ePublishing

ΕΘΝΙΚΟ ΚΕΝΤΡΟ ΤΕΚΜΗΡΙΩΣΗΣ NATIONAL DOCUMENTATION CENTRE

Mediterranean Marine Science

Vol 21, No 1 (2020)

Effect of Nutrient, Light Intensity and Temperature on the Growth Rates and Metabolism of a Stress-Resistant Bacillariophyta – Entomoneis sp. - in Izmir Bay (Aegean Sea)

SEZGI ADALIOĞLU, GÜLIZAR ÇALIŞKAN

doi: 10.12681/mms.19439

To cite this article:

ADALIOĞLU, S., & ÇALIŞKAN, G. (2020). Effect of Nutrient, Light Intensity and Temperature on the Growth Rates and Metabolism of a Stress-Resistant Bacillariophyta – Entomoneis sp. - in Izmir Bay (Aegean Sea). *Mediterranean Marine Science*, *21*(1), 1–10. https://doi.org/10.12681/mms.19439

https://epublishing.ekt.gr | **e-Publisher:** EKT | **Downloaded at:** 02/05/2024 03:18:11

Research Article

Mediterranean Marine Science
Indexed in WoS (Web of Science, ISI Thomson) and SCOPUS
The journal is available on line at http://www.medit-mar-sc.net
DOI: http://dx.doi.org/10.12681/mms.19439

Effect of Nutrient, Light Intensity and Temperature on the Growth Rates and Metabolism of a Stress-Resistant Bacillariophyta Species – *Entomoneis* sp. - in Izmir Bay (Aegean Sea)

Sezgi ADALIOĞLU [1] and Gülizar ÇALIŞKAN[2]

[1] Dokuz Eylül University, Institute of Marine Science and Technology, Izmir, Turkey
[2] Izmir University of Economics, Faculty of Engineering, Department of Biomedical Engineering, Izmir, Turkey

Corresponding author: sezgi.aoglu@deu.edu.tr

Handling Editor: Yelda AKTAN

Received: 8 January 2019; Accepted: 20 September 2019; Published online: 28 March 2020

Abstract

A unicellular marine microalga, *Entomoneis* sp. was isolated and studied as had become the dominant species compared to other bacillariophyta species in different environmental fluctuations in Izmir Bay. In effort to better understand the dynamics of this microalga that has achieved unprecedented domination, we conducted a monoculture isolation study.

In this study, experiments were planned within the annual range of the Izmir Bay temperature, and the demonstrated behavior of the species in light and nutrient conditions. The stock culture medium was illuminated by approximately 50µmol photons $m^{-2}s^{-1}$ of illumination with 14/24 daylight. The temperature of the climate chamber was set at the summer (T1 (21±1°C)), spring (T2 (17±1°C)) and winter (T3 (13±1°C)) temperatures of Izmir Bay. Experiments were also conducted with four different light intensities (L1 (50 µmol photons $m^{-2}s^{-1}$), L2 (25 µmol photons $m^{-2}s^{-1}$), L3 (5 µmol photons $m^{-2}s^{-1}$) and L4 (dark)). In this context, nutrient measurements were made on samples of the exponential, stationary and death phase of the culture and nutrient analyses were carried out. The results, which were designed according to ceteris paribus assumptions, were adapted to Michaelis-Menten kinetics. Consequently, considering the lifetime of the diatom at different temperature conditions, T3 was determined as an optimum temperature. Maximum growth rate and process time were observed at this temperature. This explains why these diatoms are abundant available in the winter. Once the light intensity was increased, the growth rate was increased at the T1 and T2 temperatures. However, T3 had a high growth rate at nearly L1 light intensity. Considering the consumption and transformation of different nutrient conditions, different results for both types of microalgae were obtained.

Keywords: microalgae; bacillariophyta; growth rate; monod kinetics; optimization; experimental design.

Introduction

Microalgae, which are one of the first steps in the food chain of the marine ecosystem, are used to supply the energy and nutrients for aquatic organisms and play an important role in the biogeochemical processes. When the ecosystem is exposed to nutrient, temperature and light fluctuations, the overall efficiency of microalgae can have a dramatic effect on quality growth rates. Having a tremendous population amongst microalgae, diatoms have a significant role on the environmental area such as approximately 20-25% carbon fixation, increasing the marine primary productivity (40%) (Lebeau &Robert, 2003), nutrient cycling in ecosystems (Mendes *et al*., 2012), and aquaculture (Brindley *et al.*, 2010). In addition, various biotechnological applications that consist of renewable energy (Cabello *et al.*, 2015), high value molecules (Brindley *et al.*, 2010), wastewater treatment (Abdelaziz *et al*., 2014) and ideal feedstock for biofuel production (Gao *et al*., 2015) can be conducted by microalgae because of their high valuable content, high growth rate and easy cultivation (Cabello *et al*., 2015; Gao *et al*., 2015).

An autotrophic diatom *Entomoneis* sp. usually twist apically, lie in girdle view and appear bilobate, with a fibulate raphe system. It is usually seen as two lobes in the cell and moves in the direction of the lobes perpendicular to the place. It changes direction with the twisting of the cell flaps. During torsion of the cell flaps, the apical axis turns into a fine wave and movement can be observed at various angles. (Dalu *et al.*, 2015; Paillès *et al.*, 2014). According to taxonomic studies, there are 44 known *Entomoneis* sp in the seas all over the world and this number is increasing (Czarnecki & Reinke, 1982; Poulin & Cardinal, 1983; Osada & Kobayasi, 1985; Paillès *et al.,* 2014; Dalu *et al*., 2015)). *Entomoneis* sp. have also been found in a wide variety of seas such as extreme saline waters, Guerrero Negro, Mexico, Marmara Sea sediment and the Adriatic Sea (Clavero & Grimalp, 1999; Paillès *et al*., 2014; Mucko *et al.,* 2017).

The optimal culture conditions of microalgae such as temperature, salinity, pH, and nutrients can obtain both high photosynthetic efficiency and high growth rate, which is an economically and ecologically important factor. Indeed, it is beneficial for control of the cultivation system in large scale production and biotechnology (Brindley *et al.*, 2010; Cabello *et al*. 2015). Breuer *et al.* (2013) submitted that optimal culture conditions could be different under nutrient depletion and repletion provision. Modelling the growth of microalgae using experimental design such as Response Surface Methodology (RSM) design is considered a well-established discipline to determine the nature of the response surface in an experimental region (Keskin Gündoğdu *et al.*, 2016). Providing a large amount of information by using a small number of experiments and being efficient have an advantage for determining the complicated response function (Isleten-Hosoglu *et al.,* 2012; Keskin Gündoğdu *et al.*, 2016). Moreover, scientists have emphasized that experiments are important to identify the response of phytoplankton to predicted future scenarios in order to define the effects on wealth, biomass and resource utilization, detection of ecosystem sensitivity and nutrient carrying capacities (Adalioğlu *et al.*, 2013; Kutlu & Buyukisik, 2014; Sommer *et al.*, 2012; Stefanidou *et al.*, 2018).

