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Growth and biochemical variability of complete and lipid extracted *Chlorella* species (application for *Artemia franciscana* feeding)

Hala Y. El-Kassas[1] · Nagwa G.-E. Mohammady[2] · Heba S. El-Sayed[3] · Basma A. ELSherbiny[1]

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Abstract This work aims at culturing different *Chlorella* species, monitoring growth and estimating the nutritional quality for further application of complete cells and lipid extracted biomass in *Artemia franciscana* feeding. We conducted experiments using *C. marina*, *C. salina*, *C. capsulata*, *C. stigmatophora* and *C. vulgaris* that were batch cultured for 14 days. *C. salina* showed the maximal count on the sixth day while *C. marina* recorded the maximum growth rate (2 ± 0.177). However, *C. capsulata* and *C. stigmatophora* recorded the minimum rate (1.5 ± 0.11). Analyses of algal biomass showed that *C. capsulata* contains maximal lipids and carbohydrates, but the minimal protein (22.8 ± 1.4 %). However, *C. salina* contained the highest protein (33.1 ± 1.4 %). After oil extraction, there were no significant losses in the other biochemical constituents of the studied *Chlorella* species. Considering algae metabolites, saturated fatty acids were the main constituent in the fatty acids methyl esters (FAMEs). Palmitic and stearic acids were dominant. Amino acid pools of the experimental marine *Chlorella* species were found to contain lysine, methionine and histidine; but were deficient in cysteine. The present investigation showed that lipids, proteins and protein to lipid ratio of *A. franciscana* napulli enriched with mixed cells of *Chlorella* species were enhanced by (22 %); 1.96 and 1.33 folds, respectively. Furthermore, the growth and survival of *A. franciscana* showed significant increases when fed on lipid extracted algae residuals, especially that of a mixed diet; which is considered as an important achievement and confirms that the residual algae biomass can be significantly used for aquaculture feeding.

Keywords *Chlorella* species · Biochemical variability · *Artemia franciscana*

1 Introduction

The nutritional quality of microalgae biomass plays a noticeable role in the diet of many living organisms including marine animals. They are required for larval nourishment, either for direct or indirect utilization as food for live prey, such as *Artemia* nauplii, which are successively used for aquaculture feeding (Muller-Feuga 2000).

The microalgae strains are known as an incredible wellspring of valuable biochemicals that can be used as food and food additives (Rocha et al. 2003) where the proteins and poly unsaturated fatty acids (PUFAs) are of principle significance (Spolaore et al. 2006), while the microalgae lipids are extremely significant at various stages of marine fish larvae of many fish, mussel and oyster species (Ronquillo et al. 2012). Algal lipids have been reported to represent a good source for nutrition in aquaculture industry (Adarme-Vega et al. 2012). The quality and quantity of lipids are of incredible significance in the nutritional value of microalgae in aquaculture as mentioned by El-Sheekh et al. (2015).

The content of polyunsaturated fatty acids (PUFA) is crucial for the use of microalgae in aquaculture (Patil et al. 2005). Omega 3 (ω3) and omega 6 (ω6) are quite

✉ Hala Y. El-Kassas
halayassin12@yahoo.com

[1] Hydrobiology Laboratory, National Institute of Oceanography and Fisheries, Alexandria, Egypt

[2] Botany and Microbiology Department, Faculty of Science, Alexandria University, Alexandria, Egypt

[3] Fish Reproduction Laboratory, National Institute of Oceanography and Fisheries, Alexandria, Egypt

compelling. In spite of that, animal lack the required enzymes to synthesize PUFA, it must be obtained from food and, therefore, is often known to be vital (Milledge 2011). Therefore, deficiency in (PUFAs) seems to be the main cause of the low survival rates of larvae (Patil et al. 2005). As a result, microalgae have been used as a dietary source for aquatic organisms, with fatty acid contents being the centric agent in the selection of microalgal species (Huerlimann et al. 2010). The complete utilization of algal biomass may involve the combination of different technologies (Wiley et al. 2011). Algae lipids can be extracted for biofuel production and the leftover solids, which are mostly carbohydrates and proteins are useful for larvae enrichment (El-Sheekh et al. 2015).

Successful algal screening mainly relies upon selecting the right species with apropos properties including biomass and fatty acid productivity. The major economic bottlenecks are algae productivity, followed by labor and then the harvesting costs as stated by (Abomohra et al. 2014).

Different green algal genera are used in aquaculture including *Chlorella* species (Muller-Feuga 2000) relying on the requirements of seafood production (Pulz and Gross 2004). Algae have also been selected in premise to their mass-culture potential of proper cellular size, digestibility and their overall essential nutritional value (Courtois de Viçose et al. 2012). Brine shrimp nauplii are non-selective particle feeders; simple methods have been emerging to incorporate various sorts and varieties of products into the *Artemia* prior to feeding to larvae. *Artemia* enrichment is widely applied in marine fish and crustacean hatcheries for enhancing the nutritional quality of *Artemia* with essential fatty acids for further aquaculture feeding (Sorgeloos et al. 2001).

Therefore, the objectives of this research were aiming to: (1) Monitor the growth and nutritional quality of five *Chlorella* species. (2) Study the application of the different *Chlorella* species for *Artemia franciscana* feeding. (3) Follow up the growth of *A. franciscana* in terms of fresh weight and survival % that fed on lipids extracted *Chlorella* species for 12 days to verify whether there is a long-term positive effect.

Five *Chlorella* species namely, *C. salina*, *C. marina*, *C. capsulata*, *C. stigmatophora* and *C. vulgaris* were grown in a batch culture. Algal growth rates, lipid production capacity, in addition to proteins as well as carbohydrate contents and ashes were determined. The algae metabolites including, fatty acids and amino acids compositions were also estimated. The algal cells were harvested by flocculation to reduce the cost of harvest. Complete algae cells and the residual algal biomass after oil extraction, were used for *A. franciscana* feeding. As *A. franciscana* nauplii are used in aquacultures within 48 h of enrichment. The proximate and FAMEs analyses of the enriched *A. franciscana* nauplii were carried out.

2 Experimental

2.1 Algae strains and growth conditions

Five species of genus *Chlorella* were chosen for this study. *C. marina*, *C. capsulata* and *C. stigmatophora* were kindly provided by Dr. I. Tzovenis, NKUA, was originally isolated from Crete, Greece. *C. salina* was obtained from the marine hatchery of the National Institute of Oceanography and Fisheries in Alexandria, Egypt. The fresh water alga *C. vulgar* was obtained from the algae culture collection, Botany department, Faculty of Science, Alexandria University. The starting inocula were: $0.3 \pm 0.03 \times 10^6$, $0.2 \pm 0.02 \times 10^6$, $0.3 \pm 0.03 \times 10^6$, $0.5 \pm 0.04 \times 10^6$ and $0.5 \pm 0.04 \times 10^6$ for *C. salina*, *C. marina*, *C. capsulata*, *C. stigmatophora* and *C. vulgaris*, respectively. The marine *Chlorella* species were grown in axenic modified F medium as described by Guillard and Ryther (1962). On the other hand, *C. vulgaris* was grown in Amaral's medium (do Amaral and Freire 2012). All growth media were prepared using analytically grade reagents supplied by sigma (St. Louis, USA). The cultures were grown at 28 °C ± 3 °C with the light intensity of 80 μ mol m^{-2} s^{-1} in a controlled culture chamber under a regime of 16:8 light/dark cycle.

