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Nutrient Effects on the Growth Rates of Marine Diatom C*haetoceros gracilis* Schütt

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Özet: *Deniz diyatomu* Chaetoceros gracilis *Schütt büyüme hızı üzerine besleyici elementlerin etkileri.* Bu çalışmanın amacı, *Chaetoceros gracilis* Schütt türünün kesikli kültür şartları altında nütrient zenginleştirme denemeleriyle, büyüme kinetikleri hakkında detaylı bilgileri ortaya çıkarmaktır. *C. gracilis* türü İzmir Körfezi'nden izole edilmiştir. Çalışmamız, incelenecek olan nütrient haricinde diğer nütrientlere doygun ve optimum ışık ve sıcaklık şartlarında gerçekleştirilmiştir. Denemeler sabit sıcaklık odasında 28°C sıcaklıkta ve "gün-ışığı" fluoresan lambalar kullanılarak 1300 ftCd ışık şiddetinde aydınlatılmıştır. Kültürlerin devamı 12:12 aydınlık:karanlık döngüsünde sürdürülmüştür. Stok kültürler ve kesikli kültür denemeleri için Guillard (1975) tarafından tanımlanan f/2 ve h/2 ortamları kullanılmıştır. Üssel büyüme hızları Guillard, 1973'e göre hesaplanmıştır. Herbir nütrient konsantrasyonuna karşılık gelen üssel büyüme hızları grafiğe geçirilmiştir. Monod eğrisi (Monod, 1942) parametreleri transformasyon ve en küçük kareler metodu kullanılarak hesaplandı. Herbir nütrient için bulunan Ks (Yarı-Doygunluk Sabiti) değerleri ise, nitrat için 0.496 µmol NO_3^-–N L^{-1}; amonyum için 0.862 µmol NH_4^+-N L^{-1}; fosfat için 1.045 µmol PO_4^{-3}-P L^{-1}ve silikat için 0.558 µmol Si L^{-1} bulunmuştur. Aynı nütrientler dikkate alındığında maksimum büyüme hızı değerleri ise gün^{-1} olarak sırasıyla, 1.611, 1.332, 1.662 ve 1.757 bulunmuştur.

Anahtar Kelimeler: *C. gracilis*, diatom, büyüme hızı, yarı-doygunluk sabiti.

Abstract: The aim of this research is to reveal the detailed informtion on growth kinetics of *Chaetoceros gracilis* Schütt from the results of nutrient enrichment experiments under batch culture conditions. *C. gracilis* Schütt was isolated from İzmir Bay. Our studies were performed under optimum light and temperature and nutrient saturated conditions except the nutrient which is investigated. Experiments were conducted in a constant temperature room at 28 °C and irradiated at 1300 ftCd (foot-candle) by daylight fluorescent lambs. Cultures were maintained on a 12:12 light:dark cycle. For stock cultures and batch culture experiments, f/2 and h/2 mediums were used described by Guillard (1975). Exponential growth rates were calculated according to Guillard 1973. The exponential growth rates were plotted against corresponding each nutrient concentrations. Monod curve (Monod, 1942) parameters were calculated by using transformations and the least square method. Ks (Half Saturation Constant) values calculated for each of nutrients were found as follows; 0.496 µmol NO_3^-–N L^{-1}; 0.862 µmol NH_4^+-N L^{-1}; 1.045 µmol PO_4^{-3}-P L^{-1} and 0.558 µmol Si L^{-1}. Concerning the same nutrients, values of maximum growth rate were found as 1.611 day^{-1}, 1.332 day^{-1}, 1.662 day^{-1} and 1.757 day^{-1} respectively.

Key Words: *C. gracilis*, diatom, growth rate, half-saturation constants.

Introduction

In the Izmir Bay, especially in the summer months when there is an increase in water column stability; small species become dominant and thus, are better able to compete with big cells (Buyukisik, 1986). Another advantage for these small species is the fact that during this time of year, nutrients in the surface water decrease to very low concentrations. With the batch culture experiments carried out using the small diatom *C. gracilis* which was isolated from İzmir Bay, it is aimed to point out the physiological properties of this species and also to form a base for model studies by parameterize its exponential growth rates, its nutrient limiting growth rates and the Michealis-Menten curves that indicate its dependence on nutrients. Being spread in a wide area of oligotrophic regions to eutrophic regions shows that this species' environmental adaptation is quite interesting.

