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RESEARCH ARTICLE

HISTOCHEMICAL AND BIOCHEMICAL STUDIES OF THE SNAKE; *PSAMMOPHIS SCHOKARI* INHABITING DIFFERENT GEOGRAPHICAL HABITATS

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ABSTRACT

Snakes of the family Psammophiidae are abundant throughout Africa and are the most commonly encountered snakes within their areas of occurrence. The Schokari sand snake, *Psammophis schokari*, is a colubrid snake distributed widely on the western Mediterranean coastal plain, widespread over much in the dune fields of the north Sinai. Sixteen specimens of the snake *Psammophis schokari* were collected from two different geographical habitats: El Daba as a coastal area and El Maghara as a desert-area. There is a variation in coloration between both snakes. They were sacrificed; liver, skeletal muscles and skin were examined. The present histological study of the liver tissue showed some changes in the architecture of the coastal and desert *Psammophis* snakes. The amount of pigments in the skin of desert *Psammophis* were greater than coastal. Variations in the amount of energy reserved in certain organs (liver, skeletal muscles, skin) was histochemically observed and confirmed by the biochemical studies in both snakes. In conclusion, the present study indicates the relation between the amounts of food available in the environment and the variation in the histology of some tissues and body reserves in coastal and desert *Psammophis* schokari.

INTRODUCTION

Snakes are prominent predators in many terrestrial, aquatic and marine communities (Rodríguez-Robles, 2002). They are enormously successful group worldwide with 2500 species and an extraordinary diversity of sizes, shapes and ecological characteristics (Pough and Groves, 1983). Snakes of the family Psammophiidae (consisting of seven genera) are mostly diurnal, active foragers that range throughout Africa and into Mediterranean Europe and southwest Asia (De Haan, 2003; Kelly *et al*., 2008). Psammophiids are abundant and often the most commonly encountered snakes within their areas of occurrence (Spawls *et al*., 2002; Luiselli *et al*., 2005). A wide ranging species, the Schokari sand racer can be found throughout Northern Africa, as far south as Chad, Somalia and Ethiopia. Its range also extends throughout the Arabian Peninsula and the Middle East to Central Asia, occurring as far north as Turkmenistan and as far east as Pakistan and north-west India (Ananjeva *et al*., 2006). *Psammophis schokari* (*P. schokari*) is Saurophagous (feeds primarily on lizards) in accordance with (Baha Din, 1998; Jongbloed, 2000), small birds, rodents and other vertebrate. The venom of this snake is not considered dangerous to humans. The jaws of this species bear two non-venomous, fang-like teeth in the upper jaw and two strongly enlarged, venomous fangs, at the level of the rear edge of the eye (Hellyer and Aspinall, 2005). These snakes are relatively common in well-vegetated areas, especially those with trees and shrubs, including areas of cultivation. Excellent climbers, they are able to catch birds in trees (Handley and Cunningham, 2000). They are also found in sand and gravel areas with low shrubs and grasses and occur from sea level to the mountain summits (Hellyer and Aspinall, 2005).

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The accumulation of energy reserves (glycogen and fat) is complicated by the fact that at the same time the snakes are undergoing several physiological processes during the time of the year (when the food is readily available and the snakes are active), normal growth and repair of body tissue must also occur (Hussein, 2007). Most studies deal with one or two main metabolites, such as glycogen (Gaffney and Fitzpatrick, 1973; Bartlett, 1975; Patterson *et al*., 1978), or focus on fat depots in fat bodies rather than the liver (Gregory, 1982). Glycogen is the main storage polysaccharide in reptiles (Abdel Raheem *et al*., 1988) and an important energy source for some turtles and snakes (Gregory, 1982). Glycogen is present in numerous cell types in different tissues and is also stored in specialized glycogen cells, e.g. hepatic cells (Fenoglio *et al*., 1992). While lipids are an important energy source for reptiles during all seasons (Costanzo, 1985). The present work aimed to demonstrate the histology of different organs in the snake *P. schokari* inhabiting different geographical habitats in Egypt (coastal and desert) to show the amount of energy reserves (glycogen and lipid) in both snakes, using histochemical and biochemical studies. We explained also the variations in their geographical habitats to understanding the relations between different environmental factors and the behavior of animal examining those data in light of the current literatures.

MATERIALS AND METHODS

Study areas

A total of 16 specimens of the snake; *P. schokari* were collected from the two following study areas as shown in maps:

El Dabaa and El Amid (Matrouh governorate)

Daba and el-Hammam are cities in Matrouh Governorate, north-west of the Arab Republic of Egypt along the Mediterranean Sea, from the most popular activities in the governorate are: grazing and planting some important crops such as Olive, wheat, watermelon, figs, dates and barleys. El Dabaa lies about 138 km from Marsa Matrouh. El Amide which was affiliated to El hammam (226 km from Marsa Matrouh) is a protected area has already been announced by UNESCO and the World Organization in 1981 reserve biosphere within the framework of rights and the Biosphere (MAB) program it contains many wild animals in addition to large numbers of reptiles. El Amide is famous for the quality of herbal products and some fruits including taste delicious figs. The mean annual temperature is 21°C. Eight specimens were collected from this coastal area (500 meter near the sea) (Fig.1a)

Source: (IDSC) Matrouh Governorate

Fig. 1a. Map showing location of coastal *Psammophish schokari* captured from El Dabaha and El Hamam (Matrouh governorate) represented by blue stars

El Maghara mountain area (Gebel Maghara), EL Arish (North Sinai governorate)

Gebel Maghara itself is one of several domes which characterize north-central Sinai. It lies about 60 km to the southwest of El Arish. The area has an elevation rate, ranging from 650-776 meter. El Maghara region is of interest to many authors who have conducted studies dealing with the area's geology, hydrogeology, and geophysical features (Khaled, 2000). This area is characterized by an arid climate; hot and dry in summer, cold with rare rainfall in winter. The mean annual temperature is 20°C and the relative humidity is 47%. (Migahid *et al*., 1959). Eight specimens were collected from desert mountain area (Fig.1b).

Source: Google map 2013.

Fig. 1b. Map showing location of desert *Psammophish schokari* captured from Gebel Maghara EL Arish (North Sinai governorate) represented by blue arrow

Experimental design

A total number of 16 adults snakes *Psammophis shokari* (8 for each species) were collected between the end of May and the beginning of June. Description, habitat and feeding of *Psammophis shokari* were recorded in our previous study (Hussien and Hussein, 2013). These snakes were hunted during the early morning and transported to the lab in Cairo where they were sacrificed by (cutting the head by scalpel). The snakes were dissected (Fig.3a); then liver; skeletal muscles and skin tissues were removed and examined:

- Histologically by using Haematoxylin and Eosin (H&E).
- Histochemically by using two types of stains:

1) Periodic acid Schiff's (PAS) for detection of glycogen in the liver, the skeletal muscle and the skin.
2) Sudan black (B) for the identification of lipids in the liver and the skeletal muscle.

- Liver, skeletal muscles and skin were used for biochemical determination of glycogen and lipids concentrations.

Histological studies

Microscopic Examination

The snakes were sacrificed; small slices of the liver, skeletal muscle and skin were fixed in 10% formalin. The specimens were dehydrated in ascending grades of ethanol, cleared in xylene and processed for the preparation of 6 µm paraffin sections. Sections for histological examination were stained by using Haematoxylin and Eosin, where the cytoplasm stained pink and the nucleus stained purple (Bancroft *et al*., 2008).

