Phytoplankton growth and microzooplankton grazing in the Homa Lagoon (İzmir Bay, Turkey)

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Abstract

Phytoplankton growth and microzooplankton grazing were investigated at one station in the Homa Lagoon from February to January in 2006-2007. Our results showed significant seasonal variations in phytoplankton dynamics. Microzooplankton was mainly composed of dinoflagellates and tintinnid ciliates and nauplii. Microzooplankton grazing increased with increasing of temperature. Grazing rate was maximum levels in spring and summer. Microphytoplankton, which dominated the total algal biomass and production, were characterized by the proliferation of several chain-forming diatoms. Small heterotrophic flagellates and aloricate ciliates were the main controllers of phytoplankton. Phytoplankton represented a significant for micrograzers, which grazing represented 20–120% of diatom and cynabacteria algal production during 2006-2007. Microzooplankton has, however, a relatively high impact on microphytoplankton, as > 45% of microalgal production was consumed throughout the year. These results suggest that the low grazing was one of the factors contributing to the development of the spring bloom. All of seasonal bases, the phytoplankton production were grazed by microzooplankton in summer, autumn and winter–spring seasons have been changing between 20%-120%**.** The seasonal variation in the microzooplankton grazing pressure seems to result from the dominant size class of the phytoplankton community of this lagoon diatom and cynabacteria.

Keywords: Grazing, Lagoons, Microzooplankton, Nutrient, Phytoplankton.

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Introduction

Phytoplankton require multiple nutrients for growth and multiple nutrients interact to limit growth is essential to understanding the causes of variation in phytoplankton (Rhee, 1978; Goldman et al., 1979). The identity of the nutrient(s) limiting biomass and primary production (Smith, 1982), and the effect of resource competition on community structure (Tilman, 1982). Of particular interest are nitrogen (N) and phosphorus (P), two macronutrients that are commonly thought to limit phytoplankton growth (Smith, 1982; Downing, 1997).

Grazing microzooplankton can control the biomass of bacteria and phytoplankton (Fenchel, 1982; McManus and Fuhrman, 1988, York et al., 2010), and are in turn a main food source for mesozooplankton (Bouley and Kimmerer, 2006; Gifford et al., 2007). Thus, microplankton are important trophic intermediaries between the microbial loop and the rest of the food web. Microzooplankton, including heterotrophic flagellates, ciliates and copepod nauplii can represent significant proportion of the total zooplankton biomass of the oceans (Odate and Maitan, 1988; Tsuda et al., 1990; Booth et al., 1993; Boyd et al., 1995). The roles of micro-zooplankton in pelagic ecosystems, include those of consumers of pico- to nano-sized phytoplankton and rapid regenerators of nutrients (Paasche and Kristiansen, 1982; Goldman and Caron, 1985). Further, the microzooplankton are considered to play an intermediary role in trophic exchanges between the pico- to nanophytoplankton and meso-zooplankton (Gifford, 1991).

Determination of effects of nutrients and microzooplankton essential to phytoplankton growth is crucial in coastal areas. The limiting nutrient can be detected using different methods, e.g., by inorganic nitrogen to phosphorus ratios (Neill, 2005), enrichment experiments (e.g., Ryther and Dunstan, 1971; Graneli, 1987), or measuring intracellular concentrations of nutrients (Hecky and Kilham, 1988).

As such complicated effects of nutrients are best studied with natural populations (Stumm and Baccini, 1983; Vaquer et al., 1996; Pedrós-Alió et al., 1999; Robinson, 2000; Morán et al., 2001; Fonda Umani et al., 2005; Sakka et al., 2007). Furthermore, the significant impacts of microzooplankton grazing on phytoplankton communities has been well documented in many regions of the world's oceans (Capriulo and Carpenter, 1983; Burkill et al., 1987; Paranjape, 1987; Gilford, 1988; Odate and Maita, 1988; Verity et al., 1993; Froneman and Perissinotto, 1996; Tsuda and Kawaguchi, 1997; Shinada et al., 2000; Suffrian et al., 2008). However, there are only a few reports on the role of microzooplankton grazing in determining in the fate of primary production coastal lagoon ecosystems (Calbet and Landry, 2004; Sakka et al., 2007).

The main objective of this study was to examine both microzooplankton grazing rates on phytoplankton and phytoplankton growth rates during various seasons of the year and discuss the balance between microzooplankton and phytoplankton in order to evaluate the

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quantitative trophic role of microzooplankton in the planktonic food web of the Homa Lagoon, İzmir, Turkey.

Materials and methods

Study Site

The study was conducted at the Homa Lagoon located at the outer part of Izmir bay (Lat: 38°31'10''N, Long: 26°49'50'' E). The Homa Lagoon located near bird sanctuary, Çamaltı salt work and agricultural area (Figure 1). Fresh water input is very restricted. There is a drainage canal irregularly opened to the lagoon.