Entomoneis sp., for which there is little information about their growth regimes, have been used for optimizing growth conditions using response surface methodology. The effect of temperature and light regimes has been well established under N and P depletion and repletion growth conditions. Internal nutrient limitation and different light intensities were developed and operated under different temperature conditions (13-17-21^{o}C) using previously isolated microalgae of *Entomoneis* sp. in synthetic seawater. Indeed, daily relative growth rates of *Entomoneis* sp. were calculated by chlorophyll a (chl-a) value.

The aim of this study was to explore the growth regime of *Entomoneis* sp. and optimization of different culture conditions. Although, *Entomoneis* sp. was not often observed in mixed culture, in most of the preliminary mesocosm experiments, it became dominant. A few studies have been carried out focused on the taxonomy of the *Entomoneis* sp., but there is no data itemperature and nutrient depletion/repletion conditions. Investigating behavior of such resistant species in different conditions will increase our understanding of interaction with the ecosystem. In addition, this microalga can be used as a source of biotechnological product because of its various metabolites and pigment content.

In this study, Box-Behnken design (BBD) and one factor design applications were used for optimization of *Entomoneis* sp. and for understanding their behavior in different culture conditions. In addition, the effect of the collective influence of three factors (temperature (T), light intensity (L) and N/P (nitrate/phosphate) was determined by using BBD and one-factor factorial design. In addition, this is the first study of obtaining the profile of an optimization of *Entomoneis* sp. culture.

Materials and Methods

The marine water samples were collected seasonally from August 2013 to December 2014 on the İnciraltı coast of Izmir Bay (lat.38,41^{o}N; long. 27,03^{o}E). During this period, the determination of resistant species and stock culture propagation studies were performed in mixed samples. *Entomoneis* sp. emerged as a resistant diatom species in each sample. The schematic diagram of the methodology is shown in Figure 1.

Strain Isolation and Identification

Water samples (minimum 60 L) taken regularly from the coast of İnciraltı were filtered through 50 and 20 µm meshes and the surface of the 20 µm mesh was examined with inverted and stereo microscopes. *Entomoneis* sp. samples were selected with a microscope, isolated by dilution method, and cultured. Then *Entomoneis* sp. was obtained in pure form by appropriate mixing and venting technique by feeding in sterile L1 media (Guillard & Hargraves, 1993).

Isolation of Entomoneis sp.

Samples of the mixed marine algal cultures were viewed morphologically in a stereo microscope for initial identification. Monoalgal culture of *Entomoneis* sp. was

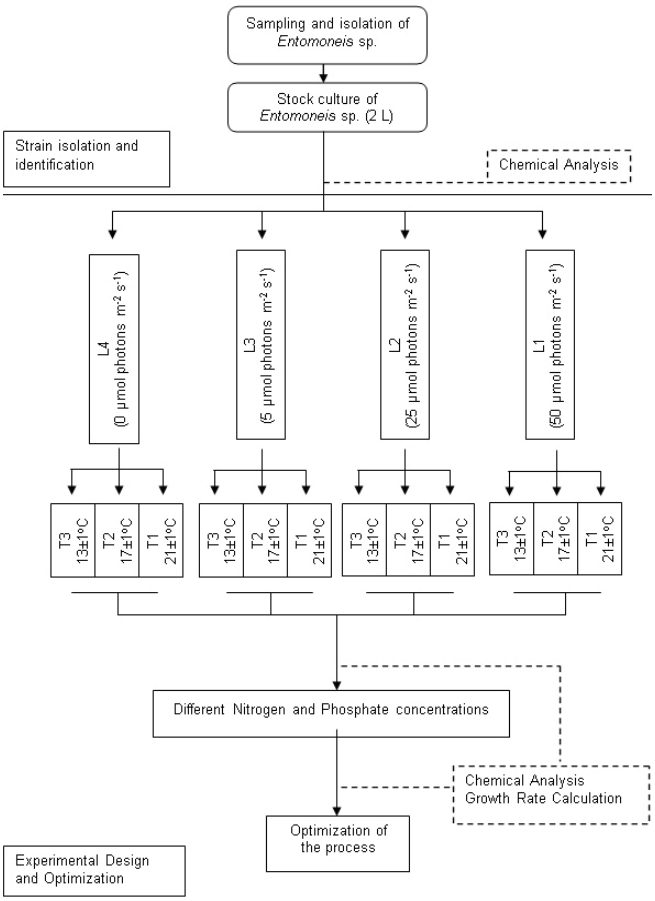

Fig. 1: Schematic diagram of methodology.

isolated by serial dilution technique in the Class II Laminar Flow Biosafety Cabinet with using NIKON SMZ25 that has high resolution and exceptionally bright specification.

Preparation of the Synthetic Seawater Medium and Stock Culture

The isolated culture from Izmir Bay was maintained in filtered and sterilized L1 medium which was suggested by Guillard & Hargraves (1993) with some modification of nitrogen and phosphate concentrations. Bioassay conditions were prepared under monoculture conditions and stock cultures of microalgae were inoculated into 300mL medium with 33±1 psu of artificial salinity in 500mL Erlenmeyer flasks. Then the microalgae stock cultures were incubated under a controlled temperature of 21±1°C and illumination of 50µmol photons $m^{-2}s^{-1}$ on the surface of the flasks and agitated by hand in a 10:14 dark:light regime. Sparging of the glass bottles was bubbled by filter-sterilized air (0.2 µm filter, Sartorius, Germany).

Experimental Design and Optimization

The experiments were carried out in two steps. In the first step, *Entomoneis* sp. was isolated and cultivated in different culture conditions. The operation parameters and growth of *Entomoneis* sp. were analyzed in this step. The second step included study of the roles of the *Entomoneis* sp. community in different nutrient concentrations and environmental factors in a lab-scale batch experiment. The parameters, including light intensity, nutrient removal and temperature were monitored in this experiment.

Design Expert software (version 7.0.0, Stat-Ease Inc., Minneapolis, MN) utilizes a 3-factor, 3-level design which is useful for investigating quadratic response surfaces and constructing second-order polynomial models. Important factors for achieving valid responses are selected using different designs. The process is usually followed by statistical analysis. One-factor design, which is made for each combination of the levels of each factor, is used for 1 numerical factor using 3 and 5 levels for a linear and quadratic model, respectively. In addition, it is a more powerful design due to reducing the error variance. Box-Behnken design (BBD) requires that factors are varied over three levels. Requiring fewer total runs may decrease the cost of experiments (Isleten-Hosoglu *et al.*, 2012; Keskin Gündoğdu *et al*., 2016).

