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GROWTH RATE OF DIATOMS IN NATURAL ENVIRONMENT FROM THE COASTAL WATERS OF PAKISTAN

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Abstract

Growth rate of diatoms were analyzed by 24 hours *in situ* incubation technique at coastal waters of Karachi, Pakistan (northern Arabian Sea). Sampling was done at two stations, station A located inside the channel and station B outside the channel during February, 2006 and at the mouth of the channel during May, 2007. The diatom species dominated in the community were *Nitzschia closterium, Nitzschia logissima*, *Pleurosigma* spp and *Thalassiosira* sp. Total community growth rate was negative -0.11d^{-1} in summer and -0.7 d^{-1} at station B but positive 0.1d^{-1} at station A in winter. The individual species have given positive response of growth even in the presence of grazers. During the summer months the differences observed from the winter months may have been due to differences in microzooplankton composition. Heterotrophic dinoflagellates have a large impact on the growth rates. Since these dinoflagellates feed on the large diatoms, it suggests that this is a major factor determine the growth rate of diatoms. Total diatom abundance at initial T0 and final T24 incubation periods coincided with the chlorophyll a values at station B in winter and summer seasons, but they did not display similar pattern at station A, suggesting that when the large grazers removed from the sample through prescreening the diatom growth increased. Presence of high concentration of nutrients near the coast could be one of the reasons of high growth rate at station A as compared to station B which was near off shore.

Introduction

The diatoms are the dominant and major part of primary production at the coastal waters of the Arabian Sea. The Arabian Sea has unique hydrographic climate which exists throughout the year (Banse, 1987, Brown *et al.*, 2002) and these climatic conditions of seasonally alternation of monsoon in the area cause heavy upwelling in the region which affects greatly on primary production, biomass and algal growth (Rhyther & Menzel, 1965; Buesseler, 1998; Barber *et al.*, 2001; Calbet & Landry, 2004). There are some reports available from western Indian Ocean and central and northern Arabian Sea (Ryther *et al.*, 1966; Smith & Bottero, 1977; Banse, 1987; Landry *et al*., 1998; Brown *et al.*, 2002) showing primary productivity in the region. The growth and abundance of phytoplankton depends on many environmental factors for example light, temperature and nutrients (Ornolfsdottir *et al.*, 2004). Any seasonal change in the environmental parameters can lead to the change in the population growth which subsequently impacts the population dynamics of consumers thereby affecting the fishery production (Kumari *et al.*, 1978). Analysis of the structure of phytoplankton community and their growth dynamics in any area is therefore important for the sustenance of commercial fisheries. When biomass and algal growth increases with the rapid proliferation of population this condition indicates that the growth rate is higher than the loss rate of population (Marra *et al.*, 1998). The community loss can be the result of different mechanisms or biological activity within the ecosystem (Brock & McClain, 1992; Leising *et al.*, 2005). The main factors involved in this process are the grazing, sinking to the ocean floor, mixing due to upwelling and mortality because of the infection of virus and parasites (Sherr & Sherr, 1994, 2002). These factors are inter-related to each other and their environment, but still little is known about their inter connection (Obayashi & Tanoue, 2002; Calbet & Landry, 2004). Manora Channel located on the estuary of Layari River is one of the major coastal areas of Karachi that receive land based pollution. The Layari discharges into the Manora Channel are a highly polluted mixture of sewage from the north and west of the city (Beg, 1979). Our investigation results showed the growth estimation of total diatom community and individual diatom species under the eutrophic conditions of this area. Further assessment of growth and grazing rate of diatom species is required for better understanding of primary productivity in this area.

Materials and Methods

Growth experiments were conducted at Manora Channel located on the estuary of Layari River. Sampling was done at two stations A (24˚49.77'N 66˚57.85'E) located inside the channel and station B (24˚47.93'N 66˚58.87'E) outside the channel during February, 2006 and May, 2007 using *in situ* incubation method (Furnas, 1990). Prior to the experiment all experimental and polycarbonate bottles were soaked in 10% HCl for at least 24 h and rinsed thoroughly with distilled water. Water sample was taken from one meter depth with 1.7L Niskin bottle. Sea water samples collected in summer were prescreened through 150µm mesh size and samples collected in winter period prescreened through 150µm mesh size and 10µm in order to remove grazers, then transfered to polycarbonate bottles (1-2L). Additional bottles filled with whole seawater in order to determine initial chlorophyll a concentration. All experimental bottles were (triplicates) incubated *in situ* for 24h, and suspended from floating buoy at the surface. After 24h of incubation, the bottles were returned to the laboratory. The samples for measurement of chlorophyll were filtered onto 25mm GF/F filters and extracted in 10mL of 90% acetone for 24h at 4ºC. Chlorophyll concentrations before and after incubation were determined and calculated (Strickland & Parson., 1972).

