

## Gene Over Expression of Glycerol-3-phosphate Dehydrogenase in Some Marine Algae of Egypt.

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**S**TUDIES focusing a survey or a comparison between freshwater and marine algae are relatively scarce. Some biochemical comparative study between three freshwater algae (*Spirogyra reinhardii*, *Cladophora glomerata* (green) and *Chara vulgaris* and six marine algae among them brown: *Sargassum dentifolium*, *Padina boryana*, *Dictyota dichotoma*; red: *Gelidium latifolium*, *Gracilaria dura* and green: *Enteromorpha intestinalis*) has been performed to study how marine algae can cope with salinity of seawater. Results obtained showed that fluorescence peaks were relatively uniform for the studied species within the same class but were different for different classes or divisions. The studied protein profile revealed the presence of three common protein bands (125, 15 and 8 kDa) only in the members of euhalopic algal group and finally the presence of one common protein band (240 kDa) only in all members of oligohalopic algae. The amount of proline showed irregular differences among the investigated marine and fresh algae. Mannitol was detected only in the members of brown algae. Moreover, the minerals (sodium and potassium) and glycerol contents of the marine algae have noticeable greater values than those of fresh algae. Glycerol-3-phosphate dehydrogenase (G3PDH) had a significant impact in the biosynthesis of glycerol. In the present study we explore the differential expression of (G3PDH) in marine algal group as compared to that of freshwater one. Results showed that, the expression level of (G3PDH) mRNA was significantly over expressed in marine algal group.

**Keywords:** Marine algae, Freshwater algae, Fluorescence emission spectra, SDS-PAGE or protein profile, Mannitol, Proline, Minerals content, Glycerol-3-phosphate dehydrogenase.

Algae are a group of chlorophyll containing, oxygen-producing, photosynthetic organisms which are genetically diverse and inhabit a wide range of environments (Peerapornpisal *et al.*, 2006). The characteristics of the organisms in each ecosystem relate directly to the conditions of the environment in that ecosystem. Algal cells are generally able to live within a certain range of enhanced salt concentrations or changing salinities since most probably all life originated in the oceans (Kirst, 1990 and Lionard *et al.*, 2005). When organisms become acclimated to a new range of conditions, they generally lose the ability to perform well under the previous conditions. In the course of evolution, this phenomenon has resulted in two nearly separate groups of organisms: freshwater and marine (Hurd *et al.*, 2014). In fact, the chemical composition of macro-algae varies considerably according to species and

habitats (Gosch *et al.*, 2012 and Jung *et al.*, 2013). Although a lot of studies were carried out on marine macro-algae (Morgan *et al.*, 1980; Castro-González *et al.*, 1996 and Shuuluka *et al.*, 2013), additional studies regarding the freshwater algae still needed to be carried out. Seaweeds are generally high in minerals contents due to their marine habitat. The mineral fraction of some seaweeds accounts for up to 36% of their dry matter (Burtin, 2003). Furthermore, studying of fluorescence emission spectra of chlorophyll may be a marker the algal environment (Topinka *et al.*, 1990)

Moreover, protein pattern might play a critical role in the adaptation of algae to their natural habitat. Some specific polypeptides might provide osmotic adjustment to the cells either by facilitating the accumulation of solutes or by providing certain metabolic alterations in the cell, which may be helpful in osmotic adjustment (Singh *et al.*, 1987 and Pareek *et al.* 1997). Algae can be counteracted different environmental stresses in their natural habitats (Lesser, 2006) through their contents from different highly compatible organic osmolytes to maintain osmotic pressure balance with the surrounding sea water (Iwamoto and Shiraiwa, 2005; Zubia *et al.*, 2008). For most, if not all compatible solutes, it is assumed that they exert a direct protective effect on biomolecules against the damage of high concentrations of inorganic ions (Bisson and Kirst, 1995; Klähn and Hagemann, 2011). In algae, the compatible solutes are restricted to four major classes of solutes: sugars and polyols, free amino acids and derivate, quaternary ammonium compounds and tertiary sulphonium compounds (Garza- Sánchez *et al.*, 2009).