Histochemical studies

Periodic acid-Schiff (PAS) staining for glycogen detection:

The form of carbohydrates in liver and skeletal muscles is mainly glycogen, thus we used Periodic acid Schiff (PAS). The glycogen inclusions gave magenta color while the nuclei were stained blue (Bancroft and Gamble, 2002). After snake dissection, tissue (liver, muscle) were fixed in 4% phosphate-buffered paraformaldehyde. Paraffin sections (6 µm) of each tissue were done, then deparaffinized and hydrated. Tissue sections were oxidized in 1% periodic acid for 5 min, rinsed in dH_2O three times, treated with Schiff's reagent for 15 min, rinsed again in dH_2O, stained with Mayer's hematoxylin for 1 min, and finally rinsed in dH_2O (Drury and Wallington, 1980). Specimens were examined under light microscope (Jenaval microscope; Carl Zeiss, Germany) at 400X magnification and photographed.

Sudan black staining for lipids detection

Lipids may be demonstrated in frozen sections by Sudan black B (Bancroft and Gamble, 2002), fat appeared as black granules. In this technique frozen sections were rinsed in 70% ethanol, then stained for up to 2 hours in saturated Sudan black B in 70% ethanol. Specimens were examined under light microscope (Jenaval microscope; Carl Zeiss, Germany) at 400X magnification and photographed.

Biochemical analyses

Liver, skeletal muscles and skin were processed for examination of metabolic reserve contents (glycogen and total lipids).

Determination of glycogen content in tissue samples

The glycogen contents of all organs were determined using the anthrone reagent according to the method of Handel (1965). The glycogen content of desired tissue was calculated from the following equation.

$$\text{Glycogen content} = \frac{A_{sample}}{Wt_L} \text{ X } \frac{\text{Std .Conc}}{A_{standard}} \text{ X } 0.9 \text{ X}100 \text{ mg /100 mg}$$

Where, A_{Sample} and $A_{standard}$, are absorbencies of sample and standard glucose, respectively. (WtL) represents the weight of liver (mg), while Std. conc. is the concentration of standard glucose. The value 0.9 represents conversion factor that converts the glucose units into glycogen.

Determination of total lipids content in tissue samples

The method of Folch *et al*. (1957) was adopted to extract total lipids from different organs. The total lipids content of desired tissue was calculated using the following equation:

Total lipids = ($A_{sample} \div A_{standard}$) x $\text{Conc.}_{Standard}$ = (mg/dl)

Where, A_{Sample} and $A_{standard}$, are absorbencies of sample and standard; Conc. Standard is the concentration of standard.

Statistical analyses

The Student's t-test was used to determine the significance variation in amount of glycogen and lipids between the coastal and desert snakes groups.

RESULTS

P. schokari displays no sexual dimorphism in body size and shape, though males tend to have larger mean body traits. Both coastal and desert Schokari snakes have a rear-striped form (Fig. 2a and 2b). The color of its smooth and slim body may vary from olive, tan or beige according to the surrounding. They have a yellow or beige color band on the ventral side of the body lined with dark dots which differed in distribution and color from coastal to desert (Fig. 2c and 2d).

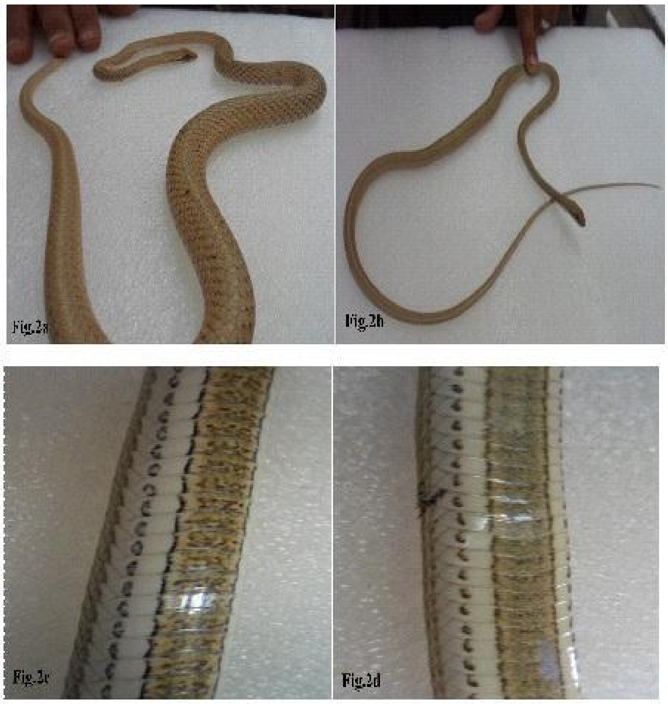

Fig. 2a and Fig. 2b. *Psammophis schokari* coastal and desert, respectively; Fig. 2c and Fig. 2d. Photographs showing the yellow or beige colored of ventral band lined with dark spots in *Psammophis schokari* coastal and desert, respectively

Histological studies

The present studies were carried out to show the histology of several tissues of the snake; *Psammophis schokari* inhabiting two different geographical habitats.

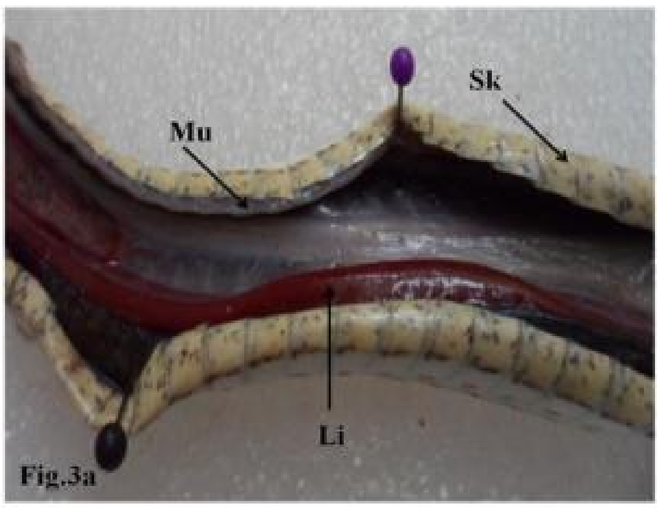

Fig. 3a. Photograph showing the liver (Li), the skeletal muscle (Mu) and the skin (Sk) of *Psammophis schokari*

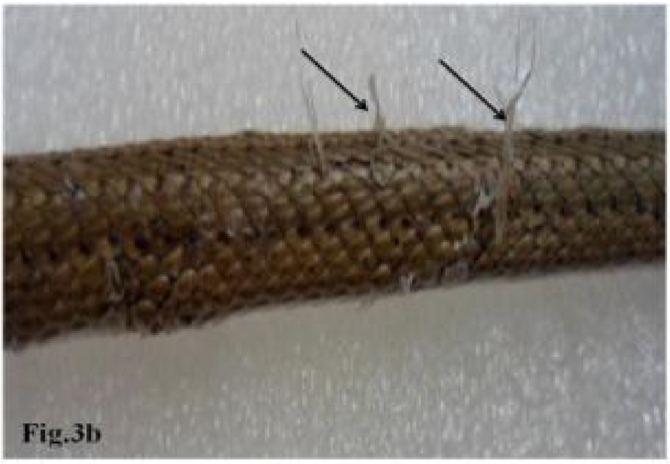

Fig. 3b. Photograph showing the skin of *Psammophis schokari* during ecdysis (arrows)