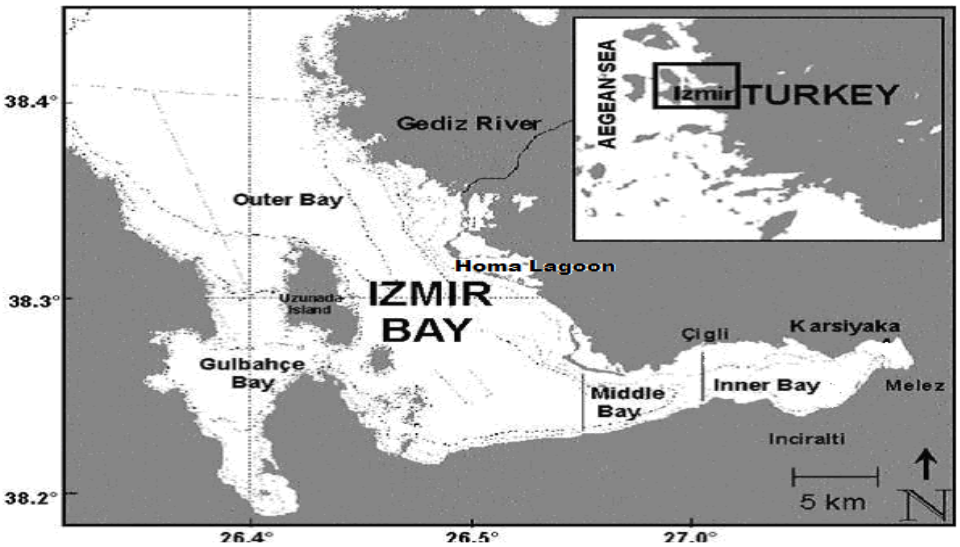

Figure 1: Map of study area

Sampling

Samples were collected from one site in the Homa Lagoon. Sea station was chosen at 1 km away from coast line. Sampling trips were carried out monthly during 2006-2007. Temperature and pH were measured by using a Hanna Model H1 8354 pH meter equipped with temperature sensor. Dissolved oxygen concentrations were measured by Winkler titration method. Salinity was determined according to Martin (1972) following Harvey's method.

Sea and lagoon water samples for determination of chlorophyll a and nutrient concentrations were collected from water surface. Water samples for dilution experiments were taken by using dyaphram pump in to the 20 L Pet carboys sieving through a 280 µm. Mesh net first to remove mesozooplankton grazers, a common procedure in such experiments (Landry et al., 1995, Downing et al., 1999). Water samples for chlorophyll a determination were filtered on to 47mm Whatman GF/C glass filter papers under reduced pressure (1/4 atm) and frozen at -20 °C.

Chemical Analysis

Chl-a concentration were determined with Hach-Lange DR-4000 spectrophotometer using tricolormatic method following

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extraction in 90% acetone according to Strickland and Parsons (1972). The filtrate was collected in 100 ml acid washed bottles and stated in a refrigerator for a few days prior to analysis. NH_4^+-N, Reactive Silicate (RSi), Reactive phosphate (RP) were analysed using spectrophotometer according to Strickland and Parsons (1972) and NO_3^--N according Wood et al. (1967).

Dilution Experiments

Total of 12 dilution experiments for lagoon water were conducted throughout the study period by using clean technique. All carboys, silicone tubing and filter cartridges were soaked overnight in 10% HCl: ultrapure water (18.2M Ω) and rinsed 3 times with ultrapure water and finally with filtered seawater. Plastic gloves were used during all steps of experiment including sub sampling and cleaning.

Duplicate incubations were carried out at 4 fractions of whole lagoon water at dilution levels D=1, 0.70, 0.45, 0.20, all enriched first with added N (NO_3^-N, NH_4^+-N), RP and RSi prior to dilution. Final nutrient concentrations were 1/100 of Guillard's f/2 medium 0.363 µMP, 8.83µ MNO_3^--N, 5 µ MNH_4^+-N, 10.7 µ MRSi). Firstly, 0.2 µm Cartridge filtered seawater was transferred to 3 L PET Carboys up to certain volume, added nutrients and then natural lagoon water was added from 20 L mixed carboys up to 2.7 L final volume. Two additional 3 L carboys were filled with unfiltered lagoon water without nutrient addition, in order to determine *in situ* growth rates of phytoplankton community. All experimental carboys (2.7 L) were incubated *in situ* for 24h.