Water parameters like salinity (refrectometer), dissolved oxygen (DO; Wrinkler's method: Hanna C100), temperature (thermometer), pH (Hanna HI9023, Italy) were measured. The data were tested statistically. Growth rates of total community and diatom species were calculated from increase and decrease of cell numbers (Smayda, 1973) by following formula:

$$\mu \text{ (doublings d}^{-1}) = (1/T) \log (T24/T0)$$

where, T is the incubation period (d) and T0 and T24 are the initial and final cell numbers. For consistency, all growth rates are expressed on a log basis (doublings d^{-1}). No growth rates were calculated when a species was not observed and net loss occured in the samples.

Diatom community structure: Acid Lugol's (1%) preserved samples were used for analysis of diatom species composition and abundance. In order to evaluate the structure of diatom community, the 250mL samples of seawater fixed in acid Lugol's solution and stored in the dark until being subjected to analysis via inverted settlement microscopy (Utermohl, 1958). Identification of diatoms was based on Tomas (1997).

Results

Growth in summer season: Growth rates of 35 diatom species were observed from *in situ* incubation experiment in the month of May, 2006. Average cell density before incubation was 8.8×10^3 and after it was decreased to 8×10^3 (Table 1). Among 35 diatom species 13 belonged to centric and 22 species belonged to pennate type. In centric group of diatom *Thalassiosira* sp., with *Chaetoceros* sp., dominated the community and *Pseudo-nitzschia* sp., with *Nitzschia closterium* dominated in pennate types.

Growth rate of diatom population showed variations among and within different species. *Rhizosolenia styliformis* and *Rhizosolenia imbricate* grew quite in low densities. *Thalassiothrix* sp., increased with high cell densities. The in *situ* rate of population growth observed in the community showed both negative and positive values (Table 1). Positive in *situ* rate of population growth was observed reaching from 1.61 and 2.60d^{-1} for *Odontella mobiliensis* and *Thalassiothrix* sp., respectively (Table 1). Population showed strong negative values reaching from –0.2d^{-1} and –2.1d^{-1} for *Guinardia striata*, *Rhizosolenia imbricata* and *Pleurosigma* sp., 2. Net population loss was observed for the diatom species *Asterionella Formosa, Chaetoceros tenussimus, Eucampia zodiacus, Leptocylindrus danicus, Navicula transitrans* and *Navicula* sp., 6 (Table 1). Obviously in the presence of grazers the community growth rate was negative -0.11d^{-1} but individual species have given positive response of growth in this incubation experiment even in the presence of grazers. The initial T0 and final T24 cell densities of dominant species are shown in Table 1.

Chlorophyll a values was estimated before T0 and after T24 incubation periods. At T0 chlorophyll a value was 0.2µg/L and after 24 hours it was decreased to 0.1µg/L (Fig. 1). Dissolved oxygen, salinity and temperature were measured before and after incubation periods. Dissolved oxygen values decreased from 11mg/L to 9mg/L before and after incubation period respectively. Salinity values remained same 38‰ during incubation periods. Temperature was 32°C to 33°C before and after incubation periods (Fig. 1).

Growth in winter season: Average cell density before incubation T0 at station B was 6.73×10^3 and after T24 it was decreased to 3.2×10^3 (Table 2). At station A average cell density before incubation was 6.567×10^3 and increased to 7.35×10^3 (Table 3). Control experiment showed similar pattern with decreasing cell abundance from 7.8×10^3 to 4.5×10^3 and 13.2×10^3 to 7.5×10^3 at T0 and T24 at station B and A (Tables 2, 3). At station **A** growth rate of seven species were observed. The community showed negative values -0.57 d^{-1} for growth in un-fractionated (control) but showed positive values for growth 0.11 d^{-1} in the samples fractionated with 150µm and 10µm. Highest growth 1.3 d^{-1} was observed for *Thalassiosira* sp., *Pleurosigma* sp., 1 and *Pleurosigma* sp., 2 also showed positive growth rate 0.6 d^{-1} and 0.4 d^{-1} in un-fractionated (control) samples but their growth rate increased to 1.1 d^{-1} and 0.9 d^{-1} in sample fractionated with 150µm. All the species present in the sample (150µm) except the diatom *Nitzscia longissima* showed positive rate of growth. This species has negative -0.4 rate of growth. *Nitzschia closterium* has shown negative growth in un-fractionated (control) and fractionated (150µm) samples at station A. In contrast this species has shown high growth 0.17 d^{-1} in the sample passed through 10µm (Table 2). The initial T0 and final T24 cell are densities shown in Table 2.

