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Weekly Fluctuation in Phytoplankton Macromolecular Composition in Response to Environmental Changes in the Eastern Harbour, Alexandria (Egypt)

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ABSTRACT

The variation in phytoplankton composition and its nutritional values were estimated in conjunction with changes in sea waters physicochemical parameters at the Eastern Harbor, Alexandria from September 2018 to August 2019. The results explain the relation between the biochemical contents and phytoplankton abundance, especially diatoms and chlorophyll-*a* concentration. Proteins as the major formed 54.55% of the total biomass, followed by much lower contribution of both carbohydrates (26.56%) and lipids (18.89%). Each macromolecular content exhibited its maximum concentration during autumn, decreasing by the order of magnitude in spring, summer, and winter seasons. The lipids/carbohydrates ratio at < 1 indicates a high nutritional content of the phytoplankton dissolved organic matter (DOM). The highest phytoplankton biopolymeric particulate organic carbon (BPC) was observed in late July (6491.95 mg C l^{-1}), and strongly connected with food material (FM) and the relative caloric content (RCC). The physical characters of the sea water contributed significantly with the BPC content rather than the measured chemical ones. The short-term monitoring may act as an important background data for further studies in a marine system to understand the potential food quality under changing environmental conditions that directly influence fish production and sustainability of marine food webs.

INTRODUCTION

Phytoplankton group is a key microorganism that serves as a primary producer for aquatic biota at higher trophic levels in the food chain. Thus, the changes in the phytoplankton community controls the quantity of food sources for higher trophic level organisms, and could consequently affect the biomass and the production of fishery resources **(Kang *et al.*, 2017)**. At the same time, phytoplankton can generate many essential biomolecules such as carbohydrates, proteins, and lipids, which are potentially highly bioactive compounds **(Jónasdóttir, 2019)**. The changes in the relative abundance and biochemical composition rely on factors such as the growth phase **(de Viçose *et al.*, 2012)**, environmental factors **(Ahn *et al.*, 2019)**, species composition

and size **(Bhavya *et al*., 2019)**. Besides, climate change can potentially contribute to the variation in algal biochemical compositions **(Kang *et al.*, 2017**). These components of either energy or structural compounds **(Novak *et al.*, 2018)** are key indicators of the physiological and nutritional status of phytoplankton and are considered valid integrators of the surrounding environments **(Ahn *et al*., 2019)**. The biochemical contents can strongly impact the trophic balance within a marine ecosystem **(Peltomaa *et al*., 2017)**, serving as an important tool to evaluate the nutritional quality of organic materials in feeding behavior **(El-Dahhar *et al*., 2021)** and the ecosystem function (**Kim *et al*. 2021**).

There are several kinds of researches on the abundance and distribution of phytoplankton in the different geographical marine areas in Egypt; however, the majority is qualitative studies that do not contain the whole elemental or biochemical content. Notably, considering the assemblages and environmental conditions, phytoplankton macromolecular analysis is a newly introduced research work in Alexandria marine waters. Previously, biochemical composition studies have been conducted on the Egyptian freshwater systems **(Abd El-Hady, 2016; Zaher *et al*., 2021)** and in some collected species from the Eastern Harbor in Alexandria **(El Zokm *et al*., 2021)**. This study aimed to investigate temporal changes in the patterns of the biochemical composition in phytoplankton assemblages regarding changing environmental conditions and phytoplankton diversity in the eastern harbor, Alexandria, Egypt. In addition, the study was conducted to improve the understanding of the physiological status of resident phytoplankton in this eutrophic marine basin to determine the best season for fish farming.

MATERIALS AND METHODS

On a weekly scale during an annual cycle (7 September 2018 to 22 August 2019), surface water samples were collected using 20µ net at a fixed station in the Eastern Harbor (Fig.1). Water temperature and salinity were determined in the field by the checked physical device HANA, Model HI 9828, and transparency by Secchi disc. Water samples were collected, sieved through large-size net (100 µm) and stored at -20°C to measure dissolved inorganic nutrients (NO_3, NO_2, NH_4, PO_4, and SiO_4) according to **Parsons *et al*. (1984)**. For chlorophyll a (Chl. *a*), water samples were filtered on GF/F filter papers, stored at -20°C until analyses according to **Jeffrey and Humphrey (1975).** The organic matter concentrations (OM) were estimated following **FAO (1976)**. The phytoplankton standing crop and community structure were investigated using a light inverted microscope. Living subsamples were first examined to avoid the disruption of delicate cells with preservation, and then the samples were preserved by the addition of 4% of neutral formalin and a few drops of Lugol's solution **(Throndsen, 1978)**, and were counted after sedimentation **(Utermöhl, 1958)**. The identification of species followed principally **Tomas (1997)** and the density was expressed as unit l^{-1}.

Fig. 1. Sampling station in Eastern Harbour, Alexandria.