The geometric phytoplankton biomass during incubations without nutrient supplements (Cm) was determined by following equations for Δt= 1day

$$Cm = \frac{Co(e^{\mu gross - gt}) - 1}{\mu gross - gt}$$

Where Co is the initial phytoplankton biomass; the determination of the net growth rate of the phytoplankton community (µ) was based on changes in concentrations at chl a

$$\mu = \frac{1}{\Delta t} In \frac{Ct}{Co}$$

Where t is the duration of the experiments Co and Ct is initial and final phytoplankton biomass, respectively. Relationship between net growth rates and grazing was described by Landry and Hassett (1982) as:

$$\mu = \mu max - gD$$

With the assumptions that µmax is density independent and g (microzooplankton grazing) is proportional to the dilution factor, D (or the grazer's density) grazing threshold (Gaul and Antia, 2001) from dilution experiments, g µgross were described as

$$\mu gross = \mu_{insitu(N-)} + g_t$$

Where $\mu_{insitu(N-)}$ is growth rate (d^{-1}) without nutrient additions and without dilution (100% natural sea water). g+ is grazing rate (d^{-1}) with nutrient additions. µmax is net infinite dilution (in case of no animals) determined by extrapolation to zero unfiltered sea water fraction (intercept of line).

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P_b=Chla biomass removed daily by grazing (%d^{-1}).

$$Pb=\frac{(Co-Coe^{g})}{Co}*100$$

Pp is the chl a production remaxed daily by grazing (%day^{-1})

$$Pp=\frac{(Coe^{\mu gross}-Co)-(Coe^{\mu insitu}-Co)}{(Coe^{\mu gross}-Co)}*100$$

Statistical analyses

Analyses were performed using SPSS 14.0 statistical software for Windows. One-way ANOVA was used to test the differences among stations for Chl a, phytoplankton, growth rates and grazing coefficients. The ANOVA was followed by multiple comparison tests (Duncan test) to identify which groups were significantly different from the others.

Results

Environmental condition and nutrient concentrations

Surface water temperature, salinity, silicate, ammonium, nitrite and nitrate concentrations are presented in Figure 2. Surface water temperature ranged from 7.0°C (in February 2006) to 26.5 °C (in August 2006). Salinity ranged from 36.45 psu to 44.71 psu. Salinity variations were due to precipitation evaporation and the water exchange with adjacent sea in relation to predominant winds. There was an increase of the pH values in March, May, July, October and November. Generally, the increase in the pH value was significant during the months when phytoplankton produced substantially. The decrease observed in phosphate concentration was due to the increase of phytoplankton biomass.

The ammonium concentration decreased in mid-spring due to the fact that phytoplankton species use ammonium in the spring bloom. The ammonium concentration reached to maximum level in August.

During the study, mean Nitrate concentration was 7.37 ± 4.84 µgat/l (0.76-16.07). Nitrate concentrations, like the other nutrients, showed an increase in mid-spring. There was no significant difference in nitrate concentrations in the summer. This could be explained by nitrate-rich waters of the bay. However, the nitrate concentrations at two stations increased at the end of autumn. During the end of autumn, nitrate concentrations decrease in the nitrate concentrations in the following months could be explained with the absence of rains and the consumption by phytoplankton.

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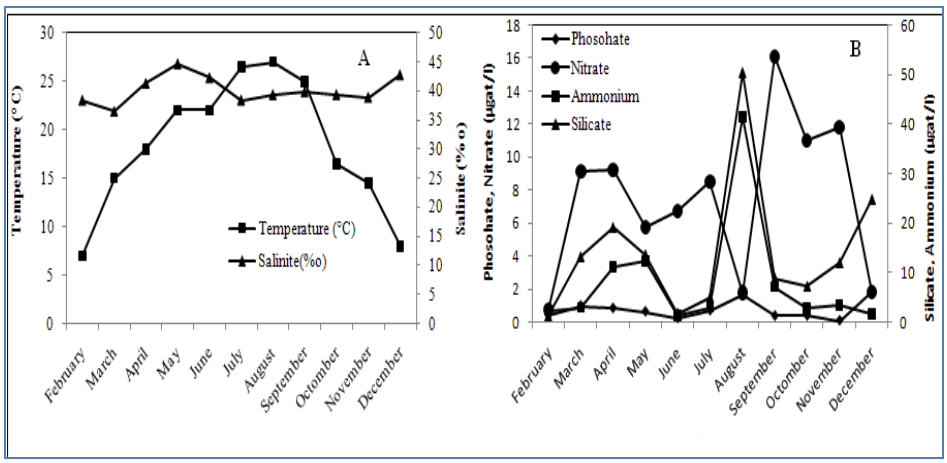

