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Comparative Study for Biomass Production and Biochemical variability of two Marine Microalgae Grown on two Water Sources for Evaluating *Artemia franciscana*

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ABSTRACT

The objective of this study was to document the growth, biomass yield, total protein, lipid, vitamin C and fatty acid contents of the diatom *Chaetoceros gracilis* F. Schütt (Bacillariophyceae) and the flagellate *Isochrysis galbana* Parke (Haptophyta) grown on two different water sources (Eastern Harbor of Alexandria-EH and El-Mex well water-El-Mex) enriched with F/2 media. Fatty acids profile of *Artemia franciscana* enriched by the two algal species was used to estimate the efficiency of these two water sources for improving mariculture in the National Institute of Oceanography and Fisheries, Alexandria, Egypt. The chemical analysis of water showed, El-Mex well water characterized by lower salinity, phosphate and higher nitrate, silicate and iron contents in contrast to EH water. The higher biomass yields, cell number, protein contents of *Ch. gracilis* was obtained in El-Mex. Owing to vitamin C content, *I. galbana* attained its maximum value in El-Mex well water (11.31 µM/g). The production of total lipid and fatty acids contents was enhanced in *Ch. gracilis* and *I. galbana* grown on El-Mex well water enriched with F/2. The percentage of saturated fatty acids (SFA) was higher than the unsaturated fatty acids (USFA) in the two algal species grown on the two water sources, especially in *Ch. gracilis* grown in EH water (85.6 %) of the total fatty acids. This increase was due to enhancement of C14:0 and C16:0 and C18:0 fatty acids. Monounsaturated fatty acids (MUFA) was increased in *Ch. gracilis* grown in El-Mex well water (31 %) due to increase the percentage of C16:1 and C22:1 (22 and 5.1 %). The higher percentage of polyunsaturated fatty acids (PUFA) was obtained when *I. galbana* grown on El-Mex well water (27 %) due to increase C20:5 and C22:6 constituting 6.9 and 8.3 %, respectively. When *Artemia franciscana* enriched with the two algal species grown on the two water sources, the percentage of SFA were decreased as compared with the USFA obtained in the two algal species, and the higher percentage of PUFA, especially in *I. galbana* (47.6 %), while in *Ch. gracilis* (24 %) that was mainly due to increase the percentage of C20:4, C20:5 and C22:6 fatty acid (8.5, 9.4 and 10.4 %) in *I. galbana* grown on El-Mex well water. Results indicated that, El-Mex well water was the best culture condition suitable for promoting PUFA content in *Artemia franciscana* enriched with *I. galbana* and *Ch. gracilis.* Also it will improve mariculture by selecting microalgae and the suitable culture condition for ideal nutritional value.

Keywords: Chaetoceros gracilis - Isochrysis galbana – Fatty acids - Water source – Mariculture - Artemia franciscana

INTRODUCTION

Microalgae culturing is likely to play an increasingly important role in aquatic food production modules, specifically to produce (or be used as) feed for fish. However, microalgae are required for larvae nutrition during a brief period either for direct consumption in the case of molluscs and penaeid shrimps, or indirectly for the live prey fed to small-larval fish (Muller-Feuga, 2000). It should be emphasized that the productivity of any hatchery is directly related to the quantity and quality of the food source used therein.

Algae can grow well on different water sources but some media may be more preferred by algae and so they grow quicker and produce more protein and fatty acids. Nitrogen is considered to be the primary limiting nutrient for algal growth in marine ecosystems (Hanisak, 1990). In the absence of nitrogen, a decrease in growth; a decrease in the contents of soluble proteins were observed (Collén et al., 2004). Phosphorus is another important nutrient in the formation of biomolecules such as nucleic acids, proteins and phospholipids. However, the most important role is in energy transfer mediated by ATP and other high energy compounds present in photosynthesis and respiration (Lobban and Harrison, 1994). Hammouda et al. (1995) showed that, nutrients in waste water are converted into protein biomass in microalgae. Besides the macro nutrients nitrate and phosphate that are essential for the growth of all algae, diatoms also depend on the availability of silicic acid (Si OH_4) to produce their frustules, and can affect their growth (Brzezinski et al., 2005; Leblanc et al., 2005). Iron requirements of different diatom species seem to be variable; Takeda (1998) showed that substantial growth of large size diatom *Chaetoceros* spp. and *Nitzschia* spp. In the iron enriched water. Thus, the iron nutritional status of the diatom appears to affect silicate utilization physiologically, and iron induced nutrient change consumption ratio in diatom.

Vitamin C or ascorbic acid (AA) is an essential vitamin for many species of fish as a co-factor in various hydroxylation reactions in living tissue, and is involved in growth and reproductive processes as well as disease resistance and immune response (Li and Robinson, 1999). Most cultured fish require vitamin C, because they are unable to synthesize it (NRC, 1993; Dabrowski, 1991). Consequently, they are dependent on constant supplies of adequate amount of AA through the feed. NRC (1993) recommended 25-50 mg AA/kg diet as a requirement to secure an optimal performance of juvenile fish, while the level of about 100 mg AA/kg diet are suggested for shrimp (D' Abramo and Conklin, 1995). As described AA was essential for most fishes, including channel catfish (*Ictalurus punctatus*) (Andrews and Murai 1975) for normal growth and function. Trout, salmon and other fish species have dietary requirements for AA (Benitez and Halver, 1982). Brown and Miller (1992) studied the ascorbic acid content of eleven species of microalgae used in mariculture, and they concluded that all species provide a rich source of ascorbic acid for maricultured animals, which can require 0.003-0.02 % of the vitamin in their diet.

Microalgae contain essential nutrients which determine the quality, survival, growth and resistance to disease of cultured animal species (Gouveia et al., 2008). Microalgae are some of the most important feed sources in aquaculture due to their nutritional value and their ability to synthesize and accumulate great amounts of n-3 polyunsaturated fatty acid (PUFA) (Patil et al., 2007), such as 22:6n-3 (Docosahexaenoic acid, DHA) and 20:5n-3 (Eicosapentaenoic acid, EPA), two of the most important essential fatty acids required for gametogenesis (Ehteshami et al., 2011). The knowledge of the chemical composition of food-species has a key role for mariculture (Rivero-Rodríguez et al., 2007), especially in larviculture (Pettersen et al., 2010).

The golden-brown flagellate *Isochrysis galbana* is a rich source of polyunsaturated fatty acids (PUFA), mainly eicosapentaenoic acid, EPA, 20:5n-3 (Molina-Grima et al., 1994; Fidalgo et al., 1998), making it a useful feed for aquatic animals (Sánchez et al., 2000). To obtain larger amounts of PUFA from *Isochrysis galbana* cultivation, different components of the culture medium such as changes in the nitrogen source (Lin et al., 2007), and iron concentration (Li et al., 2008). El-Sayed (2007) proved that the alga *Tetraselmis chuii* can synthesize great amount of PUFA by media modification through increasing Na Cl 1.5 times. Among the several diatoms strains available, *Chaetoceros* spp. is widely used in marine hatcheries as food sources as well as to maintain water quality (Riquelme and Avendaño-Herrera, 2003; Khatoon et al., 2007).

The brine shrimp *Artemia* is probably the most popular live diet in aquaculture. Zaki and Saad (2010) concluded that the nutritional adequacy of zooplankton used for feeding in newly hatched larvae in marine hatcheries depend on nutritional value of microalgae used to enrich brine shrimp *Artemia salina.* Khairy and El-Sayed (2012) evaluate enriched *Artemia salina* by the alga *Tetraselmis chuii* grown on four different culture media through feeding *Sparus aurata* larvae.

The development of new algal species-specific diet formulations supports the aquaculture (fish farming) industry as it expands to satisfy increasing demand for affordable, safe, and high-quality fish and seafood products. So, the purpose of this work was to determine the biomass and the biochemical composition of *Chaetoceros gracilis* and *Isochrysis galbana* grown on two different water sources enriched with F/2 media for the feeding of *Artemia franciscana* for improving mariculture.

MATERIALS AND METHODS

Sea water analysis

Salinity was measured in situ by using a Bechman salinometer (Model NO. R.S.10). Water sample were collected from Eastern Harbor of Alexandria (EH) and El-Mex well water (El-Mex) and kept under cold conditions until reaching the laboratory for nutrient measurement. Nitrate, phosphate and silicate were measured according to Strickland and Parsons (1972), using UV/V Spekal 1300, Analytik Jana AG Spectrophotometer. Iron concentration was measured according to APHA (1985).