About four liters of filtered seawater samples was used to measure the chemical composition. The protein content was determined by the Biuret method **(Lowry *et al.*, 1951)**, based on the colorimetric peptide-detecting assays at 750nm using bovine serum albumin as the standard for the protein concentration. Carbohydrate extraction was conducted using the method established at 490nm by **Do *et al.* (2009)**. Glucose solution was used as the standard for the carbohydrate concentration. The sulphophosphovanillin procedure estimated total lipid content utilizing cholesterol as a calibration standard **(Chabrol & Castellano, 1961)**. The food material (FM) is the sum of protein, carbohydrate, and lipid concentrations **(Danovaro *et al.*, 2000; Kim *et al.*, 2020)**. The phytoplankton relative caloric content (RCC) was measured from the proximate measurements of biochemical composition; the relative caloric coefficients for proteins, carbohydrates, and lipids were 5.56, 4.1, and 9.45, respectively **(Brett & Groves, 1979)**. Concentrations of proteins, carbohydrates, and lipids were converted to carbon equivalents using 0.40, 0.49, and 0.75 mg C l^{-1} conversion factors **(Fabiano & Danovaro, 1994)**, and the sum of protein carbon, carbohydrate C, and lipid C were referred to as biopolymeric carbon (BPC) **(Fabiano *et al.*, 1995)**.

Statistical Analyses

The regression analysis and Pearson's correlation analysis (statistical software, version 12.0; SPSS Inc.) were applied to determine the relationship between the measured biotic, and abiotic variables.

RESULTS AND DISCUSSION

This study investigated the effect of the measured abiotic and biotic factors on the physiological status of local phytoplankton in Alexandria Mediterranean waters. The estimation of biochemical compositions (lipids, proteins, and carbohydrates) serves as a valid method for verifying the origin of organic matter **(Colombo *et al*., 1996)**, and they can constitute an important fraction of particulate organic matter **(Salas *et al*., 2015)**. Besides, it is an important tool for assessing the nutritional quality of OM for consumer biota **(Joseph *et al*., 2008)**.

Physico-chemical parameters (Table 1), phytoplankton abundances, community structure and Chl. *a* content were previously published **(El-Dahhar *et al*., 2021)**. Generally, three periods of massive macromolecular content were observed in early and late of September, spring in mid-April, and at early June-August in summer (Fig. 2), under different environmental conditions accompanying the intensive phytoplankton blooms (Table 1 & Fig. 3) of different classes **(El-Dahhar *et al*., 2021)**. The previous authors recorded three distinct phytoplankton bloom peaks during three different seasons. The first bloom in the early and the last week of September with abundance $1.15x10^6$ unit l^{-1} and $1.72\ x10^6$ unit l^{-1}, respectively, mainly consisted of diatoms (66.51% and 83.56%). The second with diatoms predominance in mid-April was a minor bloom (average $450x10^3$ unit l^{-1}), and the last one with its peak on the 28th of July ($3.11x10^6$ unit l^{-1}), extended to August sustaining relatively lower densities ($545x10^3$ - $676x10^3$ unit l^{-1}), and it was dominated mainly by chlorophytes (43.25%), raphidophytes (42.53%), and associated diatoms of less frequency (14.06%).

The overall protein content was the abundant (mean 166.78 ± 141.49 µg l^{-1}); carbohydrates were the second contributory (mean 110.10 ± 82.85 µg l^{-1}), followed by lipids (mean 5.54 ± 38.79 µg l^{-1}) as illustrated in Fig. (2). This finding is consistent with the result stated by **Lee *et al*.** (**2009**). Proteins reached three major peaks; the highest on September the 7th (460 µg l^{-1}) within the intensive autumn bloom period extending to early October; diatoms and euglenophytes dominated the peak day ($0.76x10^6$ and $0.43x10^6$ unit l^{-1}, Chl. *a* 10.39 µg l^{-1}) as shown in Figs. (3 and 4). The second peak (380 µg l^{-1}) accompanied the initiation of the spring diatom bloom in late April ($0.48\ x10^6$ unit l^{-1}; mainly of diatoms). Compared to autumn, this peak occurred at low temperature (16.2°C), relatively high salinity (38.5), low nutrients, and almost the same DOM concentrations (2.1-4.7 mg O_2 l^{-1}). The bloom consumed most of the nutrients, NO_3 dropping to 0.5 µg l^{-1} on April the 29th, which seems to allow a remarkable new accumulation of lipids and carbohydrates. The present enhancement of lipid and carbohydrate accumulations instead of proteins under reduced nitrogen conditions has been the focus of numerous studies in the field and laboratory experiments **(Gao *et al*., 2019**; **Liefer *et al*., 2019)** since lipids and carbohydrates are non-nitrogenous compounds, and their concentrations are independent of nitrogenous nutrient availability **(Takagi *et al*., 2000)**. The study also explains that under sufficient nutrients, a parallel increase in protein and carbohydrate concentrations occurs simultaneously, indicating a switch in cells to anabolic rather than catabolic processes **(Finkel *et al*., 2016)**.