2.2 Monitoring of algal growth

The growth of the tested organisms was determined by cell count using the haemacytometer slide, where cell numbers were estimated at 24 h intervals. In addition to that, the growth rate was calculated using the formula proposed by Robert (1979):

$$R = \left(\frac{3.322}{t_2 - t_1}\right) \cdot \left(\log \frac{N_2}{N_1}\right)$$

where: 3.322 = growth constant, t_1 time at the beginning of the experiment, t_2 time at the end of the experiment, N_1 number of cells/ml culture at t_1, N_2 = number of cells/ml culture at t_2.

2.3 Proximate analyses of the studied *Chlorella* species

2.3.1 Determination of total lipids

For lipid extraction, the algal cells were collected at the end of the logarithmic development stage by centrifugation at 1000*g* for 5 min. Cells were homogenized with chloroform—methanol (2:1 v/v) and refluxed for a couple of minutes to inactivate the phospholipases. Purification of the extracts was performed according to Bligh and Dyer (1959). The total lipid contents were estimated for different algae cultures.

2.3.2 Estimation of carbohydrate

Total carbohydrate was evaluated using the colorimetric technique by (DuBois et al. 1959). The cells were collected by centrifugation at 5000 rpm for 10 min and measured specimens were blended with 1 mL of 5 % aqueous solution of phenol in a test tube. Therefore, 5 mL of 95 % sulfuric acid was added to the blend. Subsequently, the test tubes were permitted to remain for 10 min. They were then vortexed for a few moments and set for 20 min in a water bath at room temperature for yellow–orange color development. Light absorption was measured at 490 nm using the UV-spectrophotometer.

2.3.3 Determination of total protein

Total protein was extracted from the algal cells, according to the method of Rausch (1981). Protein content, both total and water-soluble, was determined according to Hatree (1972).

2.3.4 Ash determination

Ash contents were estimated according to AOAC (1995). Oxidation of organic matter was done using a muffle at 550 °C overnight.

2.4 Estimation of algal metabolites

2.4.1 Analysis of fatty acids methyl esters (FAMEs)

Investigation of fatty acid methyl esters (FAMEs): Total lipid portions in the distinctive green growth species were subjected to saponification. Afterwards, it was changed into methyl esters taking after the methodology of Radwan (1978). Popularities were measured and recognized utilizing gas chromatography (GC framework Hp, Germany, serial No 6890 D 1530 A serial DE 00000348) furnished with a fire ionization locator; the pressing segment material was SP-2340. The transporter gas was nitrogen and the short speed was 5 mm min^{-1}. Distinguishing proof of FAMEs was done by contrasting their maintenance times and those of the benchmarks'. Measurement depended on the inner defamed strategy.

2.4.2 Analysis of amino acid composition

Different amino acids, except for tryptophane, were extracted and determined by the method described by Spackman et al. (1958) using a Beckman 119 CL amino acid analyzer.

2.5 Enrichment of *Artemia franciscana* with the studied *Chlorella* species

Brine Shrimp *A. franciscana* was created by hatching *Artemia* cysts through a decapsulation technique. They were hatched as depicted by Lavens and Sorgloos (1996). Produced nauplii were harvested after 24 h and then washed with filtered sea water. After 6 h from hatching time, *Artemia* nauplii (instar II) were grown on their growth media in the presence of 0.5 g L^{-1} of dried biomass of the experimental *Chlorella* species. A trial to use a mixture of the dried experimental *Chlorella* species was also carried out. After 48 h, the enriched *Artemia* was harvested by plankton net (100 µm). Then, the proximate analyses were carried out by the previously described methods. Bligh and Dyre method (lipid), DuBois' method (carbohydrate) and Hatree's method (protein) were performed. Fatty acid fractions were also measured through Radwan esterification method (1978) which analyzes the fatty acid compositions of each *A. franciscana* treatment. All these methods were mentioned before in details in the algal analysis.

2.6 Following up the growth and survival of *Artemia franciscana* fed on lipid extracted *Chlorella* biomass

Artemia franciscana specimens were cultivated in 500 mL of 15 g L^{-1} artificial sea water. Approximately, 0.5 g L^{-1} of dried lipid extracted biomass of the different experimental *Chlorella* species were added to each culture which was aerated using air pump. Six treatments were decided to run the experiments for 12 days, one experiment for each *Chlorella* species and another one for a mixed diet of the different *Chlorella* species. Survival of *A. franciscana* was recorded by estimating the number of living *A. franciscana* for each experimental trial. Also, Twenty *Artemia* were sampled from each experimental trial at the beginning and end of the experiment to estimate their growth by measuring fresh weight (Pacheco-Vega et al. 2015).

2.7 Statistical analysis

All values presented in this study are the means of triplicate trials for each treatment; error bars in the figures depict the standard deviations (SD) of these triplicates.

3 Results

3.1 Monitoring of algae growth

The growths of the studied algae were compared and illustrated graphically (Fig. 1). *Chlorella* species started their stationary phase of growth on the 6th day of incubation and reached the maximum number on the 10th day. *C. salina* showed the best performance of cell division (26.5 $mL^{-1} \times 10^6$); while *C. marina* showed the lowest (10 $mL^{-1} \times 10^6$). On the other hand, *C. marina* recorded the maximum growth rate (2.0) after 3 days of incubation, followed by *C. vulgaris* (1.78). Similarly, the two algae *C. capsulata* and *C. stigmatophora* recorded the same growth rate (1.5).

3.2 Proximate analyses of the studied *Chlorella* species

3.2.1 Complete algae cells

The valuable constituents of *Chlorella* species are in Fig. 2. Lipid production potential showed that *C. capsulata* produced the highest lipid content (44.6 ± 3.4 %), followed by *C. salina* and *C. marina* (26.8 ± 2.08 and 26.7 ± 2.6 %), respectively. However, *C. vulgaris* came in last on the list (17.6 ± 2.5 %).