Growth rates of 12 diatom species were observed at station **B**. Among 12 species 8 belonged to pennate type and 4 species belonged to the centric type. In pennate type *Nitzschia closterium* dominates the community with *Pseudo-nitzschia* sp., and *Nitzschia longissima.* Growth rate of diatom population showed variation among and within different species. *Thalassiosira* sp., grew with high rate 1.6 d^{-1} even in the presence of grazers. The *in situ* rate of growth observed in sample fractionated with 150µm showed was both negative and positive values presented in Table 3. Positive in *situ* rate of population growth was observed reaching from 0.5 d^{-1} to 1.1d^{-1} for *Thalassiosira* sp., and *Pleurosigma* sp., 1 respectively. Population showed strong negative values reaching from -0.2 to -0.7 d^{-1} for *Pseudo-nitzschia* sp., and *Nitzschia closterium* respectively. Net population loss was observed for the diatom species *Rhizosolenia styliformis Pleurosigma* sp., *2 Chaetoceros* sp *Navicula* sp 6, *Rhizosolenia imbricata, pinnularia* sp., and *Navicula* sp., 3. Total growth rate observed was -0.7 d^{-1}. Growth rate of six species were observed in the sample passed through 10µm at station B. Total average cell abundance was 5.37×10^3 at T0 and after 24 hours it increased to 6.38×10^3. Total growth rate was negative -0.65 but *Pinnularia* sp., and *Navicula trasitrans* have shown positive growth 1.5 d^{-1} and 0.69 d^{-1} respectively. The initial T0 and final T24 cell densities are shown in Table 3.

Table 1. Mean cell densities/L and growth rate (d^{-1}) at time T0 and T24 of diatom population in summer season from incubation experiment.

	Morphotypes	(Mean cell densities/L)		Growth rate (d^{-1})
		T0	T24	
1.	*Amphora* sp.	20	40	0.69
2.	*Asterionella formosa*	20	0	ZERO
3.	*Coscinodiscus* sp.	27	7	-1.39
4.	*Chaetoceros affine*	233	140	-0.51
5.	*Chaetoceros* sp. 2	953	433	-0.79
6.	*Chaetoceros tenussimus*	7	0	ZERO
7.	*Chaetoceros pervianus*	53	13	-1.39
8.	*Eucampia zodiacus*	73	0	ZERO
9.	*Guinardia striata*	360	280	-0.25
10.	*Guinardia flaccida*	120	73	-0.49
11.	*Leptocylindrus danicus*	60	0	ZERO
12.	*Nitzschia longissima*	300	460	0.43
13.	*Navicula* sp. 1	13	33	0.92
14.	*Navicula* sp. 2	80	100	0.22
15.	*Navicula* sp. 6	13	0	ZERO
16.	*Navicula transitrans*	20	0	ZERO
17.	*Nitzschia closterium*	727	1147	0.46
18.	*Odontella mobiliensis*	13	67	1.61
19.	*Odontella sienensis*	73	47	-0.45
20.	*Pseudo-nitzschia* sp.	2807	1333	-0.74
21.	*Pleurosigma* sp. 1	53	220	1.42
22.	*Pleurosigma* sp. 2	53	7	-2.08
23.	*Pleurosigma macrum*	7	20	1.1
24.	*Planktoneilla sol*	7	20	1.1
25.	*Pinnularia* sp.	40	13	-1.1
26.	*Rhizosolenia setigera*	53	20	-0.98
27.	*Rhizosolenia Imbricata*	973	733	-0.28
28.	*Rhizosolenia hebitata*	113	13	-2.14
29.	*Rhizosolenia* sp.	20	47	0.85
30.	*Rhizosolenia alata*	27	7	-1.39
31.	*Rhizosolenia styliformis*	47	40	-0.15
32.	*Synedra* sp.	7	7	0
33.	*Thalassiosira* sp.	913	2000	0.78
34.	*Thalassionema-nitzschioides*	567	313	-0.59
35.	*Thalassiothrix* sp.	33	367	2.4
	Total	**8887**	**8000**	**-0.11**

Table 2. Mean cell densities/L and growth rate (d^{-1}) at time T0 and T24 of diatom population in winter season at station A from incubation experiment.