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Proline, a compatible organic osmolyte, accumulates in some microalgae (Greenway and Setter, 1979; Ahmad and Hellebust, 1988; Kalinkina and Naumova, 1992 and Singh *et al.*, 1996) and macroalgae (Edwards *et al.*, 1987; Norziah and Ching, 2000) in response to hypersaline conditions. Proline accumulation is interpreted as providing a nontoxic osmolyte, a protectant of macromolecules and as a nitrogen-storage compound (Delauney and Verma, 1993; Szabados and Savoure, 2010). Polyols are important metabolites, because they exhibit multiple functions in metabolism; osmolytes, compatible solutes, rapidly available respiration substrates, antioxidant, etc. (Seckbach, 2007). Mannitol, one of the most widely occurring polyols, acts as a compatible solute and has multiple functions, including osmoregulation, storage, and regeneration of reducing power, and scavenging of active oxygen species (Yu *et al.*, 2003; Iwamoto and Shiraiwa, 2005). Glycerol also is an important compatible solute and represents the simplest and the champion among all organic compatible solutes since it demands the least energetic cost and is the most hydrophilic compound (Rai and Gaur, 2012). However under highly saline conditions,

glycerol is reported to accumulate to approximately 85% of the algal dry weight (Calvin and Taylor, 1989). Algae regularly face the adverse effects of salinity by adjustment of metabolism and gene expression for physiological adaptation.

Molecular and genomic analyses have shown that there are different transcriptional regulatory genes controlling various biological processes that trigger cell defenses (Dimova *et al.*, 2008). Over-expression of these genes could enhance the biosynthesis of complex defense system. It was suggested that the overexpression level of G3PDH gene in the diatom cell; *Phaeodactylum tricoratum* enhanced the synthesis of glycerol (Yao *et al.*, 2014). These may indicated that, algae exhibit a wide range of acclimation in response to induced salinity. However, studies focusing a survey or a comparison between freshwater and marine algae are relatively scarce. Moreover, the adaptation of marine algae to live in seawater has not been appreciated adequately by previous investigators. Therefore the objective of the current work is to obtain a comprehensive picture about the relative contribution of the algal groups in their natural habitats to glycerol-3-phosphate dehydrogenase (G3PDH) gene expression.

### Materials and Methods

#### *Sites of collection and the selected marine and freshwater algae*

Nine algal species were investigated; six of them (*Sargassum dentifolium*, *Padina boryana*, *Dictyota dichotoma*, *Gelidium latifolium*, *Gracilaria dura* and *Enteromorpha intestinalis*) collected from marine (euhalopic) habitats; and three (*Cladophora glomerata*, *Spirogyra reinhardii* and *Chara vulgaris*) from freshwater (oligohalopic) habitats (Table 1). Four sites (Alexandria, Hurghada, Fayed and El-Qanater) were selected for algal collection to cover both euhalopic and oligoalopic ecosystem respectively.

Almost all algal samples were hand-picked and immediately washed with the surrounding water to remove extraneous matters, sand particles and epiphytes as much as possible. Then, they kept in ice box containing frozen gel cold packs to maintain the low temperature and moisture during the journey and immediately transported to the laboratory. On arrival, the algal samples were thoroughly washed with tap water and finally with distilled water. After that, the samples were spread on blotting paper to remove excess water. Only one algal species *Chara vulgaris* (Linnaeus) was laboratory tap water culture in transparent glass tanks supplemented continuously with water. Finally, cleaned algal samples were divided into two groups prior to the chemical analysis. In first group, samples were preserved in freezer for fresh weight analysis, second group was shaded air-dried, cut into small pieces and grounded into fine powder using a dry grinder and preserved for dry weight analysis.

**TABLE 1. Sites of collection, and algal groups, species and divisions.**

No.	Algal group	Division	Algal species	Collection site
1	Marine group (Euhalopic)	Phaeophyta (Brown algae)	<i>Sargassum dentifolium</i> (Turner) C. Agardh	Red Sea (Hurgada)
2			<i>Padina boryana</i> (L.) Gaill.	
3			<i>Dictyota dichotoma</i> (hudson) Lamouroux.	
4		Rhodophyta (Red algae)	<i>Gelidium latifolium</i> (Grev.) Born. et. Thur.	Mediterranean Sea (Alexandria)
5			<i>Gracilaria dura</i> (C. Ag.) J. Agardh.	Suez Canal (Fayed)
6		Freshwater group (Oligohalopic)	Chlorophyta (Green algae)	<i>Enteromorpha intestinalis</i> (Linnaeus) Link
7	<i>Cladophora glomerata</i> (L.) Kütz			River Nile (El-Qanater)
8	Charophyta		<i>Spirogyra reinhardii</i>	Pool in Borg El-Arab
9			<i>Chara vulgaris</i> (Linnaeus)	Ain Musa (Sinai)

**Sampling**

Almost all algal samples were hand-picked and immediately washed with the surrounding water to remove extraneous matters, sand particles and epiphytes as much as possible. Then, they kept in ice box containing frozen gel cold packs to maintain the low temperature and moisture during the journey and immediately transported to the laboratory. On arrival, the algal samples were thoroughly washed with tap water and finally with distilled water. After that, the samples were spread on blotting paper to remove excess water. Only one algal species *Chara vulgaris* (Linnaeus) was laboratory tap water culture in transparent glass tanks supplemented continuously with water. Finally, cleaned algal samples were divided into two groups prior to the chemical analysis. In first group, samples were preserved in freezer for fresh weight analysis, second group was shaded air-dried, cut into small pieces and grounded into fine powder using a dry grinder and preserved for dry weight analysis.