Carbohydrate content ranged from 12.8 ± 0.33 to 22.0 ± 0.57 % in *C. salina* and *C. capsulata,* respectively. Algal proteins are represented as the sum of the monomeric soluble and structural amino acids entrained in biomass; data

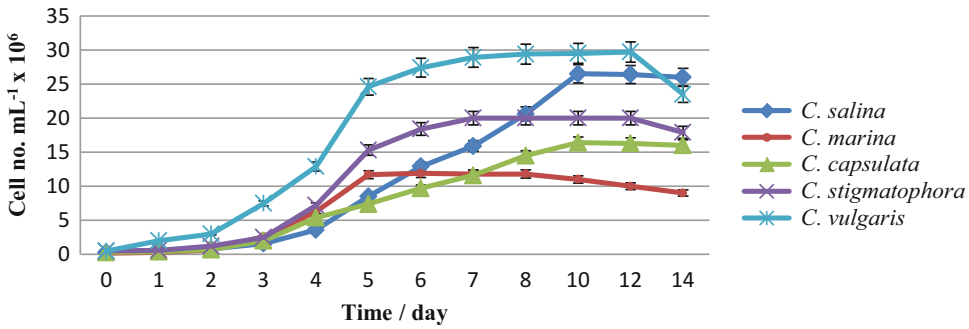

Fig. 1 Growth curve of the five experimental *Chlorella* species expressed as cell no. m $L^{-1} \times 10^6$

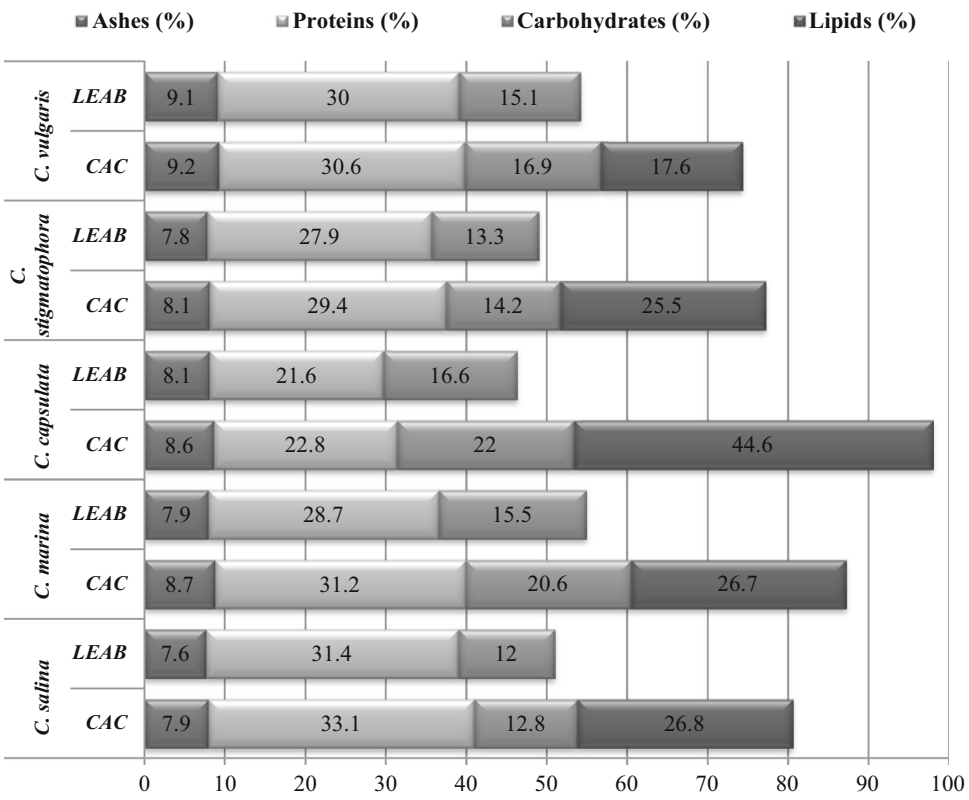

Fig. 2 Biochemical variability of complete and lipid extracted *Chlorella* species. *CAC* complete algae cells, *LEAB* lipids extracted algae biomass

recorded for different *Chlorella* species showed that proteins ranged from 22.8 ± 1.4 % in *C. capsulata* to 33.1 ± 1.4 % in *C. salina.* However, it recorded 30.6 % in *C. vulgaris.*

Ash contents present in the studied *Chlorella* species ranged between 7.9 ± 0.2 and 9.2 ± 0.2 % of the algal dry weight in *C. salina* and *C. vulgaris*, respectively.

3.2.2 Lipid extracted algae biomass

In lipid extracted algal biomass, the data recorded in Fig. 2 revealed that carbohydrate contents ranged between 15.5 ± 0.40 and 12.0 ± 0.31 % in *C. marina* and *C. salina*, respectively. It decreased by about (0.6 %) and (5.4 %) in *C. stigmatophora* and *C. capsulata*, respectively, when compared to the complete *C. stigmatophora* and *C. capsulata* cells. Protein content of *C. vulgaris* decreased by only 0.6 %. The two algae, *C. stigmatophora* and *C. capsulata* decreased by nearly 1.7 %, 1.2, respectively. However, *C. marina* and *C. salina* recorded about a 2.5 % decrease in protein contents in comparison to the complete corresponding algal cells. The ash contents present in the five studied *Chlorella* species ranged between 7.6 ± 0.2 and 9.1 ± 0.2 % of the algal dry weight in *C. salina* and *C. vulgaris*, respectively. Less than a 1 % decrease in ash contents in all the studied *Chlorella* species has been recorded.

Generally, the biochemical constituents, including proteins and carbohydrates as well as ashes in the lipid extracted *Chlorella* species were not significantly different from the complete algae cells.

3.3 Fatty acids compositions in the experimental *Chlorella* species

Fatty acids compositions in the different experimental *Chlorella* species are shown in Table 1. The highest percentages of SFA were observed for *C. salina* and *C. capsulata* followed by *C. stigmatophora* (94.2; 93.0; 87.0 %), respectively.

Different types of saturated fatty acids were detected in the different experimented *Chlorella* sp. Palmitic acid (C16:0) was found predominant in the algal lipid. The highest percentages were recorded with *C. capsulata* and *C. stigmatophora*. In addition, stearic (C18:0) and undecanoic (C11:0) acids are the most fatty acid composition of the experimented species. All of the 5 experimented *Chlorella* species contained little quantities of mono unsaturated eicosanoic acid (C20:0).

Fatty acids with different degrees of unsaturation have been recorded. The MUFAs [Oleic acid (18:1) and myristic acid (C14:1)] along with pentadecanoic acid, lauric acid and capric acid were determined. The highest values for the USFA were reported in *C. marina* and *C. vulgaris*, each containing 20 %. Also, *C. vulgaris* didn't show any contents of mono unsaturated myristoleic acid (C14:1) or saturated capric acid (C10:0). On the other hand, *C. marina* contained the highest percentage of polyunsaturated α-linolenic acid (C18:2c).

3.4 Amino acid composition of delipidated cells

The individual amino acids of delipidated *C. salina*, *C. marina*, *C. capsulata*, *C. stigmatophora* and *C. vulgaris* were recorded in Table 2. The results showed that Krebs cycle family and aliphatic amino acids family surpassed the other groups of amino acids. The total amino acids of Krebs cycle family in the examined species of *Chlorella* represented nearly 50 % of the total amino acids. The percentages of aliphatic amino acids for *C. salina*, *C. marina*, *C. capsulata*, *C. stigmatophora* and *C. vulgaris* represented by therionine, serine, glycine, alanine, valine, leucine and isoleucine were 47.24, 43.12, 85.75, 68.34 and 72.04 %, respectively. That was found to be the opposite of aromatic amino acids (Tyrosine and Phenylalanine), which were detected in traces. In addition to acidic amino acids, there were the asparatic acid and the glutamic acid for delipidated biomass of *C. salina*, *C. marina*, *C. capsulata*, *C. stigmatophora* and *C. vulgaris*; they measured at 38.05, 15.82, 3.92, 7.18 and 10.62 %, respectively. Besides that, basic amino acids (lysine and arginine) had results of 6.69, 24.27, 6.32, 2.85 and 11.97 % in the same species, respectively. Finally, sulfur containing amino acids (cysteine and methionine) and secondary amino acid (proline) were found in a few fractions.