Figure 2. Physical and chemical Parameters. A. Temperature-Salinity B. Nutrient

Phytoplankton and microzooplankton community dynamics

Chl-a concentrations were significantly different among months ($P < 0.05$) during 2006 and 2007. In the acetone extraction method, the Chl-a in May exhibited a high increase. The Chl-a (0.17-5.49µg/l) in July increased progressively and sharply decreased in January *in vivo* measurement. The relative contribution of large-sized cells (like diatoms) to total phytoplankton (412777 cells ml/L) abundance increased in July. Indeed, several chain-forming species (*Navicula* sp., *Cylindrotheca closterium*, *Licmophora abbreviate*, *Gyrosigma fasciola*, *Ceratium teres*, Tintin*nopsis* sp., *Protoperidinium brevipes*, *Dinophysis fortii*, *Prorocentrum rotundatum*, *Gyrosigma balticum*, *Gyrosigma spenceri*, *Striella delicatum*, *P. micans*, *D. fortii*, *P. rotundatum*, *P. brevipes*) proliferated intensively during spring (Fig. 3). At the beginning of summer, diatoms and dinoflagellates remarkably increased and predominant diatoms were *Rhizosolenia* sp., *N. longissima*, *Licmophora abbreviata*, *C. closterium*, *Navicula* spp.. The winter/autumn communities of phytoplankton were dominated by microzooplankton species (*Prorocentrum triestinum*, *Prorocentrum lima* nauplii, *Favella* sp., *Ceratium teres*, *P. lima*) (Fig. 4).

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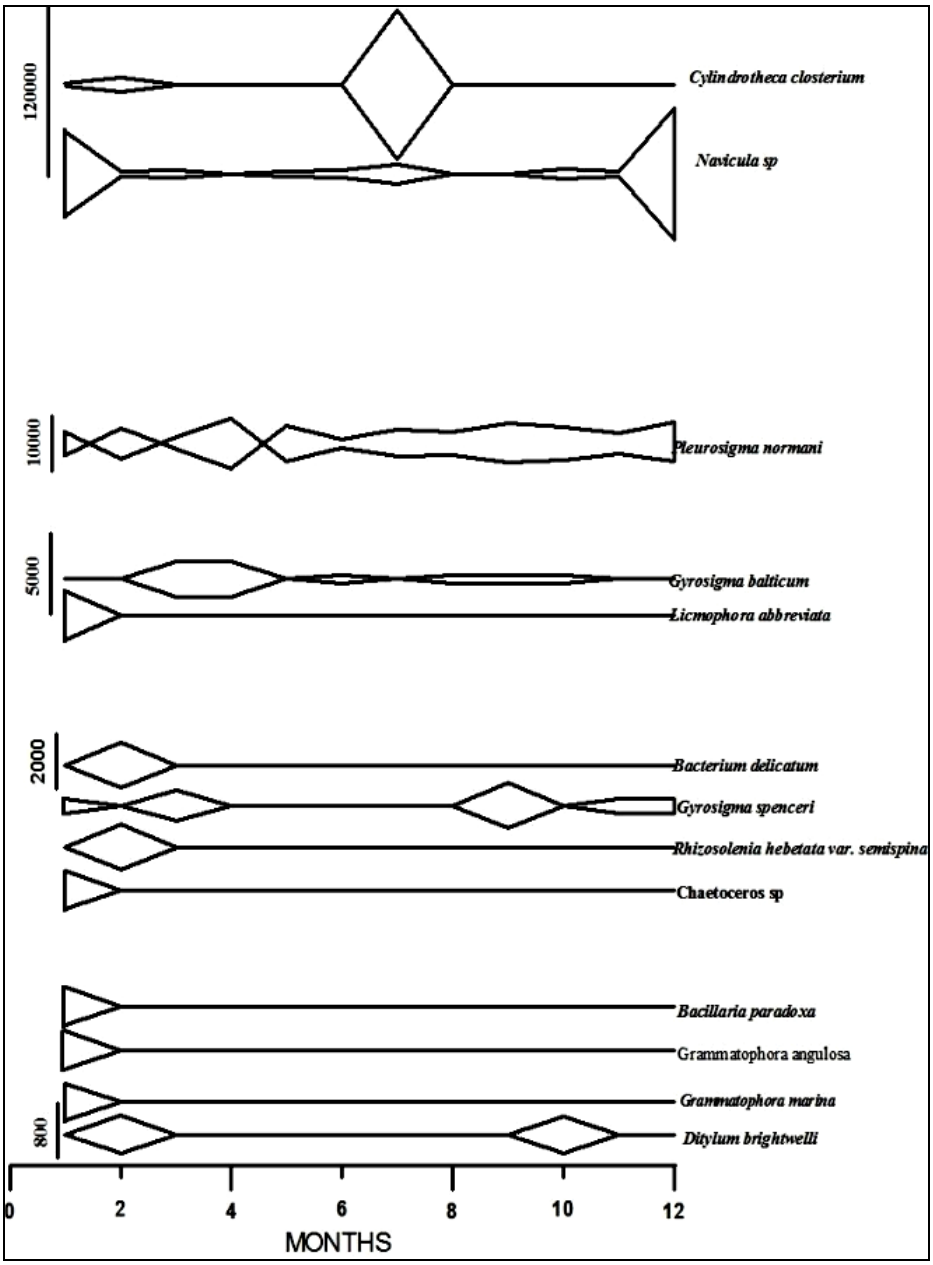

(a)