Table 1. Physico-chemical parameters of the Eastern Harbor during the study period

Parameter / Data		Temp. °C	Salinity	pH	OM mg l^{-1}	Trans.	NO_3	NO_2	NH_4	SiO_4	PO_4	Si/TN	TN/P
							µg l^{-1}						
2018	7 Sep.	28.50	33.00	8.90	4.70	1.75	2.68	0.37	0.82	2.60	0.12	0.67	32.25
	14 Sep.	27.60	35.00	8.70	2.10	1.50	2.05	0.47	1.02	1.65	0.14	0.47	25.29
	21 Sep.	26.20	38.70	8.04	2.71	2.50	1.18	0.23	1.04	1.94	0.36	0.79	6.81
	28 Sep.	24.50	37.20	8.25	4.24	1.50	1.13	0.33	2.68	2.95	1.10	0.71	3.76
	5 Oct.	25.00	37.50	8.40	3.37	2.00	4.32	0.28	1.58	8.50	0.28	1.38	22.07
	15 Oct.	23.00	37.50	8.24	2.24	1.80	1.92	0.70	3.12	2.20	0.96	0.38	5.98
	26 Oct.	23.80	35.00	8.32	2.10	2.30	6.30	0.80	4.20	8.50	2.50	0.75	4.52
	29 Oct.	21.20	37.80	8.20	2.82	1.10	1.20	0.29	2.11	0.75	2.50	0.21	1.44
	4 Nov.	26.00	37.50	8.80	2.82	2.00	2.25	0.29	2.11	1.20	1.45	0.26	3.21
	8 Nov.	21.00	39.00	8.22	2.01	2.50	1.75	0.27	1.93	0.84	1.75	0.21	2.26
	14 Nov.	18.50	38.20	8.25	2.12	2.70	1.05	0.32	2.81	0.60	1.85	0.14	2.26
	22 Nov.	18.50	38.50	8.17	2.63	2.60	0.80	0.41	2.14	1.00	1.10	0.30	3.05
	28 Nov.	18.20	38.50	8.17	2.63	0.60	0.75	0.41	2.14	1.20	1.50	0.36	2.20
	5 Dec.	16.00	39.80	8.60	2.68	2.50	0.45	0.32	2.26	0.60	0.85	0.20	3.56
	12 Dec.	15.40	39.80	8.18	2.20	3.00	0.85	0.35	1.25	0.95	1.45	0.39	1.69
	19 Dec.	14.00	39.50	8.23	2.30	3.00	0.65	0.35	1.62	0.80	1.80	0.31	1.46
	27 Dec.	13.50	39.20	8.20	2.87	2.30	0.55	0.46	1.27	0.75	2.60	0.33	0.88
2019	3 Jan.	12.40	39.50	8.25	1.95	3.00	1.60	0.42	3.20	2.60	3.20	0.50	1.63
	14 Jan.	13.50	39.90	8.26	1.88	1.50	1.60	0.39	3.70	2.30	4.20	0.40	1.35
	21 Jan.	13.00	39.00	8.29	2.05	1.20	1.60	0.47	4.51	2.60	3.20	0.40	2.06
	26 Jan.	12.40	39.00	8.29	3.05	0.50	3.20	0.47	4.51	2.60	3.20	0.32	2.56
	1 Feb.	13.00	39.00	8.25	2.17	3.00	2.30	0.50	2.67	2.25	3.90	0.41	1.40
	11 Feb.	12.50	39.80	8.23	2.31	3.20	1.30	0.52	1.45	1.80	2.30	0.55	1.42
	22 Feb.	13.00	39.60	8.37	2.41	2.50	2.50	0.35	1.44	2.00	2.00	0.47	2.15
	27 Feb.	13.60	39.80	8.30	2.35	2.00	1.80	0.32	2.45	2.20	2.00	0.48	2.29
	9 Mar.	15.50	39.00	8.25	3.11	2.50	2.00	0.20	1.18	1.80	2.10	0.53	1.61
	14 Mar.	15.00	39.40	8.80	2.39	2.00	1.20	0.35	2.10	2.40	2.20	0.66	1.66
	22 Mar.	15.20	37.50	8.70	3.35	1.80	2.40	0.27	1.80	2.00	2.00	0.45	2.24
	29 Mar.	15.40	37.00	8.70	3.85	2.00	3.20	0.37	2.13	2.00	2.20	0.35	2.59
	3 Apr.	15.60	38.00	8.60	3.82	1.70	4.50	0.30	1.97	2.20	2.80	0.32	2.42
	11 Apr.	14.70	37.20	8.70	4.02	1.70	3.20	0.25	2.03	2.60	3.80	0.47	1.44
	22 Apr.	16.20	38.50	8.60	6.50	2.20	1.20	0.25	1.58	0.90	1.30	0.30	2.33
	29 Apr.	17.00	38.00	8.25	6.50	3.00	1.20	0.24	1.61	1.00	1.50	0.33	2.03
	4 M.	19.30	38.00	8.10	5.50	1.50	0.50	0.25	1.58	0.80	0.80	0.34	2.91
	15 M.	21.50	37.50	8.23	6.50	3.00	2.60	0.56	3.62	1.80	3.00	0.27	2.26
	22 M.	21.50	37.50	7.96	3.20	3.50	2.00	0.23	2.46	1.30	2.30	0.28	2.04
	30 M.	21.20	37.50	8.30	5.40	2.50	1.80	0.51	3.71	0.90	1.30	0.15	4.63
	8 Jun.	23.50	36.00	8.30	5.50	1.50	1.40	0.18	1.88	1.60	1.70	0.46	2.04
	16 Jun.	23.70	37.50	8.60	4.40	1.80	0.70	0.14	2.07	2.20	1.20	0.76	2.43
	22 Jun.	22.80	37.80	8.60	5.50	2.90	0.60	0.51	3.71	1.80	1.30	0.37	3.71
	30 Jun.	23.60	37.50	8.50	6.00	1.50	0.80	0.15	0.62	0.50	0.90	0.32	1.74
	7 Jul.	24.80	37.00	8.43	9.40	2.00	1.20	0.39	2.16	0.80	1.10	0.21	3.41
	15 Jul.	25.60	38.00	8.40	6.20	2.50	0.85	0.30	2.50	0.80	0.90	0.22	4.06
	24 Jul.	25.80	37.50	8.60	2.92	2.00	0.80	0.21	2.11	1.80	1.30	0.58	2.40
	28 Jul.	25.80	35.40	8.80	16.92	2.50	0.80	0.31	3.55	0.50	1.80	0.11	2.59
	2 Aug.	27.20	35.00	8.60	11.00	1.80	0.50	0.41	2.40	0.50	1.80	0.15	1.84
	10 Aug.	27.20	34.20	8.70	8.10	1.50	1.50	0.39	1.97	0.40	3.20	0.10	1.21
	15 Aug.	28.80	37.00	8.80	7.70	1.50	0.82	0.43	0.58	2.60	2.00	1.42	0.92
	22 Aug.	28.80	36.70	8.70	3.20	2.30	1.08	0.48	4.21	5.20	0.58	0.90	9.95