3.5 Feeding of *Artemia franciscana* with the different studied *Chlorella* species

3.5.1 Biochemical constituents

The study was extended to apply both the complete and the lipid extracted algae cells in *A. franciscana* feeding. The results in Table 3 indicated that *A. franciscana* enriched with complete cells of the experimented *Chlorella* species resulted in improvements of their lipid contents. The increase in the percentage of lipids were 53, 20, 8 % as well as 10 and 37 % for *C. salina*, *C. marina*, *C. capsulata* as well as *C. stigmatophora* and *C. vulgaris*, respectively in comparison to the controlled *A. franciscana.* The lipid contents of *A. franciscana* increased by 1, 4, 3, 0.00 and 5 % for *C. salina, C. marina, C. capsulata* as well as *C. stigmatophora* and *C. vulgaris*, respectively, when compared with the control. When *A. franciscana* was enriched with mixed complete algae cells, the lipid contents increased by about 22 %, which was the same discovered result after the enrichment with *C. marina* complete cells.

Table 1 Fatty acids methyl esters (FAMEs) composition of total lipids mg/g fresh weight and the percent of fatty acids content of the different experimental *Chlorella* species

Fatty acids	*C. salina*		*C. marina*		*C. capsulata*		*C. stigmatophora*		*C. vulgaris*	
	mg/g	%	mg/g	%	mg/g	%	mg/g	%	mg/g	%
Saturated FAs (SFA)										
C6:0 (Hexanoic)	0.3	1.1	0.2	0.3	5.0	7.8	ND	ND	0.21	0.7
C8:0 (Caproic)	2.2	7.1	1.8	4.0	1.1	2.0	0.2	0.6	ND	0.2
C10:0 (Capric)	0.2	0.6	0.3	0.6	0.2	0.3	0.1	0.4	ND	ND
C11:0 (Undecanoic)	0.3	1.0	0.8	1.8	ND	ND	0.2	0.8	0.04	0.1
C12:0 (Lauric)	0.6	2.1	0.2	0.4	0.2	0.2	0.1	0.5	0.03	0.1
C13:0 (Tridecylic)	0.3	1.0	0.2	0.4	ND	ND	0.1	0.4	0.22	0.7
C14:0 (Myristic)	1.6	5.0	0.7	1.6	ND	ND	0.7	2.6	0.12	0.4
C15:0 (Pentadecanoic)	ND	ND	ND	ND	ND	ND	0.5	1.8	0.12	0.4
C16:0 (Palmitic acid)	15.7	49.8	19.4	41.1	32.3	51.4	15.0	55.8	3.7	11.7
C17:0 (Heptadecyclic)	ND	ND	0.5	1.1	ND	ND	0.1	0.5	0.06	0.2
C18:0 (stearic)	7.1	22.5	9.8	20.7	16.1	25.6	5.1	19.0	2.22	7.0
C20:0 (Eicosanoic)	1.3	4.0	3.4	7.3	3.6	5.7	1.4	5.0	2.68	8.5
C21:0 (Heneicosylic)	ND	ND	ND	ND	ND	ND	0.1	0.4	9.63	30.6
C22:0 (Behenic)	ND	ND	ND	ND	ND	ND	ND	ND	4.1	13.0
C23:0 (Tricosylic)	ND	ND	ND	ND	ND	ND	ND	ND	2.04	6.5
Monounsaturated FAs (MUFA)										
C14:1 (Myristoleic)	0.7	2.3	0.5	1.1	0.8	1.3	0.1	0.5	ND	ND
C15:1 (cis-10-pentadecenoic)	ND	ND	ND	ND	ND	ND	0.1	0.4	0.02	0.06
C16:1 (Palmitoleic)	ND	ND	2.3	4.8	ND	ND	0.2	0.8	0.13	0.4
C17:1 (Heptadecenoic)	ND	ND	ND	ND	ND	ND	0.1	0.4	0.04	0.1
C18:1ω9c (Oleic)	ND	ND	4.3	9.0	ND	ND	1.5	5.4	1.01	3.2
C22:1 (Docosenoic)	ND	ND	ND	ND	ND	ND	ND	ND	4.15	13.2
Polyunsaturated FAs (PUFA)										
C18:2ω6c (Linoleic)	0.3	1.0	2.7	5.8	1.6	2.5	1.0	3.5	0.60	2.0
C18:3ω3 (Alpha-Linoleic)	0.2	0.6	ND	ND	0.2	0.2	0.1	0.3	0.0	0.0
C20:4ω6 (Arachidonic acid)	ND	ND	ND	ND	ND	ND	ND	ND	0.29	1.0
C22:2 (Docosadienoic)	0.6	1.9	ND	ND	1.9	3.0	0.2	0.9	ND	ND
∑SFA	29.6	94.2	37.3	79.3	58.5	93.0	23.6	87.8	25.23	80.0
∑UFA	1.8	5.8	9.8	20.7	4.5	7.0	3.3	12.2	6.24	20.0
∑MUFA	0.7	2.3	7.1	14.9	0.8	1.3	2.0	7.5	5.35	17.0
∑PUFA	1.1	3.5	2.7	5.8	3.7	5.7	1.3	4.7	0.89	3.0
PUFAs-ω3	0.2	0.6	ND	ND	0.2	0.2	0.1	0.3	ND	ND
PUFAs-ω6	0.3	1.0	2.7	5.8	1.6	2.5	1.0	3.5	0.89	3.0

ND not detected

Results in Table 3 showed that *A. franciscana* enriched with the complete cells of the experimental *Chlorella* species resulted in the improvement of its carbohydrate content. The percentage of carbohydrate increased by 11, 9.23, 11.9 % as well as 16.3 and 9.6 % for *C. salina*, *C. marina*, *C. capsulata* as well as *C. stigmatophora* and *C. vulgaris*, respectively, when compared to the control. On the other hand, there were fewer increases in the carbohydrate contents of *A. franciscana* that had been enriched with lipid extracted biomass of the different experimented *Chlorella* species when compared to the control. The carbohydrate content in *A. franciscana* increased by 10.4, 9.8, 10.2, 12 and 7.24 % for *C. salina*, *C. marina*, *C. capsulata* as well as *C. stigmatophora* and *C. vulgaris*, respectively when compared to the controlled. In contrast to the lipid contents, when *A. franciscana* enriched with mixed complete experimented algae cells, the carbohydrate contents increased by about only 5.5 %. However, there was a lower increase level in the carbohydrate contents of *A. franciscana* that had been enriched with a mixture of lipid

