academicJournals

Vol. 13(10), pp. 1154-1159, 5 March, 2014
DOI: 10.5897/AJB10.611
ISSN 1684-5315 ©2014 Academic Journals
http://www.academicjournals.org/AJB

African Journal of Biotechnology

Full Length Research Paper

A modeling using the maximum growth capacity of *Hantzschia amphioxys* in the Homa Lagoon

Banu Kutlu* and Baha Buyukisik

Faculty of Fisheries, Tunceli University, 62000, Tunceli, Turkey.

Accepted 29 November, 2013

Homa lagoon found in the Izmir bay, Aegean Sea, is important because it is the last lagoon in which fishing activities are carried out. *Hantzschia amphioxys* species, on which this study was carried on, are benthic diatoms and they are isolated from Homa Lagoon. The Monod equation expressing the nutrient dependency of the growth rates can also be used for carrying capacity (with different parameter values; chl-a max, K_s^l). Temporal changes of the calculated maximum chl-a concentrations (carrying capacity) was 115 µg chl-a/L by determining the hyperbolical relations between the maximum biomass levels (biomass carrying capacity) and nutrient concentrations obtained from the phytoplankton growth graphs.

Key words: *Hantzschia amphioxys*, chl-a max, Homa lagoon.

INTRODUCTION

Fossil records of diatoms were found about 185 years ago (Rothpletz, 1896; Gersonde and Harwood, 1990). Diatoms are found in a wide variety of habitats including ice and soil, and they are also commonly found in both seas and freshwaters (Werner, 1977). Diatoms are the main components of phytoplankton organisms for many aquatic ecosystems.

They are named microphytobenthos in coastal areas. Moreover, they are found in high rates in the tide zones of many bays. Benthic diatoms contribute to 50% of total primary production (Perrissinotto et al., 2000; Montani et al., 2003). It is reported that diatoms have an important role in global biogeochemical cycle of nitrogen and carbon (Serodio, 2003). Furthermore, coastal waters are characterized by abundance of diatoms (Nelson et al., 1995).

In coastal waters, wave, wind, entrance of freshwater, etc. processes cause short term variations in hydrodynamic and chemical conditions of upper water column. In the subsequent process, increase of primary production is observed (Fouilland et al., 2007). Production of microphytobenthos has hourly, weekly, monthly, and seasonally changing characteristics in the tide zones of bays (Shaffer and Onuf, 1985; Pinckney and Zingmark, 1991; Smith and Underwood, 1998; Serodio and Catarino, 2000).

Microphytobenthos group is affected by environmental factors such as nutrient concentration, light intensity, salt and water temperature (Thomas, 1996; Dempster and Sammerfeld, 1998; Thessen et al., 2005).

Lagoon fields cause strong water and tide movements cause the rich water found at the bottom to flow out or cause the poor water found outside to flow in. Since water is shallow at these regions, the nutrients originating from the regeneration at the bottom are used more by benthic algae (Kutlu, 2000). Diatoms are the main components of the phytoplanktons which also serve as an important nutrient source for all aquaculture (St. John et al., 2001). Therefore, they are very important for lagoon fields.

Although, the number of the kinetic studies for the Hantzschia genus, which includes the *Hantzschia amphioxys* species, is very few, the number of studies on the *H. amphioxys* species, which forms the subject of this study, is scarce. There is no study present about the isolation of the *H. amphioxys* species and determination of the kinetic parameters on its discontinuous mono culture.

The purpose of this study was to research the maximum growth capacity and the carrying capacity of the *H. amphioxys* species in the medium with nutrient limited discontinuous culture system and optimum temperature.

MATERIALS AND METHODS

H. amphioxys was isolated from the surface sea water of the Homa Lagoon, which was filtered before f/2 was added and replicate cultures were maintained, from Izmir Bay (Aegean Sea) by using the dilution method under conditions presenting with a 11 ± 0.5°C temperature, ‰ 33 salinity, 24 L: 0D day length, 52 µmol photon.m^{2}s-1 light intensity; 40 W day light fluorescent and f/2 enrichment medium. Turner Design 10AU field fluorometre was equipped with *in vivo* Chl-a filter set and calibrated with the culture of *H. amphioxys* using Hatch -Lange Model DR4000 spektrophotometer and three coloromatic method (Strickland and Parsons, (1972).