Liver

The liver of *Psammophis* consists of only one elongated lobe in correlation with their elongated body shape. Hepatocyte is the unit structure of the liver. Hepatocytes are large polyhedral cells with round nuclei with peripherally dispersed chromatin and prominent nucleoli (Fig. 5). The extensive cytoplasm of the hepatocytes has a variable appearance depending on the nutritional status of the snakes, it is otherwise strongly eosinophilic (Figs. 4 & 5). Fig. 4 shows changes in the histological structure of the liver tissue of coastal *Psammophis shockari*. The architecture of liver tissue was lost and the hepatocytes appeared degenerated. Large phagocytic cells with ovoid nuclei could be Küpffer cells, appeared among the endothelial cells, interstitial infiltration were scattered. In the liver tissue of desert *Psammophis shockari*; the hepatic cell boundaries were lost and their homogenous cytoplasm appeared non granulated and poorly stained. Moreover, their nuclei appeared scattered in the liver tissue, some densely stained nuclei appeared more or less accumulated around the portal vein. In addition, the liver tissue were filled with irregular unstained areas appeared as location of fat droplets within the cytoplasm (Fig. 5).

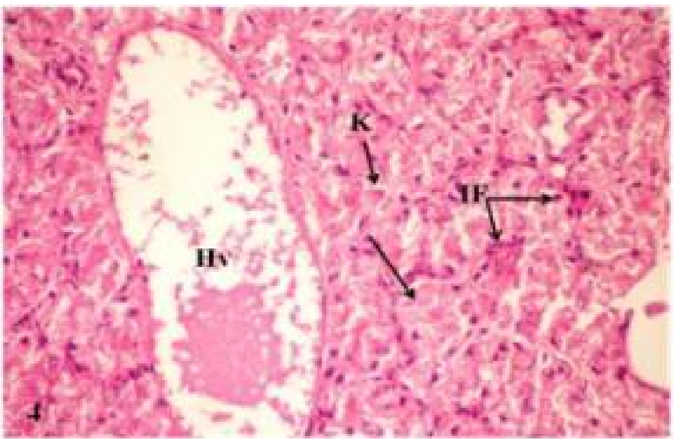

Fig. 4. Photomicrograph of the liver section of coastal *Psammophis shockari*, showing the loss of architecture of liver tissue, dilated hepatic vein (Hv) with degenerated hepatocytes (black arrow), Küpffer cells (k) and interstitial infiltration (IF). H & E. X400

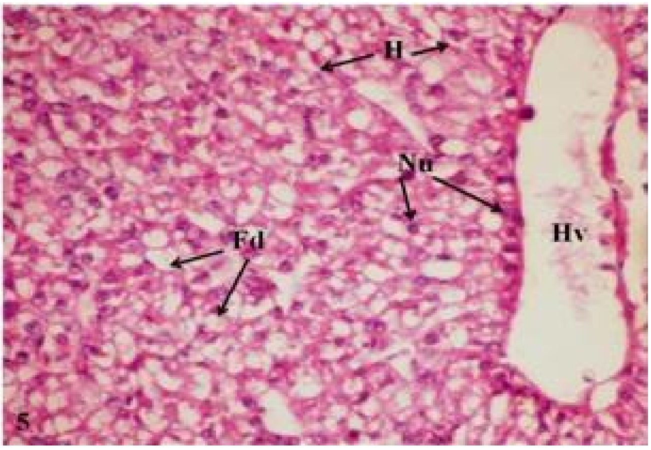

Fig. 5. Photomicrograph of the liver section of desert *Psammophis shockari* showing hepatic cells (H), the nuclei with prominent nucleoli (Nu) appeared scattered around the hepatic vein (Hv), the loss of the boundaries of hepatic cells, the deposition of fat droplets (Fd) with different sizes. H & E. X400

Skeletal muscle

The skeletal muscle fibres of coastal and desert *Psammophis shockari* did not show any histological variations.

Fig. 6. Photomicrograph of the skeletal muscle of coastal *Psammophis shockari* showing several skeletal muscle fibers (Mf) arranged in parallel and the skeletal muscle nuclei (N). H & E. X400

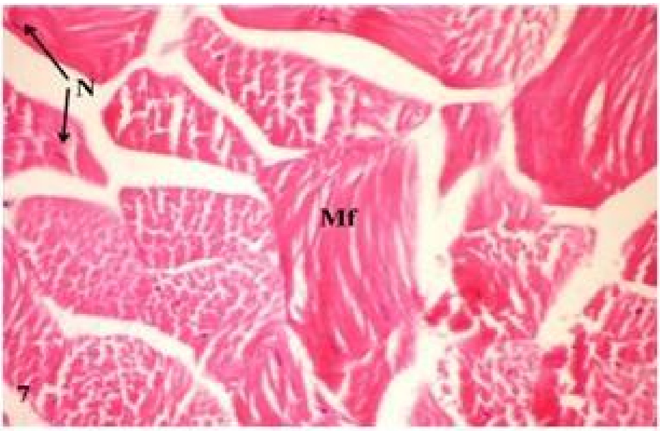

Fig. 7. Photomicrograph of the skeletal muscle of desert *Psammophis shockari* showing several polyhedral skeletal muscle fibers (Mf) with their peripheral nuclei (N). H & E. X400

In which the skeletal muscle of snakes is composed of extremely elongated multinucleated contractile cells (muscle fibres). The individual skeletal muscle fibres (mf) are grouped together into elongated bundles called fasciculi. They are arranged in parallel, with numerous flattened nuclei (N) located at regular intervals just beneath the sarcolemma as shown in Fig.6. The skeletal muscle fibres appeared polyhedral in a transverse section and their nuclei (N) appeared in peripheral location (Fig.7).

Skin

Skin tissues of both studied snakes were relatively impermeable and integumentary, glands (scent glands associated with sexual activity) were few in number. The epidermis has no blood supply, but its innermost living cells obtain their nourishment by the diffusion of substances to and from the capillaries at the surface of the dermis directly beneath them. Ecdysis is a necessary process for growth in snakes. During ecdysis the old layer of scales shed as a whole and replaced by the new layer of scales.