Temp.; Temperature, Sal.; Salinity, OM; Organic matter, Trans.; Transparency

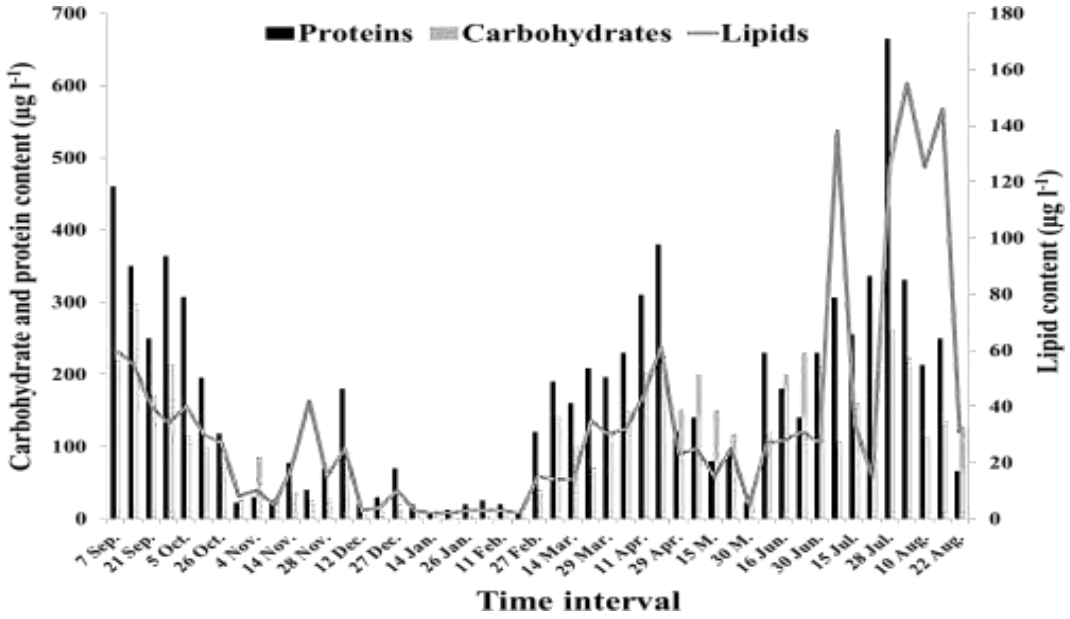

Fig. 2. Variation in protein, carbohydrate, and lipid concentrations (µg l^{-1})