Material and Method

For this study, *C. gracilis* Schütt was isolated with micropipette method described by Hoshaw and Rosowski, 1973 in Stein, 1973 from Izmir Bay (Aegean Sea). Experiments were conducted in a constant temperature room at 28 °C and irradiated at 1300 ftCd (foot-candle) by daylight fluorescent lambs. Cultures were maintained on a 12:12 light:dark cycle. For stock cultures and batch culture experiments, f/2 and h/2 mediums were used described by Guillard (1975).

The experiments were carried out in 1 liter Pyrex bottles initially containing 1 liter of seawater. For the experiment, the concentrations of nutrients in f/2 medium were changed and thus, for every nutrient a different concentration was obtained. In the experiment groups, trace elements and vitamins added to the seawater according to f/2 medium (Guillard, 1975). Ammonium, phosphate and silicate were analysed by spectrophotometric methods (Strickland & Parsons, 1972) and nitrate by the copper-cadmium reduction method of UNESCO

(1983). All of the spectrophotometric analyses were carried out by using Bosch-Lomb Spectronic 21 UVD model spektrophotometer and also chlorophyll α analysis were performed using Turner 10-AU Model Fluorometer.

For the experiment groups, the nutrients the nutrient exposured, trace elements and vitamins added to the seawater according to f/2 medium (Guillard, 1975).

Our studies were performed under non-limiting nutrient, light and temperature conditions except the nutrient exposured.

The specific growth rate was obtained from each growth curve calculating the following equation (Guillard, 1973):

$\mu=3.322*\log (N_2/N_1)/\Delta t$

where N_2 and N_1 are teh chlorophyll a concentration at the end (t_2) and beginning (t_1) of a period of time, Δt is the t_2-t_1.

The rate of growth has been shown (Monod, 1942) to be related to the concentration of substrate in the medium by the following equation.

$\mu = \mu_{max}(S/K_s+S)$

where μ is the specific growth rate, μ_{max} is the maximum specific growth rate unlimited by low concentrations of substrate, S, K_s (Half Saturation Constant) is the concentration that supports a rate equal to $\mu = \mu_{max}/2$.

Results and Discussion

According to analytical results, natural seawater concentrations are given below NO_3–N: 0.124 µmol L^{-1}, NH_4^+-N: 1.62 µmolL^{-1}; PO_4^{-3}-P L^{-1}: 0.84 µmol$L^{-1}$and Si: 4.4 µmolL^{-1}. The nitrate concentration which exposured and specific growth rates for *C. gracilis* are given at Table 1.

Table 1. The specific growth rates of C. gracilis at different nitrate concentrations (day^{-1}).

NO_3–N Concentration (µmol L^{-1})	µ
883.124	1.599
442.124	1.650
176.124	1.637
88.424	1.68
35.444	1.461
17.784	1.260
8.954	1.064
1.894	0.964
1.004	0.967
0.124	1.1

Also growth curves of different concentrations of nitrate are shown in Figure 1.

The relationship of exponential growth rates vs. nitrate concentrations was shown as a Monod curve and transformation of the data (S/µ vs. S) to obtain linearised curve (Fig. 2).

For *C. gracilis* at 28 ºC constant temperature, 1300 ftCd light intensity and by adding 10 different nitrate concentrations, that half-saturation constant value was found out as 0.496 µmolL^{-1} and the maximum growth rate as 1.661 day^{-1}.

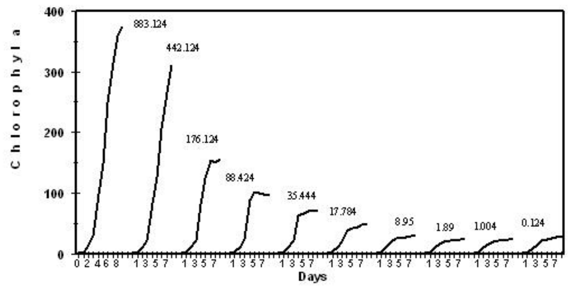

Figure 1. Growth curves of different concentrations of nitrate. Each curves are the nitrate concentrations (initial plus added) in the medium, in µmolL^{-1}.

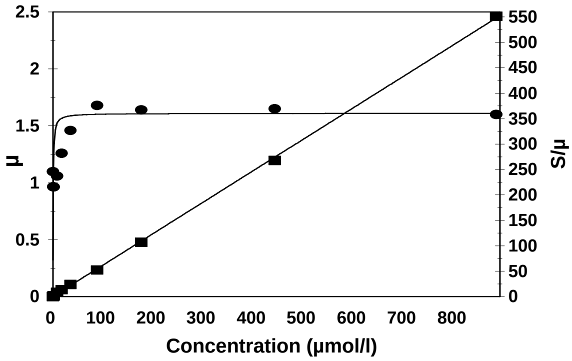

Figure 2. Specific growth rates as a function of nitrate concentration. The S/µ versus S regression line was used to calculate Ks and µmax. Circles: µ versus S, square: S/µ versus S.