The second-order polynomial function is used for correlating the independent variables and foreseeing the optimum point and response. The Equation is made for three factors:

$$Y=\beta_0 + \beta_1X_1 + \beta_2X_2 + \beta_3X_3 + \beta_{12}X_1X_2 + \beta_{13}X_1X_3 + \beta_{23}X_2X_3 + \beta_{11}X_1^2 + \beta_{22}X_2^2 + \beta_{33}X_3^2$$

where Y expresses the response variable, and X_1, X_2 and X_3 are the coded levels of the independent variables. Also, b_0 is the model constant, b_1, b_2 and b_3 are linear coefficients, b_{12}, b_{13} and b_{23} are interaction effect coefficients, and b_{11}, b_{22} and b_{33} are quadratic coefficients (interaction and quadratic terms).

After the optimization experiments, the data were analyzed by one-way analysis of variance (ANOVA) using response surface methodology. Then Duncan's Multiple Range Test (DMRT) was used for a post-hoc test to measure specific differences between pairs of means with SPSS statistical software (SPSS for Windows ver.18.0). In the statistical analyses, results based on a confidence level of $p \leq 0.05$ and 95% were evaluated as being statistically significant.

Preparation of the culture conditions

Stock cultures of *Entomoneis* sp. for inoculation were performed from an exponential growth phase (approx. 35,000 cells/mL). 125mL of this culture was diluted with sterilized artificial seawater to a final volume of 1000mL. Initial pH of batch cultivations was adjusted to 8.1-8.2 and was not controlled during the process. Changing of the illumination (L1, L2, L3, L4), temperature and nutrient concentration of the culture conditions is presented in Table 1.

Illuminations of L1, L2, L3, L4 were set as 50, 25, 5 and 0 µmol photons m^{-2} s^{-1}, respectively. No process parameters were altered during production.

Determination of the growth rates

Experiments were conducted to evaluate the effects of sectional changes of T, L and different N / P ratios on the growth rates of *Entomoneis* sp. in a synthetic seawater medium. During the process, *Entomoneis* sp. cultures were sustained in Climatic Room Laboratory conditions. Growth rates were drawn as different nutrient concentrations with *in vivo* chl-a analysis using a Turner Design Trilogy® Laboratory Fluorometer.

Daily growth rates µ (1/d) were calculated according to equation (1) (Kovárová-Kovar & Egli, 1998):

$$\mu = (x_{i+2} - x_i) / ((t_{i+2} - t_i).(x_{i+1})) \quad (1)$$

The relationship between substrate, biomass and growth rate is typically defined by equations (2) (Kovárová-Kovar & Egli, 1998):

$$d[S]/dt = k[S][B] / ([Ks] + [S]) \quad (2)$$

Where
B: Biomass concentration (mmol C m^{-3})
d: Detrital rate
µ: Growth rate
S: Substrate concentration
k: Maximum utilization rate for the substrate per unit mass of plankton [Ks]: Half saturated constant

Table 1. Preparation of the bioassay design.

	T1: 21±1 °C	T2: 17±1 °C	T3: 13±1 °C	
Code of Sample	**N/P**	**L1**	**L2**	**L3**	**L4**
N0% + P100%	0	+	+	+	+
N10% + P100%	1.10	+	+	+	+
N20% + P100%	2.20	+	+		
N40% + P100%	4.40	+	+	+	+
N60% + P100%	6.60	+	+		
N80% + P100%	8.81	+	+		
N100% + P100%	11.01	+	+	+	+
N100% + P80%	13.76	+	+		
N100% + P60%	18.34	+	+		
N100% + P40%	27.52	+	+	+	+
N100% + P20%	55.04	+	+		
N100% + P10%	110.07	+	+	+	+

Chemical Analysis

In vivo chl-a was measured daily using a Turner Design Trilogy® Laboratory Fluorometer. The method is based on the measurement of chlorophyll fluorescence in living cells (Holm-Hansen *et al*., 1965). However, there was extractive chl-a analysis *(in vitro)* in each life phase according to standard chl-a methodology (Strickland & Parsons, 1972). Experiments were ended when the algal population had decreased to approximately 200 µg/L of *in vivo* chl-a. The cultures were checked for contamination with inverted and stereo microscopes.

Samples were taken for the dissolved inorganic nutrient in every phase of the bioassay. Samples were filtered through pre-combusted Whatman GF/F filters. All the different conditions of the experiments were collected in 100mL polyethylene bottles. Then the samples were kept in a deepfreeze (-20°C) until the chemical analysis. The colorimetric methods adopted were performed by T80 Plus UV/VIS Spectrophotometer.

Nitrogen and phosphor (N and P) nutrient concentration were measured spectrophotometrically. NO_2-N was analyzed using the method described by Grasshoff *et al*. (2007), and Nitrate(NO_3)+Nitrite(NO_2), NH_4 following Strickland & Parsons (1972) and Helder & De Vries, (1979). *O*-phosphate phosphorus (*o*.PO_4-P) was measured spectrophotometrically using the molybdosilicate method (Strickland & Parsons, 1972). The values were used for N/P ratio calculation.

Results

In the set of experiments, different methods of classification were applied with different temperature, light intensity and nutrient content. Various kinds of varieties and classification methods were made separately for the comparisons and installation reliability.

Within the scope of this study, microbial dynamics of *Entomoneis* sp. culture related to different nutrient and light intensity levels were investigated. Nitrogen and phosphorus are macro elements that can be consumed by *Entomoneis* sp. cultures or form compounds in the oxidation stages from chemical routes. In addition, these nutrients are necessary for living and for multiplying living cells in different oxidation levels.

Environmental factors such as nutrient concentration, light intensity, pH, temperature, and concentration of inoculation are parameters affecting nutrient uptake of microalgae. The inoculation values for the interaction of these parameters and for the optimization of environmental conditions of the *Entomoneis* sp. cultures were kept constant at about $35x10^3$ cells/mL.

In vitro chl-a samples were extruded within 24 hours. Concentration values afterwards were determined by Parsons *et al.,* (1984) using the T80 Plus UV/VIS Spectrophotometer. In the coming years, one way to predict the effect of these changes in environmental conditions on *Entomoneis* sp. culture structure, such as changes in chlorophyll, is to model development processes under the changing environmental conditions using the ecophysiological differences. Temperature and light intensity are some of the potential ecophysiological properties to be used in such a statistical model. These properties can relate growth rate, nutrient consumption and harvesting

processes to each other and can help to observe biogeochemical effects (Aysel & Aysel, 2002; Sabancı & Koray, 2001).