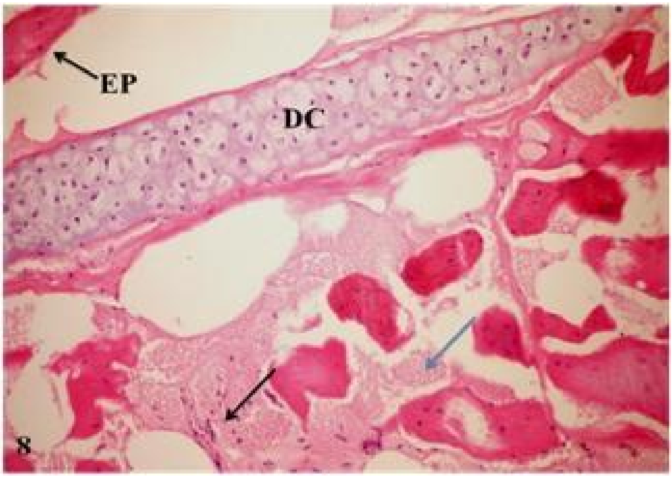

Fig. 8. Photomicrograph of the skin of coastal *Psammophis shockari* showing the epidermis (EP), dermal cartilage (DC) and the muscular layer with intermuscular edema (blue arrow) and inflammatory cells (black arrow). H&E X 400

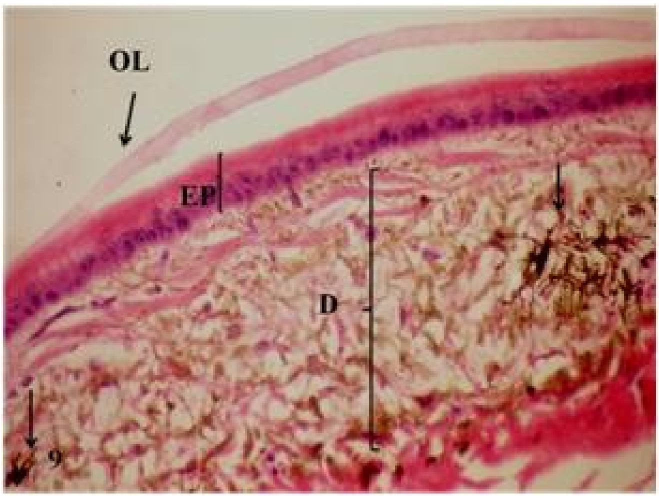

Fig. 9. Photomicrograph of the ecdysing skin of the desert *Psammophis shockari* showing the epidermal layer (EP), the accumulation of pigment cells (arrows) covering the dermal layers (D) and the shading skin (old layer) (OL). H & E. X400

Shedding started with the skin of the head, the snake then laterally crawled out of its old skin, the dermis, the deeper layer of connective tissue consisted of fibrous and reticulated layers and the pigment cells were mainly found in (Fig. 9). Skin Sections of coastal *Psammophis shockari* showed the epidermis, the dermal cartilage and the muscular layer with intermuscular edema and some inflammatory cells (Fig. 8). While the skin of desert *Psammophis shockari* showed ecdysis (Fig. 3b), thus the epidermal layers appeared well distinct (Fig. 9). The pigment cells were distributed densely as a complete net covering the dermal layers.

Histochemical studies

These studies were carried out on *Psammophis schokari* inhabiting two different geographical habitats to show variation in glycogen and lipids content in the different studied tissues.

Tissue glycogen

Liver glycogen

The hepatic tissue of the coastal *Psammophis shockari* showed heavy accumulation of glycogen in the cytoplasm of the degenerated hepatocytes (Fig. 10).

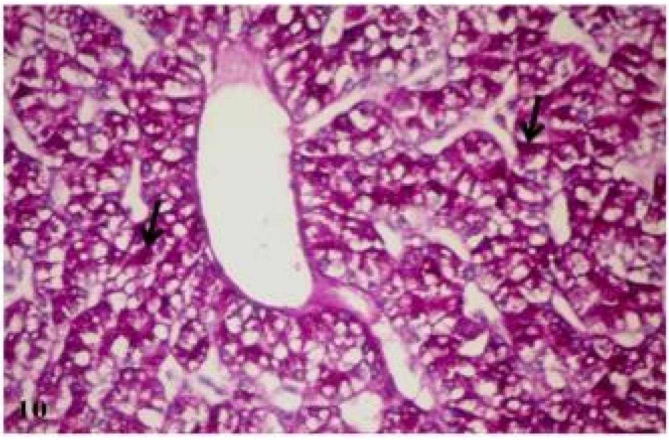

Fig. 10. Photomicrograph of the liver section of coastal *Psammophis shockari* showing heavy accumulation of glycogen in the cytoplasm of hepatocytes stained magenta (arrows), the nuclei were counterstained blue. PAS. X400

While the liver tissue of the desert *Psammophis shockari* (filled with irregular unstained areas) showed low content of glycogen inclusions, fine granules of glycogen remaining inside some hepatocytes, with accumulation of glycogen in the wall of blood vessel as shown in Fig. 11.

Skeletal muscle glycogen

Skeletal muscle contained minute quantities of glycogen diffused through the myofibrils in comparison with liver glycogen. Regardless to the pattern of glycogen distribution in the skeletal muscles, the difference between coastal and desert *Psammophis* was unexceptional. The section in skeletal muscle of coastal *Psammophis shockari* showed moderate amount of glycogen contents (Fig. 12).

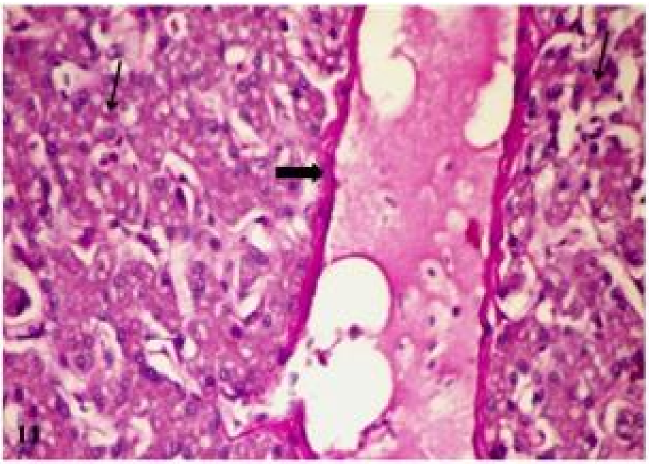

Fig. 11. Photomicrograph of the liver section of desert *Psammophis shockari* showing the hepatic cells exhibited weak PAS reaction, fine granules of glycogen remaining inside some hepatocytes (arrows), accumulation of glycogen in the wall of blood vessel (thick arrow). PAS. X400

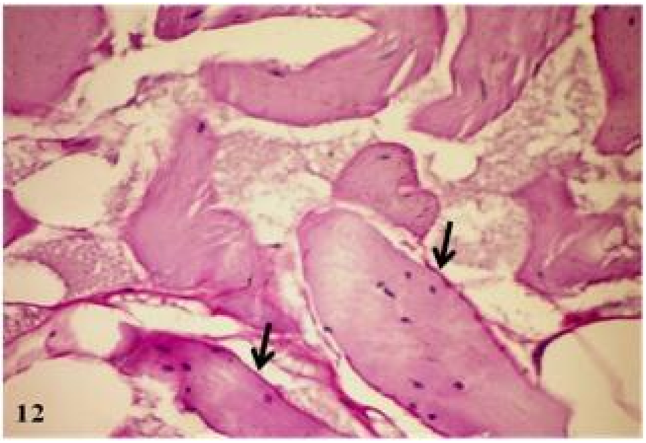

Fig. 12. Photomicrograph of the skeletal muscle of coastal *Psammophis shockari* showing the glycogen content appearing on the periphery of the muscle fibers (arrows). PAS. X400

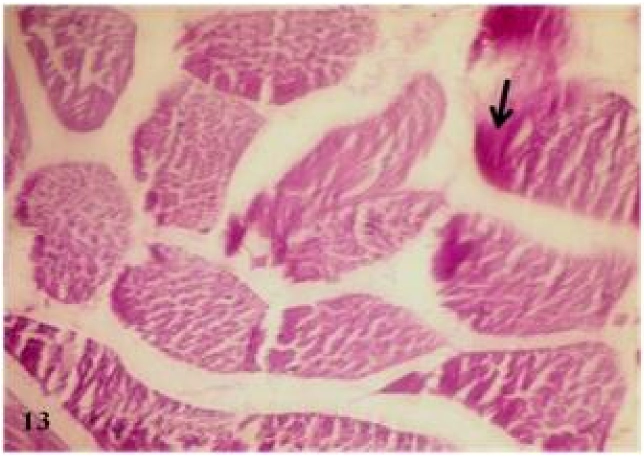

Fig. 13. Photomicrograph of the skeletal muscle of desert *Psammophis shockari* showing mild glycogen inclusions in the muscle fibers (arrow). PAS. X400

The glycogen content appeared more accumulated in the peripheral muscle fibres than central ones. Whereas the skeletal muscle of the desert *Psammophis shockari* showed mild glycogen inclusions, which appeared at the tissue periphery as shown in Fig. 13.