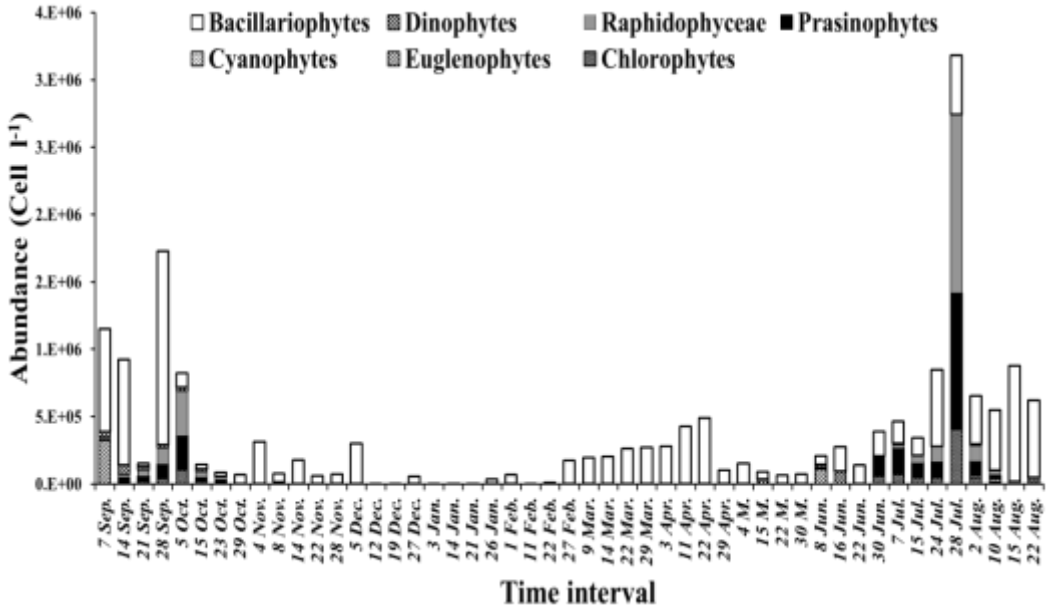

Fig. 3. Variations in phytoplankton abundance (unit l^{-1}) during the study period

The third peak in protein content accompanied the major bloom between mid-June and mid-August. Protein (665 µg l^{-1}) and Chl. *a* (16.75 µg l^{-1}) reached the maximum all the year round on July the 28th (Fig. 4), with the highest abundance of both raphidophytes and chlorophytes ($1.32x10^6$ and $1.36x10^6$ unit l^{-1}), as well as a pronounced proportion of diatoms ($0.33x10^6$ unit l^{-1}). The initiation of this bloom in early July, dominated by diatoms, raised the protein content to 306 µg l^{-1}, while the termination on August the 22nd

under reduced NO_3 (0.82 µg l^{-1}) was significantly coupled with enhanced concentrations of both carbohydrates and lipids. Temperature (23.6–28.8°C), salinity (34.2–37.5), PO_4 (0.9–3.2 µg l^{-1}), and DOM (2.92-16.9 mg O_2 l^{-1}) seem to significantly affect the protein variability. About half of the DOM concentrations were <3 mg O_2 l^{-1}, while three major peaks of 9.4-16.92 O_2 l^{-1} occurred on several days in early and late July. Its variability seems to depend on the abundance of raphidophytes and chlorophytes (r = 0.67 and 0.72, at $P < 0.01$), and to a much less extent on diatoms (r = 0.27 at $P < 0.05$). The changes in phytoplankton abundance, Chl. *a*, carbohydrates, and lipids showed positive significant association with protein (r = 0.83, 0.87, 0.81, and 0.51, respectively at $P < 0.1$). Generally, the harbor is considered as a protein-dominant system. However, the contributions of other chemical components and their ratios were more significant at certain times, depending on the phytoplankton composition, mainly diatoms that can affect variations in proteins by 7%, carbohydrates by 5%, and lipids by 1%. The spring diatom bloom offers a good example of the changes in chemical composition under the interaction of growth phases of multiple small size species (*Chaetoceros decipiens*, *Asterionella glacialis*, *Skeletonema costatum*), and environmental conditions. The excess nutrients, probably due to the new intake, contributed to the increased proteins; however, carbohydrates and lipids became dominant after the bloom collapse under almost complete NO_3 depletion. The rapid changes in the production of each of the biochemical components were previously reported to vary by species, even on a daily scale **(Morán *et al*., 2010)**, and different phytoplankton size **(Kang *et al*., 2017; Ahn *et al*., 2019)**.