*Algal identification*

Algal identification was based on morphological observations and/or sometimes on microscopical examination using microscope fitted with built-in digital camera. Samples were identified according to Knight and Park (1931),

Zinova (1967), Papenfuss (1968), Jaasund (1976), Fedarov (1977), Abbott and Dawson (1978), Cribb (1983) and Aleem (1993).

#### *Biochemical analysis of algae*

Room temperature fluorescence emission spectra of pigments were performed using total pigments extracted from algal samples using Perkin Elmer LS50B spectrofluorometer according to Middleton *et al.*, 1996. The determination, identification and characterization of different protein fractions were obtained using one-dimensional sodium dodecyl sulphate polyacrylamide gel electrophoresis. Polyacrylamide slab gel (12.5%) was prepared according to Laemmli (1970). The destained gel was analyzed by Gel Documentation System (GDS). Gel analysis was performed in a GDS model UVP's GDS 8000 from UVP Inc. (California 91786 USA), to analyze the band pattern, molecular weights and band percentages. Additionally, sodium, potassium, and calcium were estimated according to Ranganna (1977) using atomic absorption spectrophotometer (Perkin Elmer USA 3100).

Moreover, mannitol and free proline were determined according to the method described by Cameron *et al.* (1948) and Bates *et al.* (1973) respectively. Glycerol was extracted from algal tissues according to the method adopted by Kochert (1978). Moreover, glycerol content was estimated according to Lambt and Neish (1950). The name and sequence of the Oligonucleotide primers were reported according to the list of Hu *et al.* (2014). The fold change in G3PDH gene expression was calculated by normalizing with UBQ10 gene expression according to the following formula (Livak and Schmittgen, 2001)

#### *Statistic Analysis*

Data were subjected to an analysis of variance and the mean were compared using the least significant difference (LSD) test at the 0.05 level.

### **Results and Discussion**

The feasibility of distinguishing macro-algal classes by their fluorescence peaks were investigated using narrow-wave band light to excite groups of accessory pigments in brown, red, and green macro-algae and measuring fluorescence emission. Members of Phaeophyta, Rhodophyta and Chlorophyta had distinctive fluorescence emission spectra when samples were excited at 435 nm.

The results (Fig. 1, a-i) indicated that, the excitation of the studied algal extracts at 435 nm causes fluorescence emission spectra of different peaks around 550, 680 and 700 nm. Two Rhodophyta genera showed two main peaks; red chlorophyll fluorescence (RF) with emission around 700 nm and another larger peak around 550 nm (green fluorescence). These were more pronounced in comparison to the rest of the algal divisions. Excitation of Chlorophyta genera resulted in two peaks around 550 and 680 nm in both the freshwater (Fig. 1, a-c) and the marine algal group (Fig. 1, d-e). In Phaeophyta (Fig. 1, f-h), three peaks were recorded; two main peaks around 680 and 700 nm and one smaller peak

around 550 nm. The differences can be attributed to differences in accessory pigments between these divisions (Kancheva *et al.*, 2005).

Results suggested that the changes in protein pattern might play a critical role in the adaptation of either group to their natural habitat.