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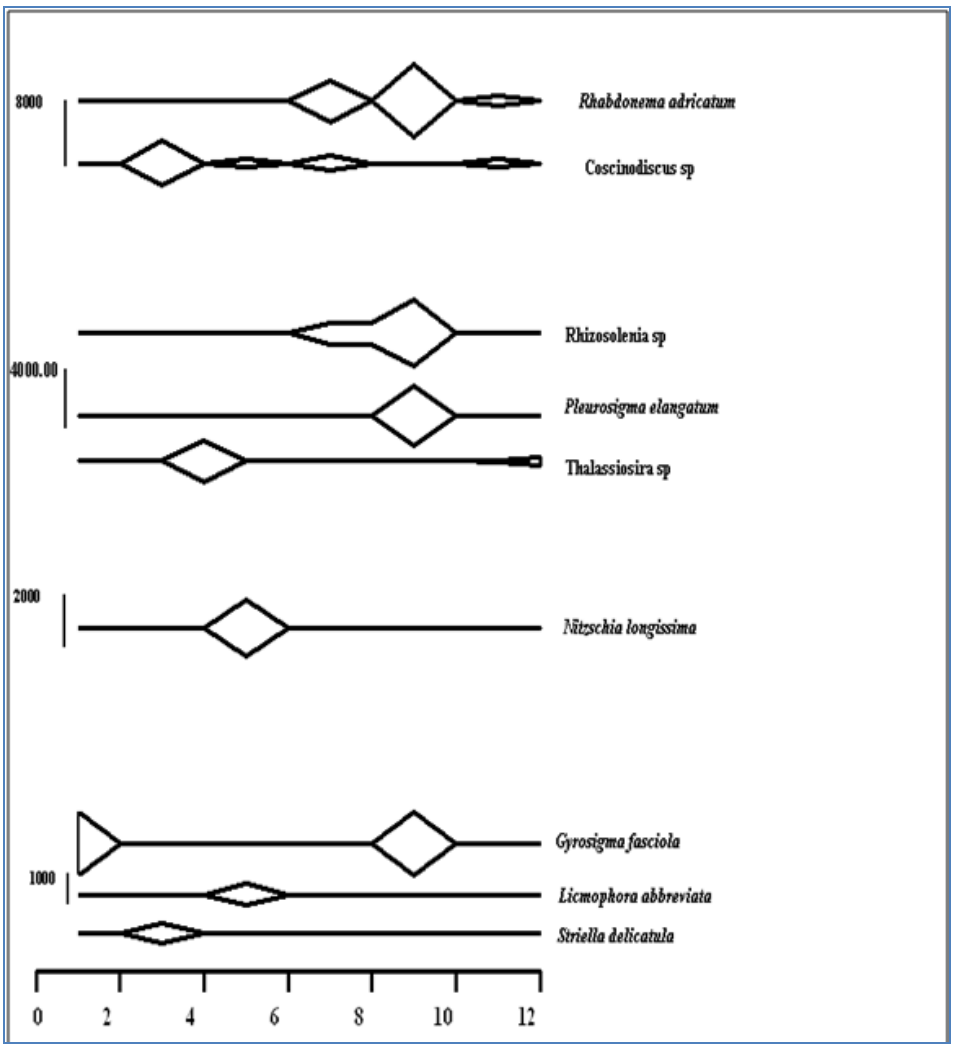

(b)

Figure 3: Succession of diatom species in the Homa lagoon of Agean Sea (a, b)