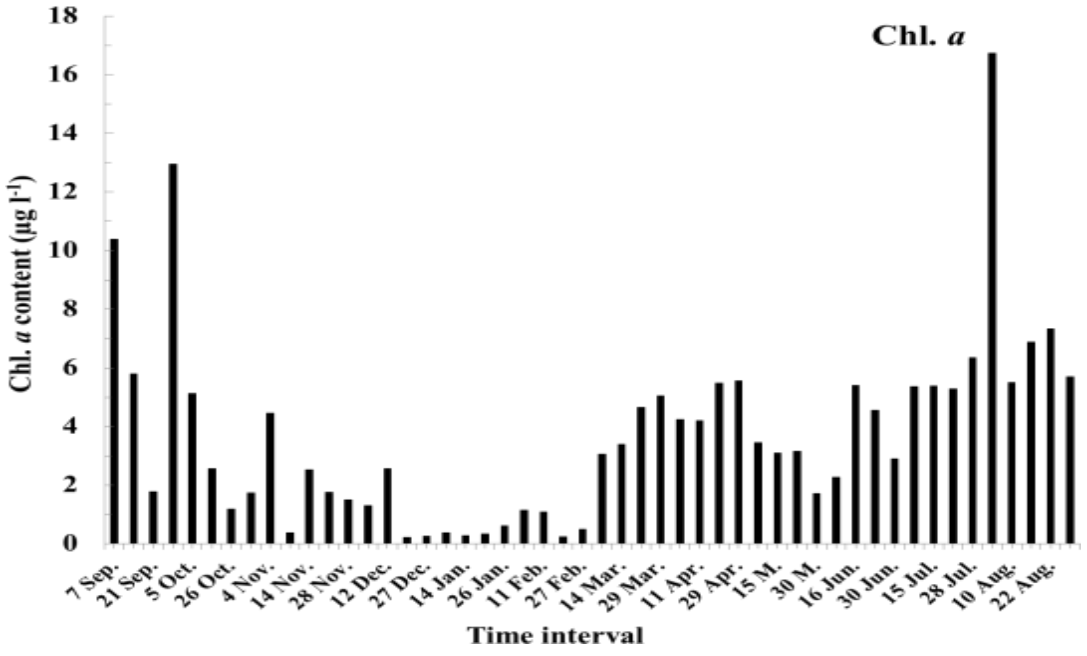

Fig. 4. Changes in Chl. *a* content (µg l^{-1}) during the study period

The ratio of protein to carbohydrate is a practical parameter since it corresponds to the availability of the most common limiting nutrients, phosphorus and nitrogen **(Ganf *et al*., 1986)**. The maximum peak in the ratio was detected in late January and early February (Table 2), which necessarily mean an abundanceof nitrogenous nutrients and/or the presence of newly-produced material or the presence of aged DOM that would result in water mixing under rough sea conditions **(Lee *et al*., 2009)**. While, the lowest PROT/CHO ratio was observed in early November (0.35), reflecting the depletion in nitrogenous water content **(Yun *et al*., 2015)**. The present ratios were more or less similar to the obtained ratio (1 ± 0.3) of **Kim *et al*.** (**2021**) and (0.7 ± 0.6) **Bhavya *et al*. (2019)**, but higher than those reported in the northeastern Mediterranean Sea (0.09) in the study of **Danovaro *et al*. (2000)**. During the study period, the ratio was usually higher than 1, suggesting the high production of the area with massive algal blooms. On the other hand, the ratio < 1 certainly indicates a nitrogen deficiency for phytoplankton growth as stated by **Lee *et al*. (2009)** and **Kim *et al*. (2021)**.

The temporal carbohydrate distribution showed different facets; lowest (<10 µg l^{-1}) in late January-February; increased levels over the proteins with the predominance of diatoms, and other nine peaks >200 µg l^{-1} on scattered days in September, April, June, July and August (Fig. 2). The dominance of carbohydrates over protein estimated as 1.11–1.92 fold was achieved in 12 cases. The increased carbohydrate during spring diatom- dominated bloom over the protein levels might be attributed to the active sharing of dinoflagellates. Phytoplankton abundance and Chl. *a* correlated significantly with the temporal variations of carbohydrate, particularly with the predominance of raphidophytes, chlorophytes, and euglenophytes in summer (r = 0.55, 0.53, and -0.43, respectively).

Despite the much lower contribution of lipids, its flux content was observed to be 0.5-2.97fold over that of carbohydrates during the July-August bloom, with the predominance of co-occurred chlorophytes and diatoms (r = 0.63, P < 0.05). The ratio of lipids/carbohydrates was <1 during the study period, indicating a high nutritional concentration of DOM in phytoplankton **(Abd El-Hady, 2016)**. The increased contribution of lipids over carbohydrates was observed with the overgrowth and/or decline of diatom blooms. The reduction in nutrient concentrations also predicted differences in the energy flux **(Anthony *et al*., 2000)**. The maximum levels of the lipid content were determined from late July to mid-August (126-155 µg l^{-1}), concomitantly to the different stages of the massive multi-specific summer blooms. Lipids, among other components showed a high positive correlation with phytoplankton abundance and Chl. *a* (r = 0.57 and 0.61, respectively). The protein/lipid ratio (mean 6.24 ± 3.43) indicated that 85.71% of all values were in the range between 10% and 30%. Seasonally, the ratio with low phytoplankton production in winter exhibited a wide range of variation between <1 and >20.