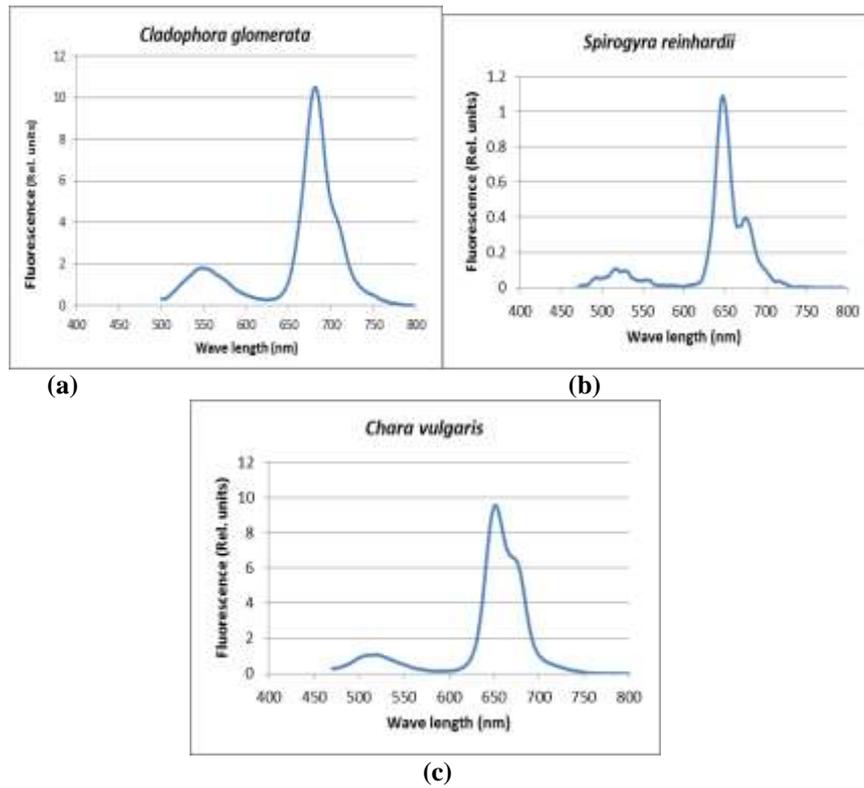
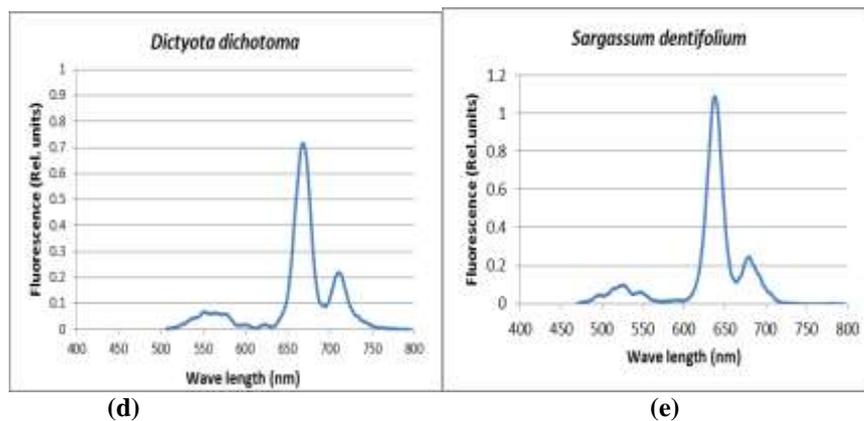
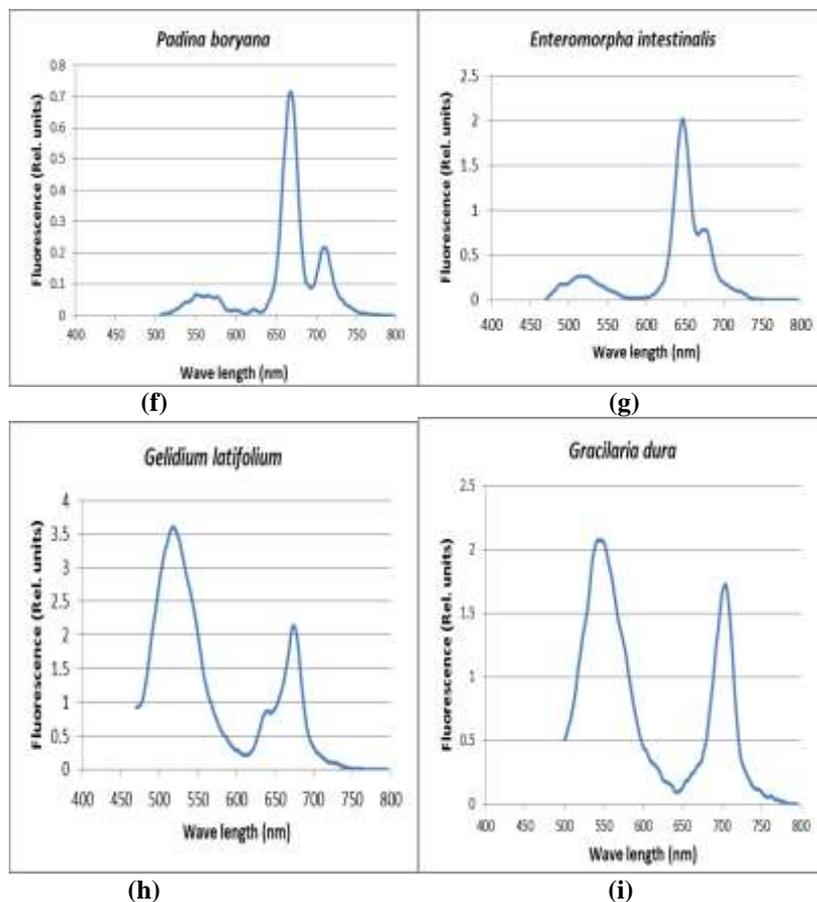