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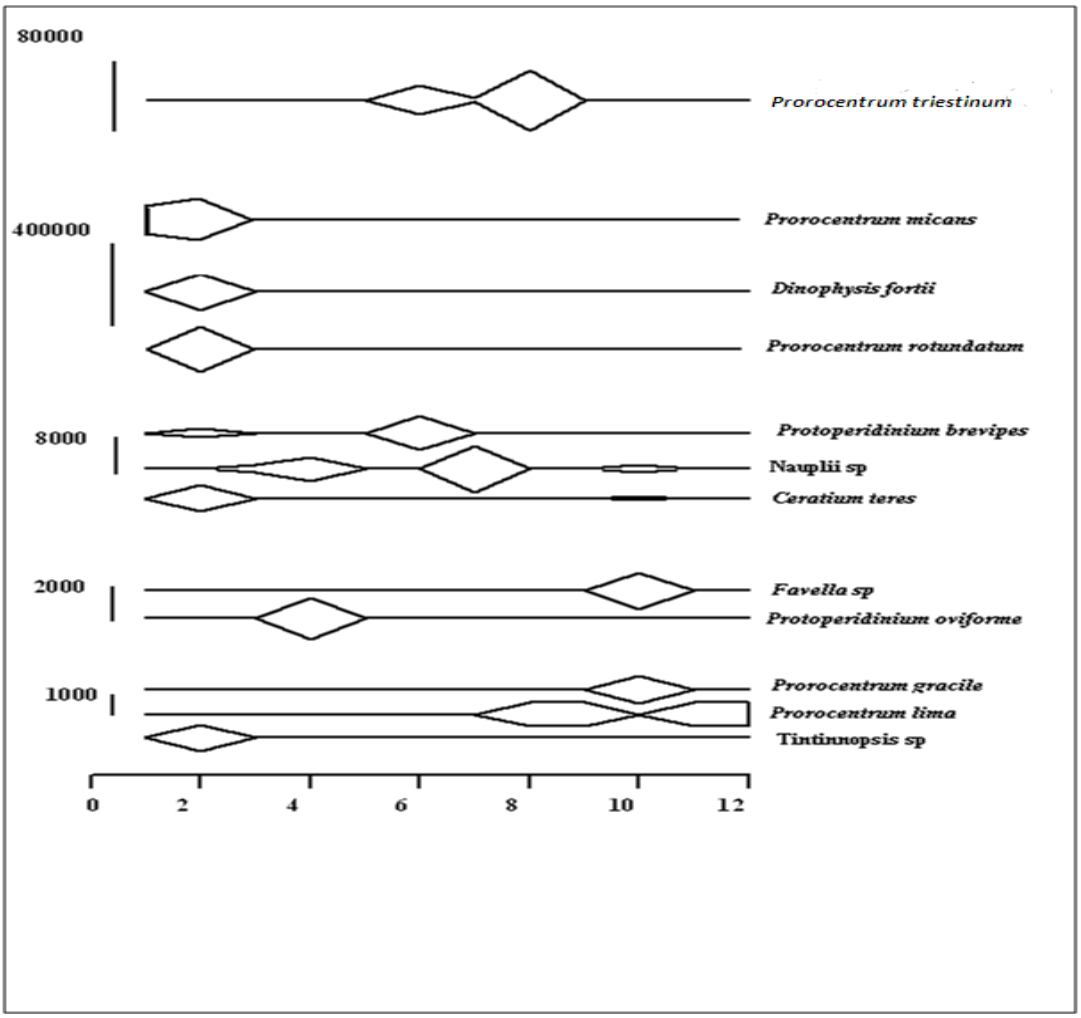

Figure 4: Succession of dinoflagellate species in the Homa Lagoon of Agean Sea

Phytoplankton growth and microzooplankton grazing

Saturation values were detected in the 20%, 45% and even 70% as dilution experiments in March. Grazing was found to be 0.80 day^{-1}, kmax was calculated as 1.32 day^{-1}. There was an increase of abundance in the phytoplankton population community with the increase in water temperature early spring bloom. Primary production was consumed at a rate of 58%. Grazing rates on total phytoplankton were significantly different among months ($P < 0.05$) during 2006-2007. It was observed that phytoplankton abounded in the Homa Lagoon in March and April and microzooplankton grazing reached the point of saturation in these months. Grazing reached saturation point even in the samples which were subjected to 70% dilutions. In April, grazing and kmax were 1.19 day^{-1} and 2.44, respectively (Fig. 5). The increase in March was maintained. As a result, both grazing and the primary production increased. There was also an increase in the diversity of species in the

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medium. The 97% of the biomass was consumed by grazing from April to the end of the year. Grazing in May was 3.34 day^{-1} and kmax was 4.28 day^{-1}. There was an increase in phytoplankton species composition (Fig. 5). The quantity of phytoplankton in May is not so much as to render the microzooplankton grazing at saturation level. All the values in June are maximum levels. During the period of spring, grazing rates prominently increased with increasing the growth rate of community and it was observed that maximum growth rates were significantly higher than the grazing rates. The situation can be explained in proportion to the duration and intensity of light. In March, significant increases in growth rate due to grazing weak limitation. In April and May, is provided for increasing in growth rate of phytoplankton community.

The 99% of the biomass was consumed by grazing in June. During first half of July, grazing, kmax, r^2 were1.12 d^{-1}, 4.509 day^{-1} and 78%, respectively. The grazing percentages progressively decreased from 99% to 66% from July onwards (Fig. 5). In early summer, the growth rates reached to the maximum level while the lowest grazing rates were recorded. In August, very high grazing rate is indicated a temporary over-grazing in a narrow. Therefore, the situation is important in terms of reflecting the period.

The community composition showed changes in September and October. The grazing, kmax, r^2 were 1.34 day^{-1}, 3.84 day^{-1} and 94%, respectively (Fig. 5). At the beginning of October, the changes in primary production, potential primary production and grazing showed similar trend. Grazing increased as primary production increased and grazing was more than production in the medium from the end of summer onwards (Fig. 5). Grazing percentage increased in September and reached the maximum point in November. All data showed decreasing in grazing percentage during the winter. Early autumn, grazing rates of phytoplankton were in lower levels while community grazing rates of phytoplankton increased. In contrast, in mid to late autumn, grazing rates were higher levels.