Microorganisms

The *Chaetoceros gracilis* and *Isochrysis galbana* strain used in this study were obtained from the microalgae culture collection of marine hatchery laboratory, National Institute of Oceanography and Fisheries, Alexandria, Egypt.

Culture media

The culture was grown on two different water sources from Eastern Harbor of Alexandria (EH) and El-Mex well water (El-Mex), and enriched with F/2 media (Guillard, 1975), Table 1 . Two daylight fluorescent tubes of 20 W were used to maintain constant illumination at culture temperature about 25±1 ºC.

Brine Shrimp (Artemia) Culture

Artemia franciscana was produced by hatching *Artemia* cysts through decapsulation technique. They were incubated to hatch as described by Lavens and Sorgloos (1996). The produced nauplii were harvested after 24 hrs, then washed with filtered sea water, and after 6 hrs from hatching time, *Artemia* nauplii (instar II) enriched with *Ch. gracilis* and *I.galbana* grown on two different water sources enriched with F/2 media. After 24 hrs, enrichment *Artemia* was harvest by plankton net (100 µm), and the fatty acids content were estimated.

Table1: ***Guillard F/2 media used to culture marine microalgae (Guillard, 1975).***

Major nutrient	Chemical formula	Concentration (g/l)
1-Nitrate	$NaNO_3$	75.0 g/L
2-Phosphate	$NaH_2PO_4.H_2O$	5.0 g/L
3-Silicate	$Na_2SiO_3.9H_2O$	30.0 g/L
4-Trace Metals		
	$FeCl_3.6H_2O$	3.5 g
	Na_2EDTA	4.36 g
Dissolve in 900 ml of distilled H_2O. **Add 1 ml of each of the following trace metal solutions.**		
	$CuSO_4.5H_2O$	0.98 g/100 ml
	$ZnSO_4.7H_2O$	2.20 g/100 ml
	$CoCl_2.6H_2O$	1.00 g/100 ml
	$MnCl_2.4H_2O$	18.00 g/100 ml
	$Na_2MoO_4.2H_2O$	0.63 g/100 ml
Make up the volume to 1 liter with distilled H_2O. **Add 1 ml per liter of seawater of the above solutions 1−4.**		
5-Vitamins		
	Biotin	1.0 mg
	B_{12}	1.0 mg
	Thiamin HCl	20.0 mg
Dissolve vitamins in 1 liter of distilled H_2O. Store frozen. Add 0.5 ml of vitamin solution for every 1 liter of seawater.		

Brine Shrimp (Artemia) Culture

Artemia franciscana was produced by hatching *Artemia* cysts through decapsulation technique. They were incubated to hatch as described by Lavens and Sorgloos (1996). The produced nauplii were harvested after 24 hrs, then washed with filtered sea water, and after 6 hrs from hatching time, *Artemia* nauplii (instar II) enriched with *Ch. gracilis* and *I.galbana* grown on two different water sources enriched with F/2 media. After 24 hrs, enrichment *Artemia* was harvest by plankton net (100 µm), and the fatty acids content were estimated.

Algal cell numbers of *Ch. gracilis* and *I. galbana* were determined every 2 days by placing an aliquot of well-mixed culture suspension on a Neubauer haemocytometer (Assistant, Germany). The cell number in the culture was calculated by dividing the number of cells counted by the volume and the dilution.

Algal optical density (OD) of *Ch. gracilis* and *I. galbana* was measured at 540 and 750 nm, respectively by using UV/V Spekal 1300, Analytik Jana AG Spectrophotometer.

Algal biomass was calculated by measuring dry weight. For dry weight measurement homogenous suspensions of known quantity of *Ch. gracilis* and *I. galbana* sample were filtered through glass fiber filter paper, then oven dried at 75ºC for 4 to 6 hours. The dried filter paper containing algal biomass were cooled and weighed. The difference between the initial and final weight were taken as the dry weight of algal biomass (mg/l).

Chemical analysis of algae and Artemia salina

Algae were harvested at late exponential growth stage, triplicate samples of algal material were allocated to each assay (protein, lipid, fatty acids and vitamin C).

Total protein in the two algal cells were determined by the Folin-phenol method of Lowery et al. (1951), and total lipid contents were analyzed gravimetrically after extraction with chloroform–methanol (2:1) using the Folch method as modified by Bligh and Dyer (1959). Fatty acid methyl esters were analyzed in the algal cells and *Artemia* using gas liquid chromatography (HP-6890 gas–liquid chromatography).

Vitamin C concentration was measured by using the 2, 6-dichlorophenol-indophenol (DCPIP) photometric method of (Guri, 1983).

Statistical analysis

The collected data were analyzed using one-way analysis of variance (ANOVA). Significant differences among the different treatments were determined using the Duncan multiple range test at $P < 0.05$ level of probability. Statistical analysis was done using SPSS software program version 15.

RESULTS

The chemical analysis of two water sources was observed in Table 2. The results showed that, EH water was characterized with higher salinity and phosphate value, in contrast El-Mex well water had higher nitrate, silicate and iron (Fe) value.

The initial cell density for the two algal species was 0.5 X 10^6 cell/ml, and it can be seen from the growth curve of culture time versus cell density (Figure 1 and 2, for *Ch. gracilis* and *I. galbana*, respectively), cell density of *Ch. gracilis* was improved significantly at higher nitrate silicate and Fe concentration (El-Mex well water enriched with F/2 media). The maximum cell density (8.47 X 10^6 cell/ml) at day 10 was achieved (Figure 1), which was almost 18 times greater relative to the initial cell count as compared to *I. galbana* (1.57 X 10^6 cell/ml) at day 8, this was illustrated by a threefold greater cell density as compared with the initial cell density (Figure 2).

Table 2: ***Water analysis of two different water sources (Eastern Harbor of Alexandria (EH) and El-Mex well water (El-Mex).***

Location	Parameter				
	Salinity (‰)	Nitrate (mg/l)	Phosphate (mg/l)	Silicate (mg/l)	Fe (µ/l)
EH	39.1± 0.37	78.3± 0.95	32.4± 0.22	4.89± 0.1	35.0±1.0
El-Mex	29.8± 0.21	890.7± 3.3	12.8± 0.3	274.3± 2.02	136±5.4.0

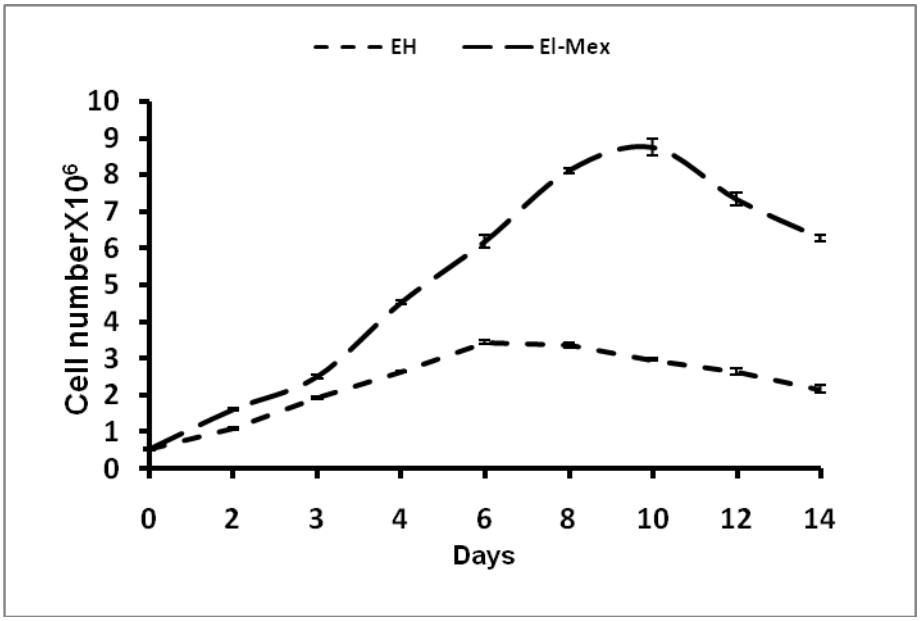

Figure 1. ***Cell number (x 10^6) of Ch. gracilis grown on EH and El-Mex water sources enriched with F/2 media.***