Skin glycogen

The skin of coastal *Psammophis shockari* revealed PAS reactivity due to high accumulation of glycogen in the dermal cartilage layer (Fig. 14). While the skin of desert *Psammophis shockari* (where ecdysis occurred) showed mild glycogen accumulation in the dermal and muscular layer appeared as pink colored granules as shown in Fig. 15.

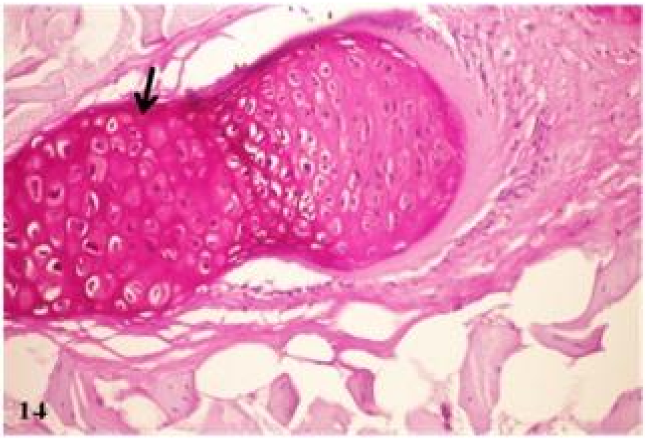

Fig. 14. Photomicrograph of the skin of coastal *Psammophis shockari* showing accumulation of glycogen in the dermal cartilage layer (arrow). PAS. X400

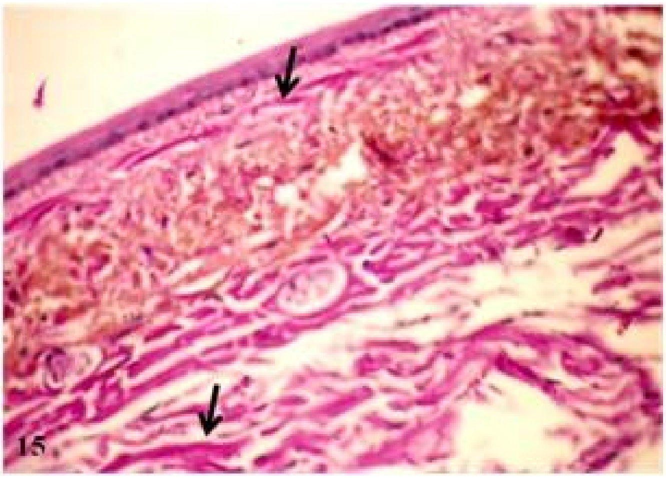

Fig. 15. Photomicrograph of the skin of desert *Psammophis shockari* showing mild glycogen accumulation in the dermal and muscular layer (arrows). PAS. X400

Tissue Lipids

Liver lipids

The lipids exhibited different forms in the liver of snakes, in some hepatocytes the lipids precipitated along the hepatic meshwork in lumps, in others; they occurred as amorphous or fine granules stained black. The liver section of coastal *Psammophis shockari* showed moderate content of lipids appeared scattered in the hepatic cells (Fig. 16).

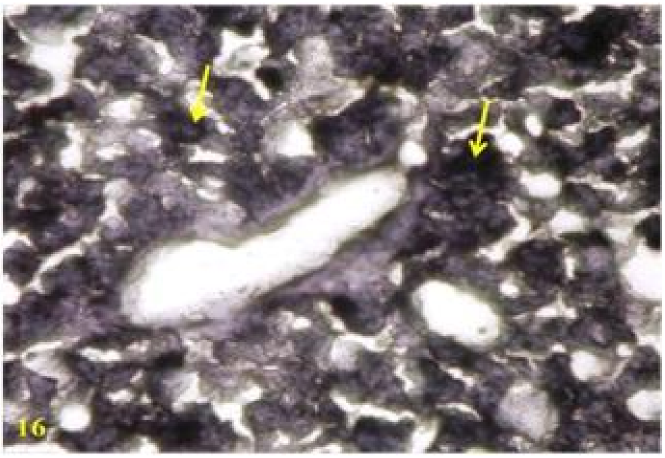

Fig. 16. Photomicrograph of the liver section of coastal *Psammophis shockari* showing moderate fat accumulation in hepatocytes (arrows). Sudan black. X400

Similar to the coastal *Psammophis* snakes the lipid content in the liver of the desert *Psammophis shockari* showed also mild fat accumulation (Fig.17). Some fat soluble droplets appeared as empty vacuoles in both coastal and desert *Psammophis* (Figs. 16&17).

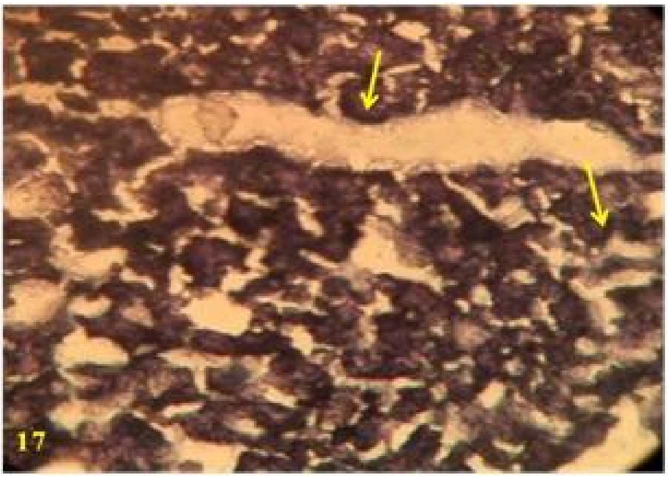

Fig. 17. Photomicrograph of the liver section of desert *Psammophis shockari* showing mild fat accumulation (arrows). Sudan black. X400

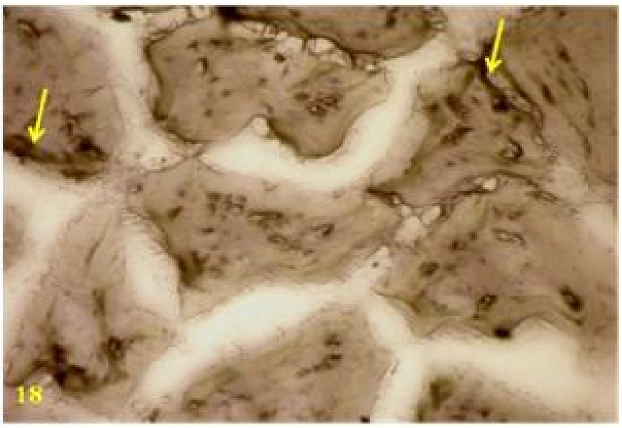

Fig. 18. Photomicrograph of the skeletal muscle of coastal *Psammophis shockari* showing moderate lipid inclusions deposited in the muscle fibers (arrows). Sudan black. X400

Skeletal muscle lipids

The lipids content in skeletal muscles of both coastal and desert *Psammophis* were nearly similar. The liver of these two snakes contained more lipids than the skeletal muscles. The skeletal muscle of coastal *Psammophis shockari* showed moderate lipid inclusions deposited to the periphery of the skeletal muscle fibers (Fig.18). While the skeletal muscle of desert *Psammophis shockari* showed mild lipids content appeared as a dark line surrounding the muscle fiber as shown in Fig.19.