The culture medium prepared was incubated under constant (25, 45, 52, 58 and 65 µmol/m^{2}s) light intensities at 6, 12, 15, 16.5, 18, 20 and 25°C temperatures and the exponential phase was monitored. The measurements were estimated as the amount of chl a using the Turner Designs 10-AU Field Floumeter (Guillard, 1975; Guillard and Rather 1962; Brand and Guillard, 1981). The chlorophyll based specific growth rates were calculated following Guillard (1973):

$$\mu = 1/\,(t_2\text{-}t_1)*\log_2\,(N_2/N_1)$$

Firstly, their growth rates in response to temperature were plotted. Each temperature value in the chart represented the experimental point for five different light intensities. The optimum growth rate was obtained under the conditions of 1.711 day^{-1} and 18°C and 52 µmol/m^{2}s light intensity. This result was defined as µopt. A curve was fitted that matched the experimental points in the chart. In order for this curve to include growth inhibition, the growth rates were obtained. Vollenweider (1965)'s formula describing the photosynthesis/light curves was modified and used to represent temperature curves (µ= µ max*f(T)*h(T)). h (T)= is a function describing inhibition. The whole Equation is as follows:

$$\mu = \mu_{max} * \frac{\mathbf{aT}}{\sqrt{\mathbf{1}+(\mathbf{a}T)^2}} * \frac{\mathbf{1}}{(\sqrt{\mathbf{1}+(\alpha T)^2})^{\,2}}$$

Maximum Chl-a value in each growth curve (Chl-a/time) was recorded as biomass carrying capacity (BCC). Relationships (like µ/properties, BCC/temperatures, BCC/I, BCC/nutrient concentrations) were investigated statistically. The maximum biomass carrying capacity (BCC_{max}) and the half-saturation constants were calculated with the help of the Michaelis-Menten Equation. All the half saturation constants for BCC/I curves (for seven different temperatures) were not statistically significant (p = 0.55). But the calculated values of BCC max were statistically significant (p<0.005), expect for 16.5 and 20°C.

The maximum biomass carrying capacity (BCCmax) and the half-saturation constants were calculated with the help of the Michaelis-Menten Equation. All the half saturation constants for BCC/ I curves (for seven different temperatures) were not statistically significant (p = 0.55) but the calculated values of BCCmax were statistically significant (p<0.005), except for 16.5 and 20.0°C.

RESULTS

Temperature

The research was made on seven different temperature values (6, 12, 15, 16.5, 18, 20 and 25°C) with the purpose of determining the optimal temperature of the *H. amphioxys* species used in the study. Test temperatures of the research were chosen considering the seasonal properties of the Homa Fishery.

Exponential growth phase of the *H. amphioxys* species was reached quicker than expected at 6°C in discontinuous culture system. At 12°C in the discontinuous culture system of the *H. amphioxys* species, it was observed that the species did not enter the lag phase and they went into the exponential growth phase. When the temperature was raised to 18°C, the *H. amphioxys* species was observed to enter the lag phase in the 1st day and in the following days it was observed to enter the exponential growth phase.

When the temperature was raised to 20°C, the cells of the *H. amphioxys* species were observed to enter the lag phase in the 1st day. When the effects of the discontinuous culture system and nutrient limited conditions on the growth of *H. amphioxys* are examined at the intensity of light, which is the highest temperature, it was observed that the species enter the growth phase faster than expected like all temperatures.

The carrying capacity of the lagoon water for the *H. amphioxys* species changed in a polynomial relation with the temperature (Figure 1). When the temperature decreased below 10°C, the maximum chl-a value obtained rapidly decreased. It was found to be constant between 10 and 20°C, and again an increase was observed between 20 to 25°C.

---

*Corresponding author. E-mail: banukutlu@tunceli.edu.tr or kutlubanu@gmail.com. Tel: +90.94282131784.