Table 2 Amino Acids profiles in the different experimental *Chlorella* species

Amino acids	*C. salina*	*C. marina*	*C. capsulata*	*C. stigmatophora*	*C. vulgaris*
Arginine	3.57	14.74	3.01	2.13	8.85
Lysine	2.75	9.53	3.68	0.72	3.12
Alanine	2.74	3.31	9.27	1.82	3.84
Threonine	0.50	1.16	10.15	1.51	2.36
Glycine	2.03	9.61	9.91	1.29	7.39
Valine	1.90	3.46	11.66	1.82	4.43
Serine	ND	9.83	ND	ND	ND
Proline	1.09	3.66	2.23	1.85	2.07
Isoleucine	78.58	15.75	ND	61.90	54.02
Phenylalanine	0.94	ND	1.16	18.40	0.46
Glutamic	1.91	8.61	22.41	4.88	6.64
Aspartic	2.01	7.21	15.64	2.30	3.98
Cystine	0.01	3.92	0.98	0.03	0.06
Tyrosine	1.75	9.21	3.66	1.36	2.79

ND not detected

Table 3 Lipids, carbohydrates as well as proteins and protein: lipid ratios in *Artemia franciscana* enriched with the different experimental *Chlorella* species

Enrichments with experimental *Chlorella* species	Lipids %	Carbohydrates %	Proteins %	Protein: lipid ratio
Artemia franciscana	37 ± 1.0	10 ± 0.3	31.00 ± 0.8	0.84
A. franciscana + *C. salina*	90 ± 2.3	21 ± 0.5	58.20 ± 1.5	0.65
A. franciscana + *C. salina*[a]		20.4 ± 0.5	42.90 ± 1.1	
A. franciscana + *C. marina*	57 ± 1.5	19.23 ± 0.5	57.10 ± 1.5	1
A. franciscana + *C. marina*[a]		18.9 ± 0.5	48.40 ± 1.3	
A. franciscana + *C. capsulate*	45 ± 1.2	21.9 ± 0.6	53.13 ± 1.4	1.18
A. franciscana + *C. capsulata*[a]		20.2 ± 0.5	47.13 ± 1.2	
A. franciscana + *C. stigmatophora*	47 ± 1.2	26.3 ± 0.7	67.26 ± 1.7	1.43
A. franciscana + *C. stigmatophora*[a]		22 ± 0.6	53.00 ± 1.4	
A. franciscana + *C. vulgaris*	74 ± 1.9	19.6 ± 0.5	70.33 ± 1.8	0.95
A. franciscana + *C. vulgaris*[a]		17.24 ± 0.4	59.83 ± 1.6	
A. franciscana + Mixed experimental chlorellas	59 ± 1.5	15.5 ± 0.4	65.28 ± 1.7	1.12
A. franciscana + Mixed experimental chlorellas[a]		14.5 ± 0.4	60.78 ± 1.6	

[a] Lipids extracted *Chlorella* species

extracted algae meal (4.5 %) when compared to that of the controlled. Generally, the carbohydrate content in *A. franciscana* that was enriched with different experimental *Chlorella* species was more or less the same when compared to those that were enriched with the lipid extracted ones.

By detecting the protein content in tested *A. franciscana*, whether enriched with complete algal biomass or the lipid extracted biomass; both were found to have protein ranging from 43 to 70 %. This refers to the highest protein percentage of *A. franciscana* enriched with *C. vulgaris* and *C. stigmatophora* as recorded in Table 3. The results also indicated that the enrichment of *A. franciscana* on the complete cells of the experimented *Chlorella* species improved their protein contents. The percentages of protein increased to 27.2, 26.1, 22.13 % as well as 36.26 and 39.33 % for *C. salina*, *C. marina*, *C. capsulata* as well as *C. stigmatophora* and *C. vulgaris*, respectively, when compared with control. On the other hand, there were fewer increases in the protein contents of *A. franciscana* that had been enriched with lipid extracted biomass of the different experimented *Chlorella* species when compared to the control. The *A. franciscana* protein contents increased by 11.9, 17.4, 16.13, 22 and 28.83 % for *C. salina*, *C. marina*, *C. capsulata* as well as *C. stigmatophora* and *C. vulgaris*, respectively, when compared to the control. The protein contents of *A. franciscana* which had been enriched with mixed complete algae cells, increased by

about 34.28 %. On the other hand, lower increases in the protein contents of *A. franciscana* that were enriched with mixtures of lipid extracted algal residues (29.78 %) were recorded. Upon comparing the protein content of *A. franciscana* that had been enriched with complete algal cells, and that enriched with the same lipids extracted residues, it appeared obvious that the protein contents of *A. franciscana* were significantly higher in those enriched with the complete experimental algal cells than that enriched with lipid extracted cells. The protein contents decreased by 15.3, 8.7, 6, 14.26 % as well as 10.5 and 4.5 % for *C. salina*, *C. marina*, *C. capsulata*, *C. stigmatophora* as well as *C. vulgaris* and those enriched with the mixed species of algae.

The results presented in Table 3 revealed that protein/lipid ratios of *A. franciscana* that had been enriched with the different experimental *Chlorella* species were highly improved when compared to the controlled. The ratio had increased by about 0.16, 0.34, 0.59, 0.11 and 0.28 % for those enriched with *C. marina*, *C. capsulata*, *C. stigmatophora*, *C. vulgaris* and mixed algae, respectively.

3.5.2 Fatty acid composition

From *A. franciscana* fatty acid composition shown in Table 4, saturated fatty acids were found to be abundant in *A. franciscana* that was enriched with complete algal cells of the five experimental *Chlorella* species. Upon

Table 4 Fatty Acids Methyl Esters (FAMEs) composition (mg/g dry weight) of *Artemia franciscana* enriched with the different experimental *Chlorella* species