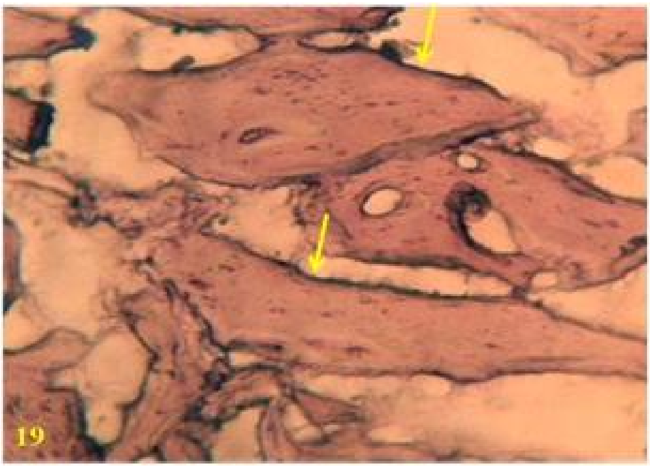

Fig. 19. Photomicrograph of the skeletal muscle of desert *Psammophis shockari* showing Lipids appeared as a dark line surrounding the muscle fiber (arrows). Sudan black. X400

Biochemical analyses

The previous histological and histochemical investigations were confirmed by the biochemical changes, which reflect immediate environmental changes. The sequence of the studied parameters was stated on the same sequence as the histochemical study. Based on glycogen content analysis (Fig. 20a), it was found that the liver, the skeletal muscles and the skin of *Psammophis shockari* inhabited coastal area (El Dabaa and El Amid); contained more glycogen than *Psammophis shockari* inhabited desert mountain area (El maghara mountain area). While the total lipid content analysis (Fig.20b), showed slight difference in fat content in liver and skin of both coastal and desert *Psammophis shockari*.

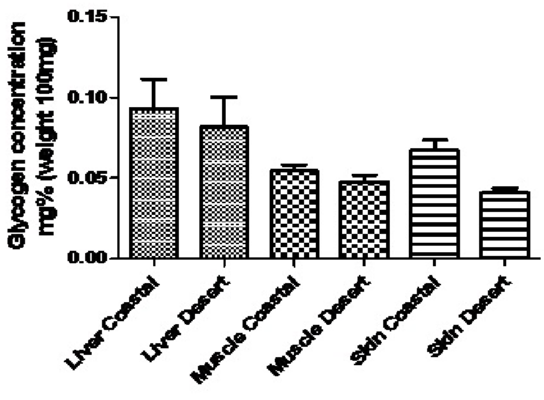

Fig. 20a. The glycogen content in the liver, skeletal muscle and skin of coastal and desert snake *Psammophis shockari*

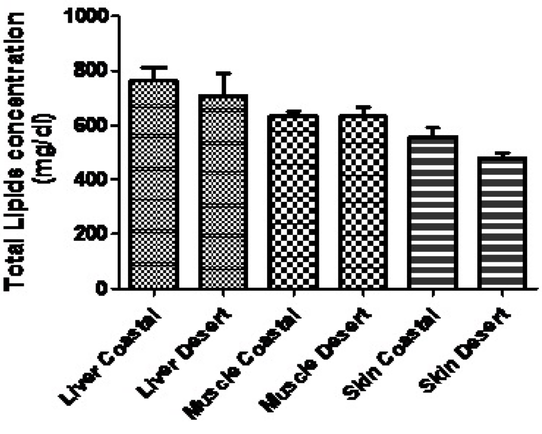

Fig. 20b. The total lipids content in the liver, skeletal muscle and skin of coastal and desert snake *Psammophis shockari*

DISCUSSION

Psammophis species are often the most common and conspicuous snakes throughout their ranges in Africa and Palearctic Asia (Jongbloed, 2000; Kelly *et al*., 2008). In our previous study (Hussien and Hussein, 2013); we reported that, there is a few morphological variations present between coastal and desert *P. schokari*

Histological studies

The histological changes occurred in the liver tissue of both studied snakes were highly related to the amount of the glycogen and total lipids. The liver acts as an intermediate site for the storage and biochemical processing of lipids before they were ultimately stored in the perivisceral adipose tissue (Starck and Beese, 2002). The present histological study of the liver tissue showed some changes in the architecture of the coastal and desert *Psammophis shockari*, these changes could be in correlation with the changes in metabolic concentration (glycogen and lipids) and the arousal of the snake which undergone sudden changes in the environmental climate as also demonstrated by (Hussein, 2007).

Also the liver tissue of the desert snake were filled with irregular unstained areas appeared as location of fat droplets within the cytoplasm, Starck and Beese (2002) stated that hepatocytes size also increased with the incorporation of lipid droplets after feeding. No histological change occurred in the architecture of all muscular tissues of both snakes. This was in agreement with Starck and Beese (2002), they stated that changes in the tissues of garter snakes (*Thamnophis sirtalis parietalis*) were specific and restricted to those tissue components that contributed to the specific function. The present study revealed that the skin of both snakes appeared in the non-striped morphology, the skin of both snakes was relatively impermeable and heavily pigmented. Skin pigments acted as thermoregulators and helped in protection from enemies and bright illumination. This was in agreement with the observation of several authors (Akef *et al*., 1995; Hussein, 2000; Hussein, 2007). In general, the amount of pigments in the skin of the desert *Psammophis* was greater than that of coastal. This could be explained on the fact that the desert snake was more subjected to the sunlight than coastal snake.

Snakes undergo cyclical shedding of the entire epidermis (ecdysis or moulting) to allow the skin to follow seasonal periods of rapid body growth or to restore skin functions such as gas exchange (Maderson *et al*., 1998; Lillywhite, 2006). The present study shows that both coastal and desert snakes shed their skin, the frequency of shedding depends upon many factors, such as age, environmental temperature, amount of food, activity level, reproductive status and humidity. This was in agreement with the observations of Woerpel and Rosskopf (1988) who stated that good nutrition and proper humidity enhanced ecydsis and the shedding process preceded mating and birth giving. Older snake may shed its skin only once or twice a year, but a younger, still-growing snake, may shed up to four times a year. Newborn snakes undergo ecdysis within a short period following birth or hatching (Greene *et al*., 2002; Tu *et al*., 2002). In the present work we recorded several histochemical and biochemical variations occurred in both coastal and desert snake's tissue. Among these of great relevance was variation in glycogen and total lipids content. The energy could be stored in the form of fat and glycogen (Hussein, 2007). Furthermore, the data presented here demonstrated that variation in body reserves has been related to the amount of food available in the environment. The present result was in agreement with other previous studies (Derickson, 1976; Forsman and Lindell, 1991; Shine and Madsen, 1997; Madsen and Shine, 1998; 1999; Lourdais *et al*., 2002).