	Morphotypes (control)	(Mean cell densities/L)		
		T0	T24	Growth rate (d^{-1})
1.	*Nitzschia closterium*	12853	7113	-0.59
2.	*Nitzschia longissima*	180	33	-1.69
3.	*Pleurosigma* sp. 1	13	27	0.69
4.	*Thalassiosira* sp.	100	273	1.01
5.	*Licmophora* sp.	7	0	zero
6.	*Pleurosigma* sp. 2	33	53	0.47
7.	*Pseudo-nitzschia* sp.	30	0	ZERO
	Total	**13217**	**7500**	**-0.57**
	150 µm			
1.	*Nitzschia closterium*	6393	6967	0.09
2.	*Nitzschia longissima*	100	67	-0.4
3.	*Pleurosigma* sp. 1	7	20	1.1
4.	*Thalassiosira* sp.	60	227	1.33
5.	*Pleurosigma* sp. 2	27	67	0.92
6.	*Pinnularia* sp.	7	10	0.41
	Total	**6567**	**7353**	**0.11**
	10µm			
1.	*Nitzschia closterium*	**5373**	**6383**	**0.1**

Chlorophyll a: At station A chlorophyll a values at T0 control (un-fractionated) samples was 10.25µg/L and at T24 it decreased to 7.3µg/L. The chlorophyll a values in 150µm was 9.7µg/L and after incubation period it was decreased to 5.7µg/L. The values observed in 10µm were 8.8µg/L at T0 and 5.0µg/L at T24. At station B chlorophyll a values at T0 control (un-fractionated) samples was 4.2µg/L and at T24 it was decreased to 1.7µg/L. The values observed in 10µm were 3.7µg/L at T0 and 1.5µg/L at T24 (Fig 1).

Water parameters: Temperature, salinity, pH and transparency were measured before and after incubation periods. At station A initial temperature was 24°C and final was 24.5°C. At station B initial temperature was 23°C and final was 25°C. Salinity was 35‰ at T0 and 37‰ at T24 at station A and at station B remained same 40‰ before and after incubation period. pH values were observed 7.4 to 7.5 at T0 to T24 respectively at station A and at station B it was 7.7 to 7.9 at T0 to T24 respectively (Fig. 1).

Discussion

These investigations reporting for the first time estimation of growth rate of diatoms species in natural environment from coastal waters of Karachi, Pakistan. There is no previous data available for the comparison of growth rate of diatom community from this region. Two experiments were done for the estimation of growth rate at the mouth of Manora Channel. One experiment was done in summer season and other was in winter to observe the difference in growth rate and mortality or loss of population in the samples by passing through the size fractions of 150µm and 10µm. The overall growth rate was negative in both seasons showed the high grazing pressure on diatom population but comparatively high growth rates in summer for individual species. The growth or production of phytoplankton indirectly showed the pressure of grazers on the microbial community because micro zooplanktons play a significant role in the marine food web and these are the organisms graze on phytoplankton. During the summer months, the differences observed from the winter months may have been due to differences in microzooplankton composition (Personal communications). Heterotrophic dinoflagellates have a large impact on the growth rates. Since these dinoflagellates feed on the large diatoms it suggests that this is a major factor which determined the growth rate of diatoms.

Barber *et al.*, 2001 estimated the primary productivity from Central Arabian Sea, Oman. The values of primary production was expected higher because the period of inter monsoon has high rate of production. The values they observed were less than expectations because of intense grazing pressure of mesozooplanktons which was not allowing the diatoms community to grow (Barber *et al.*, 2006). Goericke, (2002) & Schiebel *et al.*, (2004) also reported that during upwelling the cell abundance of diatoms decreased because of low nutrient availability and grazing. Brown *et al.*, (2002) reported exceeded loss of population because of the grazing over growth rates from Oman, central Arabian Sea. The similar higher

grazing rate over growth observed from Dabob Bay, Washington, USA is reported by Leising *et al.*, (2005). When grazing exceeds the growth it suggests that microbial community shifted from autotrophic to heterotrophic population. For the justification of higher grazing rates there are two mechanisms describe by Leising *et al.*, (2005) one is the limiting nutrients and other inclusion of large mesozooplanktons (copepods) in the samples. A convincing explanation is given by Barber *et al.*, (2001) from central Arabian Sea Oman, those large copepods for instance *calanoids carinatus* present in the upwelled waters during the period between southwest monsoons. These hungry copepods are in large numbers and grazing together with the microzooplankton prevented the accumulation of diatom biomass.