Fatty acids	Controlled *A. franciscana*		*A. franciscana* + *C. salina*		*A. franciscana* + *C. marina*		*A. franciscana* + *C. capsulata*		*A. franciscana* + *C. stigmatophora*		*A.franciscana* + *C. vulgaris*	
	mg/g	%	mg/g	%	mg/g	%	mg/g	%	mg/g	%	mg/g	%
Saturated FAs (SFA)												
C6:0 (Hexanoic)	0.03	0.1	ND	ND	ND	ND	0.32	1.0	0.3	0.7	ND	ND
C8:0 (Caproic)	0.03	0.14	0.04	0.1	ND	ND	0.03	0.1	ND	ND	0.04	0.2
C10:0 (Capric)	0.03	0.14	0.4	1.0	0.1	0.2	0.1	0.3	0.04	0.1	0.2	1.1
C11:0 (Undecanoic)	0.05	0.2	0.4	1.0	0.3	0.6	0.35	1.0	0.1	0.3	0.1	1.0
C12:0 (Lauric)	0.4	1.6	2.9	7.2	2.7	6.5	1.6	4.8	1.1	2.6	0.5	3.1
C13:0 (Tridecylic)	0.7	2.8	3.2	8.0	4.0	9.6	1.0	2.9	0.7	1.7	0.8	4.8
C14:0 (Myristic)	1.4	5.8	6.0	15.1	7.7	18.2	1.8	5.2	1.7	4.2	2.3	14.0
C15:0 (Pentadecanoic)	0.9	3.8	4.7	11.9	5.7	13.6	1.0	2.9	1.0	2.5	1.8	11.1
C16:0 (Palmitic)	3.8	15.6	9.5	23.8	8.7	20.7	14.3	41.7	10.0	24.4	4.8	29.3
C17:0 (Heptadecyclic)	0.3	1.1	0.5	1.3	0.5	1.2	0.2	0.5	0.1	0.2	0.3	1.6
C18:0 (Octadecanoic)	2.1	8.8	3.6	9.0	3.0	7.1	4.1	12.1	3.5	8.5	1.4	8.7
C20:0 (Eicosanoic)	ND	ND	0.8	2.1	0.8	2.0	0.3	0.9	0.2	0.5	ND	ND
Monounsaturated FAs (MUFA)												
C14:1 (Myristoleic)	0.3	1.5	1.6	4.1	2.1	4.9	0.3	0.8	0.4	0.9	0.7	4.1
C15:1 (cis-10- pentadecenoic)	0.7	2.9	2.3	5.7	2.9	7.0	0.4	1.2	0.5	1.1	0.9	5.4
C16:1 (Palmitoleic acid)	0.7	3.0	1.0	2.4	1.1	2.7	0.1	0.4	0.5	1.3	0.3	2.0
C17:1 (Heptadecenoic)	0.7	2.7	0.9	2.3	0.8	1.9	0.2	0.5	0.2	0.5	0.3	2.1
C18:1ω9c (Oleic)	4.8	19.7	0.3	0.8	0.9	2.2	2.3	6.8	5.4	13.2	0.7	4.1
Polyunsaturated FAs (PUFA)												
C18:2ω6c (Linoleic)	5.7	23.5	0.7	1.7	0.7	1.6	2.4	7.0	10.0	24.5	0.9	5.6
C18:3ω3 (Alpha-Linoleic)	ND	ND	ND	ND	ND	ND	3.2	9.2	4.7	11.4	ND	ND
C20:2 (Eicosadienoic)	0.6	2.5	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
C22:2 (Docosadienoic)	0.4	1.7	1.0	2.5	ND	ND	0.2	0.7	0.2	0.5	0.3	1.8
∑SFA	10.1	41.7	32.0	80.5	33.5	79.7	25.1	73.4	19.1	46.6	12.2	74.9
∑UFA	14.1	58.3	7.8	19.5	8.5	20.3	9.1	26.6	21.9	54.4	4.1	25.1
∑MUFA	7.2	29.8	6.1	15.3	7.8	18.7	3.3	9.7	7.0	17.0	2.9	17.7
∑PUFA	6.9	28.5	1.7	4.2	0.7	1.6	5.8	16.9	14.9	36.4	1.2	7.4
PUFAs-ω3	ND	ND	ND	ND	ND	ND	3.2	9.2	4.7	11.4	ND	ND
PUFAs-ω6	5.9	24.3	0.7	1.7	0.7	1.6	2.4	7.0	10.0	24.5	0.9	5.6

ND not detected

comparing results, it has been reported that palmitic acid represents the most abundant with low concentrations of capric, undecyclic, lauric, tridecyclic, myristic, pentadecenoic, heptadecyclic and octadecanoic acids. Results in Table 4 indicated that the SFA increased to 80 % in *A. franciscana* that was enriched with *C. salina* and *C. marina*. Also, enrichment of *A. franciscana* with the same two *Chlorella* species resulted in an improvement of C18:3ω3 (Alpha-Linoleic) by nearly 9.2 and 11.4 folds, respectively. *A. franciscana* was enriched with a mixture of the five experimental *Chlorella* species. The results in Table 5 indicated that the SFA increased to 72.6 %. The contents of PUFAs-ω3 had increased by nearly 7.9 folds in *A. franciscana* that was enriched with the mixture of the studied *Chlorella* species. This gave them a good chance to be used in *A. franciscana* feeding.

3.5.3 Growth and survival follow up

Growth and survival of *A. franciscana* that fed on the lipid-extracted biomass were monitored to verify whether there is a long-term positive effect of exhausted *Chlorella* biomasses which could be an important achievement. After 12-day feeding trials, the results (Fig. 3) revealed that the survival and growth of *A. franciscana* that fed on a mixed diet of the residual algae biomass yielded the superior survival and average fresh weight of *A. franciscana* recording 91.8 ± 4 % and 4.2 ± 0.4 mg, respectively. However, using the residual biomass of the different marine *Chlorella* species, the survival of *A. franciscana* recorded moderate values ranging between 68 ± 3.3 and 78 ± 3.2 % for *C. capsulata* and *C. salina*, respectively. Furthermore, the lowest survival accompanied with fresh

Table 5 Fatty Acids Methyl Esters (FAMEs) composition (mg/g dry weight) of *Artemia franciscana* enriched on a mixture of the complete experimental *Chlorella* species

Fatty acid	Controlled *A. franciscana*		*A. franciscana* + mixed Chlorellas	
	mg/g	%	mg/g	%
Saturated FAs (SFA)				
C6:0 (Hexanoic)	0.03	0.1	2.2	29.5
C8:0 (Caproic)	0.03	0.14	0.6	7.8
C10:0 (Capric)	0.03	0.14	0.03	0.4
C11:0 (Undecanoic)	0.05	0.2	0.03	0.4
C12:0 (Lauric)	0.4	1.6	0.0	0.0
C13:0 (Tridecylic	0.7	2.8	0.04	0.6
C14:0 (Myristic)	1.4	5.8	0.05	0.7
C15:0 (Pentadecanoic)	0.9	3.8	0.06	0.7
C16:0 (Palmitic)	3.8	15.6	1.1	14.6
C17:0 (Heptadecyclic)	0.3	1.1	0.1	1.3
C18:0 (Octadecanoic)	2.1	8.8	1.1	14.6
C20:0 (Eicosanoic)	ND	ND	0.07	0.9
Monounsaturated FAs (UFA)				
C14:1 (Myristoleic)	0.3	1.5	0.01	0.1
C15:1 (cis-10-pentadecenoic)	0.7	2.9	0.08	1.1
C16:1 (Palmitoleic)	0.7	3.0	0.05	0.7
C17:1 (Heptadecenoic)	0.7	2.7	0.18	2.4
C18:1ω9c (Oleic)	4.8	19.7	0.7	10.2
Polyunsaturated FAs (PUFA)				
C18:2ω6c (Linoleic)	5.7	23.5	0.6	7.9
C20:2 (Eicosadienoic)	0.6	2.5	ND	ND
C22:2 (Docosadienoic)	0.4	1.7	ND	ND
∑SFA	10.1	41.7	5.46	72.6
∑UFA	14.1	58.3	2.02	27.4
∑MUFA	7.2	29.8	1.42	19.5
∑PUFA	6.9	28.5	0.6	7.9
PUFAs-ω3	ND	ND	0.6	7.9
PUFAs-ω6	5.9	24.3	ND	ND