Glycogen

Glycogen is present in numerous cell types in different tissues and is also stored in specialized glycogen cells, e.g. hepatic cells (Fenoglio *et al*., 1992). Studies performed on the coastal and desert *Psammophis shockari* indicated that the storage of glycogen was not uniform in all the studied tissues. This was in agreement with the observations of Hussein (2007). The present histochemical study supported by the biochemical study, showed accumulation of glycogen in the cytoplasm of the degenerated hepatocytes of the coastal *P. shockari* in comparison with that of the desert *P. shockari* which showed low content of glycogen inclusions. This result may be attributed to the direct food intake, moderate climate and much more annual rainfall and variety of vegetations in the coastal area as demonstrated by (Dugan and Hayes, 2012) who worked on Rattlesnakes. The hepatocytes boundaries of the coastal and desert *Psammophis shockari* were completely lost may be due to the accumulation of glycogen as demonstrated by (Starck and Beese, 2002) or the change in the environmental factors which could affect the reptiles' phenotype (Bonnet *et al*., 2001). Skeletal muscle of both studied snakes contained smaller amount of glycogen than liver. The present biochemical studies confirmed by the histochemical results revealed that both coastal and desert *P. shockari* showed moderate amount of glycogen with unremarkable difference in glycogen content. Kostaropoulos and Loumbourdis (2002) found that stored skeletal muscle glycogen could be used as readily available source of energy enabling the activation of the animal soon after emergence, in search of food and predator avoidance. The present histochemical and biochemical study dealing with the amount of glycogen in the skin showed that the skin of coastal *P. shockari* showed more glycogen content than desert snake. Hong *et al*. (1968) observed that there was a correlation between the glycogen stored in skin and seasonal variation.

Lipids

Lipids are an important energy source for reptiles during all seasons (Costanzo, 1985). The present histochemical study supported by the biochemical study, showed the little difference between total lipid content in the liver section of coastal and desert *Psammophis shockari*. The liver sections of both snakes showed the places of fat droplets exhausted appeared in the present histochemical sections as empty vacuoles. The use of stored total lipids may be exclusively for reproduction as demonstrated in snakes in previous studies (Saint Girons and Kramer, 1963; Wharton, 1966; Gibbons, 1972; Hussein, 2007). The amount of lipids content in the liver of both snakes was nearly more than that in the skeletal muscle. Santos and Liorente (2004) stated that the lipids content in the skeletal muscles of viperine snake *(Natrix maura)* represented a small part of the overall lipids content. Moreover, Graves *et al*. (1991) reported that fresh lipids would be deposited on the surface of the new epidermal generation after each shedding cycle. They reported also that skin total lipids appeared to be a source of pheromones. The histochemical observation confirmed these findings, that lipid inclusions deposited to the periphery of the skeletal muscle fibers have nearly similar amount in coastal and desert *Psammophis.* This was in agreement with the observations of Santos and Liorente (2004), they reported that the lipids stored in skeletal muscle turned out to be a supplementary energetic resource to fuel reproductive effort. The total lipids content in the skin was studied biochemicaly only and showed slight difference between coastal and desert *Psammophis shockari*. In animals, fat was often deposited subcutaneously and protected against extreme environmental conditions particularly at lower temperatures (Tanaka, 1995).

Conclusion

In this context, we concluded that the variation in coloration between both snakes is an adaptation to the particular environment that individual specimens occupy and the variation in histology and body reserves has been related to the amount of food available in the environment. Further contributions from genetics, morphology, physiology, ecology and evolutionary biology will be required to declare further similarity and dissimilarity between *Psammophis schokari* inhabiting different geographical habitats.

Conflict of interest

The author alone is responsible for the content and writing of this article.

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REFERENCES

Abdel-Raheem, K., Okasha, S., El-Deib, S., Shaalan, S., El-Daly, E. 1988. Hibernation in reptiles: 3-Carbohydrate metabolism in (*Eryx colubrinus* and *Eumeces schneideri*). *J. Therm. Biol*. 14: 133-137.

Akef, M. S. A., Abdel-Mageid, S. S. and Tawfik, A. A. 1995. Seasonal comparison of carbohydrate localization in certain tissues and organs of two sympatric anuran species (*Bufo regularis* and *Bufo kassasii*) (Bufonidae, Amphibia). *J. Egypt Ger. Soc. Zool*. 18: 201-218.

Ananjeva, N. B., Orlov, N. L., Khalikov, R. G., Darevsky, I. S. and Barabanov, A. 2006. The Reptiles of Northern Eurasia: Taxonomic Diversity, Distribution, Conservation Status. Pensoft Publishers, Sofia, Bulgaria.

Baha el Din, S. *Psammophis schokari* (Schokari sand snake) 1998. *Diet Herpetol. Rev*. 29: 104.

Bancroft, J. D. and Gamble, M. Theory and practice of histological techniques. Chapter lipids: Jones, M. L., Mucins, C. and Tolly, B. A 2002. 5th edition Churchill Livingstone, London, Edinburgh., New York, Philadelphia, St Louis Sydney, Toronto.

Bancroft, J. D., Stevens, A. and Turner, D. R. 2008. Theory and practice of histological techniques: Churchill Livingstone New York.

Bartlett, P. Winter energy requirements of Sceloporus occidentalis in the Mojave desert 1975. *Comp. Biochem. Physiol*. 55: 159–181.

Bonnet, X., Shine, R., Naulleau, G. and Thiburce, C. 2001. Plastic vipers: influence of food intake on the size and shape of Gaboon vipers (*Bitis gaponica*). *J. Zool. Lond*. 255: 341-351.

Costanzo, J. P. 1985. The bioenergetics of hibernation in the eastern garter snake (*Thamnophis sirtalis*). *Physiol. Zool*. 58: 682-692.

De Haan, C. C. 2003. Extrabuccal infralabial secretion outlets in Dromophis, Mimophis, and Psammophis species (Serpentes, Colubridae, Psammophiini). A probable substitute for ‘‘self rubbing’’ and cloacal scent gland functions, and a cue for a taxonomic account. Comptes. *Rendus. Biologies*. 36: 275–286.

Derickson, W. K. 1976. Lipid storage and utilization in reptiles. *Am. Zool*. 16: 711-723.

Drury, R. A. B. and Wallington, E. A. 1980. Carleton’s Histological Technique. 5th ed. Oxford, UK, Oxford University Press.

Dugan, E. A. and Hayes, W. K. 2012. Diet and Feeding Ecology of the Red Diamond Rattlesnake, Crotalus ruber (Serpentes: Viperidae) *Herpetologica*. 68(2): 203-217.

Fenoglio, C., Bernocchi, G. and Barni, S. 1992. Frog hepatocyte modifications induced by seasonal variations: A morphological and cytochemical study. *Tissue Cell*. 24: 17-29.

Folch, J., Lees, M. and Stanley, G. H. S. 1957. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem*. 226: 497-509.

Forsman, A. and Lindell, L. E. 1991. Trade – off between growth and energy storage in male *Vipera berus* (L.) under different prey densities. *Funct. Ecol.* 5: 717-723.