Experimental Design and Optimization

Response surface methodology is an important statistical and mathematical techniques that is useful for developing, improving and optimizing a process (Keskin Gündoğdu *et al*., 2016). In this study, the Box–Behnken model for three variables (temperature, light intensity and N/P ratio), along with their minimum and maximum levels, was used as the experimental design model for optimization of growth of *Entomoneis* sp. In the experimental design model, temperature (13–21 °C), light intensity (0–50 µmol photons m^{-2} s^{-1}) and N/P ratio (0–110) were taken as input variables. The statistical analysis of the chl-a model is shown in Table 2. Temperature, light intensity and N/P ratio were accomplished by means of analysis of variance (ANOVA). As shown in Table 2, the model with the F-value of 23.09 was significant ($p<0.05$). In terms of the ANOVA test, there was a significant interaction between temperature and light intensity on chl-a concentration of *Entomoneis* sp. ($p<0.05$).

The interaction effects of the light intensity and temperature on the chl-a concentration are shown with the 3-dimensional plot in Fig. 2. Light intensity is directly proportional to the chlorophyll concentration while N/P ratio is fixed at 11. The maximum chlorophyll a concentration was seen when light intensity (43.15 µmol photons m^{-2} s^{-1}) and temperature value (13.7°C) were at the maximum and minimum level in that order, while the N/P ratio was kept at the central level of 11. Optimization results also showed that the growth of *Entomoneis* sp. was inhibited by increasing temperature value. As shown in Fig. 2, when the intensity of light decreased and the temperature increased, the chlorophyll levels decreased in the culture.

Significant studies have been carried out on diatomic metabolism, mostly under the N restriction. Our trial results also showed that chlorophyll a concentration might be associated with seven different nitrate concentrations

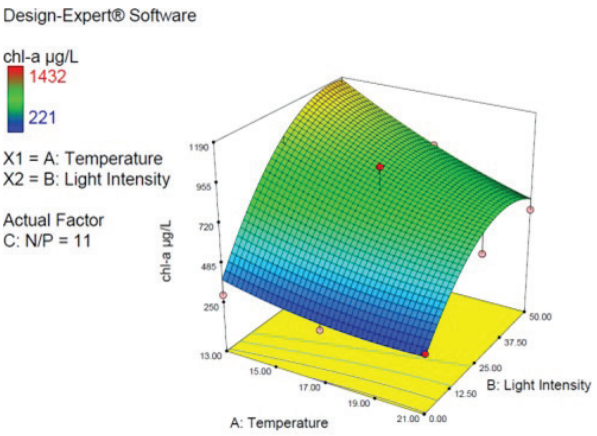

Fig. 2: 3D response surface plot and contour line of Box–Behnken Design showing the mutual effect of temperature and light intensity on chlorophyll a concentration (µg/L) of Entomoneis sp. using an N/P ratio of 11.

Table 2. Analysis of variance for response surface optimization of N/P ratio, temperature and light density for *Entomoneis* sp. on chlorophyll a concentration.

Source	SS	DF	MS	F-value	p>F	
Model	8.433E+006	23	3.667E+005	23.09	<0.0001	**significant**
A-Temperature	1.885E+006	1	1.885E+006	118.74	<0.0001	
B-Light density	3.029E+006	1	3.029E+006	190.80	<0.0001	
C- N/P	1.265E+006	6	2.108E+005	13.27	<0.0001	
Temperature x Light density	2.742E+005	1	2.742E+005	17.27	<0.0001	
Temperature x N/P	1.367E+005	6	22789.31	1.44	0.2093	
Light density x N/P	1.689E+006	6	28147.24	1.77	0.1129	
Temperature x Temperature	55632.06	1	55632.06	3.5	0.0643	
Light density x Light density	1.647E+006	1	1.647E+006	103.71	<0.0001	
Residual	1.508E+006	95	15877.62			
Lack of Fit	1.508E+006	39	38676.26			
Pure Error	0.000	56	0.000			
Core Total	9.941E+006	118				
Std. Dev.	126.01		**R-Squared**	0.8483		
Mean	617.28		**Adj R-Squared**	0.8115		
C.V.%	20.41		**Pred R-Squared**	0.7233		
Press	2.751E+006		**Adeq Precision**	20.056		

SS, sum of squared; DF, degrees of freedom; MS, mean square

as 0-1.1-4.4-11-27-110-247 N/P range applied to *Entomoneis* sp. (Collos *et al.,* 1980; Geider *et al.*, 1993; Lomas & Glibert, 2000). The effect of the proportions of inorganic nutrients has been studied in both a laboratory culture and an outdoor mesocosms. Most work on the subject focuses on changes in the inorganic N/P ratio.

Growth rate optimization is shown in Fig. 3. Light intensity and temperature directly affected the growth rate and when the temperature was below and above 17 degrees, the growth rate was decreased. The maximum growth rate was seen when light intensity was up to 25 µmol photons m^{-2} s^{-1} and temperature value was approximately 17°C, while the N/P ratio was kept at the low level of 4.4 and chl-a value was approximately 1002 µg/L. Optimization results also showed that the growth of *Entomoneis* sp. was not inhibited by high light intensity. In this study, N/P ratio was kept at 4.4 for the statistical experimental design, because some microalgae species have been found to be more adaptive to growing in nitrogen-limited cultures (Isleten-Hosoglu *et al.,* 2012). This optimization results of µ is 0.41 day^{-1} at 14.6°C and 40 µmol photons m^{-2} s^{-1}. If we increase the desirability of optimization the values are changed as seen in Fig. 4.

The interaction effects of the light intensity and temperature on the growth rate are shown with the 3-dimensional plot in Fig. 4. The maximum chlorophyll a concentration was seen when light intensity (43 µmol photons m^{-2} s^{-1}) and temperature value (13.7°C) were at the maximum and minimum level in that order, while the N/P ratio was kept at the central level of 27. Optimization result is clear where the growth rate is 0.396 day^{-1} and chl-a value is 1108 µg/L. The optimization results also showed that the growth of *Entomoneis* sp. was inhibited by increasing temperature value. As shown in Fig. 4, when the intensity of light increased, and the temperature decreased, the growth rate levels increased in the culture.

The interaction effects of the all the factors with two groups are determined and discussed for Entomoneis sp.