Eppley & Thomas (1969), found Ks values as 0.2 µg at L^{-1} (=0.2µmolL^{-1}), maximum growth rate as 3.2 doubling/day at 21 ºC and under batch culture conditions.

Also Eppley, Roggers and McCarthy (1969), found Ks values between 0.1-0.3 µgat L^{-1} (=0.1-0.3 µmolL^{-1}) at the experiments has been carried out. The experiments mentioned above were carried out between 18 ºC and 21 ºC.

Finding the different Ks values amd maximum growth rates under different temperature conditions is expectible.

Different environmental conditions that isolated same are the reason. According to literatures, the half-saturation constant values of the species from the “nutrient poor” environment are generally lower than from the “nutrient rich” environments.

Eppley and Thomas (1969), has been reported that the Ks values of nitrate between 0.4-5.1 µgat L^{-1} (=0.4-5.1 µmolL^{-1}) in neritic areas.

The ammonium concentrations which exposured and specific growth rates for *C. gracilis* are given at Table 2.

Growth curves of different concentrations of ammonium are shown in Figure 3.

As it can be seen in the graphics, the specific growth rate of *C. gracilis* between 51.62 µmolL^{-1} NH_4^+-N and 6.62 µmolL^{-1} NH_4^+-N didn’t show significant changes. But from 6.62 µmolL^{-1} NH_4^+-N concentration downwards a gradual decreased in specific growth rate can be observed.

The relationship of exponential growth rates vs.

ammonium concentrations was shown as a Monod curve and transformation of the data (S/μ vs. S) to obtain linearised curve (Fig. 4).

For *C. gracilis* at 28ºC constant temperature, 1300 ftCd light intensity and by adding 11 different ammonium concentrations,Ks value was found out as 0.862 μmolL^{-1} and the maximum growth rate as 1.322 day^{-1} (Fig. 4).

Table 2. The specific growth rates of C. gracilis at different ammonium concentrations (day^{-1}).

NH_4^+-N Concentration (μMOL L^{-1})	μ
501.62	1.613
201.62	1.583
101.62	1.483
51.62	1.305
26.62	1.304
6.62	1.305
3.62	1.249
2.62	0.898
2.12	0.792
1.72	0.695
1.62	0.990

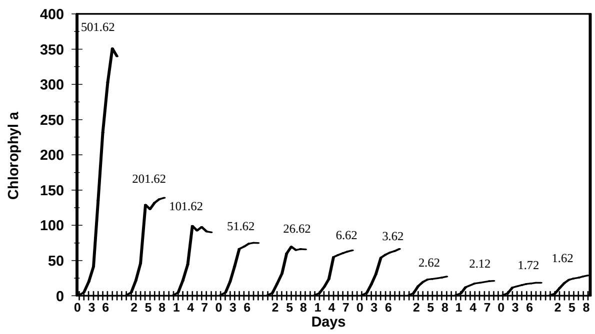

Figure 3. Growth curves of different concentrations of ammonium. Each curves are the ammonium concentrations (initial plus added) in the medium, in μmolL^{-1}

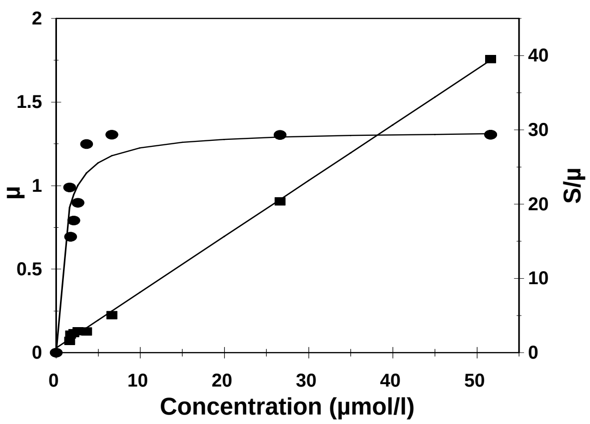

Figure 4. Specific growth rates as a function of ammonium concentration. The S/μ versus S regression line was used to calculate Ks and μmax. Circles: μ versus S, square: S/μ versus S.

