result was ascertained from the hierarchical clusters analysis that separated the brackish from marine genera at similarity of 50%.

Both sexes of marine and brackish water showed increase in water content as the length and weight increased. However, their moisture percent declined with them. Female *C. glaucum* was the only exception that showed a positive correlation. This may mean that females' *C. glaucum* became more watery at the larger size than males.

Generally, variation in the water content between sexes was not significant. This was previously noticed by Jayabal and Kalyani (1987), who stated that different body fractions of both male and female *M. meretrix* showed similar trend in water content. So, this data was pooled and compared with the other genera. The present results revealed monthly significant variation between *T. carinifera* and *F. fragilis* from one side and between them and the other bivalves from the other hand. This was clear from the highest water content that recorded in *T. carinifera* followed by *F. fragilis* then *C. glaucum*. Hence, the maximum water content was recorded in brackish shellfishes (*T. carinifera*, *F. fragilis* and *C. glaucum*), while, the least water content was estimated in the marine bivalves (*D. semistriatus* and *D. trunculus*). We can relate this to the surrounding environment. As *D. semistriatus* and *D. trunculus* live in higher salinity (43 pus), it may accumulate less water than that leave in the brackish water (36 pus) according to their osmoregulation. A similar result was previously recorded by Jayabal and Kalyani (1987). They mentioned that, at lower salinity, water accumulated into the body.

Generally the present study revealed a distinct monthly pattern of variation in the water content of all studied species. Some authors related this variation to the spawning season. Ogogo (2004) attributed the fall in moisture to the spawning of the animal. Meanwhile, Manhas *et al*. (2013) observed that moisture content was high during the peak spawning. Taylor and Venn (1979) previously interpreted this. The rapid increase in moisture weight corresponds with the animal absorbing more water to fill the lumen created in the gonad after the release of the gamete. Recently, Udoh and Abiaobo (2014) confirmed that as they found the shellfish species absorbed more water after spawning. The present studied species characterized by continuous spawning. Spawning of *C. glaucum* occurred most of the year, without prolonged periods of reproductive inactivity (Mohammad, 2002; Machreki-Ajmi *et al*., 2013). The continuity of spawning was also recorded for the other shellfishes *F. fragilis* (Mohammad, 2002), *T. carinifera* (Radwan *et al*., 2009),