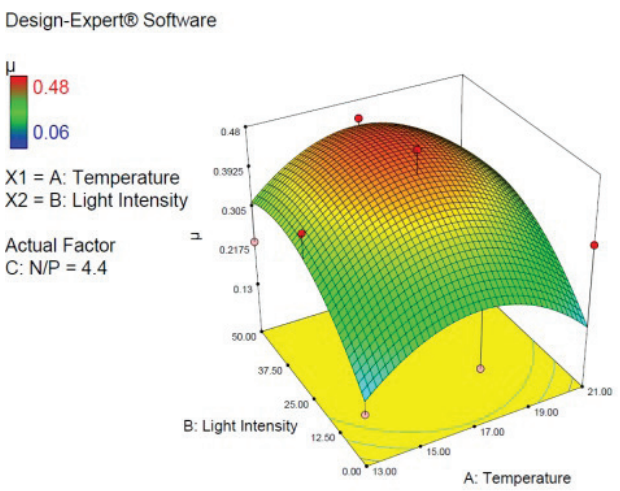

Fig. 3: 3D response surface plot and contour line of Box–Behnken Design showing the mutual effect of temperature and light intensity on growth rate (day^{-1}) of Entomoneis sp. using an N/P ratio of 4.4.

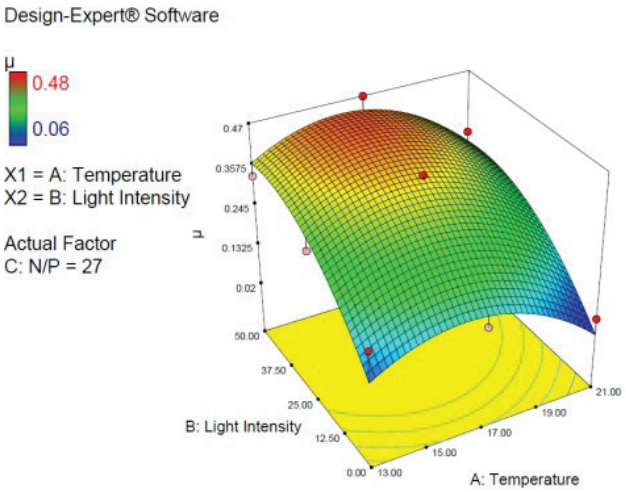

Fig. 4: 3D response surface plot and contour line of Box–Behnken Design showing the mutual effect of temperature and light intensity on growth rate (day^{-1}) of Entomoneis sp. using an N/P ratio of 27.

culture. As shown in Table 2 and post hoc test to measure specific differences between pairs of means, light intensity vs. temperature had a significant difference for both chlorophyll and growth rate responses. However, differences of N/P ratio vs. temperature and N/P ratio vs. light intensity had fewer mean differences than the corresponding Rp, which is the critical value for comparing differences in Duncan's Multiple Range Test. This means that the differences of N/P ratio were not significant with changing of temperature and light intensity, separately.

Determination of the growth rates

At the beginning of the experiment, the diversity of the experiment was achieved by considering the inorganic N/P ratio in the medium calculated on molar basis (Table 1).

Growth rates depending on the light intensity, temperature and N/P ratios are shown in Fig.5. All experiments were started to measure the growth rate under the light cycle of 14:10 L:D. In order to observe nutrient changes in the experiments, a blank medium was also prepared in which no nutrients were added. Also, a different concentration of dissolved inorganic nitrogen or phosphate was added on the first day. The effect of different temperatures, N/P ratio and light intensity on phases was observed in the growth curve formation in which we examined the variation of chlorophyll a *in vivo* with time. The growth curve shown in Fig. 5 gives information on how many days the culture will complete the lag phase in, and on what days it will be in the logarithmic phase and will pass to the death phase. According to the study, the increase of the *in vivo* chl-a approached saturation on the 25th-30th days.

When considering the amount of chl-a production seen as a control of cell viability and activity of different

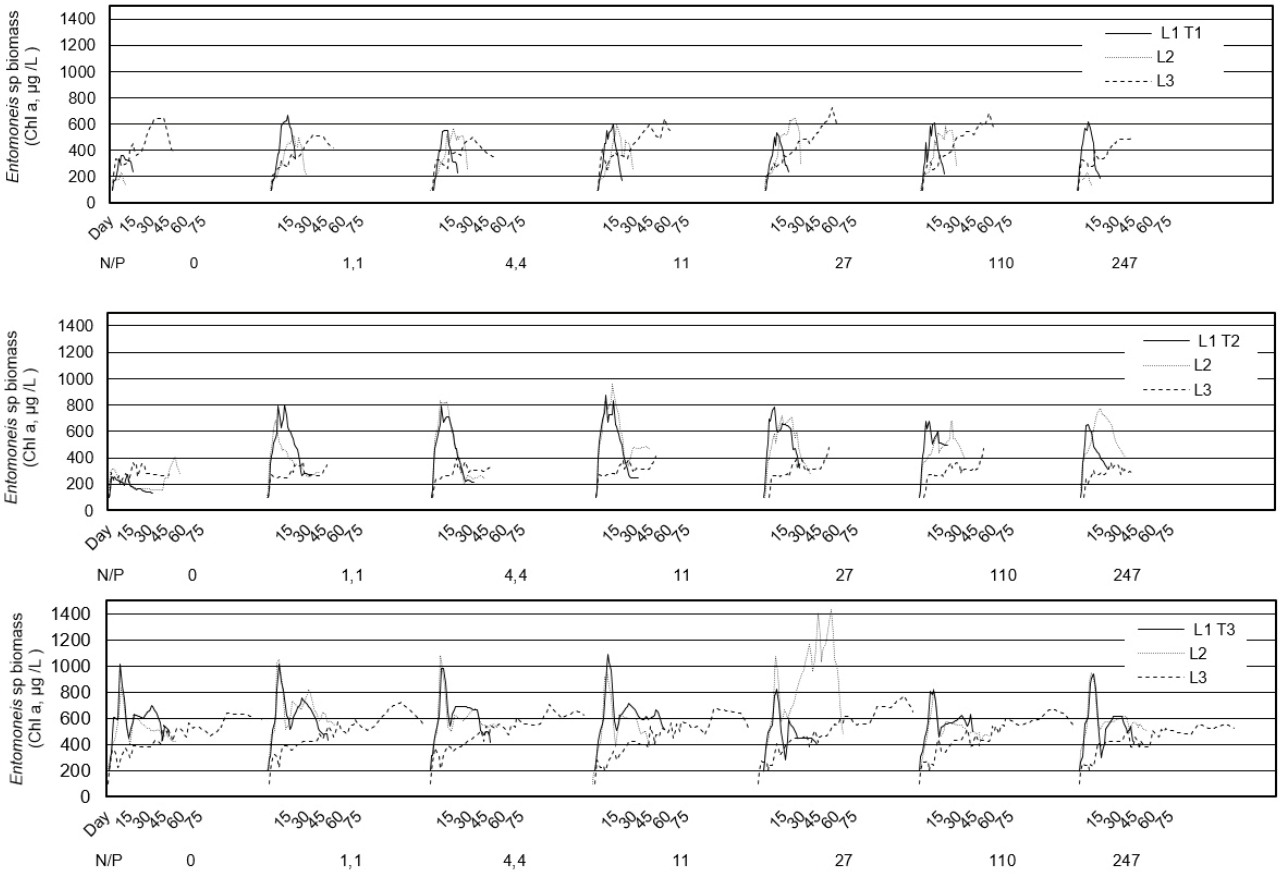

Fig. 5: Entomoneis sp biomass (Chl a, µg /L) under different N/P ratios and light intensities (a) representing growth under T1°C (b) T2°C (c) and T3°C.