Due to the studies, the Half-Saturation Constant values were found between 0.5-9.3 μmol NH_4^+-N L^{-1} (Eppley, 1969). According to these results, the values we obtained are within limits.

Thomas & Dodson (1972), found Ks values of ammonium as 0.1 μM for *C. gracilis* which isolated from Tropical Pacific Ocean. This value was obtained with the result of uptake experiment.

Having a lower Half-Saturation Constant value than ammonium shows that the *C. gracilis* is able to uptake the low nutrient concentration in its medium. But in oligotrophic conditions the species will prefer both nutrients.

Throughout our studies, *C. gracilis* has been exposured to 11 different phosphate concentrations under optimum temperature (28ºC) and optimum light intensity (1300 ftCd) conditions.

For phosphate, the concentrations which *C. gracilis* has been exposed to and the specific growth rates obtained are as shown in Table 3.

Growth curves of different concentrations of phosphate are shown in Fig. 5.

Table 3. The specific growth rates of C. gracilis at different phosphate concentrations (day^{-1})

PO_4^{-3}-P Concentration(μmol l^{-1})	μ
37.14	1.599
18.99	1.539
8.1	1.430
4.47	1.289
2.665	1.233
1.566	1.063
1.203	0.768
0.985	0.864
0.913	0.808
0.876	0.765
0.84	0.608

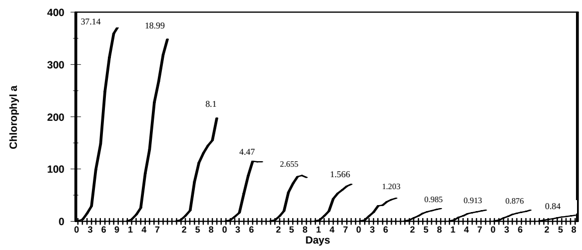

Figure 5. Growth curves of different concentrations of phosphate. Each curves are the phosphate concentrations (initial plus added) in the medium, in μmolL^{-1}

Considering the specific growth rates of. *C. gracilis* in all the 11 different concentrations of phosphate which it has exposured to, we see that these nutrients is being effected by the levels of the concentrations chosen for these experiments. Thus, according te Liebig Minimum Rule low phosphate together with nitrate is the most important nutrient for the growth of phytoplanktonic organisms, this results is expectable.

The relationship of exponential growth rates vs. phosphate concentrations was shown as a Monod curve and transfomation of the data (S/µ versus S) to obtain linearised curve (Fig. 6).

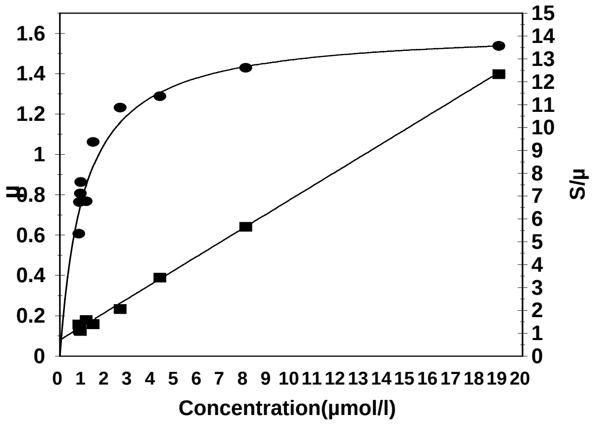

Figure 6. Specific growth rates as a function of phosphate concentration. The S/µ versus S regression line was used to calculate Ks and µmax. Circles: µ versus S, square: S/µ versus S.

For *C. gracilis* at 28 ºC constant temperature, 1300 ftCd light intensity and by adding 10 different phosphate concentrations, Ks value was found out as 1.045 µmol L^{-1} and the maximum growth rate as 1.622 day^{-1} (Fig. 6).

Thomas and Dodson (1969), has been estimated the half-saturation constant for *C. gracilis* as 0.12 µgat PO_4^{-3}-P L^{-1}. This difference depends on two reasons.

Both experiments have been carried out under batch culture conditions. But the temperatures were very different. Thomas ve Dodson (1969) carried out their experiment under 20 ºC. This experiment however was made under 28ºC optimum temperature. Temperature has an effect on as maximum growth rate as half-saturation constant values.

The characteristics of the area, which *C. gracilis* was isolated from, have a great difference. Thomas & Dodson (1969) isolated the species from Tropical Pacific Ocean, which has a low nutrient level. We isolated *C. gracilis* from Izmir Bay which has a higher nutrient level compared with the Tropical Pacific Ocean. The Ks values can be changed significantly by differences of clones.