ND not detected

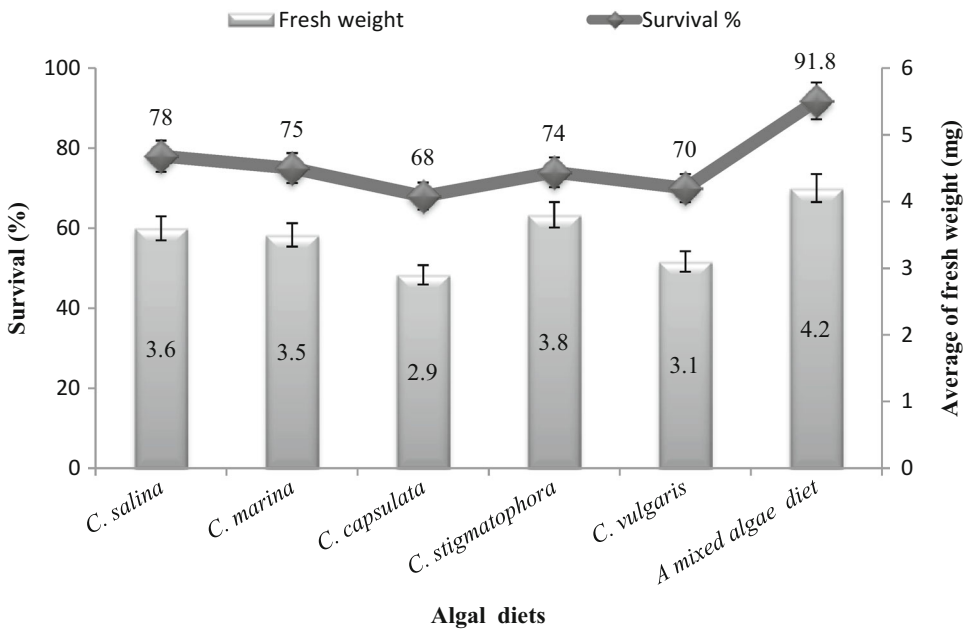

Fig. 3 Survival % and fresh weight of *Artemia franciscana* in artificial sea water enriched with different residual algae biomass

weight of *A. franciscana* was recorded upon using *C. capsulata*: 68 % and 2.9 mg, respectively, after the same period of growth.

4 Discussion

In this study, we are interested to know about the five experimental *Chlorella* species for their growth and the characterization of their biomass contents for further applications in *A. franciscana* feeding.

Monitoring of the algae growth was determined during the exponential phase that mainly relied upon the inocula sizes, culture conditions and the composition of the growth media as revealed by Jayasankar and Valsala (2008). On the 10th day, the five studied *Chlorella* species reached their maximum cell number and the alga *C. salina* was superior in growth, while *C. marina* was the lowest grown alga. The growth rate of *C. marina* was recorded for its maximum growth rate (2.0) after 3 days of incubation, followed by *C. vulgaris* after 4 days. Under this administration, Mandalam and Palsson (1997); and Scarsella et al. (2010) reported that *C. vulgaris* growth rate was equal to 1.4 cell 10^4/h, with a biomass productivity of 0.27 g L^{-1}.

Growth monitoring, in terms of cell numbers or biomass, and the biochemical composition are two essential attributes to evaluate the prospective of a species as promising candidates for different applications as demonstrated by Araújo and Garcia (2005).

In this study, *Chlorella* species began their stationary phase of growth on the sixth day of incubation and achieved the maximum number on the 10th day, in conjunction with the most elevated lipid content. *C. capsulata* had accumulated the highest lipid content (44.6 ± 3.4 %) followed by *C. salina* (26.8 ± 2.08 %) and *C. marina* (26.7 ± 2.6 %). Comparable results were recorded by several past studies [Huerlimann et al. (2010), Muthukumar et al. (2012) and Moheimani (2013), Sudha et al. (2013)]. Besides, microalgae produced and stored lipids in the form of phospholipids and glycolipids which can be used as feed in aquaculture as indicated by Muthukumar et al. (2012). Ilavarasi et al. (2011) discussed lipid production as a process organized by algae growth. However, Feng et al. (2011) discussed the lipid accumulation on the bases that after day 8, the growth of algae nearly ceased, thus resulting in the increased lipid synthesis.

The total lipid extracted from different experimental *Chlorella* species, *C. salina* and *C. capsulata* followed by *C. stigmatophora* was found to have accumulated enormous saturated fatty acids (94.2; 93.0; 87.0 %), respectively. However, Shahar (2014) reported that the major fraction of fatty acids belonging to *C. salina* was PUFAs. The main components of SFAs in the studied *Chlorella* species were palmitic acid (C16:0) as well as stearic acid (C18:0). In this respect, El-Sheekh and Hamouda (2016) reported that palmitic acid is the major constituent in the crude lipids of the green alga *Ankistrodesmus falcatus.* Moreover, Bakhtiarvandi et al. (2014) stated that SFAs are utilized as energy substrate and, therefore, SFAs are needed for nutrition.

Several researches concluded the presence of UFAs in algae lipids; they have been considered as wellsprings of PUFAs for aquaculture industry as stated by Patil et al. (2005).

Oleic acid was recorded in *C. marina*, *C. stigmatophora* and *C. vulgaris*. These outcomes are additionally in accordance with those reported by (Ötleş and Pire 2001). Furthermore, Gerasimenko et al. (2010) stated that algal lipids could be a source of polyunsaturated fatty acids (PUFAs) of ω-3 and ω-6 series. In our study, PUFAs-ω6 has been detected with nearly low concentrations in all the studied *Chlorella* species. These results are also in line with findings of Brown et al. (1997) who revealed that the PUFAs contents in marine *Chlorella* sp. were very low. It is important to draw attention that the GC mass device might miss some essential fatty acids within the algal lipid that may be essential for nutrition because of the coating material of the device.

Carbohydrate production in algae is crucial since it acts as structural components in the cell wall and the compounds stored intracellular (Markou et al. 2012). They added that the output of carbohydrate content rely on the microalgal species, the cultivation parameters and the environmental parameters. Carbohydrate content of the studied *Chlorella* species ranged from 12.8 ± 0.33 to 22.0 ± 0.57 % in *C. salina* and *C. capsulata*, respectively. In this appreciation, it was reported that distinctive strains of *Chlorella* spp. displayed different behaviors and accumulated compounds, including carbohydrates, in variable quantities (Barsanti and Gualtieri 2006).

Different metabolic studies have confirmed the capacities of microalgae as a novel source of protein. The average quality of most of the studied algae is equal or even superior to that of other traditional high quality plant proteins (Spolaore et al. 2006). Protein contents of the studied *Chlorella* species ranged from 24.8 ± 1.4 % in *C. capsulata* to 33.1 ± 1.4 % in *C. salina.* However, in the lipids extracted from *C. marina* cells, the protein content was observed to have decreased by about (8 %). In this respect, green algae were reported to contain proteins in addition to various nutrients (El-Sheekh and El-Kassas 2014). Additionally, the recorded data lies within the range reported by Guccione et al. (2014) on their study using *Chlorella* for protein and biofuels. On the contrary, Grigorova (2006) showed that *Chlorella* protein content was 55 % of the alga dry weight.

Considering amino acids contents in the experimental *Chlorella* species, proteins from four trial marine *Chlorella* species were found to be deficient in cysteine and moderately possessing methionine and histidine; however, they contain enough obvious quantities of lysine. Similarly, Fabregas and Herrero (1998) presumed that lysine concentration in marine micro algae species surpassed that in beef or a whole egg. Therefore, it seems logical to assume that *Chlorella*'s protein, and possibly cells, may serve as an acceptable source of amino acids in animal enrichment, and if supplemented with cysteine, methionine and histidine, it would be identical to other proteins of high nutritional quality.