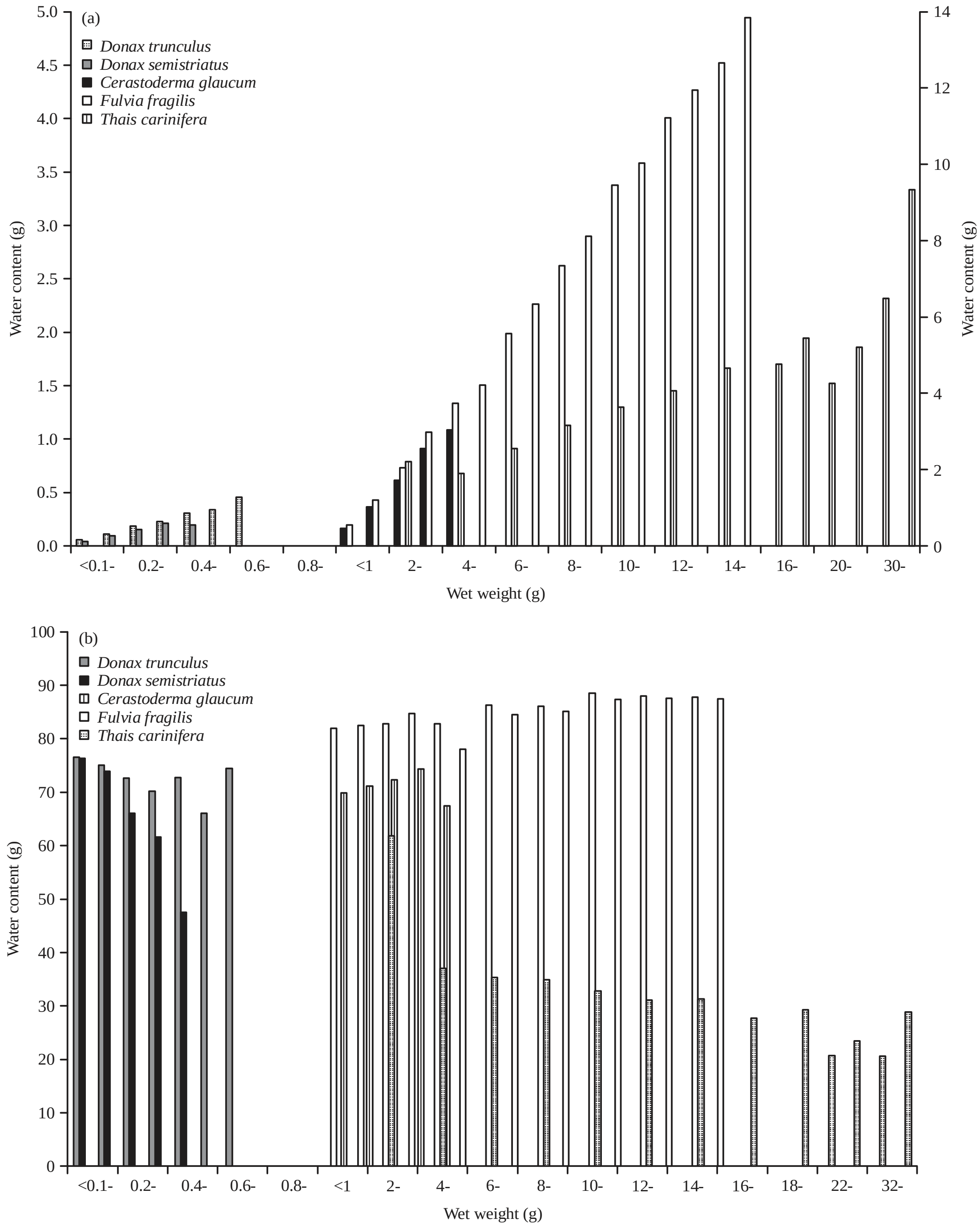

Fig. 14(a-b): Variation in (a) Water content and (b) Its percentage with wet weight in the studied species

D. semistriatus and *D. trunculus* (Ali *et al*., 2009; Alkaradawe, 2014). The irregular annular trends in the water content of the present studied species did not coincide with the spawning season. So, the present results disagree with the previous assumptions but consistent with Gharsallah *et al*. (2010). They mentioned that among the biochemical component analyzed in the *Hexaplex truculus*, the protein, lipid and carbohydrate contents showed marked variations throughout the reproductive cycle, whereas, the moisture content did not show significant oscillations. Moreover, we assume that the gonad did not represent a large fraction of the body and hence, the change in its water content did not impact on the total water content. This assumption coincides with the early finding of Jayabal and Kalyani (1987). They estimated water content in four selected body parts (digestive diverticula, adductor muscle, foot and gonad) of *M. meretrix* and concluded that gonad had the minimum water content among the four body fractions. Accordingly, we can attribute the