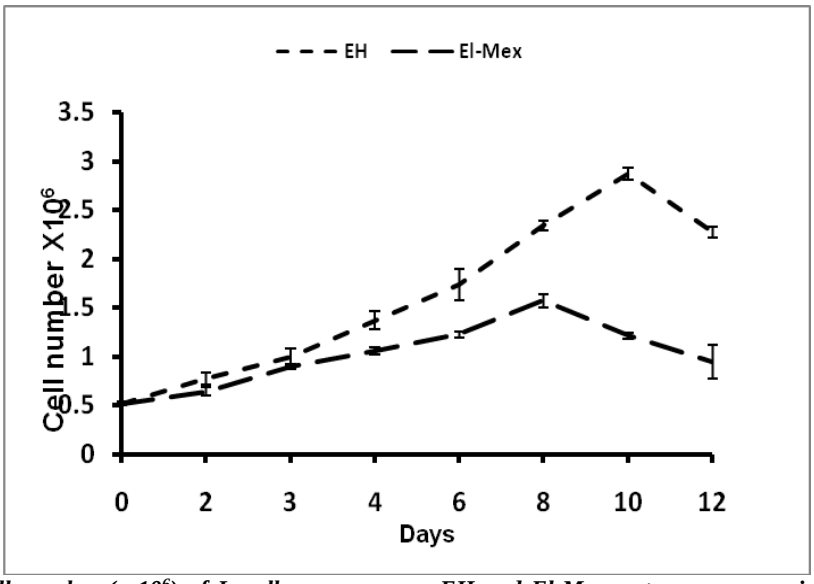

Figure 2. ***Cell number (x 10^6) of I. galbana grown on EH and El-Mex water sources enriched with F/2 media.***

Cell numbers and optical density were significantly different between the two tested species at the two water sources ($P < 0.05$). The higher optical density of *Ch. gracilis* (Figure 3) was obtained in El-Mex well water, while its minimum optical density was obtained in EH as compared with *I. galbana* (Figure 4). As shown in Figure 5, the maximum biomass yield as dry weight (9.6 mg/l) was recorded for *Ch. gracilis* at day 10 grown in El-Mex well water. In contrast, *I. galbana* had a significant highest growth as biomass yield (Figure 6) in EH as compared with El-Mex. On the other hand, observations of morphology of the two species showed that, cell size became larger than normal during the late period of cultivation, and also the dark brown colour was observed in case of *Ch. gracilis* grown on El-Mex well water.

As shown in Table 3, a significant differences ($P < 0.05$) in the chemical composition of the two tested species in the two water sources. The higher protein and total lipids contents of *Ch. gracilis* was observed in El-Mex well water enriched with F/2 (lower salinity, phosphate contents and higher nitrate, silicate and iron contents, Table 2). In contrast the higher protein and lipids contents of *I. galbana* were observed in EH water enriched with F/2 (higher salinity, phosphate and lower nitrate, silicate and iron contents). As regarded to vitamin C, *I. galbana* attained its maximum content in El-Mex well water enriched with F/2..

Table 3: ***Total protein, lipid (mg/l) and vitamin C (µM/g) of Ch. gracilis and I. galbana grown on EH and El-Mex water sources enriched with F/2 medium***.

Location	Parameter					
	T. Protein		T. Lipids		Vit. C	
	Ch. gracilis	*I. galbana*	*Ch. gracilis*	*I. galbana*	*Ch. gracilis*	*I. galbana*
EH	35.1±2.52	56±3.76	4.59±0.34	3.60±0.22	6.32±0.1	7.46±0.1
El-Mex	90.98±2.89	42±0.63	6.23±0.05	4.52±0.24	4.37±0.1	11.31±0.1

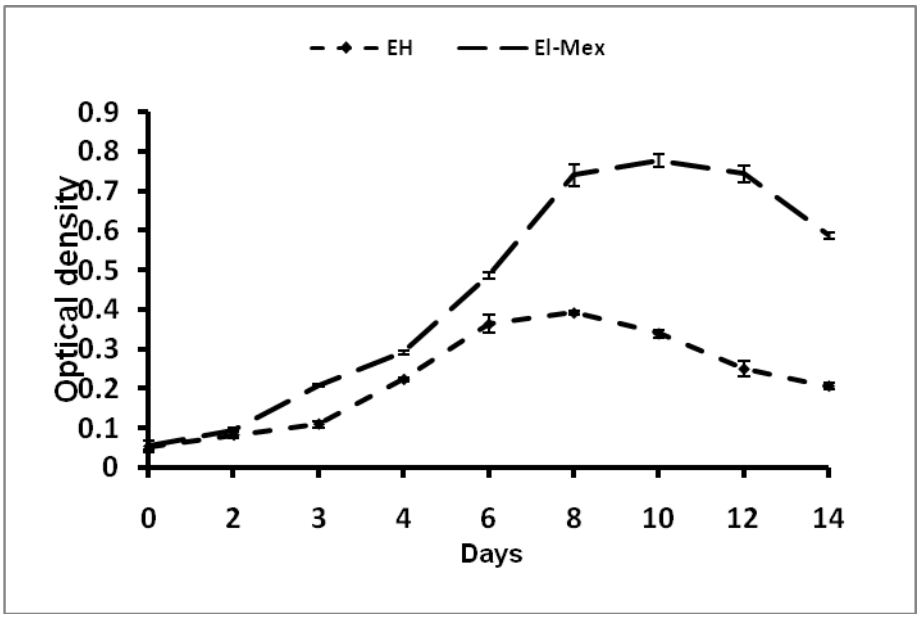

Figure 3. Optical density of Ch. gracilis grown on EH and El-Mex water sources enriched with F/2 media.

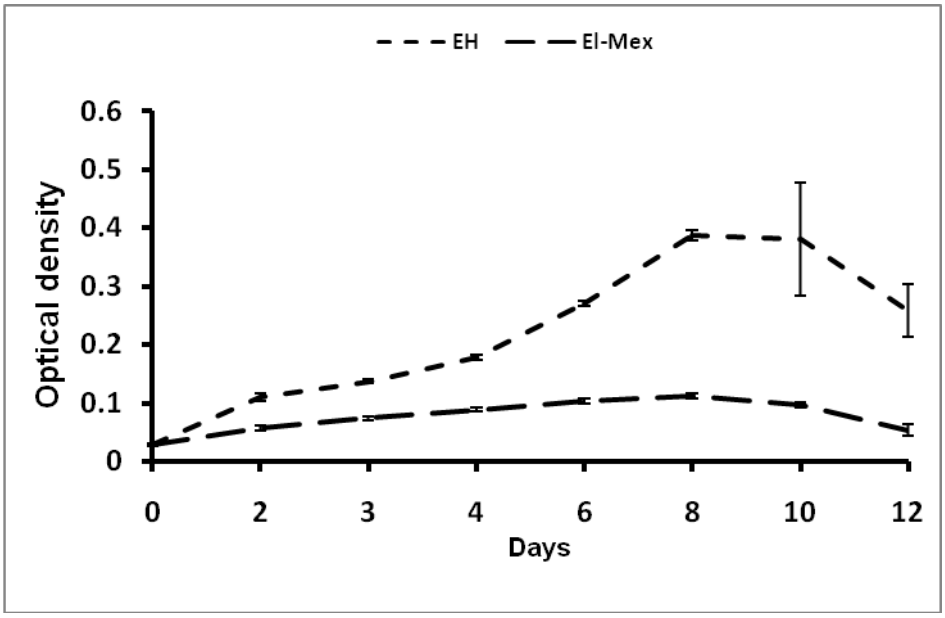

Figure 4. Optical density of I. galbana grown on EH and El-Mex water sources enriched with F/2 media.

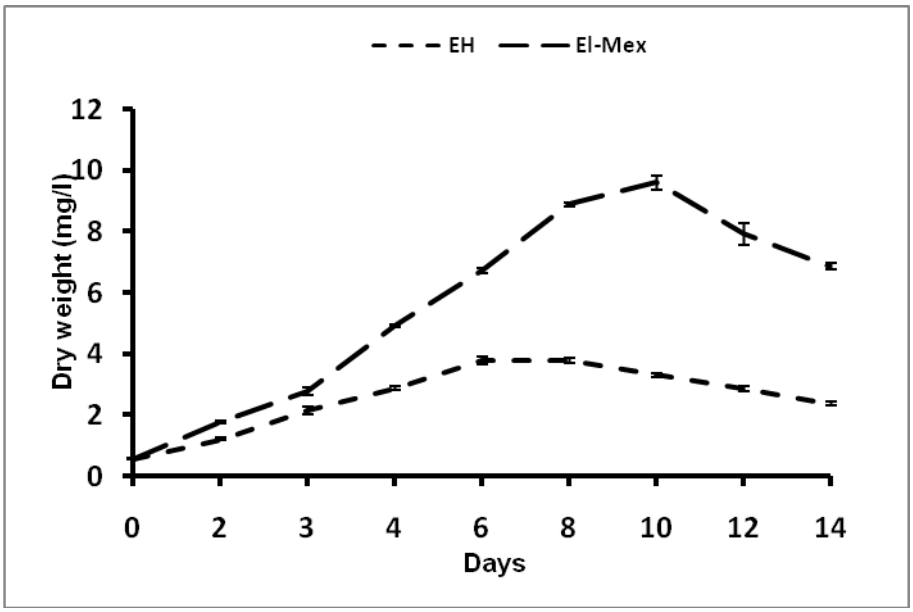

Figure 5. Dry weight (mg/l) of Ch. gracilis grown on EH and El-Mex water sources enriched with F/2 media.