In January, grazing and kmax were increased. Grazing was found to be 0.20 day^{-1} in the experiment carried out in February (Fig. 5). Grazing had the least percentage since production was low in February. There was a decrease in phytoplankton activity in the medium, and the grazing had the least percentage value. During the first half of the season, nutrient limitation was observed. In growth rates between nutrient addition and without nutrient addition groups were observed difference. In January, growth and grazing rate of phytoplankton community were nearly equal and relatively higher level although the variation value was significantly high. At the end of winter, both grazing rate and growth rate were significantly declined and grazing was relatively low.

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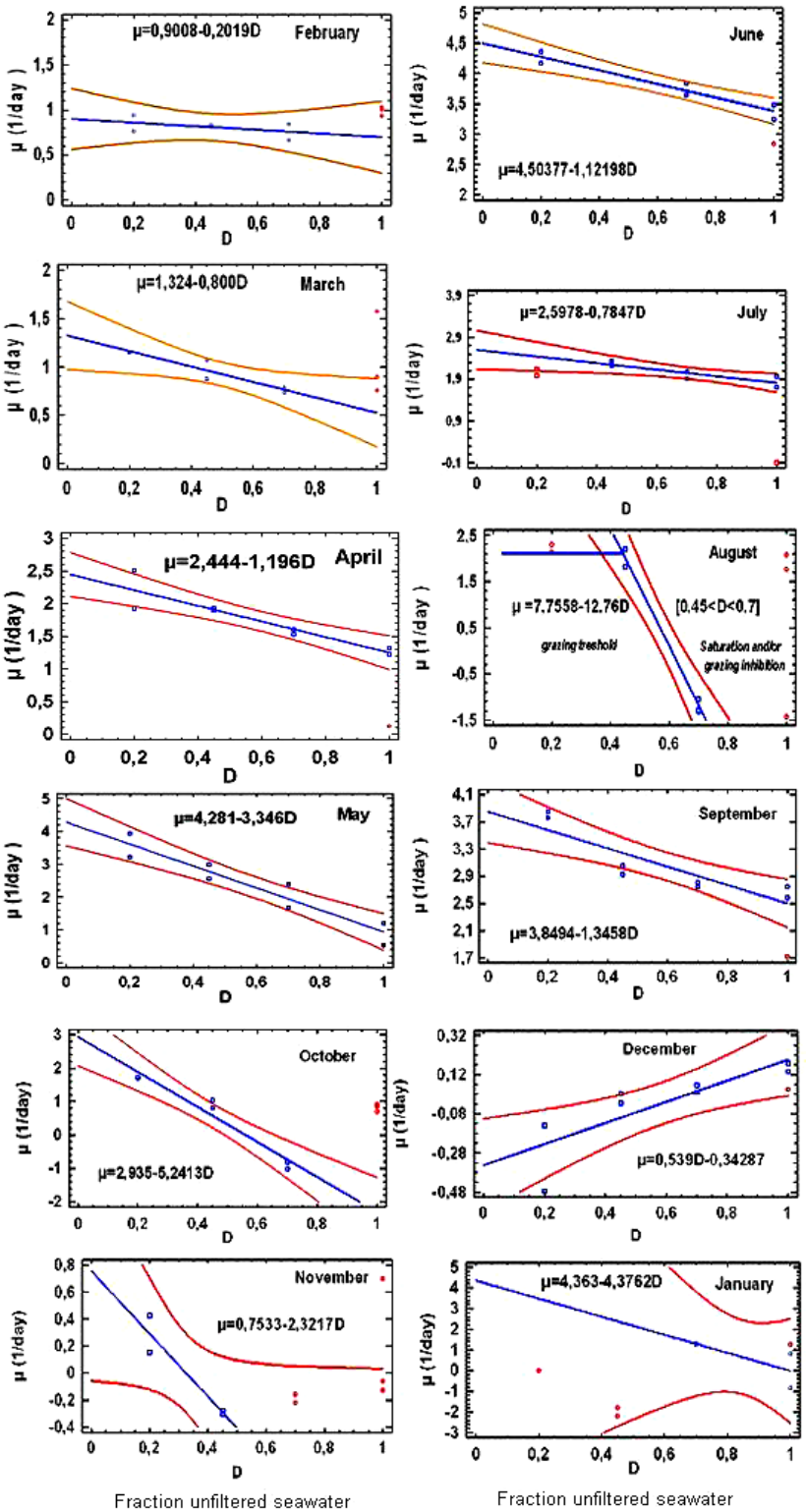

Figure 5. Relation between net growth rate of phytoplankton and fraction of unfiltered sea water.