Statistically calculated carrying capacity parameters

Nitrate

Potential maximum chl-a values for nitrate were found to be 158.83 ± 113.7 µg chl-a/L and the half-saturation coefficients ($K_s^{'}$) were found to be 190.11 ± 184.7 (Figure 2). According to the results of the statistical analysis made by the least squares method, the variation in the nitrate concentration gives the carrying capacity of water to be 98%.

For the *H. amphioxys* species, in adequately high concentrations, nitrate substantially increased the carrying capacity of water with regards to reactive phosphate (Table 1 and Figure 2).

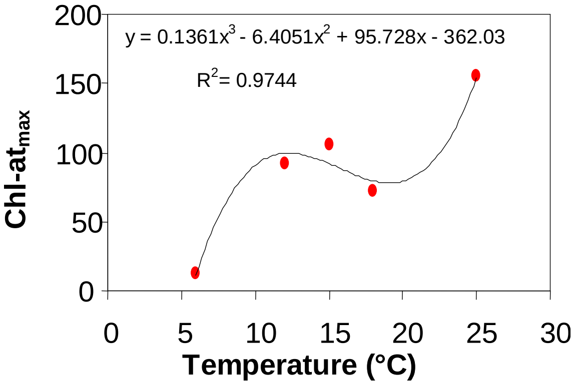

Figure 1. Relationship of potential carrying capacity (in light and nutrient saturation) versus temperature.

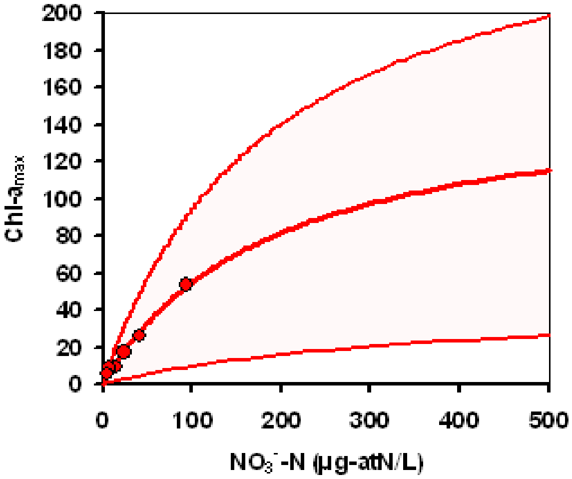

Figure 2. NO_3^--N chla max carrying capacity of the *H.amphioxys* species R^2=0.98 r=0.991

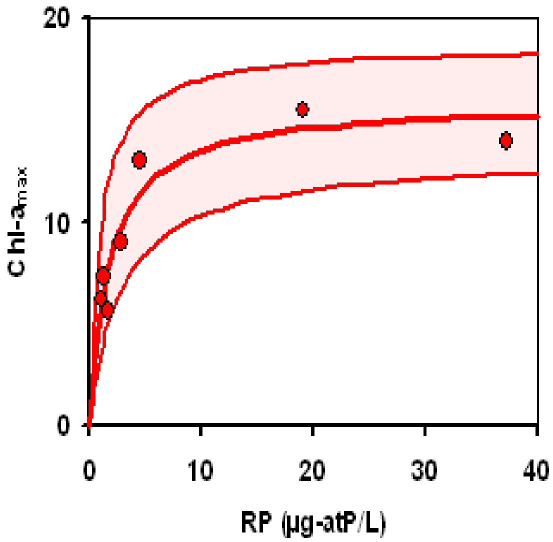

Figure 3. RP chla max carrying capacity of the *H. amphioxys* species. R^2 = 0.90 r = 0.951.

Reactive phosphate

Potential maximum chl-a values for reactive phosphate were found to be 15.90 ± 2.73 µg chl-a/L and the half-saturation coefficients ($K_s^{'}$) was found to be 1.798 ± 0.974. According to the results of the statistical analysis made by the least squares method, the variation in the phosphate concentration gives the carrying capacity of water as 90% (Figure 3).