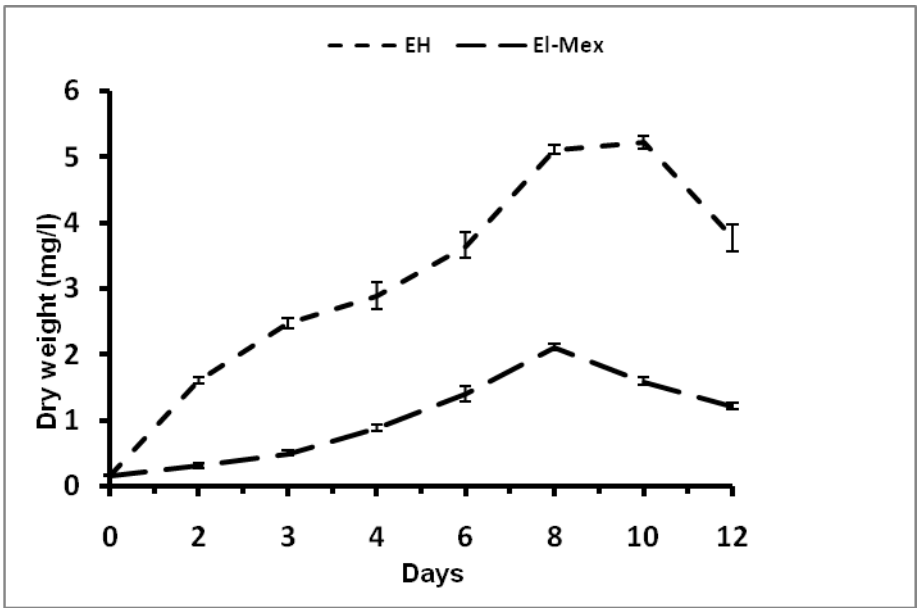

Figure 6. Dry weight (mg/l) of I. galbana grown on EH and El-Mex water sources enriched with F/2 media.

Table 4: ***Fatty acids analysis (mg/l) of Ch. gracilis and I. galbana grown in EH and El-Mex water sources enriched with F/2 media.***

	Ch. gracilis		***I. galbana***	
Fatty acid (FA)	**EH**	**El-Mex**	**EH**	**El-Mex**
Saturates (SFA)				
C 6:0	1.57	0.71	7.3	0.99
C 8:0	16.68	0.27	1.57	18.85
C 10:0	1.76	0.01	2	1.75
C 11:0	5.1	0.05	1.18	45.54
C 12:0	13.07	7.25	2.66	0.69
C 13:0	4.9	1.22	3.26	3.66
C 14:0	48.82 (26 %)	113.74 (14.4 %)	12.53 (6.1 %)	96.41 (13.2 %)
C 15:0	2.69	4.58	1.59	1.02
C 16:0	52.24 (27.8 %)	184.97 (23.4 %)	53.88 (26.4 %)	105.81 (14.5 %)
C 17:0	0.87	2.54	1.83	0.011
C 18:0	10.66 (5.7 %)	108.21 (13.7 %)	38.37 (18.8 %)	59.83 (8.2 %)
C 20:0	1.23	0.03	9.24	11.13
C 21:0	1.32	11.51	0.83	3.8
C 23:0	0.02	0.04	0.41	0.01
Sum	**160.93**	**435.13**	**136.65**	**349.501**
% to total FA	**85.6**	**55.1**	**66.9**	**48**
Monounsaturates (MUFA)				
C 14:1	0.46	2.91	2.45	1.86
C 15:1	0.13	2.79	1.28	3.22
C 16:1	1.4 (0.7 %)	173.75 (22 %)	0.81 (0.4 %)	86.96 (11.9 %)
C 17:1	0.06	4.78	3.37	3
C 18:1	0.12	10.47	2.68	17.85
C 20:1	0.11	10.05	6.71	28.85
C 22:1	1.45 (0.8 %)	40.24 (5.1 %)	2.49 (1.2 %)	40.57 (5.6 %)
Sum	**3.73**	**244.99**	**19.79**	**182.31**
% to total FA	**2**	**31**	**9.7**	**25**
Polyunsaturates (PUFA)				
C 18:2	3.2	5.28	18.23	1.92
C 18:3	2.47	2.91	1.49	0.8
C 20:2	2.08	2.27	2.86	17.8
C 20:3	0.11	2.33	1.49	7.88
C 20:4 n6 (AA)	5.26 (2.8 %)	15.79 (2 %)	6.28 (3.1 %)	4.74 (0.7 %)
C 20:5 n3 (EPA)	2.47 (1.3 %)	41.06 (5.2 %)	7.76 (3.8 %)	50.08 (6.9 %)
C 22:2	3.53	6.05	1.38	53.11
C 22:6 n3 (DHA)	4.32 (2.2 %)	34.12 (4.3 %)	8.37 (4.1 %)	60.34 (8.3 %)
Sum	**23.44**	**109.81**	**47.86**	**196.67**
% to total FA	**12.4**	**13.9**	**23.4**	**27**
Total fatty acids (m/l)	**188.1**	**789.93**	**204.3**	**728.48**

As represented in Table 4, total lipids and fatty acids content were enhanced in *Ch. gracilis* than *I. galbana* grown in El-Mex well water enriched with F/2 media constituting 789.93 and 728.48 mg/l, respectively. As regarded to saturated fatty acids (SFA), C14:0; C16:0 and C18:0 was the dominant fatty acid, constituting 51.5 % in *Ch. gracilis* which more than in *I. galbana* (35.9 %) grown on El-Mex well water. The monounsaturated fatty acids (MUFA) C

16:1 and C22:1 in the two algal species attained the maximum values when grown on El-Mex well water (27.1 and 17.5 % in *Ch. gracilis* and *I. galbana*, respectively. Finally, the total polyunsaturated fatty acid (PUFA) especially C20:4, C20:5 and C22:6 were higher in *Ch. gracilis* and *I. galbana* grown on El-Mex well water and forming 11.5 and 15.9 %, in the two species respectively.

Table 5: ***Fatty acids analysis (mg/l) of Artemia franciscana fed on Ch. gracilis and I. galbana grown EH and El-Mex water sources enriched with F/2 media.***

	Ch. gracilis		***I. galbana***	
Fatty acid	**EH**	**El-Mex**	**EH**	**El-Mex**
Saturates (SFA)				
C 6:0	0.69	34.71	22.43	12.04
C 8:0	32.2	41.95	10.39	10.39
C 10:0	0.76	30.58	10.22	14.37
C 11:0	2.61	35.41	11.31	15.73
C 12:0	2.88	34.41	29.44	12.21
C 13:0	1.98	34.45	12.3	2.96
C 14:0	10.67 (6.4 %)	23.08 (2.1 %)	28.74 (4.1 %)	48.23 (5.6 %)
C 15:0	1.13	4.71	21.64	13.01
C 16:0	23.74 (14.3 %)	154.1 (13.9 %)	47.27 (6.7 %)	83.27 (9.6 %)
C 17:0	1.5	22.39	22.77	16.96
C 18:0	22.15 (13.3 %)	76.56 (6.9 %)	74.51 (10.6 %)	41.08 (4.7 %)
C 20:0	1.53	28.23	29.6	14.26
C 21:0	5.78	31.52	42.03	15.11
C 23:0	0.01	10.1	23.9	10.03
Sum	**107.63**	**562.2**	**386.55**	**309.65**
% to total FA	**65.4**	**50.9**	**55**	**35.7**
Monounsaturates (MUFA)				
C 14:1	0.42	0.97	3.42	3.39
C 15:1	2.29	5.76	2.57	3.93
C 16:1	8.88 (5.3 %)	51.46 (4.7 %)	18.93 (2.7 %)	4.26 (0.5)
C 17:1	1.36	12.03	4.4	13.89
C 18:1	0.02 (0.01 %)	157.23 (14.2 %)	85.38 (12.1 %)	13.55 (1.6 %)
C 20:1	0.01	26.6	13.49	56.41
C 22:1	8.01 (4.8 %)	23.82 (2.2 %)	39.83 (5.7 %)	49.61 (5.7 %)
Sum	**20.99**	**277.87**	**168.02**	**145.04**
% to total FA	**12.6**	**25.1**	**23.9**	**16.7**
Polyunsaturates (PUFA)				
C 18:2 n6	5.08	13.26	3.2	60.7
C 18:3 n3 (ALA)	1.18	11.69	2.22	14.62
C 20:2 n6	3.28	19.55	31.79	2.37
C 20:3 n6	0.03	4.43	14.6	48.02
C 20:4 n6 (AA)	4.08 (2.5 %)	40.91 (3.7 %)	20.4 (2.9 %)	73.76 (8.5 %)
C 20:5 n3 (EPA)	8.47 (5.1 %)	76.43 (6.9 %)	31.01 (4.4 %)	84.8 (9.6 %)
C 22:2 n6	0.61	0.04	2.32	38.83
C 22:6 n3 (DHA)	13.8 (8.3 %)	99.07 (9.0 %)	42.8 (6.1 %)	89.8 (10.4 %)
Sum	**36.53**	**78.97**	**148.34**	**412.9**
% to total FA	**22**	**24**	**21.1**	**47.6**
Total fatty acids (ppm)	**166.15**	**1105.45**	**702.91**	**867.59**