nutrient conditions, *Entomoneis* sp. reached the maximum amount of average chlorophyll a concentration (1200±200 µg/L chl-a) at the temperature of 13°C (T3) degrees and at the light intensity of L2. At the other T1 and T2 degrees, 700±50 and 800±100 µg/L chl-a were reached in L3 and L2 light intensity, respectively (Fig. 5).

A similar phenomenon was observed in N/P ratio of up to 11 experiments where the maximum chlorophyll was observed at L2 light intensity. A concentration of 1400 µg/L chl-a was obtained with 27.5 of N/P ratio at temperature T3 where the maximum amount of chlorophyll was obtained in L2 light intensity in both sets of N and P variations. This situation proves that these species are found more frequently in the winter months and that production increases due to temperature. At all temperatures, chl-a concentration of L3 light intensity is lower than other light intensities and the maximum amount of chl-a is achieved for longer periods. L4 light intensity did not make a significant difference for each temperature and gave the lowest amount of 250 µg/L chl-a and growth rate of 0.25 day^{-1}.

At the T3 temperature, *Entomoneis* sp. grew rapidly and reached its maximum growth rate. The growth rate of *Entomoneis* sp. was higher in the experiments where the temperature was T3 and the light intensity was L2. However, the longest exponential phases were determined at light intensity of L3. The L3 growth curves of *Entomoneis* sp. were similar under varying N/P ratios (Fig.5). There were no significant differences in growth rates of *Entomoneis* sp. cultures grown on combinations of nitrate and ammonia.

In the monod equation, in which all N/P values set at temperature T1 are considered, the maximum growth rate reached (μ_{max}) was calculated as 0.3 day^{-1} at L1 and L3, and 0.25 day^{-1} at L2 and L4 (Fig. 6). However, in experiments in which N/P values were greater than 11, half-saturation constant (K_s) ranged from 0.03 to 0.59, while in experiments in which N/P values were below 11, they ranged between 0.02 and 0.68. The K_s sequence showed similarity in almost all experimental sets, decreasing as light intensity increased ($K_{s\_L2}<K_{s\_L1}<K_{s\_L3}<K_{s\_L4}$).

At temperature T2, the μ_{max} reached (0.5 day^{-1}) was greater compared with the experimental sets at T1. At this temperature, growth rates were in the order of $\mu_{max\_L1}>\mu_{max\_L2}>\mu_{max\_L3}>\mu_{max\_L4}$. In experiments having N/P values of 0-11, the K_s sequence was calculated as $K_{s\_L1}<K_{s\_L2}<K_{s\_L3}<K_{s\_L4}$. In cases where values were higher than

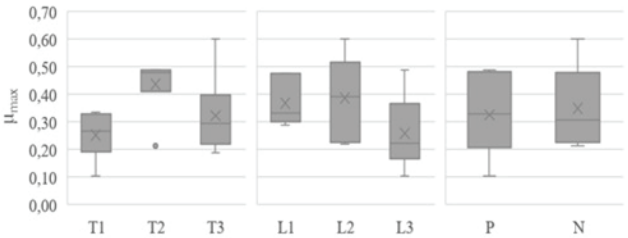

Fig. 6: Maxiumum growth rate determination of all temperatures, light intensities and nutrient concentrations.

11, no significant difference in K_s values was observed.

In the experiments conducted, at temperature T3, *Entomoneis* sp. reached the death phase at a later time by remaining at the stationary phase for longer. Particularly at L3 light intensity, cells remained in the exponential phase for a long period and did not progress to the death phase. In the monod equation, in experiments where N/P values were greater than 11, the highest μ_{max} value that was determined at L3 light intensity was calculated as 0.72 day^{-1}, while K_s reached 0.88 units. In the same experimental set, while no significant sequence was observed in μ_{max} or K_s values according to light intensity, the μ_{max} value was observed in the L1 and L2 experimental sets when N/P values were 27.5 and in L3 when N/P value was 18.3. In all experiments, a decrease in growth rate was observed when N/P value was 11. In experiments where N/P values were lower than 11, the sequence of μ_{max} values was $\mu_{max\_L3}>\mu_{max\_L2}>\mu_{max\_L1}>\mu_{max\_L4}$. In the L1, L2 and L3 experimental sets, K_s was calculated as 0.036, 0.0023 and 0.76 respectively. μ_{max} values were around 0.3; 0.4-0.5 and 0.6-0.7 day^{-1} at T1, T2 and T3 temperatures respectively.

According to the experimental sets and the optimization studies made for these experimental sets, the values found when chl-a values were maximized matched the results of the experiments. While the maximum growth rate determined was 0.72 d^{-1}, the mean maximum growth rate for all experimental sets was calculated as 0.37 d^{-1}. In the study conducted by Sarthou *et al.* (2005), in a light-filled environment and under nutrient-sufficient conditions, the maximum specific growth values obtained were calculated between 0.2 d^{-1} and 3.3 d^{-1}. In this optimization study, it was found that the determined growth rates also ranged between these values. In another similar study, variations in growth rate of *Entomoneis* sp. resulting from addition of bird droppings to its growth environment were examined (Jauffrais *et al.*, 2015). Growth rate obtained with enrichment was measured as 0.9 day^{-1}. In this study, in which optimization was carried out according to seasonal variations in its own ecosystem, the growth rate of 0.72 day^{-1} is close to those data.