Temporal changes of the calculated maximum chl-concentrations (carrying capacity) are given in Figure 4. In Figure 4, the curve found in the upper part shows the effect of temperature on the carrying capacity, and the carrying capacity of lagoon water for the species in question reaches the maximum value of 115 µg chl-a/L from June to the end of September. However, the observed values (in situ) were substantially low (Figure 4). Moreover, the *in situ* chl-a concentrations denote the biomass of the community. Biomass of the *H. amphioxys* species is less in this total biomass. The Monod equation expressing the nutrient dependency of the growth rates can also be used for carrying capacity (with different parameter values; chl-a max, $K_s^{'}$). Nutrients substantially decrease the carrying capacity of lagoon water all year around and while the nitrogenous compounds are the carrying capacity limiting nutrients, they are also found to be limiting the growth rate (Table 1).

Medium concentrations of nutrients decrease the carrying capacity of the lagoon water for the *H. amphioxys* species below 5 µg/L (<5 µg/L). *In situ* chl-a values are smaller than the carrying capacity that is limited by the nutrients. This is due to the contributions of the possible loss terms, which are advective transportation and grazing, because they are statistically calculated from the values obtained from the carrying capacity. Therefore, they do not include grazing and advective transportation components.

DISCUSSION

The fact that lagoons are shallow and deep, water mixes with water column results in the nutrients’ turning back into the medium very fast. The rich deep water’s rapidly transferred to the surface means a significant increase in the amount of production. The temporal variations of the growth rate and the maximum chl-concentrations that the species can reach were calculated, considering the temporal variations of the temperature and nutrient concentrations in the lagoon. With the water temperature decreasing to 7°C in February and December, substantial negative effects of water temperature was found. Limiting effects of nutrients have been clearly observed. Small increases were observed in growth rates in March, April, July, September and October. Growth rates substantially decreased in other months. In response to temperature and more significant nutrient concentrations, the carrying capacity of the lagoon water increased in March, July,

Table 1. Temporal changes of the calculated maximum chl-a concentrations (carrying capacity).

Month	*In situ* nutrient concentrations				Temperature (°C)	Chl-a tmax	Chl-a max *in situ*			Limiting nutrient	
	Ammonium (µg at/l)	Nitrate (µg at/l)	Phosphate (µg at/l)	Silica (µg at/l)			NO_3	PO_4	Liebig		Riley
February	2.35	0.27	0.40	1.20	7	38.86	0.05	7.07	0.005	N	0.05
March	2.94	7.26	0.98	13.15	15	72.22	2.66	25.44	2.66	N	2.40
April	11.14	6.65	0.82	19.05	18	45.13	1.52	14.15	1.52	N	1.38
May	12.31	4.03	0.62	13.55	22	30.01	0.62	7.72	0.62	N	0.58
June	1.27	6.50	0.21	1.72	26.3	92.95	3.07	9.72	3.07	N	2.34
July	2.93	8.05	0.69	4.90	26.5	98.87	4.02	27.42	4.02	N	3.50
August	41.43	1.60	1.68	140.38	27	115.06	0.96	55.49	0.96	N	0.95
September	7.11	15.75	0.37	8.77	25	61.72	4.72	10.42	4.72	N	3.25
October	2.73	10.57	0.36	7.16	16.5	58.60	3.09	9.80	3.09	N	2.35
November	3.48	11.68	0.08	11.92	14.5	76.34	4.42	3.18	3.18	RP	1.85
December	1.70	1.36	1.80	24.72	8	60.53	0.43	30.28	0.43	N	0.42
January	2.65	1.96	0.81	7.28	12	89.41	0.91	27.77	0.91	N	0.88
February	0.91	10.77	0.08	0.64	13.5	83.37	4.47	3.63	3.63	RP	2.00