In this study, *C. gracilis* has been exposured to 9 different silicate concentrations. The specific growth rate obtained according to experiment results are shown in Table 4.

As it can be seen in the table, the specific growth rates were found out between 1.99 and 0.928 day^{-1}.

Growth curves of different concentrations of silicate are shown in Fig. 7.

The relationship of exponential growth rates vs. silicate concentrations was shown as a Monod curve and transformation of the data (S/µ vs. S) to obtain linearised curve (Fig. 8).

For *C. gracilis* at 28 ºC constant temperature, 1300 ftCd light intensity and by adding 9 different silicate concentrations, Ks value was found out as 0.558 µmol L^{-1} and the maximum growth rate as 1.757 day^{-1} (Fig. 8).

Thomas & Dodson (1975), found out the Ks values for the same species as 0.47 µmol Si L^{-1}. According to the same study, maximum growth rate was estimated as 3.21 doublings/day.

Table 4. The specific growth rates of *C. gracilis* at different silicate concentrations (in day^{-1}).

Silicate Concentration(µmol l^{-1})	µ
123.3	1.991
101.9	1.904
69.79	1.641
37.69	1.640
26.99	1.559
19.5	1.041
17.36	1.381
16.82	1.259
16.29	0.928

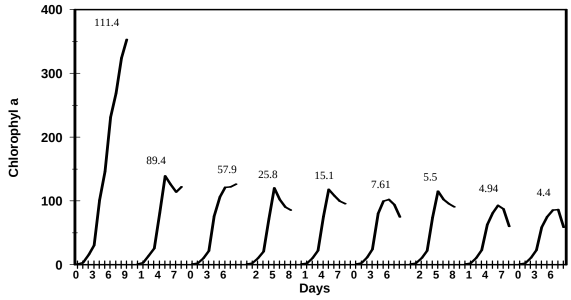

Figure 7. Growth curves of different concentrations of silicate. Each curves are the silicate concentrations (initial+added) in the medium, as µmol L^{-1}.

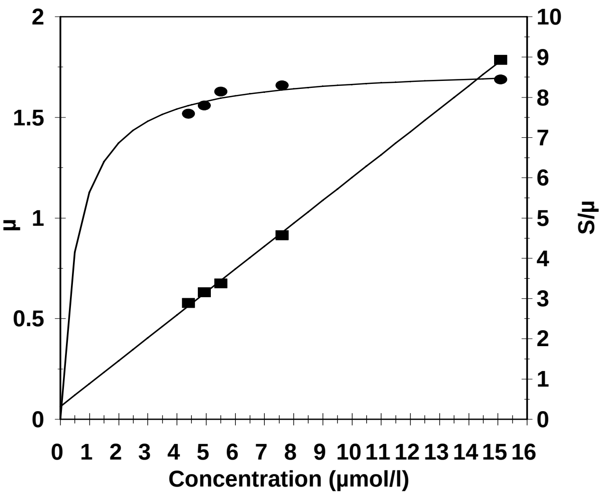

Figure 8. Specific growth rates as a function of silicate concentration. The S/µ versus S regression line was used to calculate Ks and µmax. Circles: µ versus S, square: S/µ versus S.

Nelson et al. 1976, Paasche 1973a, b, Guillard et al. 1973 found out different values of 0.19-2.13 µmol $Si(OH)_4$ L^{-1} for different clones of *Thalassiosira pseudonana*. Likewise for *Skeletonema costatum* the Half-Saturation Constant value changing between 0.42-1.13 µmol L^{-1} was stated by Conway et al. (1976), Conway and Harrison (1977), Paasche (1973 b).

Great differences between he given values in these studies may be due to differences in the size of species, their growth rates, clones and whether the species are oceanic or neritic forms.

In summary the Half-Saturation Constant values of *C. gracilis* which have been isolated from Izmir Bay, are given in Table 5.

Table 5. Ks values of *C. gracilis* for different nutrients.

	Ks
Ammonium	0.862 µmol L^{-1}
Nitrate	0.496 µmol L^{-1}
Phosphate	1.045 µmol L^{-1}
Silicate	0.558 µmol L^{-1}

As a result when the Ks values we obtained are compared with other studies we concluded that our data have not been exactly the same but showed great similarities. In this case we pointed out that this species has an adaptation ability from oligotrophic conditions to eutrophic conditions.

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