Individual diatom species and total community have variations in their growth rate. Both pennate and centric species responses differently in *in situ* incubation experiment. Diatom species *Nitzschia longissima* showed positive growth rate 0.4 d^{-1} in summer and negative rate of growth in winter season. This may be due to some special safety measures taken by the diatom species like this species form balls of needles to increase their cell size (Buck & Chavez, 1994) which is supposed to be an anti grazing strategy against microzooplanktons grazing present in the environment in that particular season.

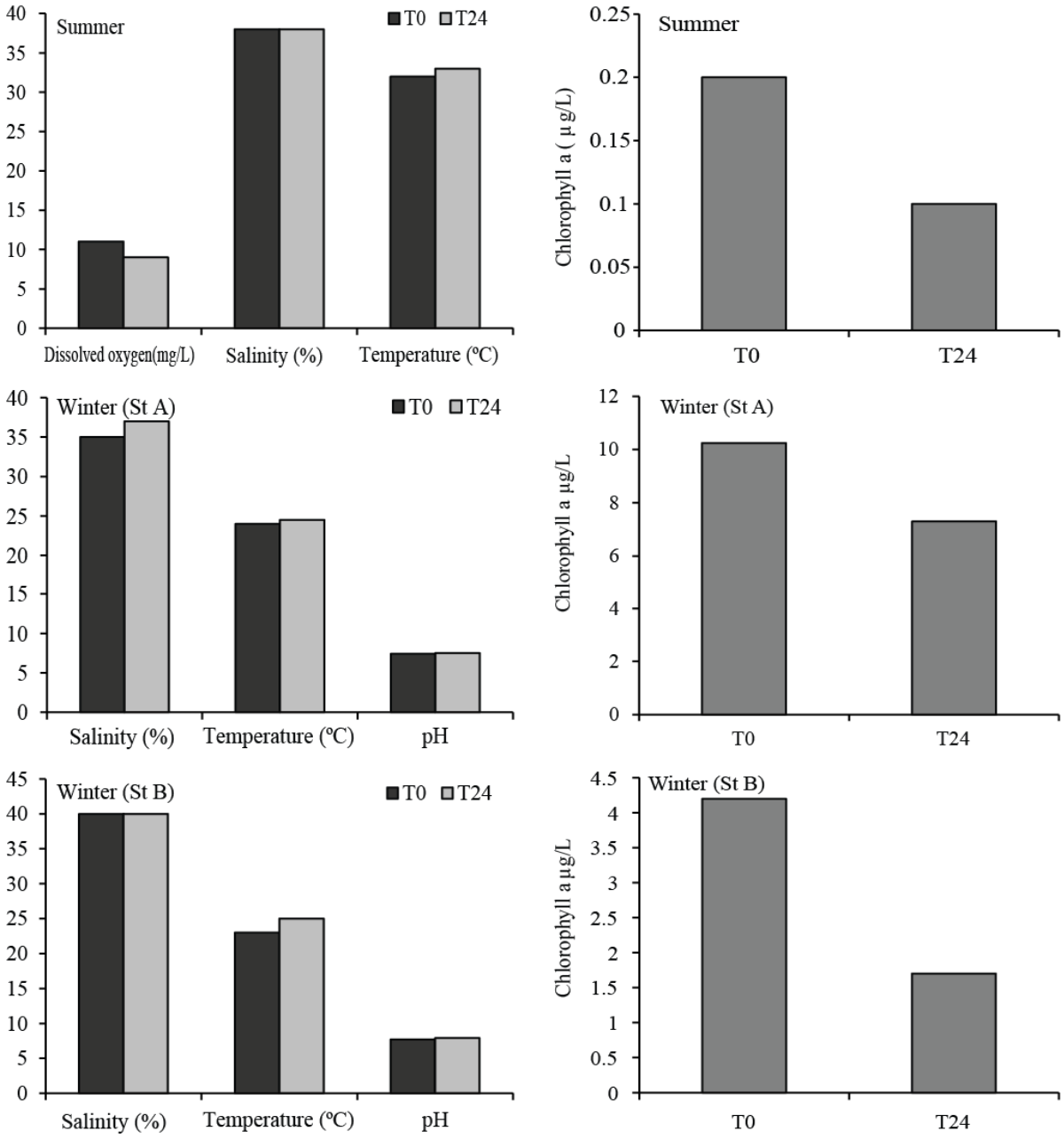

Fig. 1. Water parameters including temperature (°C), salinity (psu), pH, dissolved oxygen (mg/L) and chlorophyll a (µg/L) at time T0 and T24 in summer and winter seasons.