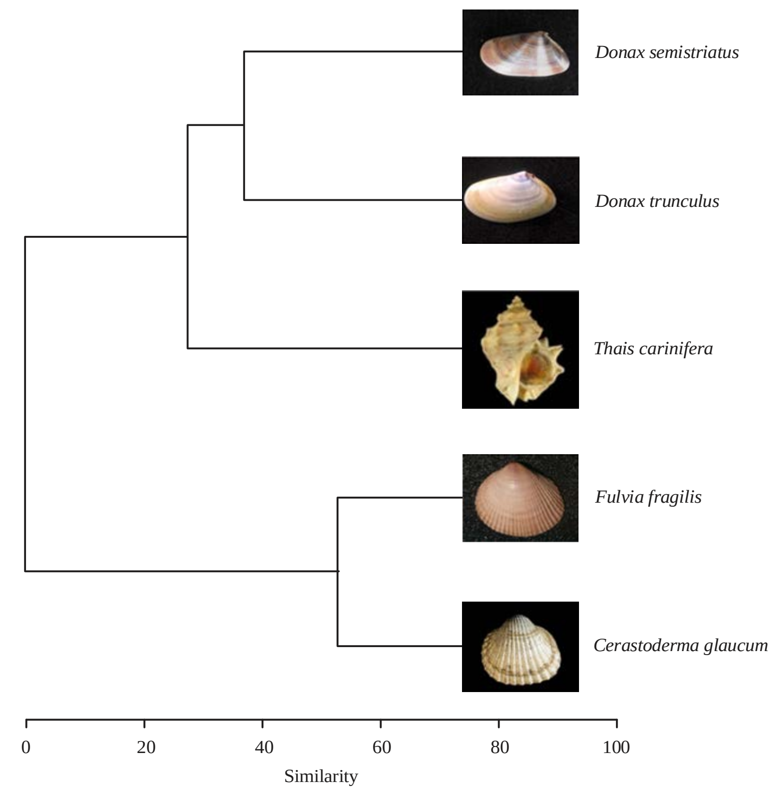

Fig. 15: Dendrogram for hierarchical clusters analysis shows the similarity in water content between the studied species based wet weight

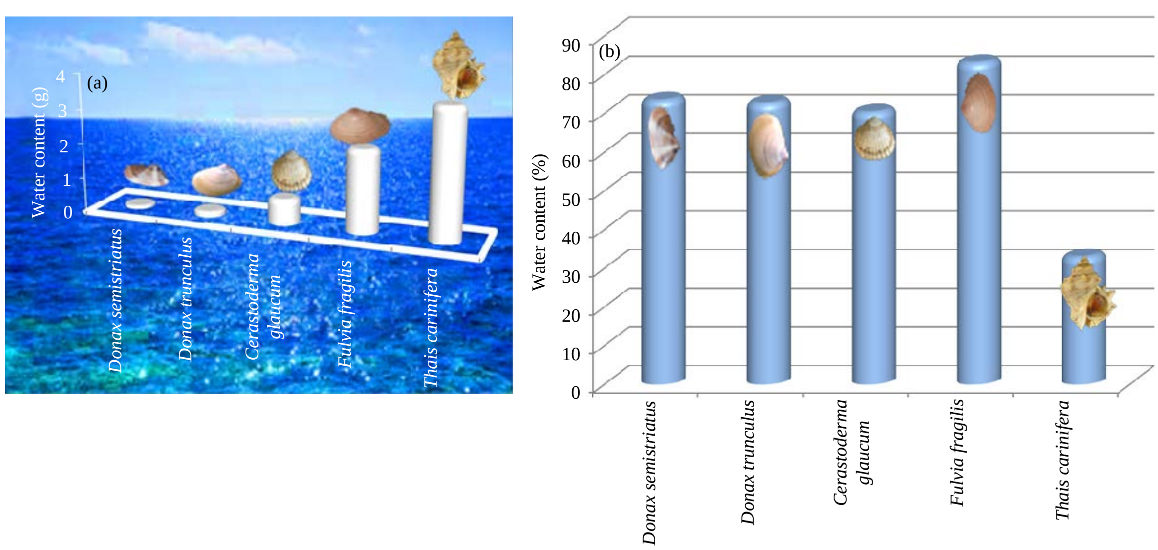

Fig. 16(a-b): Average variation in (a) Water content and (b) Its percentage in the studied species

variation in the water content of the present studied shellfishes to the environmental factors rather than spawning seasons. Water content was influenced by food availability and salinity (Jayabal and Kalyani, 1987). Starvation was also among factors that affected water content. Riley (1976) mentioned that starvation involved dry weight losses and a general increase in the water content of the body of *Crassostrea gigas*.

CONCLUSION

In a conclusion, demand of water varies according to the surrounding environment and genera. The marine species contained lower water content than the brackish species. The gastropod had higher water content than the bivalves and the variation was not significant between the species of the same genus or between sexes. Allah has created every (living) creature from water and of them are those that move on their bellies and of them are those that walk on two legs and of them are those that walk on four. Allah creates what He wills. Indeed, Allah is over all things competent (Surat An-Nūr (The Light), 45).

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