Table 2. Variations in the ratio of phytoplankton biochemical composition

Date	PROT/CHO	PROT/LIP	LIP/CHO
7 Sep.	2.091	7.667	0.273
14 Sep.	1.167	6.364	0.183
21 Sep.	1.462	6.250	0.234
28 Sep.	1.709	10.706	0.160
5 Oct.	2.647	7.675	0.345
15 Oct.	1.950	6.500	0.300
26 Oct.	1.532	4.370	0.351
29 Oct.	0.815	2.750	0.296
4 Nov.	0.349	3.000	0.116
8 Nov.	0.833	5.000	0.167
14 Nov.	2.139	4.278	0.500
22 Nov.	1.538	0.952	1.615
28 Nov.	2.500	4.667	0.536
5 Dec.	2.143	7.200	0.298
12 Dec.	2.500	6.667	0.375
19 Dec.	3.000	7.500	0.400
27 Dec.	3.500	7.000	0.500
3 Jan.	2.000	6.667	0.300
14 Jan.	1.000	5.000	0.200
21 Jan.	2.000	6.000	0.333
26 Jan.	4.000	6.667	0.600
1 Feb.	5.000	8.333	0.600
11 Feb.	2.500	6.667	0.375
22 Feb.	2.000	5.000	0.400
27 Feb.	3.000	8.000	0.375
9 Mar.	1.329	13.571	0.098
14 Mar.	1.600	11.429	0.140
22 Mar.	2.930	5.943	0.493
29 Mar.	1.581	6.533	0.242
3 Apr.	1.533	7.188	0.213
11 Apr.	1.520	7.045	0.216
22 Apr.	1.689	6.230	0.271
29 Apr.	0.800	5.217	0.153
4 M.	0.704	5.600	0.126
15 M.	0.533	5.333	0.100
22 M.	0.761	3.560	0.214
30 M.	0.688	4.400	0.156
8 Jun.	1.917	8.519	0.225
16 Jun.	0.905	6.429	0.141
22 Jun.	0.609	4.516	0.135
30 Jun.	1.085	8.519	0.127
7 Jul.	2.860	2.217	1.290
15 Jul.	1.594	7.500	0.213
24 Jul.	1.688	22.400	0.075
28 Jul.	2.529	5.278	0.479
2 Aug.	1.484	2.135	0.695
10 Aug.	1.885	1.704	1.106
15 Aug.	1.838	1.712	1.074
22 Aug.	0.520	2.129	0.244

The scarce work made in the Mediterranean Sea on annual fluctuations of organic macromolecules indicates a pattern similar to that found in the present study, but the maximum peaks of lipids were restricted to winter-spring period **(Modica *et al*., 2006; Bhavya *et al*., 2019)**. **Finkel *et al*. (2016**) concluded that, the relative amount of each biochemical composition differs among the phytoplankton classes and species, and even within a single type of organism in response to environmental conditions.

The overall predominance of proteins denies the assumption of nitrogen limitation in the harbor, which receives continuous and/or pulsed nutrient supply. Thus, our results suggest that nitrogen was not strong enough to limit phytoplankton growth but could induces changes in the chemical composition. Differences in the biochemical compositions of phytoplankton can lead to differences in nutritional qualities for potential consumers **(Jo *et al*., 2017).**

The ratio of proteins: carbohydrates: lipids (Table 2), based on average values is typically similar to the approximate values (5:3:2) reported for phytoplankton **(Jónasdóttir, 2019)**. The significant positive relationship between them at $P < 0.01$, particularly in winter, indicates importance of high carbohydrate and lipid contents for protein synthesis to face phytoplankton growth **(Lee *et al*., 2020)**. The data could support/or contradict others with respect to the importance of carbohydrate and lipid contents in protein synthesis, and the timing of increased phytoplankton abundance and biomass **(Suárez & Marañón, 2003)**; evidence on how protein synthesis is related to production and growth in natural phytoplankton assemblages remains contradicting **(Jo *et al*., 2017)**.