Gaffney, F. G. and Fitzpatrick, L. C. 1973. Energetics and lipid cycles in the lizard; Cnemidophorus tigris. *Copeia*. 446 – 452.

Gibbons, J. W. 1972. Reproduction, growth and sexual dimorphism in the canebrake rattlesnake (*Crotalus horidus atricaudatus*). Copeia. 222-226.

Graves, B. M., Halpern, M. and Friesen, J. L. 1991. Snake aggregation Pheromones: source and chemosensory mediation in Western ribbon snakes (*Thamnophis proximus*). *J. Comp. Psychol*. 105: 140-144.

Greene, H. W., May, P. G., Hardy, D. L., Sr Sciturro, J. M. and Ferrell, T. M. 2002. Parental care by vipers. In Biology of the Vipers (ed. Schuett G.W., Höggren M., Douglas M.E. & Greene H.W.), 179-206. Eagle Mountain (UT): *Eagle Mountain Publishing*.

Gregory, P. T. 1982. Reptilian hibernation. In: Biology of the Reptilia. Physiological ecology (Edited by Gans, C, Pough, FH.), 13: 53-154.

Handel, E. V. 1965. Estimation of glycogen in small amounts of tissue. *Analyst. Biochem*. 11: 256-265.

Handley, D., Cunningham, P. L. 2000. Sand Snake *Psammophis schokari schokari* (Forskål 1775): an arboreal hunter.10:2 (Autumn / winter 2000). 27-28.

Hellyer, P. and Aspinall, S. 2005. The Emirates: A Natural History. Trident Press Limited, London.

Hong, S. K., Park, C. S., Park, Y. S. and Kim, J. K. 1968. Seasonal changes of antidiuretic hormone action on sodium transport across frog skin. *Am. J. Physiol*. 215: 439-443.

Hussein, R. A. 2000. Cyclic histochemical changes in energy metabolism and mucopolysaccharides in tissues of some geckos from naturally occurring habitats. M.Sc. Thesis, Zoology Department, Faculty of Science, Cairo University.

Hussein, R. A.(2007. Histological, histochemical and biochemical changes in energy reserved of certain tissues of the *Psammophis sibilans* and *Natrix tessellata*: Seasonal profiles of fuel reserved in a wild- caught population. Ph.D. Thesis, Zoology Department, Faculty of Science, Cairo University.

Hussien, N. A. and Hussein, R. M. 2013. Morphological and Molecular Polymorphism of Snake *Psammophis schokari* (Colubridae) in the Desert-Mountain and Coastal Areas of Egypt. *World Appl. Sci. J.* 27(8): 996-1004.

Jongbloed, M. 2000. Wild About Reptiles – Field Guide to Reptiles and Amphibians of the UAE. London, Barkers Trident Communications.

Kelly, C. M. R., Barker, N. P., Villet, M. H., Broadley, D. H. and Branch, W. R. 2008. The snake family Psammophiidae (Reptilia: Serpentes): phylogenetics and species delimitation in the African sand snakes (Psammophis Boie, 1825) and allied genera. *Mol. Phylogenet. Evol*. 47: 1045–1060.

Khaled, A. A. 2000. Geophysical study to determine the impact of structural elements on the groundwater occurrence in El-Maghara area, North Sinai, Egypt. PhD thesis, Faculty of Science, Al Azhar University, Cairo.

Kostaropoulos, I. and Loumbourdis, N. S. 2002. Seasonal alterations of glycogen, lactate and total protein concentration in the muscles of (*Agama stellio stellio*) (Sauria Agamidae). *Amph. Rept*. 23: 523-528.

Lillywhite, H. B. 2006. Review Water relations of tetrapod integument. *J. Exp. Biol*. 209: 202-226.

Lourdais, O., Bonnet, X. and Shine, R. 2002. Capital breeding and reproductive effort in a variable environmental, a longitudinal study of a viviparous snake. *J. Anim. Ecol*. 71: 470-479.

Luiselli, L., Filippi, E., Capula, M. 2005. Geographic variation in diet composition of the grass snake (*Natrix natrix*) along the mainland and an island of Italy: the effects of habitat type and interference with potential competitors. *Herpetol J* 15: 221–230.

Maderson, P. F. A., Rabinovitz, T., Tandler, B. and Alibardi, L. 1998. Ultrastructural contributions to an understanding of the cellular mechanisms in lizards skin shedding with comments on the function and evolution of unique lepidosaurian phenomenon. *J. Morphol*. 236: 1-24.

Madsen, T. and Shine, R. 1998. Quantity or quality? Determinants of maternal reproductive success in tropical pythons (Liasis fuscus). *Proc. R. Soc. Lond*. B. 265: 1521-1525.

Madsen, T. and Shine, R. 1999. The adjustment of reproductive threshold to prey abundance in a capital breeder. *J. Anim. Ecol*. 68: 571-580.

Migahid, A. M., El-Shafei, A. M. and Abdel-Rahman, A. A. 1959. Ecological observation in the western and southern Sinai. *Bull Soc. Geogr. Egypt* 32: 105-165.

Patterson, J. W., Davies, P. M. C., Veasey, D. A., Griffiths, J. R. 1978. The influence of season and glycogen levels in the lizard (lacerta vivipara). *Comp. Biochem. Physiol*. 60: 491–493.

Pough, F. H. and Groves, J. D. 1983. Specialization in the body form and food habits of snakes. *Am. Zool*. 23: 443-454.

Rodríguez-Robles, J. A. 2002. Feeding ecology of North American gopher snakes (*Pituophis catenifer*, Colubridae). *Biol. Linn. Soc*. 77: 165- 183.

Saint Girons, H. and Kramer, E. 1963. Le cycle sexual chez (Vipera berus) (L.) en Montagne. *Revue Suisse Zool*. 70: 191-211.

Santos, X. and Liorente, G. A. 2004. Lipid dynamics in the viperine snake (*Natrix maura*), from the Ebro Delta (NE Spain). Oikos. 105: 132-140.

Shine, R. and Madsen, T. 1997. Prey abundance and predator reproduction: rats and pythons on a tropical Australian floodplain. Ecol. 78: 1078-1086.

Spawls, S., Howell, K., Drewes, R. and Ashe, J. 2002. A Field Guide to the Reptiles of East Africa. Academic Press, San Diego.

Starck, J. M. and Beese, K. 2002. Structural flexibility of the small intestine and liver of garter snakes in response to feeding and fasting. *J. Exp. Biol*. 205: 1377-1388.

Tanaka, K. 1995. Seasonal changes in glycogen and inositol/sorbitol contents of the house spider (*Achaearanea tepidariorum*) (Araneae: Theridiidae). *Comp. Biochem. Physiol*. 110: 539-545.

Tu, M. C., Lillywhite, H. B., Menon, J. G. and Menon, G. K. 2002. Postnatal ecdysis establishes the permeability barrier in snake skin: New insights into lipid barrier structures. *J. Exp. Biol*. 205: 3019-3030.

Wharton, C. H. 1966. Reproduction and growth in the cottonmouth (*Agkistrodon piscivorous*) Lacepede, of Cedar Keys, Florida. *Copeia*. 149-161.

Woerpel, R. W., Rosskopf, W. J. Jr 1988. Avian- Exotic Animal care guides. *American Veterinary Publication.*

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