As shown in Table 5, when *Artemia franciscana* enriched with the two algal species grown on the two water sources, the percentage of saturated fatty acids were decreased as compared with the saturated fatty acids obtained in the two algal species (Table 4). The results in Table 5 showed that, the saturated fatty acids (SFA), especially C14:0, C16:0, C18:0 was the main saturated fatty acid, and the percentage of them in *Ch. gracilis* and *I. galbana* grown on EH water was higher than El-Mex well water which amounting of 34 and 21.4 % of the total fatty acids in EH and 22.9 and 19.9 % El-Mex, respectively. The higher percentage of monounsaturated fatty acids (MUFA) in *Artemia franciscana* enriched with *Ch. gracilis* grown on El-Mex well water (25.1 % of the total fatty acid) was due to mainly increase the percentage of C18:1 fatty acid (14.2 %). In contrast, total polyunsaturated fatty acids (PUFA) in *Artemia franciscana* enriched with *I. galbana* grown on El-Mex well water (47.6 %), this mainly due to increase the percentage of C20:4 (8.5 %); C20:5 (9.8 %) and C22:6 (10.4%) fatty acids.

DISCUSSION

The algae tested were selected on the basis of their potential suitability as food for feeding of *Artemia salina*. In this experiment, the growth of microalgae was monitored through the measurement of the biomass, cell count and optical density.

The results indicated that, the maximum biomass yield as dry weight (9.6 mg/l) was recorded in *Ch. gracilis* at day 10 presented in El-Mex well water enriched with F/2 media. Cell number and OD were significantly different between the two tested species at the two water sources ($P < 0.05$). The cell density of *Ch. gracilis* was improved significantly (18 times greater than the initial cell count) at lower salinity and phosphate content (29.8‰ and 12.8 mg/l, respectively), and higher nitrate; silicate (890, 274.3 mg/l, respectively) and iron concentration (136 µ/l) in El-Mex well water enriched with F/2 media. On the other hand, *I. galbana* attained its higher cell density at day 8 which was threefold greater than the initial cell density. These results are in harmony with those obtained by many authors who cleared that in the presence of nitrogen fractions the growth of many algae increased, and increase in nitrogen may overcome some of the inhibitory effect of many factors (El-Said 1990; El-Maghraby, 1997). El-Sayed (2007) revealed that, nitrate concentration at 1.5 times that of the basal medium was highly significant for optimization of the growth of *Chlorella salina* to two times than that of the control. Also the higher optical density of *Ch. gracilis* was obtained in El-Mex, while its minimum optical density was obtained in EH. In contrast, *I. galbana* had a significant highest growth as biomass yield, cell number and optical density in EH as compared with El-Mex. Recent studies showed that diatoms in all size classes were able to benefit from iron fertilization, and high silicate concentrations enhanced maximum cell numbers 14.9 times in *Ch. debilis* and 5.5 times in *Ch. dichaeta* (Hoffmann et al., 2007). Brzezinski et al. (2005) concluded that, low silicate (Si) concentrations can limit diatom growth, besides the effect on cell growth, iron fertilization increases the maximum specific uptake rates of silicic acid in diatoms. Takeda (1998) concluded that, iron should be considered not only as a factor directly limiting phytoplankton growth, biomass, but also as a factor indirectly controlling the biogeochemical cycling of nutrients particularly silicate.

Observations of morphology of the two studied species showed that, cell size became large than normal during the late period of cultivation, and also the dark brown color was observed in case of *Ch. gracilis* grown on El-Mex well water. In addition, the cells grown under nitrate depletion became large, which was further evidence of nitrogen stress (Thomas et al., 1981). Hoffmann et al. (2007) reported that, iron and silicate both had an effect on cell morphology in *Chaetoceros* species, while cells

grown under iron replete conditions had a healthier appearance, iron limitation led to a visible loss in cellular chlorophyll concentrations in *Ch. dichaeta* and *Ch. debilis.*

The biochemical composition of algae varies between species and according to culture conditions (Martínez-Fernández et al., 2006). Protein is typically the major biochemical component of algae (Wikfors et al., 1992) although the growth medium and growth stage will affect biochemical composition (Leonardos and Lucas 2000). The results of the present study showed that, the higher protein content of *Ch. gracilis* was obtained in El-Mex well water enriched with F/2 which characterized by low phosphorus content, while the higher protein content of *I. galbana* was observed in EH water enriched with F/2 (higher salinity, lower nitrate, silicate and iron contents). Leonardos and Lucas (2000) reported that phosphorus limitation affected to decrease protein content of a microalga *Ch. muelleri*. Feng et al. (2011) showed that, the protein content *I. zhangjiangensis* was the lowest due to nitrogen deficiency. El-Sayed (2007) proved that, *Dunaliela salina* can synthesize protein more than control by 29.4 % with decreasing sodium chloride 50 % of the basal medium.

As regarded to vitamin C, the results indicated that, the higher vitamin C content (11.31 µM/g) was obtained from *I. galbana* grown on El-Mex well water enriched with F/2 media. In contrast increased salinity caused increase in vitamin C content in *Isochrysis* sp. as recorded by (Chakraborty et al. 2007), they observed that, ascorbic acid ranged between 0.12 mg/100g to 0.04 mg/100g. Brown and Miller (1992) concluded that the differences in the composition of the eleven microalgae from ascorbic acids were from 0.11 to 1.62 %. Lovell and Lim (1978) reported that channel catfish fed diets deficient in ascorbic acid were how more susceptible to bacterial infection than those fed diets supplemented with ascorbic acid. Transfer of ascorbic acid (and other vitamins) between trophic levels is important for fish larvae and late larval/early juvenile crustaceans that are reared on algal-fed zooplankton. Hapette and Poulet (1990) showed that, fed microalgae to previously starved populations of the copepods *Calanus helgolandicus* and *Acartia clausi*, increases in the ascorbic acid levels of 50% and 60%, respectively, for the animals was observed. Their results supported the hypotheses that ascorbic acid from the microalgae can be incorporated with high efficiency through direct feeding.

Lipids are considered very important during gametogenesis for gonad maturation and especially in females to provide an energy source for subsequent embryo development (Pollero et al., 1983). The results showed that, total fatty acids were enhanced in *Ch. gracilis* than *I. galbana* grown in El-Mex well water (low salinity, iron and high nitrate phosphate concentration) enriched with F/2 media constituting 789.93 and 728.48 mg/l, respectively in the two species. Feng *et al.* (2011) indicated that the addition of nitrate is one of the factors promoting cell division of *Isochrysis zhangjiangensis*, and it can accumulate lipids under nitrogen-repletion conditions. Fidalgo et al. (1998) reported that, high lipid content in *I. galbana* (38.5%) was attained at a high concentration of 4 mg atom N/l ($NaNO_3$).