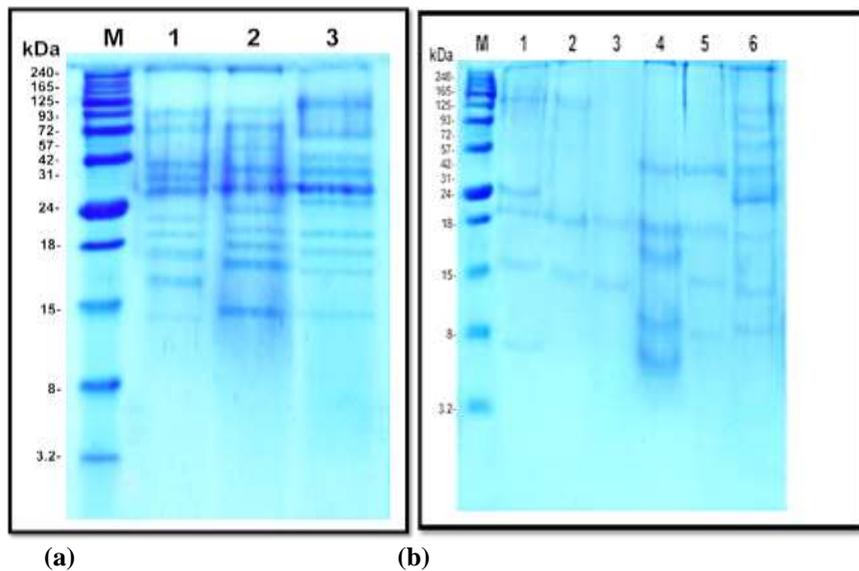
Figure (1, a-c): Emission spectra of the freshwater algae.  $E_x \lambda=435\text{nm}$





**Fig. (1, d-i): Emission spectra of the marine algae.  $E_x \lambda=435\text{nm}$**

The SDS-gel recorded the occurrence of three common protein bands (M.wt: 125, 15 and 8 kDa) only in the majority of the marine algal members which were absent from the members of freshwater group (Fig. 1, a – i). According to Pareek *et al.* (1997), it is proposed that some proteins that accumulate in response to salinity may be considered as adaptive proteins. The presence of the low molecular weight protein (8 kDa) in most of the marine algal species might explain the better performance of marine algae under saline conditions (Dunn, 1993). On the other hand, one specific protein band (M.wt: 240 kDa) was observed only in all members of the freshwater group which may be specific for this group. Also, the presence of certain characteristic protein banding pattern for each genus was noticed. Such results were consistent with the work carried out by Rouxel *et al.* (2001) and their proposal for species identification by SDS-PAGE of red algae.



**Fig. (2, a and b):** (a) Electrograph SDS-PAGE of total soluble proteins extracted from the freshwater algal taxa, (b) Electrograph SDS-PAGE of total soluble proteins extracted from the marine algal taxa (M: Marker).

It is clearly shown that, the mineral contents (Table 2) particularly sodium, potassium and calcium of the marine algal taxa had a noticeable greater value ( $P < 0.05$ ) than those belonging to the freshwater group. The maximum sodium level ( $P < 0.05$ ) was recorded in *Gracilaria dura* ( $30.44 \text{ mg.g}^{-1} \text{ DW}$ ) followed by *Enteromorpha intestinalis* ( $20.72 \text{ mg.g}^{-1} \text{ DW}$ ) and *Sargassum dentifolium* ( $16.04 \text{ mg.g}^{-1} \text{ DW}$ ). The highest ( $P < 0.05$ ) potassium value was recorded in *Sargassum dentifolium* ( $46.00 \text{ mg.g}^{-1} \text{ DW}$ ) followed by *Gracilaria dura* ( $45.24 \text{ mg.g}^{-1} \text{ DW}$ ). The greatest calcium level ( $P < 0.05$ ) was detected in *Sargassum. Dentifolium* ( $12.98 \text{ mg.g}^{-1} \text{ DW}$ ). All the previous mentioned algal taxa belonging to the marine group.

According to previous studies by Arasaki and Arasaki (1983) and Nisizawa (2002) marine algae are known to be high in mineral content. This may be due to their marine habitat and the diversity of the minerals they absorb is wide (MacArtain *et al.*, 2007). In addition, marine algae have the capacity to retain inorganic marine substances due to the characteristics of their cell surface polysaccharides (Rupérez, 2002; Bocanegra *et al.*, 2009).