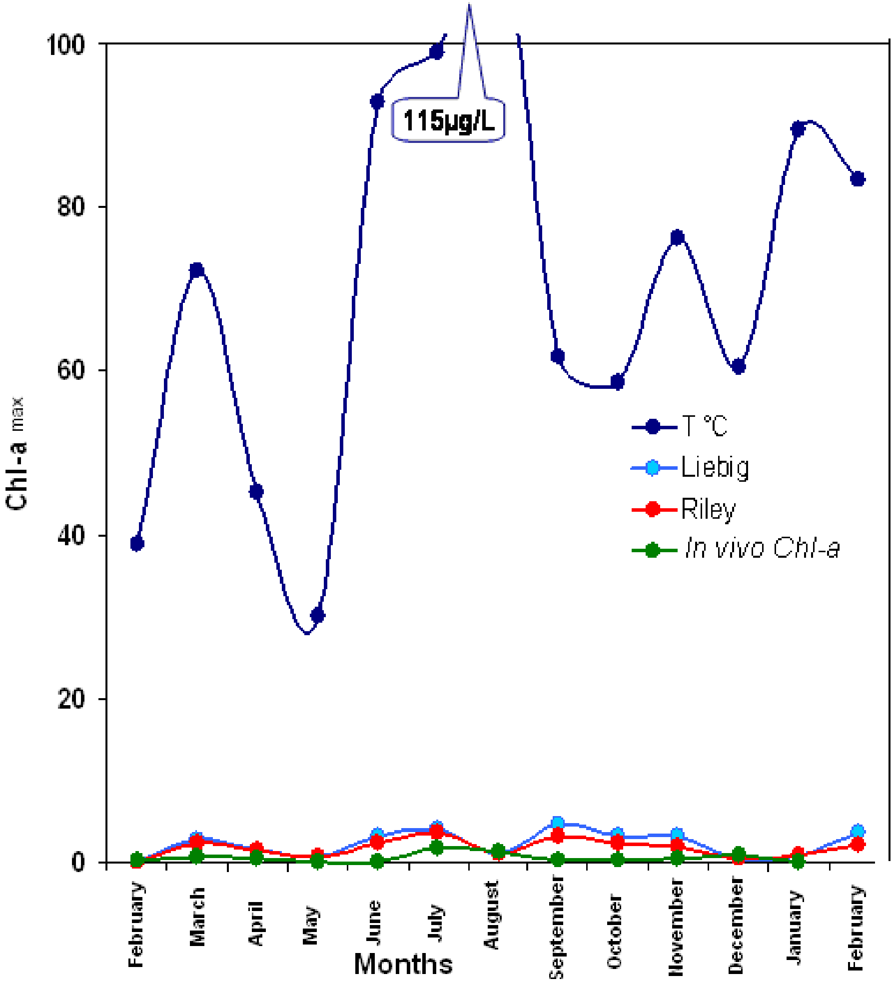

Figure 4. Temporal changes of the calculated maximum chl-a (carrying capacity).

September, October and November. However, in March and July, while the *in situ* chl-a values were observed to be lower than the values determined from the model, small increases was observed.

Reproduction of the *H. amphioxys* species all the year around in the lagoon is limited by *in situ* nutrient concentrations and by the temperature in very cold months of winter. They can be observed in the lagoon in March, July and September. The period we found shows analogy with the study made by Sabanci (2008). Nutrient limited growth for this species has been reactive limited phosphate in February, while it was limited by nitrogenous compounds in the other months. Due to the lipid synthesis observed in silicate limited growth, the surface layer of the sediment will be rich in lipids. This situation shows that, they can have a great role in nutrition of sea bream seed fish, which is a demersal fish and gastropods (Xing et al., 2008) with bottom mud.

A detailed research has not been made yet on the benthic diatoms, which have a great role in nutrition of crustaceans (Pena de la, 2007) and gastropods (Xing et al., 2008). With this study, by revealing the growth kinetics of the benthic diatoms, contribution will be made to dynamic applications related to nutrition of crustaceans and fish.

With this study, including application of the carrying capacity to the Homa Lagoon, it can be concluded that the carrying capacity can be also applied to fish breeding farms (fisheries). At the same time, in demersal fish farming, lipid production of the *H. amphioxys* species at the bottom would have positive contributions on the species nourished in net cages, which also cover the bottom.

By uptake of mineralization products in the sediment by these species, eutrophication will be prevented to some extent (by keeping the produced nutrients in the sediment by algae). Our goal was to determine the growth rate of all nutrients and the maximum levels of the phytoplankton biomass (the maximum biomass carrying capacity) in the case of how full growth can be reached and at what level the nutrient restrictive growth keeps the biomass (biomass carrying capacity) and the nutrient(s) that sustain year long growth. By determining the hyperbolical relations between the maximum biomass levels (biomass carrying capacity) and nutrient concentrations obtained from phytoplankton growth graphs, we tried to apply flexible models which will restrict the levels phytoplankton can reach.