Elenkov et al. (1996) reported that high salinity affected increasing lipid content of *Cladophora vagubunda*. Also Pernet et al. (2003) reported that the total lipid of *Ch. mueleri* diatom increased was related to silicon-depletion. In contrast to our study, the diatom *Cyclotella cryptica* can accumulate lipids during the silicon deficiency. Chen (2012) cultivated 12 species of marine diatoms in three different environments and concluded that these conditions altered the fatty acid composition of the diatom species, emphasizing that production and storage of lipids is species-specific. Whereas, lipid accumulation of *Ch.* cf. *wighamii* diatom is usually was triggered by nutrition deficiency (Araújo et al. 2005). Liu and Lin

(2001) observed that, the lipid contents of *Isochrysis* sp. increased with an increase of salinity.

The total of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) in microalgae can be changed due to differences in culture media and environmental conditions (Rousch et al., 2003). The present study showed SFA was the dominant fatty acid compared to MUFA and PUFA. SFA, especially C14:0; C16:0 and C18:0 was the dominant fatty acid, constituting 51.5 % in *Ch. gracilis* which more than in *I. galbana* (35.9 %) grown on El-Mex well water. In most organisms, fatty acid biosynthesis culminates in the formation of either C16:0 or C18:0 saturated fatty acids and these fatty acids are modified through a sequence of desaturates and elongates to produce unsaturated fatty acids and PUFAs (Ratledge, 2004). The MUFA, C 16:1 and C22:1 in the two algal species attained the maximum values when grown on El-Mex well water (27.1 and 17.5 % in *Ch. gracilis* and *I. galbana*, respectively). Finally, the total polyunsaturated fatty acid (PUFA) especially C20:4 (ALA), C20:5 (EPA) and C22:6 (DHA) were higher in *Ch. gracilis* and *I. galbana* grown on El-Mex well water and forming 11.5 and 15.9 %, in the two species respectively. In accordance, Liu and Lin (2001) showed lipid content was higher (DHA and PUFA were 11.2 % and 38.6 %, respectively) at low salinity (3.2 %). Most of these fatty acids recorded in the previously in *Isochrysis galbana* (Lin et al., 2007) and *I. zhangjiangensis* (Feng et al., 2011). Coincide with our results, Mortensen et al. (1988) observed that, omega-3 fatty acids decreased in *Ch. gracilis* with decreasing silicate availability**,** and the change in lipid content in diatom species due to nitrogen depletion appears to be species-specific (Shifrin and Chisholm, 1981). The ratio of omega-3 polyunsaturated fatty acid to the sum of saturated and monounsaturated fatty acid in *I. galbana* increased as the nitrogen load increased (Sukenic and Wahnon, 1991).

Fatty acids play a key role in aquaculture, since they are very important for the growth of marine organisms. Because of this, some microalgal species may be potentially indicated as useful for feeding marine animals, while others show to be unsuitable as food-species, if the essential PUFA lack in them. Physiological studies demonstrated that fish cannot synthesize omega-3 fatty acids (kanazawa 1985) but accumulate them via the food chain where algae are the primary source of these fatty acids.

Increasing the nutritive value of *Artemia salina* may intern increase the survival and growth of larvae to which they fed (Zaki and Saad, 2010). In the current study, the percentage of saturated fatty acids in *Artemia franciscana* enriched with the two algal species grown on the two water sources, were decreased as compared with the saturated fatty acids obtained in the two algal species. The results showed that, the SFA, especially C14:0, C16:0, C18:0 was the main fatty acids, and the percentage of them in *Ch. gracilis* and *I. galbana* grown on EH water was higher than El-Mex well water which amounting of 34 and 21.4 % of the total fatty acids in EH and 22.9 and 19.9 % El-Mex, respectively. The higher percentage of MUFAs in *Artemia franciscana* enriched with *Ch. gracilis* grown on El-Mex well water (25.1 % of the total fatty acid) was due to mainly increase the percentage of C18:1 fatty acid (14.2 %). In contrast, PUFAs in *Artemia franciscana* enriched with *I. galbana* grown on El-Mex well water (47.6 %), this mainly due to increase the percentage of C20:4 (8.5 %); C20:5 (9.8 %) and C22:6 (10.4%) fatty acids. Arts et al. (1997) indicated that period of nutrient deficiency may intensify lipid synthesis in some phytoplankton sp. and thereby enhance the rate of lipid biomass that is transferred from phytoplankton to zooplankton. PUFAs are considered essential for growth, development, and cellular function (Holland, 1978; Chu and Greaves 1991). In addition, many marine bivalves show a low capacity for desaturating fatty acids to PUFAs; thus, they satisfy most of their essential fatty acid requirements through

dietary sources (Zhukova et al. 1998). Like other aquatic organisms, fish larvae and shrimp will require dietary lipids (unsaturated and saturated fatty acids) to provide precursors for chain elongation of essential highly unsaturated fatty acids, synthesis of hormones, and as an energy source in developing embryos and larvae (Cohen et al. 1988; Napolitano et al. 1993).

CONCLUSION

El-Mex well water may consider as a model culture with salinity (29.8 ‰), nitrate (890 mg/l), phosphate (12.8 mg/l) and iron (136 µ/l) when enriched with F/2 media representing relatively ideal culture condition was excellent nutrient stress medium for enhancement of the production and synthesis of certain biomass, protein, lipid, vitamin C and fatty acids by *Ch. gracilis* and *I. galbana* especially increasing of PUFA (two folds than EH). So, the two algal species tested in this study seem to be potentially useful as food-species for *Artemia franciscana* which may achieved specific requirements for the fish larvae and improving mariculture in the National Institute of Oceanography and Fisheries, Alexandria, Egypt.

REFERENCES

Andrews, J.W., Murai, T., 1975. Studies on the vitamin C requirements of channel catfish *(Ictalurus punctatus*). J. Nutr. 705: 557-561.

APHA (American Public Health Association/AWWA/WPCF), 1985. Standard methods for the examination of water and wastewater. 60th. Ed. APHA / AWWA / WPCF. Washington, 1217 pp.

Araűjo, S.deC., Maria, V., Garcia, T., 2005. Growth and biochemical composition of the diatom *Chaetoceros* cf. *wighamii* brightwell under different temparature, salinity and carbon dioxide levels. I. Protein, carbohydrates and lipids. Aquacul., 246: 405-412.

Arts, M.T., Richard, D.R., Evans, M.S., 1997. Seasonal changes in particulate and dissolved lipids in a eutrophic prairie lake. Freshwater Biol., 38: 525–537.

Benitez, L.V., Halver, J.E., 1982. Ascorbic acid sulfate sulfohydrolase (C_2 sulfatase): The modulator of cellular levels of L-ascorbic acid in rainbow trout. Proc. Natl. Acad. Sci., 79: 5445-5449.

Bligh, E.G., Dyer, W.J., 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol., 37: 911–917.

Brown, M.R., Miller, K.A., 1992. The ascorbic acid content of eleven species of microalgae used in mariculture. J. App. Phycol., 4: 205-215.

Brzezinski, M.A., Jones, J.L., Demarest, M.S., 2005. Control of silica production by iron and silicic acid during the Southern Ocean Iron Experiment (SOFeX), Limnol. Oceanogr., 50 (3): 810–824.

Chakraborty, R.D., Chakraborty, K., Radhakrishnan, E.V. 2007. Variation in fatty acid composition of *Artemia franciscana* nauplii enriched with microalgae and baker's yeast for use in larviculture. J. Agri. Food Chem., 55: 4043-4051.

Chen, Y.C., 2012. The biomass and total lipid content and composition of twelve species of marine diatoms cultured under various environments. Food Chem., 131:211-219.

Chu, F.L.E., Greaves. J. 1991. Metabolism of palmitic, linoleic, and linolenic acids in adult oyster, *Crassostrea virginica*. Mar. Biol., 110: 229–236.

Cohen, Z., Vonshak, A., Boussiba, S., Richmond, A., 1988. Effect of environmental conditions on fatty acid composition of the red alga *Porphyridium cruentum*: correlation to growth rate. J. Phycol., 24: 328–332.

Collén, P.N., Camitz, A., Hancock, R.D., Viola, R., Pedersén, M. 2004. Effect of nutrient deprivation and resupply on metabolites and enzymes related to carbon allocation in *Gracilaria tenuistipitata* (Rhodophyta). J. Phycol., 40: 305-314.

Dabrowski, K., 1991. Administration of gulonolactone does not evoke ascorbic acid synthesis in teleost fish. Fish Physiol. Biochem., 9: 215-221.