Discussion

Environmental conditions and nutrient concentrations

Inorganic nitrogen and phosphorous (NO_2^- + NO_3^- + NH_4^+, PO_4^{-3}) in the Homa Lagoon is characterized by relatively

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normal concentrations compared to previous reports for other Mediterranean coastal systems, as lagoon of Thau (Vaquer et al., 1996) and Gulf of Trieste (Fonda Umani et al., 2003), Bizeert lagoon nutrient concentrations of inorganic nitrogen and phosphorous, (Sakka et al., 2007). The nitrite was low concentrations from the end of February to the end of the year. The increase in nitrite concentrations may explain the nitrification. In the range of Chl-a concentrations levels reported for the three coastal ecosystems (Fonda Umani et.al., 2003; Bec et al., 2005; Sakka et al., 2007). This study illustrated that species-specific information on critical N:P ratios are requires for accurate predictions of the effect of nutrient additions on a phytoplankton community. Furthermore, consideration should be given to the prokaryotic or eukaryotic species of phytoplankton competitors as likely exhibit different physiological properties and reactions (Hudson, 2007).

Chl-a specific μ over a year in the surface water of the Homa Lagoon were estimated as 1.12 day^{-1}. Similarly, μ values were reported in previous studies (Paranjape, 1989; Tsuda and Kawaguchi, 1997; Goericke and Welschmeyer, 1998; Lessard and Murrell, 1998; Shinada et al., 2000; Odate and Saitoh, 2001).

Phytoplankton and microzooplankton community dynamics

In previous studies, diatoms predominated when the surface water temperature was low in winter (9°C) (Odate and Maita, 1988; Shinada et al., 1999). μ in winter and spring were comparable to reported values based on cultures for diatoms at low temperature (Smith and Sakshaug, 1990). Raven (1986) reported the summer phytoplankton community of lagoon was dominated by cyanobacteria at 20°C. On the other hand, microzooplankton was dominated by heterotrophic dinoflagellates and ciliates during summer.

The present results showed that phytoplankton abundance and biomass were in lower levels during the winter season. During the winter period, the ambient phytoplankton biomass may be sufficiently low that microzooplankton may cease grazing, thus exhibiting a de facto threshold feeding response (Frost, 1975; Campbell and Carpenter, 1986), or they may only graze minimally. Additionally, the low significant grazing observed during the period of year, in particular in December and February 2002, would be the grazing of the phototrophic *P. lima*, *P. micans*, *P. Rotundatum* and *P. triesstrium*.

Phytoplankton growth and microzooplankton grazing

Microzooplankton grazing (g) averaged 71% of phytoplankton growth (μ) on a worldwide basis (Calbet and Landry, 2004). They stated that microzooplankton grazing accounted for a cross-system average of 64% of primary production within a range of 59 to 74%. In contrast, the present results indicated that in 2006-2007, and the average ratio of g: μ in the Aegean Sea Homa Lagoon in year was 1.71. The impact of microzooplankton on the phytoplankton blooms in the upper estuary might significant.

In this study, there was no significant difference in the mean μ between summer and winter–spring seasons, although higher μ often occurred in summer.

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Similarly, μ in surface waters were not varied systematically over the year, in spite of seasonally changing concentrations of nitrate (Goericke and Welschmeyer, 1998). The present results showed that g was less than μ in spring. This suggests that the onset of the spring bloom results from increased phytoplankton growth, as observed at the beginning of March, coupled with a continuously low grazing pressure in spring. At this stage, we cannot identify what factor(s) affect the increase in phytoplankton growth. However, the factors seem to be most likely associated with phytoplankton growth are light and mixing depth. Phytoplankton has a high potential growth rate throughout the year.

It has been found that phytoplankton growth rates were low due to selective grazing by the microzooplankton community on different phytoplankton groups, which were evaluated by company the composition of the phytoplankton community after incubation in the presence and absence of grazing (Lionard et al., 2005).

Based on dilution experiments, we found a clear seasonal change of microzooplankton grazing as potential loss factor for phytoplankton impact between spring, summer and autumn in a shallow coastal area. Our study demonstrated high seasonal variability in microzooplankton grazing. Microzooplankton grazing controlled phytoplankton dynamics in summer, presumably retaining a high percentage of summer phytoplankton biomass in the pelagic food web. Microzooplankton grazing was not controlled the diatom spring bloom: from the build-up until the decay of the diatom bloom no grazing was observed. Bloom dynamics were mainly controlled by light and nutrients and that most of the biomass entered benthic food webs by sedimentation and benthic assimilation (Loebl and Beusekom, 2008).

In conclusion, our study showed the high impact of microzooplankton grazing on phytoplankton production throughout the year in Homa Lagoon. Grazing control was particularly pronounced in summer and autumn when the large algae (i.e., diatoms) contributed a high fraction of the primary production. As phytoplankton production was mostly channelled to the microzooplankton, an important component of the microbial food web, the flux of material to the seafloor, would be reduced. Results showed significant correlations between phytoplankton growth and microzooplankton grazing rates between phytoplankton and ciliate abundance (Zhou et al., 2012).

This should be considered when modelling the carbon cycling in coastal environments and under conditions of diatom dominance. Our study demonstrated that the ciliate community of the restricted Homa Lagoon was the more active grazer of the large algae (diatoms) than the heterotrophic dinoflagellates, which is contrary to finding in open coastal waters.

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