Concentrating on the well-known importance of N as a limiting nutrient (Carthy and Carpenter, 1983; Dugdale and Goering, 1967), those models tracked the supply of nitrate (NO_3^-) via upwelling and mixing, its uptake by phytoplankton and biological regeneration of N as ammonium (NH_4^+) in surface waters.

Other modeling efforts, beginning with the pioneering work of Walsh (1975) have explicitly considered growth limitation by light, and by two or more nutrients. They have simulated the cycling of up to four elements (C, N, P and Si) in surface waters, and allowed for the depletion of up to four nutrients (NO_3, NH_4^+, PO_4 and $Si(OH)_4$) to limiting concentrations. Almost all, recent multielement models (Dugdale and Wilkerson, 1998; Pondaven et al., 1998; Spitz et al.,2001) have included two or more phyto-plankton groups with qualitatively different nutrient requirements, such as diatoms (which take up $Si(OH)_4$ and require Si for growth) and non-siliceous picoplankton (which do not take up $Si(OH)_4$). This study experimented four different nutrient groups and nutrient requirements of *H. amphioxys* were determined, and a simple model was comprised of nutrient requirements of phytoplankton in the Homa Lagoon.

Mongin et al. (2003) showed that this has the effect of causing chlorophyll a to be limited by cellular N when N limits phytoplankton growth (for example, in surface waters in summer) and by cellular C when light limits growth (for example, within the DCM). In our study, it was found that the *H. amphioxys* species in the community in the Homa Lagoon was N restrictive for all the study period except two months.

REFERENCES

Brand LE, Guillard RR, Murphy S (1981). A method for the rapid and precise determination of acclimated phytoplankton reproduction rates. J. Plankton Res. 3:193-201.

Dempster TD, Sommerfeld MR (1998). Effects of environmental conditions on growth and lipid accumulation in Nitzschia communis (Bacillariophyceae), J. Phycol. 34:712-21.

Dugdale RC, Goering JJ (1967). Uptake of new and regenerated forms of nitrogen in primary productivity. Limnology and Oceanography. 12:196-206.

Dugdale RC, Wilkerson FP (1998). Silicate regulation of new production in the equatorial Pacific upwelling. Nature 391:270-273.

Fouilland E, Raymond GEL, Ken J, Slater J, Calleja A (2007). The response of a planktonic microbial community to experimental simulations of sudden mixing conditions in temperate coastal water importance of light regime and nutrient enrichment, J. Exp. Mar. Biol. and Ecol. 35:211-225.

Gersonde R, Harwood DM (1990). Lower cretaceous diatoms fom odg leg 113 site 963 Weddel Sea. Part1, Vegettaive Cells. In Barker PF, Kennet JP et. al., (Eds) Proceedings of the Ocean Drilling Program. Vol.113. Ocean Drilling Program, College station, Teexas.pp.403-25.

Guillard RL, Rhther JH (1962). Studies of marine Plantonic diatoms I. Cyclotella nana Hustedt and Detonula conferuacea Cleve. Can. J. Microbial, 8:229-239.

Guillard RRL (1973). Culture of phytoplankton for feeding marine invertebrates in: Smith, W.L. and M.H. Chanley (eds), Culture of Marine Invertebrate Animals, Plenum pres, New York, NY.

Guillard RRL(1975). Culture of phytoplankton for feeding marine invertebrates in: Smith, W.L. and M.H. Chanley (eds), Culture of Marine Invertebrate Animals, Plenum pres, new York, N.Y.Journal of Marine Systems 17:587-619.

Kutlu B (2000). Simulated community culture on water of Izmir Bay (Homa Lagoon), Mba thesis, Izmir, 91pp.