The FM concentration represents the quantity of food transferred to higher trophic levels **(Kim *et al*., 2018)**. The weekly FM values (mean 310.86 µg l^{-1} ± 240.84, range 17-1054 µg l^{-1}) were characterized by several facets: significant temporal changes ($P < 0.05$) of no distinct trend, higher concentrations in summer (mean 514.5 ± 206.69 µg l^{-1}), and minimum in winter (6.25 ± 7.1 µg l^{-1}). Relatively high content on scattered days were detected (e.g., early December, 298 µg l^{-1}) with high diatom abundance (293.2 × 10^3 cell l^{-1}, Fig. 3). Statistically, significant correlation of FM was found with diatoms (r = 0.64, $P < 0.01$), relatively less with chlorophytes (r = 0.57, $p < 0.05$) and raphidophytes (r = 0.53, $P < 0.01$). The FM temporal variations were thought to result mainly from changes in the phytoplankton biomass, abundance, composition, and environmental conditions. **Kim *et al*. (2020**) reported similar results in a euphotic system (294.4 µg l^{-1}).

The temporal trend of caloric content typically followed that of the FM with an average value of 321.1 ± 366.6, ranging between a minimum of 18.9 in January and the maximum of 1464 in early August (Fig. 5). Overall, the data confirmed that protein content was the major energy source (54.55%), followed by carbohydrates (26.56%) and lipids (18.89%). While, salinity seems to affect strongly the caloric content (r = -68, $P < 0.05$).

The BPC varied between 27.58 mg C l^{-1} in late October and 6491.95 mg C l^{-1} in late

almost similar temporal variations (Fig. 5), arranged as summer > spring > winter > autumn. The physical parameters have a direct effect on BPC, whereas pH (r = 0.53), salinity (r=0.51), and temperature (r=0.50) influence the BPC variability at $P < 0.05$. While, NH_4, PO_4, and nutrient ratios seem to be of a negligible effect, the OM concentrations positively correlated with BPC (r = 0.84, $P < 0.01$). The values of BPC were significantly correlated with lipids and proteins (r = 0.73 and 0.70, at $P < 0.01$), and relatively less with carbohydrates (r = 0.63, $P < 0.05$). A very close relationship was detected between Chl. *a* and FM (r = 0.86, $P < 0.01$), CC and BPC (r = 0.61, and 0.64, p < 0.05, respectively).

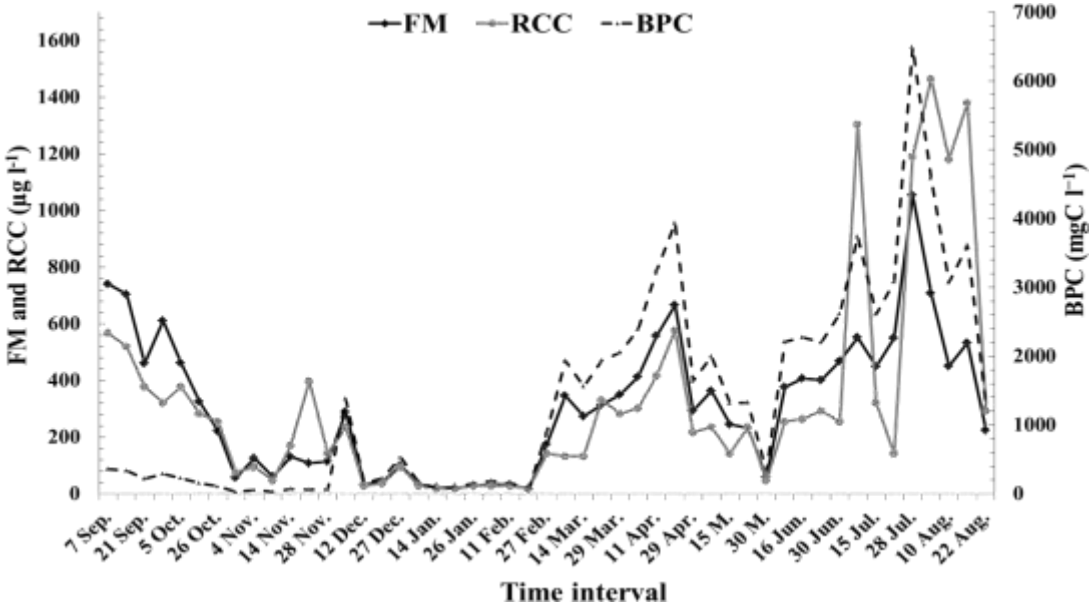

Fig. 5. Variations in the biopolymeric carbon (BPC), food material (FM), and relative caloric content (RCC) during the study period.

CONCLUSION

The short-term scale study of phytoplankton structure, composition and ambient physico-chemical parameters provides a baseline to understand changes in phytoplankton macromolecules, which certainly affect changes in the nutritional quality, reproductive periods, and the survival strategy of higher trophic levels, acting as biomarkers for the changes in the ecosystem. Protein is the principal constituent of the phytoplankton macromolecules in the harbor, followed by carbohydrate and lipid. The massive algal proliferation of different classes, and the bloom termination periods stay behind great variability in macromolecular composition; diatom group was the master class influencing BPC, FM, and RCC. It is important to incorporate the chemical composition of phytoplankton into routine environmental management programs.

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