Discussion

Few studies are found in the literature obtaining the profile of a growth rate of *Entomoneis* sp. and its optimization study. What is actually found in the studies of many research groups are the analyses of their chemical content in freshwater microalgae and physical properties and focus on the marine mixed culture studies. The analyses were carried out by the inhibition of growth and the evaluation of changes in the photosynthetic pigments (Dudkowiak *et al.*, 2011; Jauffrais *et al.*, 2015; Satoh *et al.*, 2005; Soares *et al.*, 2012). Jauffrais *et al.* (2015) conducted a study on the Atlantic French coast in the Gulf of Burgas at the same time as our experiment. This study shows that coastal bird feces can significantly affect growth of diatom and biochemical composition by addition of dissolved matter. In media fortified with bird feces, the maximum growth rate reached at least 35% higher compared to our study.

The current results show that the growth rate of *Entomoneis* sp. is affected by temperature. Jauffrais *et al.* (2017) investigated the effect of bacteria on growth, biomass, elemental (C & N) and biochemical composition, and extracellular polymeric substances (EPS) excretion by two marine benthic diatoms, *Halamphora coffeaeformis* and *Entomoneis paludosa*. In the study, in which a growth curve for diatoms in environments containing and not containing antibiotics was created, batch cultures of *Entomoneis* sp. reached a maximum growth rate of 1.80 ± 0.10 day^{-1} at a temperature of 17°C, a salinity of 35 and a light:dark cycle of 14:10 in an antibiotic-free environment. Under the same conditions but in an environment in which antibiotics were applied, however, a maximum growth rate of 1.50 ± 0.10 day^{-1} was determined (Jauffrais *et al.,* 2017). In untreated cultures, high bacterial abundance promoted cell division, increased growth rate and cellular abundance of *E. paludosa*. The 0.72 day^{-1} growth rate determined in the present study was found to be low when also compared with the data in the literature in terms of the likelihood of bacteria being present. Since the possibility of presence of bacteria was not focused on in the study, experiments were conducted via an *Entomoneis* sp. culture environment realized as a monoculture. According to these results, to ensure an increase in growth rate, ideas can be created for future studies on suitable bacterial combinations for *Entomoneis* sp. suited to interdependent life.

Furthermore, the effect of suitable temperature and light intensity on growth rate was observed. The minimum and maximum temperature and light intensity ranges created for the *Entomoneis* sp. monoculture isolated on the İnciraltı coast in the Aegean Region by considering the seasonal temperatures of the sea were applied for optimization specific to that region.

For algae to display their best performance, the correct ecosystem environment is needed and only in such a situation will growth rates be high. In this study, the optimum temperature was found for development of *Entomoneis* sp. and a laboratory model was created to determine how the seasonal transition temperature in Izmir Bay will affect the development of the diatom *Entomoneis* sp. diatom in the euphotic zone depending on the changing light intensity over depths.

Conclusions

Examining the different environmental conditions for the *Entomoneis* sp. culture isolated in İnciraltı, Izmir, the environmental optimization and research into the effect of the temperature changes and differences in light intensity in Izmir Bay on the growth, behavior and metabolic production of *Entomoneis* sp. have been carried out for the first time in this region. The direct effect of these independent variables was thus determined and subsequently used to make predictions about the response as growth rate and chl-a for given levels of each factor.

This essay has focused on three factors as nutrient,

light and temperature affecting the chl-a and growth rate of *Entomoneis* sp. on the İnciraltı coast of Izmir Bay. This is the first regional optimization of *Entomoneis* sp. for Izmir Bay.

The major outcome of our experiments was the fact that *Entomoneis* sp., which is one of the most resistant to environmental stress among diatoms, showed a significant sensitivity to temperature. This study provides sufficient insights into achieving an increase in process growth rate, which could make the approach appealing to various biomass and pigment production and to the understanding of regional conditional differences for the monoculture study and optimization of condition for *Entomoneis* sp.

Acknowledgements

This work was supported by the Scientific and Technological Research Council of Turkey (TÜBİTAK) [grant number 113Y112]. The data presented in this article were produced within the projects above; however, it is only the authors of this article who are responsible for the results and discussions made herein.

References

Abdelaziz, A.E.M., Leite, G.B., Belhaj, M.A., Hallenbeck, P.C., 2014. Screening microalgae native to Quebec for wastewater treatment and biodiesel production. *Bioresource Technology*, 157, 140-148.

Adalioğlu, S., Buyukisik, B., Yasar, D., 2013. Microplankton growth in response to nutrient enrichments in Gerence Bay, Izmir, Western Turkey. *Indian Journal of Marine Sciences*, 42, 859-867.

Aysel, V., Aysel, F., 2002. Barutçu Gölü' ün (Selçuk, İzmir, Türkiye) mikro ve makro algleri, E*ge Üniversitesi Su Ürünleri Dergisi*, 19, 1-11.

Breuer, G., Lamers, P.P., Martens, D.E., Draaisma, R.B., Wijffels, R.H., 2013. Effect of light intensity, pH, and temperature on triacylglycerol (TAG) accumulation induced by nitrogen starvation in *Scenedesmus obliquus*. *Bioresource Technology*, 143, 1-9.

Brindley, C., Acian, F.G., Fernandez-Sevilla, J.M., 2010. The oxygen evolution methodology affects photosynthetic rate measurements of microalgae in well-defined light regimes. *Biotechnology and Bioengineering*, 106, 228-237.

Cabello, J., Toledo-Cervantes, A., Sanchez, L., Revah, S., Morales, M., 2015. Effect of the temperature, pH and irradiance on the photosynthetic activity by Scenedesmus obtusiusculus under nitrogen replete and deplete conditions. *Bioresource Technology*, 181, 128-135.

Clavero, E., Grimalp, J., Marine, M.H., 1999. *Entomoneis vertebralis* sp. nov. (Bacillariophyceae); a new species from hypersaline environments. C*ryptogamie Algologie*, 20, 223-234.

Collos, Yves; Slawyk, G., 1980. Nitrogen uptake and assimilation by marine phytoplankton. *Primary Productivity in the Sea*, 19, 195-211.

Czarnecki, D.B., Reinke, D.C., 1982. Nomenclatural changes among some Kansas diatoms. *Transactions of the Kansas Academy of Science*, 85, 174-176.

Dalu, T., Taylor, J.C., Richoux, N.B., William Froneman, P., 2015. A re-examination of the type material of entomoneis paludosa (W Smith) Reimer and its morphology and distribution in African waters. *Journal of the Czech Phycological Society*, 15 (1), 11-25.

Dudkowiak, A., Olejarz, B., Łukasiewicz, J., Banaszek, J., Sikora, J. *et al.,* 2011. Heavy metals effect on cyanobacteria *Synechocystis aquatilis* study using absorption, fluorescence, flow cytometry, and photothermal measurements. *International Journal of Thermophysics*, 32, 762-773.

Gao, F., Yang, Z.H., Li, C., Zeng, G.M., Ma, D.H. *et al.,* 2015. A novel algal biofilm membrane photobioreactor for attached microalgae growth and nutrients removal from secondary effluent. *Bioresource Technology*, 179, 8-12.