Mongin M, Nelson D, Pondaven P, Brzezinski M,Trieguer P (2003). Simulation of upper-ocean biogeochemistry with a flexible-composition phytoplankton model: C, N and Si cyclingin the western Sargasso Sea. Deep-Sea Research I 50: 1445-1480

Montani S, Magni P, Abc N (2003). Seasonal and interannual patterns of intertidal microphytobenthos in combination with laboratory and areal production estimates, Mar.Ecol.Prog.Ser.249:79-91.

Nelson DM, Trequer P, Brzezinski MA, Leynaert A, Pena de la MR (2007). Cell growth and nütrient value of the tropical benthic diatom, Amphora sp., at varying levels of nütrients and light instensity, and different culture locations. J. Appl. Phycol. 19:647-655.

Perrissinotto R, Nozais C, Kibirige I, (2000). Spatio-temporal Dynamics of phytoplankton and microphytobenthos in a South African temporarily-open estuary, Estuar. Coast. Shelf. Sci. 55: 47-59.

Pinckney J, Zingmark RG (1991). Effects of tidal stage and sun angles on intertidal benthic microalgae productivity mar. Ecol. Prog. Ser. 76:819

Pondaven P, Fravalo C, Ruiz-Pino D, Treguer, P Queguiner B, Jeandel C (1998). Modelling the silica pumping the permanently open ocean zone of the Southern Ocean.

Rothpletz A (1896), Uber die flyysch-fuco iden und eniqe andre fossilse algen, sowie uber liasische, Diatomeen Fubrend Horrenchwamme, Z.D Sch, Geol. Ges. 48:854-914.

Sabancı ÇF (2008). Homa Lagoon (Izmir Bay, Aegean Sea) Epipelic Intertidal zone, epiphytic and epilithic diatom kommunitelerinin taksnomik environment as a factor to be studied and its relationship. Phda thesis, Izmir,

Serodio J (2003). A chlorophyll fluorescence index to estimate short-term rates of photosynthesisi by intertidal microphytobenthos, J. Phycol. 39:33-46.

Serodio J, Catarino F (2000). Modelling the primary productivity of intertidal microphytobenthos: time scales of variability and effects of migratory rhythms Mar. ecol. Prog. Ser. 192:13-30.

Shaffer GP, Onuf CP (1985). Reducing the error in estimating annual production of benthic microflora: hourly to monthly rates, patchiness in space and time, Mar. Ecol. Prog. Ser. 26:221-31.

Smith DJ, Underwood GJC (1998). Expolymer production by intertidal epipelic diatoms, Limnol. Oceanogr. 43:1578-1591

Spitz HY, Moisan JR, Abbott MR (2001). Configuring an ecosystem model usingdata from the Bermuda Atlantic Time Series (BATS). Deep-Sea Res. II 48:1733-1768.

St. John MA, Clemmesen C Lund, Koster T (2001). Diatom production in the marine environment: Implications for larval fish growth and Condition. Ices J. Mar. Sci. 58:1106-13.

Strickland, J.D.H., Parsons, T.R., 1972, A practical handbook of seawater analysis, fisheries research boards of Canada. Bull, 167, Ottawa. p. 310.

Thessen AE, Dortch Q, Parsons ML, Mossion S (2005). Effects of salinity on pseudo-nitzshia species (Bacillariophyceae) growth and distribution. J. Phycol. 41:21-9.

Thomas JH (1996). Effects of temperature and illuminance on cell division rates of three species of tropical oceanic phytoplankton, Appl. Phycol. 2:17-22.

Vollenweider, R. A. 1965, Calculation models of photosynthesis-depth curves and some implications regarding day rate estimates in primary production measurements. Mem. Ist. Ital. Idrobiol., 18Suppl.:425-457.

Walsh JJ (1975). A spatial simulation model of the Peru upwelling ecosystem. Deep-Sea Research I. 22:201-236.

Werner D (1977). Silicate metabolism, in werner, d (Ed.), The biology of Diatoms, Botanical Monograph. Vol.13. university of California Pres, Berkeley, pp.110-49.

Xing R, Wang C, Cao X, Chang Y (2008). Settlement, growth and survival of abalone, Haliotis discus hannai, ina response to eight monospecific benthic diatoms, J. Appl. Phycol. 20:47-53.