D' Abramo, L.R., Conklin, D.E., 1995. New developments in the understanding of the

nutrition of penaeid and caridean species of shrimp. In: Browdy, C.L., Hopkins, J.S. (Eds.), Swimming through Troubled water. Proceedings of the Special Session on Shrimp Farming, Aquaculture 1995. World Aquaculture Society, Baton Rouge, LA, USA, pp. 95-107.

Ehteshami, F., Christianus, A., Rameshi, H., Harmin, S.A., Saad, C.R., 2011. Proximate and fatty acid composition of the gonads of wild versus hatchery-conditioned *Pinctada margaritifera* broodstock. Aquacult. Nutr., 17:675-682.

Elenkov, I., K. Stefanov, D., Dimitrova, K., Popov, S., 1996. Effect of salinity on lipid composition of *Cladophora vagabunda*. Phytochem., 42: 39–44

El-Maghraby, D.E., 1997. The Biotechnology of culturing *Dunaliella salina* for the production of some voluble metabolites: M. Sc. Thesis, Faculty of Science, Alexandria University.

El-Said, A.F. 1990. Interactive effects of salinity and natural nitrogen sources on the growth and metabolic processes in algae. M.Sc. Thesis, Faculty of science, Alexandria University, Botany Department.

El-Sayed, H.S., 2007. Ecophysiological studies on some algal species used in feeding of newly hatched larval of fishes. Ph.D. Thesis, Fac. Sci. Alex. Univ. Egypt, p. 355.

Feng, D., Chen, Z., Xue, Z.W., 2011. Increased lipid production of the marine oleaginous microalgae Isochrysis zhangjiangensis (Chrysophyta) by nitrogen supplement. Bioreso. Technol., 102: 6710–6716.

Fidalgo, J.P., Cid, A., Torres, E., Sukenik, A., Herrero, C., 1998. Effects of nitrogen source and growth phase on proximate bio-chemical composition, lipis classes and fatty acid profile of the marine microalga *Isochryis galbana*. Aquacul., 166:105-116.

Gouveia, L., Batista, A.P., Sousa, I., Raymundo, A., Bandarra, N.M., 2008. Microalgae in novel foods products. In: Food Chemistry Research Developments (ed. KN Papadopoulos). Nova Science Publishers, 75-111.

Guillard, R.R.L. 1975. Culture of phytoplankton for feeding marine invertebrates. pp. 29−60 in: W.L. Smith and H.H. Chanley (eds.). *Culture of Marine Invertebrate Animals.* Plenum Press, New York, New York.

Guri, A., 1983. Variation in glutathione and ascorbic-acid content among selected cultivars of Phaseolus-vulgaris prior to and after Exposure to ozone. Can. J. Plant Sci., 63: 733–737.

Hammouda, O., Gaber, A., Abdel-Raouf, N.1995. "Microalgae and wastewater treatment," Ecotoxicol. Environ. Saf., 31: 205-210.

Hanisak, M.D., 1990. The use of *Gracilaria tikvahiae* (Gracilariales, Rhodophyta) as a model system to understand the nitrogen nutrition of cultured seaweeds. Hydrobiol., 204/205: 79-87.

Hapette, A.M., Poulet, S.A., 1990. Variation of vitamin C in some common species of marine plankton. Mar. Ecol. Prog. Ser., 64: 69-79.

Hoffmann, L.J., Peeken, I., Lochte, K., 2007. Co-limitation by iron, silicate, and light of three Southern Ocean diatom species. Biogeosciences Discuss., 4: 209–247.

Holland, D.L., 1978. Lipid reserves and energy metabolism in the larvae of benthic marine invertebrates. In: Malins D.C. and Sargent J.R. (eds), Biochemical and Biophysical Perspectives in Marine Biology, 4. Academic Press, New York, 85–123.

Kanazawa, A. S., 1985. Essential fatty acid and lipid requirement of fish. In C.B. Cowey, A.M. Mackie & J.G. Bell (eds), Nutrition and Feeding in Fish. Academic Press, London: 281-294.

Khairy, H.M., El-Sayed, H.S. 2012. Effect of enriched *Brachionus plicatilis* and *Artemia franciscana* nauplii by microalga *Tetraselmis chuii* (Bütcher) grown on four different culture media on the growth and survival of *Sparus aurata* larvae. Afr. J. Biotech., 11(2):. 399-415.

Khatoon, H., Yusoff, F.M., Banerjee, S., Shariff, M., Mohamed, S. 2007. Use of periphytic cyanobacterium and mixed diatoms coated substrate for improving water quality, survival and growth of *Penaeus monodon* Fabricius postlarvae. Aquacul., 271: 196-205.

Lapointe, B.E., 1986. Phosphorus-limited photosynthesis and growth of *Sargassum natans* and *Sargassum fluitans* (Phaeophyceae) in the

western North Atlantic. Deep-Sea Res. 33: 391–399.

Lavens, P., Sorgeloos, P., 1996. Manual on the production and use of the live food for aquaculture. FAO, Fisheries Technical paper, Rome, 360: 361.

Leblanc, K., Hare, C.E., Boyd, P.W., 2005. Fe and Zn effects on the Si cycle and diatom community structure in two high and low-silicate HNLC areas, Deep-Sea Res. I, 52: 1842–1864.

Leonardos, N., Lucas, I.A.N., (2000). The nutritional value of algae grown under different culture conditions for *Mytilus edulis* L. larvae. Aquacul., 182:301-315.

Li, Y.Q., Horsman, M., Wang, B., 2008. Effects of nitrogen sources on cell growth and lipid accumulation of green alga Neochloris oleoabundans. Appl. Microbiol. Biotechnol., 81: 629–636.

Li, M.H., Robinson, E. H., 1999. Effect of reducing dietary digestible energy to protein ratio on weight gain and body fat of juvenile channel catfish (*Ictalurus punctatus*). J. World Aquacult. Soc., 30:123-127.

Lin, Y.H., Chang, F.L., Tsao, C.Y., Leu, J.Y., 2007. Influence of growth phase and nutrient source on fatty acid composition of *Isochrysis galbana* CCMP 1324 in a batch photoreactor. Biochem. Eng. J. 37: 166–176.

Liu, C.P., Lin, L.P., 2001. Ultrastructural study and lipid formation of *Isochrysis* sp. CCMP1324. Bot. Bull. Acad. Sin., 42: 201-214.

Lobban, C.S., Harrison, P.J., 1994. Seaweed Ecology and Physiology. Cambridge: Cambridge University Press.

Lovell, R.T., Lim, C., 1978. Vitamin C in pond diets for channel catfish. Trans. Am. Fish. Soc. 107: 321-325.

Lowry, O.M., Rosebrough, N.J., Farr, L.A., Randall, R.J., 1951. Protein measurements with the folin phenol reagent. J. Biol. Chem., 193: 265-275.

Martínez-Fernández, E., Acosta-Salmón, H., Southgate, P.C., 2006. The nutritional value of seven species of tropical microalgae for Black-lip pearl oyster (*Pinctada margaritifera*, L.) larvae. Aquacul., 257: 491–503.

Molina Grima, E., Sanchez Perez, J.A., Garcia Camacho, F., Garcia Sanchez, J.L., Fernandez Sevilla, J.M., 1994. Variation of fatty acid profile with solar cycle in outdoor chemostat culture of *Isochrysis galbana* AL II4. J. Appl. Phycol., 7:129-134.

Mortensen, S.H., Børsheim, K.Y., Rainuzzo, J.R., Knutsen, G., 1988. Fatty acid and elemental composition of the marine diatom *Chaetoceros gracilis* Schütt. Effects of silicate deprivation, temperature and light intensity. J. Exp. Mar. Biol. Ecol., 122 (2): 173-185.

Muller-Feuga, A., 2000. "The role of microalgae in aquaculture: situation and trends," J. Appl. Phycol., 12: 527-534.

Napolitano, G.E., Ackman, R.G., Silva-Serra, M.A., 1993. Incorporation of sterols by the sea scallop *Placopecten magellanicus* (Gmelin) fed on microalgae. Mar. Biol., 117: 647–654.

NRC (National Research Council), 1993. Nutrient requirements of fish. National Academy Press. Washington DC, USA, 114 pp.

Patil, V., Källqvist, T., Olsen, E., Vogt, G., Gislerød, H.R., 2007. Fatty acid composition of 12 microalgae for possible use in aquaculture feed. Aquacul. Int., 15:1-9.

Pernet, F., Tremblay, R., Demers, D., Roussy, M., 2003. Variation of lipid class and fatty acid composition of *Chaetoceros muelleri* and *Isochrysis* sp. Grown in a semicontinuous system. Aquacul., 221: 393–406.