Table 3. Mean cell densities/L and growth rate (d-^{1}) at time T0 and T24 of diatom population in winter season at station B from incubation experiment.

	Morphotypes (control)	(Mean cell densities/L)		Growth rate (d-^{1})
		T0	T24	
1.	*Nitzschia closterium*	6847	4033	-0.53
2.	*Rhizosolenia styliformis*	40	0	ZERO
3.	*Pseudo-nitzschia* sp.	447	153	-1.07
4.	*Pleurosigma* sp. 2	20	0	ZERO
5.	*Pleurosigma* sp. 1	13	33	0.92
6.	*Chaetoceros* sp.	20	0	zero
7.	*Nitzschia longissima*	387	160	-0.88
8.	*Navicula* sp. 6	13	0	zero
9.	*Rhizosolenia imbricata*	13	0	zero
10.	*Thalassiosira* sp.	27	140	1.66
11.	*Thalassiothrix* sp.	20	27	0.29
12.	*Pinnularia* sp.	33	13	-0.92
	Total	**7880**	**4560**	**-0.55**
	150µm			
1.	*Nitzschia closterium*	6053	2840	-0.76
2.	*Rhizosolenia styliformis*	0	0	
3.	*Pseudo-nitzschia* sp.	220	173	-0.24
4.	*Pleurosigma* sp. 2	7	0	zero
5.	*Pleurosigma* sp. 1	7	20	1.1
6.	*Chaetoceros* sp.	0	0	zero
7.	*Nitzschia longissima*	333	80	-1.43
8.	*Navicula* sp. 6	7	0	zero
9.	*Rhizosolenia imbricata*	0	0	zero
10.	*Thalassiosira* sp.	87	147	0.53
11.	*Thalassiothrix* sp.	0	0	ZERO
12.	*Pinnularia* sp.	20	0	zero
	Total	**6733**	**3260**	**-0.73**
	10µm			
1.	*Nitzschia closterium*	4640	2440	-0.64
2.	*Navicula* sp. 3	27	0	zero
3.	*Pseudo-nitzschia* sp.	133	80	-0.51
4.	*Pinnularia* sp.	7	30	1.5
5.	*Nitzschia longissima*	340	120	-1.04
6.	*Navicula transitrans*	7	13	0.69
	Total	**5153**	**2683**	**-0.65**

Pleurosigma species and *Thalassiosira* species were reported as a dominant component of the diatoms community from the coast of Pakistan (Naz *et al.*, 2010). Their growth rate has also showed positive values in all the samples. These species are characterized by thick frustules. It suggests that species with thick silicified cell wall have anti grazing strategy against the grazers (Gomez *et al.*, 2007) and in an environment with heavy grazing pressure heavily silicified diatoms may be favored. *Nitzschia closterium* grow very actively in the summer but in winter has given negative rate of growth in all samples except in the samples fractionated with 150µm and 10µm at station A. This is a small size pennate diatom species and in our studies high growth rates 0.4 d^{-1} observed in summer. The relationship between grazers and diatoms prey are size dependent. Dominancy and high growth of this small diatom in the samples fractionated with 10µm confirmed the absence of larger protist which can be easily grazed on it (Landry *et al.*, 2000). Similar observation was made by Furnas, (1991) from Australia GBR (Great Barrier Reef) subtropical waters and Landry *et al.*, (2000) from eastern Pacific Ocean.

Total diatom abundance at initial T0 and final T24 incubation periods coincided with the chlorophyll a values at station B in winter and summer seasons but they did not display similar pattern at station A suggesting that when the large grazers removed from the sample by prescreening with 150µm and 10 µm the diatom growth increased.

The present study demonstrates the growth rates of total diatom community and individual species. Further additional work is needed to explain the grazing impact on diatom community and how the abundance and number of species vary in their growth rate and given response differently in various regions.

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