**TABLE 2. Minerals, Mannitol, Proline, Glycerol and level of Glycerol-3- phosphate dehydrogenase (G3PDH) transcript in freshwater and marine algal groups**

Algal group	Algal Species	Minerals (mg.g <sup>-1</sup> dw)				Mannitol mg.g <sup>-1</sup> dw	Proline μg.g <sup>-1</sup> fw	Glycerol μM.g <sup>-1</sup> fw	Fold change 2 <sup>-ΔΔCT</sup>	Mean
		Sodium (Na <sup>+</sup> )	Potassium (K <sup>+</sup> )	Calcium (Ca <sup>+2</sup> )	Na / K					
Marine (Euthalopic) algae	<i>Sargassum dentifolium</i>	16.04 <sup>e</sup> ±0.32	46.00 <sup>a</sup> ±0.73	12.98 <sup>a</sup> ±0.25	0.35 <sup>e</sup> ±0.008	5.25 <sup>b</sup> ±0.05	2.04 <sup>d</sup> ±0.03	144.04 <sup>b</sup> ±1.2	55.70 <sup>c</sup> ±1.58	53.605
	<i>Padina boryana</i>	5.00 <sup>f</sup> ±0.04	15.40 <sup>d</sup> ±0.42	5.26 <sup>d</sup> ±0.03	0.32 <sup>e</sup> ±0.004	5.10 <sup>b</sup> ±0.05	2.25 <sup>d</sup> ±0.03	148.47 <sup>b</sup> ±2.2	61.82 <sup>b</sup> ±1.70	
	<i>Dictyota dichotoma</i>	5.60 <sup>e</sup> ±0.02	37.50 <sup>c</sup> ±0.51	7.84 <sup>c</sup> ±0.08	0.15 <sup>f</sup> ±0.004	5.80 <sup>a</sup> ±0.04	2.22 <sup>d</sup> ±0.04	238.51 <sup>a</sup> ±2.9	106.89 <sup>a</sup> ±3.06	
	<i>Gelidium latifolium</i>	9.96 <sup>d</sup> ±0.07	7.50 <sup>b</sup> ±0.30	3.21 <sup>f</sup> ±0.02	1.33 <sup>b</sup> ±0.016	-	12.58 <sup>a</sup> ±0.63	136.35 <sup>c</sup> ±3	46.52 <sup>d</sup> ±1.33	
	<i>Gracilaria durra</i>	30.44 <sup>a</sup> ±0.61	45.24 <sup>b</sup> ±0.82	2.64 <sup>g</sup> ±0.03	0.67 <sup>e</sup> ±0.007	-	3.47 <sup>c</sup> ±0.12	129.49 <sup>d</sup> ±0.9	39.39 <sup>e</sup> ±1.14	
	<i>Enteromorpha intestinalis</i>	20.72 <sup>b</sup> ±0.43	9.30 <sup>f</sup> ±0.21	4.52±0.04	2.23 <sup>a</sup> ±0.017	-	4.33 <sup>b</sup> ±0.22	52.23 <sup>f</sup> ±0.4	11.31 <sup>f</sup> ±0.33	
Freshwater (Oligohalopic) algae	<i>Cladophora glomerata</i>	2.20 <sup>g</sup> ±0.03	4.97 <sup>h</sup> ±0.18	2.48 <sup>g</sup> ±0.01	0.44 <sup>d</sup> ±0.016	-	2.24 <sup>d</sup> ±0.01	42.71 <sup>g</sup> ±0.3	2.77 <sup>g</sup> ±0.04	2.03
	<i>Spirogyra reinhardii</i>	0.98 <sup>h</sup> ±0.01	11.36 <sup>c</sup> ±0.33	0.87 <sup>i</sup> ±0.01	0.08 <sup>g</sup> ±0.002	-	0.63 <sup>e</sup> ±0.10	9.48 <sup>h</sup> ±0.05	1.00 <sup>g</sup> ±0.02	
	<i>Chara vulgaris</i>	0.15 <sup>i</sup> ±0	4.39 <sup>hi</sup> ±0.19	10.77 <sup>b</sup> ±0.15	0.03 <sup>h</sup> ±0.003	-	1.24 <sup>e</sup> ±0.04	40.88 <sup>g</sup> ±0.6	2.34 <sup>g</sup> ±0.06	

The minimum Na<sup>+</sup>/K<sup>+</sup> value was recorded in the oligohalopic *Spirogyra* spp. (0.03), whereas the marine *Enteromorpha intestinalis* had the highest value (2.23). Sodium and potassium play an important role in the electrolyte balance (Krishnaiah *et al.*, 2008). Osmoregulation in marine algae are mainly maintained by Na<sup>+</sup> and K<sup>+</sup> pump operating between seawater and cell sap to attain high Na<sup>+</sup> to K<sup>+</sup> ratio (Eisler, 1980). It is interesting to note here that the Na<sup>+</sup>/K<sup>+</sup> were below 0.8 in most algal taxa under investigation. From the nutritional point of view, the intake of sodium chloride in diets with a high Na<sup>+</sup> to K<sup>+</sup> ratio, have been related to the incidence of hypertension (Dahl *et al.*, 1972). Such result indicates that the algal taxa in the present investigation can be used as a concentrated form of mineral supplements (with a low Na<sup>+</sup>/K<sup>+</sup>) in comparison with land vegetables (Dawczynski *et al.*, 2007; Astorga-España *et al.*, 2015 ).