Pettersen, A.K., Turchini, G.M., Jahangard, S., Ingram, B.A., Sherman, C.D.H., 2010. Effects of different dietary microalgae on survival, growth, settlement and fatty acid composition of blue mussel (*Mytilus galloprovincialis*) larvae. Aquacul., 309:115-124.

Pollero, R.J., Irazu, C.E., Brenner, R.R., 1983. Effect of sexual stages on lipids and fatty acids of *Diplodon delodontus*. Comp. Biochem. Physiol., 76: 927–931.

Ratledge, C., 2004. Fatty acid biosynthesis in microorganisms being used for single cell oil production. Biochim., 86, 807–815.

Riquelme, C,E., Avendaño-Herrera, R.E., 2003. Microalgae and bacteria interaction in the aquatic environment and their potential use in aquaculture. Rev. Chil. Hist. Nat., 76: 725-736.

Rivero-Rodríguez, S., Beaumont, A.R., Lora-Vilchis, M.C., 2007. The effect of microalgal diets on growth, biochemical composition, and fatty acid profile of *Crassostrea corteziensis* (Hertlein) juveniles. Aquacul., 263:199-210.

Rousch, J.M., Scott, S.E., Sommerfeld, M.R., 2003. Changes in fatty acid profiles of thermo-intolerant and thermo-tolerant marine diatoms during temperature stress. J. Exp. Mar. Biol. Ecol., 295:145-156.

Sánchez, S., Martinez, M.E., Espinola, F., 2000. Biomass production and biochemical variability of the marine microalga *Isochrysis galbana* in relation to culture medium. Biochem. Eng. J., 6: 13–18.

Shifrin, N.S., Chisholm, S.W., 1981. Phytoplankton lipids: inter specific differences and effects of nitrate, silicate and light-dark cycles. J. Phycol., 17: 374-384.

Strickland, J.D.H., Parsons, T.R., 1972. A practical handbook of seawater analysis. Fisheries Research Board of Canada Bulletin 167: 310 pp.

Sukenik, A., Wahnon, R., 1991. Biochemical quality of marine unicellular algae with special emphasis on lipid composition: I. *Isochrysis galbona.* Aquacul., 97, 61-72.

Takeda, S., 1998. Influence of iron availability on nutrient consumption ratio of diatoms in oceanicwaters. Nature, 393: 774-777.

Thomas, W.H., Seibert, D.L.R., Alden, M., Neori, A., 1981. Effects of light intensity and nitrogen deficiency on yield, proximate composition, and photosynthetic efficiency of *Phaeodactylum.* SERI/CP-624-1228ed, In: Proceedings of the Subcontractors' Review Meeting-Aquatic Species Program. Solar Energy Research Institute, US Department of Energy, 33–58.

Wikfors, G.H., Ferris, G.E., Smith, B.C. 1992. The relationship between gross biochemical composition of cultured algal foods and growth of the hard clam, *Mercenaria mercenaria* (L.). Aquacul., 108: 135–154.

Zaki, M.I., Saad, H., 2010. Comparative study on growth and survival of larval and juvenile *Dicentrarchus labrax* rearing on rotifer and *Artemia* enriched with four different microalgae species. Afr. J. Biotech., 9 (24): 3576-3588.

Zhukova, N.V., Imbs, A.B., Yi, L.F. 1998. Diet-induced changes in lipid and fatty acid composition of *Artemia salina*. Comp. Biochem. Physiol., 120B: 499–506.

دراسه مقارنه لأنتاج الكتله الحيويه والمكونات البيوكيميائيه لنوعين من الطحالب البحريه الدقيقه المرباه على مصدرين للمياه لأستخدامها فى تقييم الأرتيميا فرانسيسكانا

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يهدف هذا البحث الى دراسه النمو والكتله الحيويه والمحتوى الكلى من البروتين والدهون وفيتامين سى وأيضا كميه الأحماض الدهنيه لنوعين من الطحالب البحريه أحدهما من الدياتومات البنيه (*Chaetoceros gracilis*) والأخر من الطحالب البنيه الذهبيه وهو الطحلب السوطى *Isochrysis galbana*. تم تنميه هاذان الطحلبان على مصدرين من مصادر المياه المستخدمه للأستزراع البحرى بالمعهد القومى لعلوم البحار والمصايد بالأسكندريه (مياه الميناء الشرقى بالأسكندريه EH ومياه بير المكس El-Mex well water) وذلك بعد اضافه الوسط الكيميائى (F/2). وبعد استزراع هاذان الطحلبان تم استخدامهما فى غذاء الأرتيميا سالينا وذلك لعمل اثراء لها لكى يتم تغذيه الأسماك بها فيما بعد. وتم هذا الأستزراع لقياس مدى كفاءه هاذان المصدران لتحسين وزياده وجوده الأستزراع البحرى بالمعهد. تم تحليل مياه المصدرين المستخدمين وأوضحت النتائج وجود كميات منخفضه من الملوحه والفوسفات وكميات عاليه من النترات والسليكات والحديد وذلك فى مياه بير المكس وعلى العكس فى مياه الميناء الشرقى. وأوضحت النتائج المعمليه أنه قد تم الحصول على أعلى انتاج من النمو والكتله الحيويه والبروتين الكلى لطحلب *Ch. gracilis* الذى تم تربيته على مياه بير المكس المزوده بالوسط الكيميائى F/2 وعلى العكس بالنسبه لطحلب *I. galbana* الذى حقق أعلى نمو والكتله الحيويه والبروتين الكلى فى حاله تنميته على مياه الميناء الشرقى المزوده بالوسط الكيميائى. أما بالنسبه لكميه فيتامين سى فأن أعلى كميه له ظهرت فى *I. galbana* (١١,٣١ ميكرومول لكل جرام) الذى تم تربيته على بير المكس المزوده بالوسط الكيميائى F/2 .

بالنسبه للأحماض الدهنيه الكليه فأوضحت النتائج أنه تم تحفيز كلا من الطحلبين على انتاج كميات عاليه من الأحماض الدهنيه عند تنميتهم على مياه بير المكس المزوده بالوسط الكيميائى F/2 وكانت نسبه الأحماض الدهنيه المشبعه أعلى من نسبه الأحماض الدهنيه الغير مشبعه وخصوصا *Ch. gracilis* النامى على مياه الميناء الشرقى وكانت نسبتها (٨٥,٦%) من نسبه الأحماض الدهنيه الكليه. وهذه الزياده ترجع الى زياده نسبه C14:0 , C16:0 , C18:0 . بالنسبه للأحماض الدهنيه وحيده التشبع فكانت أعلى كميه لها فى طحلب *Ch. gracilis* النامى على مياه بير المكس (٣١ %) وذالك يرجع لزياده نسبه C16:1 , C22:1 التى كونتا 22 و ٥,١ % من نسبه الأحماض الدهنيه الكليه. و بالنسبه للأحماض الدهنيه عديده عدم التشبع أوضحت النتائج أن أعلى نسبه منها ظهرت فى *I. galbana* (27 %) الذى تم تنميته على مياه يئر المكس وذلك لزياده نسبه C22:6 , C20:5 التى كونتا ٦,٩ و ٨,٣ % على التوالى. فى حاله تغذيه *Artemia franciscana* بالطحلبين بعد تنميتهم على مياه المصدرين المختلفين وجد انه عند تحليل الأحماض الدهنيه لها أن نسبه الأحماض الدهنيه المشبعه بها قلت بالمقارنه بالأحماض الدهنيه الغير مشبعه وكانت أعلى زياده فى الأحماض الدهنيه عديده عدم التشبع فكانت أعلى نسبه مئويه فى الأرتيميا المغذاه على *I. galbana* (٤٧,٦ %) وهى أعلى من النسبه التى وجدت فى *Ch. gracilis* وهى (٢٤ %) بالنسبه لمجموع الأحماض الدهنيه الكليه وهذا يرجع الى زياده نسبه C20:5, C20:4 C22:6 (٨,٥ ، ٩,٤ ، ١٠,٤ % على التوالى من نسبه الأحماض الدهنيه الكليه) فى *Artemia franciscana التى تم تغذيتها على* طحلب *I. galbana* الذى تم تنميته على مياه بير المكس. وأخيرا نستنتج من هذا البحث أن مياه بير المكس بيئه مناسبه لأستزراع كلا الطحلبين لتحقيق أعلى قيمه غذائيه للأرتيميا وذلك بأنتاج أعلى قيمه من الأحماض الدهنيه عديده التشبع وهذه النتيجه تؤدى الى تحسين انتاجيه الأستزراع البحرى.