The ash contents of the studied *Chlorella* species was in the extent reported by Grigorova (2006), who showed that ash content of *Chlorella* was 8.7 %. On the contrary, other studies revealed that green algae including *C. salina* contained significantly higher contents of ashes [Wong and Chan (1980); (Bi et al. 2013)].

Surprisingly, the results revealed that there weren't any significant differences between complete and lipid extracted algae residue. This finding reflects a significant economic value of the five studied *Chlorella* species for aquaculture feeding at the level of a commercial scale. Besides that, the determination of the chemical composition of food plays a key role in larviculture (Pettersen et al. 2010). Therefore, the experimental algal meal of the delipidated *Chlorella* species, including the recorded nutritional components, may serve as viable feed stuff in aquaculture.

Feeding marine creatures under culture conditions is one of the most important challenges to improve performance and ensure production processes (Cisneros and Vinatea 2009). *Artemia* (brine shrimp) is probably the most popular live eating regimen in aquaculture (Khairy and El-Sayed 2012). Therefore, this study was extended to use the different, both complete and delipidated experimental *Chlorella* species for *A. franciscana* feeding.

In this study, the enriched *A. franciscana* has gained significant improvements in its biochemical composition. The carbohydrate contents of *A. franciscana* increased by values that ranged between 9.23 and 16.3 % when compared to the controlled. Moreover, the protein contents of *A. franciscana* were doubled in those fed on the complete experimented algal cells. In these contexts, Sun and Wang (2009) revealed that the nutritive quality of microalgae is related to their biochemical composition. Earlier studies by Brown (2002) and Becker (2007) suggested that microalgae have a vital role in aquaculture for providing protein (essential amino acids). *Chlorella* has high protein contents with a balanced amino acid composition. They can be transferred up through the food chain to improve nourishments of young larvae, ornamental fish, shell fish and bivalves [Muller-Feuga (2000), Cho et al. (2007)]. Furthermore, marine algae can be used as an enrichment media instead of certain commercial media that give the same nutritional level as reported by Senthil et al. (2012). Recently, El-Sheekh et al. (2015) revealed that there were significant increases in the biochemical composition of the *A. franciscana* (carbohydrate, total protein, total lipid, and omega3 fatty acids), after 24 h of enrichment with the complete *Tetraseilimus chuii* cells cultured on optimized media ($P \leq 0.001$).

The lipids of the studied *Chlorella* species had been extracted for biodiesel production and the lipid extracted

residue of the different experimental *Chlorella* species was used for *A. franciscana* feeding. These residues of the different experimented *Chlorella* species exerted significant improvements in the constituents of *A. franciscana,* i.e.; that is a possible technique to re-cycle/up-cycle the exhausted biomasses, and would be an important achievement. This is critical in the view of the recent analysis that has shown that to achieve a positive energy steadiness and to produce economically viable biofuels, the residue after extraction must be used for co-products as stated by Wijffels et al. (2010) and Prommuak et al. (2013).

A trial to use a mixed diet of the five experimental algae has been carried out. The contents of PUFAs-ω3 increased by nearly 7.9 folds in *A. franciscana* that had been fed on the mixture of the different lipid extracted *Chlorella* species, giving it a decent opportunity to be used in *A.franciscana* feeding. Correspondingly and in concurrence with our outcomes, Zaki and Saad (2010) concluded that a deliberately chosen mixed diet of microalgae can offer excellent nutrition for larvae, either directly or indirectly (through enrichment of zooplankton). They added that the nutrition sufficiency of live food provided to the newly hatched larvae improves their growth and enhances their survival rate. Furthermore, the use of an algal meal could be a nutritious, economic and environmentally sustainable substitution for animal protein as stated by Dib (2012).

During the experimental work, the protein/lipid ratio of *A. franciscana* that had been enriched with the complete cells of different experimented *Chlorella* species was highly improved when compared to the controlled. In this respect, Olsen et al. (2000) reported that imbalances in the lipid composition of the diet, either quantitative or qualitative, resulted in poor larval growth and performance. Large scale research and product development has gone into improving rotifer and brine shrimp nutritional quality by manipulating their diet e.g., by microalgal strain selection or by incorporating dried microalgal biomass into formulated inert diets (Shields and Lupatsch 2012).

The recorded improvements in the *A. franciscana* survival and fresh weight when feeding on the dried algae residues were similar to that reported by Abomohra et al. (2014) during their study using the dried *S. obliques* for different *Artemia* species feeding. The study results were also in accordance with Kim et al. (2002) using dietary supplementation of *Chlorella ellipsoidea.* Kim et al. (2002) reported higher weight gain and improved feed adequacy and protein efficiency ratios in juvenile Japanese flounders (*Paralichthys olivaceus*). Furthermore, (Godínez et al. 2004) suggested that the energy content of other studied species of microalgae can affect the growth of *Artemia.* However, Maldonado-Montiel and Rodríguez-Canché (2005) revealed that biochemical composition of the various algae strains could not be correlated with survival and feed conversion rate values could confirm the best algal feed to *Artemia* sp. Here in this study, the unexpected improvements in the fresh weight and survival % of *A. franciscana* that fed on a mixed exhausted algae biomass may be attributed to the nutritional contents of the delipidated dried algae species which complete each other forming an absolute balanced diet that encourage *A. franciscana* growth and survival.

5 Conclusions

The main goals of this investigation were successfully accomplished. This work examines batch scale cultivation of five *Chlorella* species as promising microalgae for *A. franciscana* feeding in simple, efficient and economical methods. The lipids, carbohydrates and protein contents of the experimental *Chlorella* species were estimated. The alga *C. capsulata* produced the highest lipid content. FAMEs of total lipids of the experimental *Chlorella* species showed that *C. salina*, *C. capsulata* and *C. stigmatophora* accumulate enormous saturated fatty acids while unsaturated fatty acids were present in *C. marina* and *C. vulgaris*. The study's results showed that, after lipid extraction, there weren't any significant losses in the amounts of the algal proteins, carbohydrates and ashes when compared to the values recorded for the complete algae cells. In addition, the amino acid composition of delipidated cell residue was found in agreement with previous studies. The enrichment of *A. franciscana* with complete cells of the different experimental *Chlorella* species resulted in an increase of its lipids, carbohydrates and protein contents when compared to the controlled. The enrichment with a mixture of the experimental *Chlorella* species improved the lipid content by 22 %, protein content as well as Protein/Lipid ratio by about 1.96 and 1.33 folds, respectively. However, the enrichment with the mixture of the delipidated experimental *Chlorella* species attained the detection of PUFAs-ω3 by 7.9 % in the enriched *A. franciscana*. Survival and fresh weight of *A. franciscana* were highly improved when feeding on the mixed dried lipid extracted algae meals. Therefore, the study recommends feeding of *A. franciscana* with mixed algal residues.

Compliance with ethical standards

Conflict of interest There are no conflicts of interest.

Research involving human participants and/or animals All procedures followed were in accordance with the ethical and there are no Human Participants or Animals.

Informed consent Written Informed consent was obtained from all participants.

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