It was expected that all marine algal species under investigation accumulate high proline contents (Table 2); however it is apparent that this is especially true only for the marine red alga; *Gelidium latifolium*. Similarly, Gressler *et al.* (2010) recorded the high proline content in several red algae. The current results

revealed also that, despite the scarcity of proline in *spirogyra reinhardii* and *Chara vulgaris*, some marine algae as *Dictyota dichotoma* and *Sargassum dentifolium* exhibited also low proline content. This observation weakens the hypothesis that proline is the major osmolyte in marine algae inhabiting sea water.

Our Results also showed that, mannitol was recorded only in members of brown algae (Table 2). It is worth to note here that mannitol is the primary product from photosynthesis in brown algae (Thomas and Subbaramaiah, 1991). In addition, it is the main reserve polysaccharide (Horn *et al.*, 2000). Further, accumulation of low molecular weight carbohydrates such as mannitol in various brown algal species under salinity has strongly suggested their involvement in the process of osmotic adjustment and osmo-protection (Karsten, 2012 and Groisillier *et al.*, 2014).

The present study revealed also that, the highest glycerol contents were measured in all the members of marine group dominated by brown algae followed by red algae and finally green algae (Table 2, Fig 3). In contrast, freshwater members have the lowest glycerol contents as presented by *reinhardii*. The highest glycerol content was detected in *Dictyota dichotoma* (238.51  $\mu\text{M.g}^{-1}$  fw) followed by *Gelidium latifolium* (136.35  $\mu\text{M.g}^{-1}$  fw) and finally *Enteromorpha intestinalis* (52.23  $\mu\text{M.g}^{-1}$  fw). On the other hand, glycerol content represented the lowest values in the oligohalopic alga *Spirogyra reinhardii*. (9.48  $\mu\text{M.g}^{-1}$  fw). It was reported that, the accumulation of glycerol was activated by extreme salinity of seawater thus glycerol may act as osmoregulator (Borowitzka and Borowitzka, 1988) to enable marine algae to survive at high salinities of seawater. The observed high content of glycerol (Table 2) in the investigated marine algae may be due to the protective effect of glycerol on the stability of photosystem II protein complexes (Khristin and Simonova, 1998; Allakhverdiev *et al.*, 2000). In addition, accumulation of glycerol allows survival at high sodium concentration (Borowitzka and Borowitzka, 1988). Brown and Borowitzka (1979) reported that, glycerol are capable of adjusting the osmotic balance without affecting enzyme activities.

The osmotic acclimation effect of glycerol has been recognized early in few members of marine algae (Ben-Amotz and Avron, 1980; Saut *et al.*, 2011; Yao *et al.*, 2014). However, how it is involved in defense responses and adaptation of marine algae has not been investigated. Allam (1994) algae which can cope with wide range of salinity levels may have specific or characteristic genes which can show their expressions only under environmental stresses. The same author reported that most or all of these genes are suppressed again when the external stresses disappeared. In this respect, our study aims to look for specific genes that have high expression only in members of marine algae in their natural habitat. Moreover, one of the important tasks we chose is to gain preliminary insights about the transcriptional responses of glycerol-3-phosphate dehydrogenase (G3PDH is the key enzyme in glycerol biosynthesis).

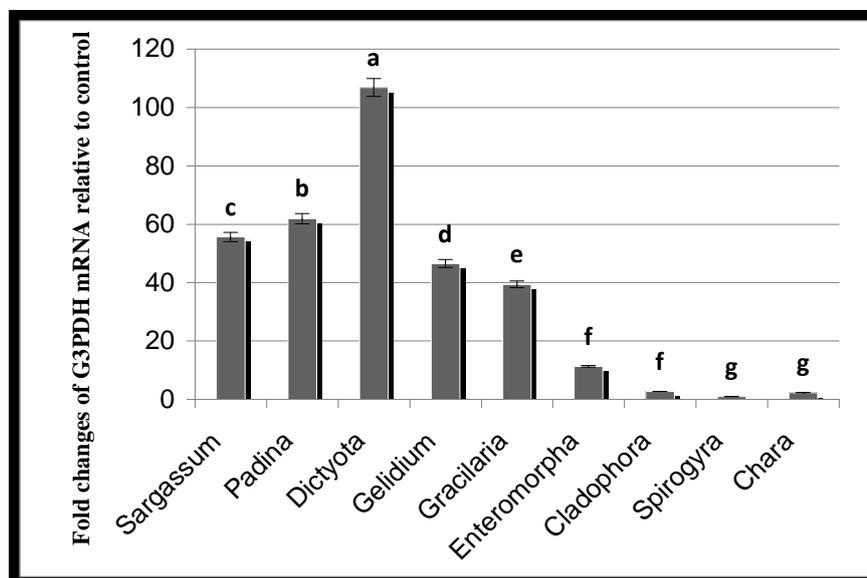


Fig. 3. Expression level of G3PDH mRNA in freshwater and marine algal groups compared to UBQ10.