Geider, R.J., La Roche, J., Greene, R.M., Olaizola, M., 1993. Response of the photosynthetic apparatus of Phaeodactylum tricornatum (Bacillariophycea) to nitrate, phosphate, or iron starvation. *Journal of Phycology*, 29 (6), 755-766.

Grasshoff, K., Kremling, K., Ehrhardt, M., 2007. Methods of Seawater Analysis: Third, Completely Revised and Extended Edition. Methods Seawater Anal. Third, Complet. Revis. Ext. Ed. 1-600 pp.

Guillard, R., Hargraves, P., 1993. *Stichochryis immobilis* is a diatom, not a chrysophyte. *Phycologia*, 32, 234-236.

Helder, W., De Vries, R.T.P., 1979. An automatic phenol-hypochlorite method for the determination of ammonia in sea- and brackish waters. *Netherlands Journal of Sea Research*, 13, 154-160.

Holm-Hansen, O., Lorenzen, C.J., Holmes, R.W., Strickland, J.D.H, 1965. Fluorometric determination of chlorophyll. *ICES Journal of Marine Science*, 30 (1), 3-15.

Isleten-Hosoglu, M., Gultepe, I., Elibol, M., 2012. Optimization of carbon and nitrogen sources for biomass and lipid production by *Chlorella saccharophila* under heterotrophic conditions and development of Nile red fluorescence based method for quantification of its neutral lipid content. *Biochemical Engineering Journal*, 61, 11-19.

Jauffrais, T., Agogué, H., Gemin, M.P., Beaugeard, L., Martin-Jézéquel, V., 2017. Effect of bacteria on growth and biochemical composition of two benthic diatoms Halamphora coffeaeformis and *Entomoneis paludosa*. *Journal of Experimental Marine Biology and Ecology*, 495, 65-74.

Jauffrais, T., Drouet, S., Turpin, V., Méléder, V., Jesus, B. *et al.,* 2015. Growth and biochemical composition of a microphytobenthic diatom (*Entomoneis paludosa*) exposed to shorebird (*Calidris alpina*) droppings. *Journal of Experimental Marine Biology and Ecology*, 469, 83-92.

Keskin Gündoğdu, T., Deniz, İ., Çalışkan, G., Şahin, E.S., Azbar, N., 2016. Experimental design methods for bioengineering applications. *Critical Reviews in Biotechnology*, 36, 368-388.

Kovárová-Kovar, K., Egli, T., 1998. Growth kinetics of suspended microbial cells: from single-substrate-controlled growth to mixed-substrate kinetics. *Microbiology and Molecular Biology Reviews*, 62, 646-66.

Kutlu, B., Buyukisik, B., 2014. A modeling using the maximum growth capacity of *Hantzschia amphioxys* in the Homa La-

goon. *African Journal of Biotechnology*, 13, 1154-1159.

Lebeau, T., Robert, J.-M., 2003. Diatom cultivation and biotechnologically relevant products. Part II: Current and putative products. *Applied Microbiology and Biotechnology*, 60, 624-632.

Lomas, M.W., Glibert, P.M., 2000. Comparisons of nitrate uptake, storage, and reduction in marine diatoms and flagellates. *Journal of Phycology*, 36, 903-913.

Mendes, L.F., Vale, L.A.S., Martins, A.P., Yokoya, N.S., Marinho-Soriano, E. *et al.,* 2012. Influence of temperature, light and nutrients on the growth rates of the macroalga Gracilaria domingensis in synthetic seawater using experimental design. *Journal of Applied Phycology*, 24, 1419-1426.

Mucko, M., Bosak, S.,Orlić, S.,Gligora, U.M., Peharec, Š.P. *et al.,* 2017. *Entomoneis tenera* sp. nov., a new marine planktonic diatom (Entomoneidaceae, Bacillariophyta) from the Adriatic Sea. *Phytotaxa*. 292 (1).

Osada, K., Kobayasi, H., 1985. Fine structure of the brackish water pennate diatom *Entomoneis alata* (Ehr.) Ehr. var. japonica (Cl.) comb. nov. *Japanese Society of Phycology*, 33, 215-224.

Paillès, C., Poulin, M., Boudouma, O., Pierre, C., Paddock, M. *et al.,* 2014. Entomoneis calixasini sp . nov ., a new fossil diatom from the Turkish Marmara Sea sediments. *Diatom Research*, 29, 411-422.

Parsons, T.R., Maita, Y., Lalli, C.M., 1984. Determination of chlorophylls and total carotenoids: spectrophotometric method. A Man. *Chemical Biology Methods Seawater Analysis*, 101-104.

Poulin, M., Cardinal, A. 1983. Sea ice diatoms from Manitounuk Sound, southeastern Hudson Bay (Quebec, Canada).III. Cymbellaceae, Entomoneidaceae, Gomphonemataceae, and Nitzschiaceae. *Canadian Journal of Botany*, 61, 107-118.

Sabancı, F.Ç., Koray, T., 2001. İzmir Körfezi (Ege Denizi) mikroplanktonunun certikal ve horizontal dağılımına kirliliğin etkisi. *Aquatic Sciences,* 18, 187-202.

Sarthou, G., Timmermans, K.R., Blain, S., Tréguer, P., 2005. Growth physiology and fate of diatoms in the ocean: A review. *Journal of Sea Research*, 53, 25-42.

Satoh, A., Vudikaria, L.Q., Kurano, N., Miyachi, S., 2005. Evaluation of the sensitivity of marine microalgal strains to the heavy metals, Cu, As, Sb, Pb and Cd. *Environment International*, 31, 713-722.

Soares, B.M., Vieira, A.A., Lemões, J.S., Santos, C.M.M., Mesko, M.F. *et al.,* 2012. Investigation of major and trace element distribution in the extraction-transesterification process of fatty acid methyl esters from microalgae *Chlorella* sp. *Bioresource Technology*, 110, 730-734.

Sommer, U., Adrian, R., Bauer, B., Winder, M., 2012. The response of temperate aquatic ecosystems to global warming: Novel insights from a multidisciplinary project. *Marine Biology*, 159, 2367-2377.

Stefanidou, N., Genitsaris, S., Lopez-Bautista, J., Sommer, U., Moustaka-Gouni, M., 2018. Effects of heat shock and salinity changes on coastal Mediterranean phytoplankton in a mesocosm experiment. *Marine Biology,* 165.

Strickland, J.D.H., Parsons, T.R., 1972. A Practical Handbook of Seawater Analysis. A Pract. *Handbook of Seawater Analysis*, 167, 185pp.