The qRT-PCR analysis revealed that, the expression level of G3PDH mRNA was significantly up-regulated ( $p < 0.05$ ) in marine algal groups, as compared to that of the freshwater algae (Table 3, fig. 4, a and b). However, the changes in G3PDH expression levels of euhalopic was about 23-fold higher than that observed in the freshwater taxa. The results (Table 2) revealed two different patterns in the level of G3PDH transcript ranged from 9.19 to 106.89 in the marine group while it ranged from 1.0 to 2.77 in members of freshwater group. In the marine group, maximum levels of G3PDH transcript were measured in *Dictyota dichotoma* (106.8) followed by *Padina boryana* and *Sargassum dentifolium* (61.8 and 55.7 respectively). The lowest levels were detected in *Enteromorpha intestinalis* (11.31), while moderate levels were noticed in the members of red algae; *Gelidium latifolium* and *Gracilaria dura* (46.5 and 39.3 respectively). On the other hand, only small changes in G3PDH expression were observed in members belonging to the freshwater group; where the highest level was observed in *Cladophora glomerata* (2.77), however the lowest expression was found in *Spirogyra reinhardii* (1.0). Comparing with the increased expression level of G3PDH in all marine taxa under investigation, we found decreased level in freshwater taxa indicating that Glycerol-3-phosphate dehydrogenase (G3PDH) was found to play a role in osmo-adaptation (Yao *et al.*, 2014).

Overall, the current work represented an important step toward understanding the mechanisms involved in salinity adaptation of the marine algae in their natural habitat. However, more studies are needed to investigate the other metabolic pathways which regulate the accumulation of glycerol to enable marine algae to

survive in seawater. It is still open question whether all algae thriving in saline environments exhibit overexpression of glycerol-3-phosphate dehydrogenase (G3PDH).

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(Received 31 / 8 / 2016;  
accepted 26 / 11 / 2016)

## التعبير الجيني الزائد للجلسرول ثري فوسفات ديهيدروجينيز في بعض طحالب المياه المالحة لمصر

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هناك القليل من الدراسات التي أهتمت بالمقارنة بين طحالب المياه العذبة والمياه المالحة. لذلك تهدف هذه الدراسة إلى مقارنة بعض التركيب البيوكيميائي لثلاث أنواع من طحالب المياه العذبة: اسبيروجيرا رينهاردى، كلادوفورا جلوميراتا (طحالب خضراء) و كارا فولجاريز، وست أنواع من طحالب المياه المالحة: سارجاسوم ديتيفوليوم، بادابنا بوريانا، ديكتيوتا دايكوتوما (طحالب بنية)، جيليديوم لاتيفوليوم، جراسيلاريا دورا (طحالب حمراء)، انتيرومورفا انتيستيناليس (طحلب أخضر) من مصر. و ذلك لمعرفة الكيفية التي تتغلب و تتمكن بها طحالب المياه المالحة من المعيشة طبيعيا في مياه البحار. فقد أثبتت الدراسة أن بصمة الانبعاث الضوئي لأنواع الرتبة الواحدة متشابهة و تختلف البصمة باختلاف الرتب و الأقسام الطحلبية المختلفة. و مع استخدام التفريد الكهربائي لأنمات البروتين تبين وجود ثلاثة حزم (١٢٥، ١٥، ٨ KDa) تميز طحالب المياه المالحة، أما طحالب المياه العذبة تميزت بوجود حزمة بروتينية واحدة (٢٤٠ KDa). أظهرت هذه الدراسة أيضا وجود اختلاف هام في محتوى البرولين بين أفراد المجموعتين قيد الدراسة. هذا ولقد تميزت طحالب المياه المالحة البنية عن باقى طحالب نفس البيئة و البيئات الأخرى بوجود مادة المانيتول. هذا و قد أثبتت الدراسة أيضا الإرتفاع النسبى لأيونات الصوديوم و البوتاسيوم، و كذلك التعبير الجيني للإنزيم الجلسرول ثري فوسفات ديهيدروجينيز الخاص بتخليق الجلسرول و بالتالى تراكمه فى طحالب المياه المالحة بالمقارنة بوجوده فى طحالب المياه العذبة. و أظهرت النتائج على أن التعبير الذائد لهذا الإنزيم هو ما قد يمكن و يساعد الطحالب البحرية من المعيشة و التأقلم فى مياه البحار.