Annual Cycle of Phytoplankton Community Structure in the Gulf of Aqaba Detected by HPLC Analysis of Pigments

Tariq Al-Najjar and Mohamed Rasheed *

ABSTRACT

The variation of the phytoplankton community in the Gulf of Aqaba over one year was determined by HPLC at biweekly intervals between January and December 2001. Prochlorophytes dominated during the stable summer stratification period through late fall. They comprised 55% of the total phytoplankton biomass with a maximum concentration which occurred during the end of May (75%) and a minimum concentration (30%) during the period December-February. Eukaryotic algae dominated during winter mixing period and comprised about 60% of the total phytoplankton biomass. Dinophyceae showed a low biomass throughout the entire year and did not exceed 3 ng l⁻¹. Cryptophyceae found only in November and at the end of July. Prymnesiophyceae were found in winter and spring, while Chrysophyceae reached their maximum biomass in spring. One peak of Chlorophyceae was recorded during winter. Bacillariophyceae was detected at the beginning of the winter period and remained <5 ng l⁻¹ for the rest of the year.

KEYWORDS: HPLC, Phytoplankton, Seasonality, Gulf of Aqaba.

INTRODUCTION

Phytoplankton biomass is generally low in the Red Sea, rarely exceeding 1mg Chl a m⁻³ (Genin et al., 1995) with Trichodesmium and other net- (> 65 µm) or micro- (>20 µm) (Kimor, 1971; Levanon-Spanier et al., 1979; Lindell and Post, 1995). The bulk of phytoplankton biomass is composed of picophytoplankton (< 2 µm in cell diameter) represented by a small number of species belonging to 3 major taxonomic groups: cyanobacteria, prochlorophytes and eukaryotic algae (Lindell and Post, 1995). There have been several studies describing the seasonal dynamics of the micro-, nano- and picophytoplankton communities in the Red Sea (Kimor, 1971; Levanon-Spanier et al., 1979; Lindell and Post, 1995; Al-Najjar, 2000), but a comprehensive assessment encompassing the entire phytoplankton size range simultaneously to the deeper water (up to 400 m) of the Gulf is still needed.

Advanced High Performance Liquid Chromatography (HPLC) pigment method has been developed in recent years to obtain both accurate chlorophyll a data and detailed information about the composition of phytoplankton communities (Mantoura and Llewellyn, 1983). The method is based on the premise that different algal classes have specific signature, or marker pigments. For example, fucoxanthin, zeaxanthin, and chlorophyll b have been selected as a taxonomical pigments for bacillariophyta (diatoms), cyanobacteria (blue-green algae), and chlorophyta (green algae), respectively (Stauber and Jeffrey, 1988; Millie et al., 1993; Jeffrey et al., 1997). Each marker pigment concentration can be further expressed as a percentage of the chlorophyll a value for a specific algal class, with a suitable conversion factor used to estimate the relative distribution of each algal class in the sample (Wright et al., 1996; Obayashi et al., 2001). As compared to the tedious and sophisticated enumeration by epifluorescence microscopy or electron microscopy, HPLC analysis requires no specific preparation techniques for the various size groups, enabling bulk analysis of the samples at the class level (Hooks et al., 1988). This advantage as well as the speed, sensitivity and accuracy of the method has rendered HPLC a powerful tool for the quantitative assessment of phytoplankton communities over the last two decades (Gieskes and Kraay, 1983; Klein and Sournia, 1987; Bidigare et al., 1990; Althuis et al., 1994).

This report presents the results of a study which we know as one of the few studies on different phytoplankton taxa dynamics using High Performance Liquid Chromatography (HPLC) technique down to 400 m of the water column in the Gulf of Aqaba, Red Sea. We hope that the result will help understand the role of the various taxonomic and size groups in the functioning of this unique oligotrophic system.

**Materials and Methods**

**Sampling**

Phytoplankton were sampled from long-term monitoring station A (29°27.362N-34°57.238E, Fig. 1) located at the northern tip of the Gulf of Aqaba (Klinker et al., 1978; Lindell and Post, 1995). Daytime biweekly samples were taken from January to December 2001. Water was sampled with 5-liter plastic Niskin bottles at discrete depth 0, 25, 50, 75, 100 and 125, 200, 300, and 400 m, transferred to acid-cleaned 5 liter plastic containers, and kept cool and shaded during the transport to the laboratory.

Water temperature, depth and salinity were recorded concurrently using a self-recording Ocean Sensor OS 200 CTD. Subsamples of 4 liters were filtered under vacuum (max. 0.2 bar) on 0.7 µm GF/F-filters (Whatman, 25 mm). Filters were folded, put into Eppendorff-caps and kept in an ultrafreezer at –80 °C.

**Pigment Extraction**

Filters were ground in 2.5 ml acetone (90 %), and shaken by a cell homogenizer. The holder for the tube was cooled in a freezer before use. The extract was centrifuged for ten minutes at 5000 rpm, cooled at –10°C. The supernatants were taken by a medical syringe and put back into an Eppendorff-cap through a pre filter of 0.2 µm pore size.

**HPLC-Run**

100 µl of the samples were injected into a HPLC system. The column was a 3µm Shando Hypersil MOS2 (endcapped), C-8 (6.2 to 6.8 % carbons), 120 Å pore size, and 100x4.6 mm and maintained at 30 °C. Pigments were separated at a flow rate of 1 ml min⁻¹ by a linear gradient programmed as follows (minutes; % solvent A; % solvent B): (0;75;25), (1;50;50), (20;30;70), (25;0; 100), (32;0;100). The column was then reconditioned to original conditions over a further 7 min. Solvent A consisted of 70:30 (v: v) methanol: 1M ammonium acetate and solvent B was 100% methanol.

Pigments were detected by spectrophotometric detector. Chl a and b standards were obtained from Sigma Chemical Co., and standards for other pigments were from the Water Quality Institute (VKI), Høgsholm, Denmark. The limits of detection for all analyses were of the order of 1.0 ng l⁻¹.

**Results**

The waters of the Gulf of Aqaba undergo stronger seasonal fluctuation than do other subtropical seas. During summer, the water column is stratified, however, in winter the thermocline deteriorates and deep convective mixing persists for several months. Depending on the stability of the water column, the seasons were identified in which winter refers to December-March, spring April- May, summer June- September and fall from October- December (Fig. 2).

The concentrations of pigments in the upper (0-200 m) and lower (200-400 m) water column of the Gulf of Aqaba are shown in Table (1). The change of the phytoplankton community over one year (Fig. 3) shows that the Prochlorophytes dominated during the stable summer stratification period through late fall, comprising 55 % of the photosynthetic biomass. Maximum concentrations occurred during the end of May (75%). Prochlorophyte concentrations declined to 30 % during winter (December and February) once mixing reached the maximum (Fig. 1, 3). Eukaryotic algae dominated during the winter mixing period counting about 60 % of the total photosynthetic pigments.

Fig. 4 shows the seasonal variations in absolute concentrations of the different phytoplankton groups. Dinophyceae had a low biomass throughout the entire year which never exceeded 3 ng l⁻¹. Cryptophyceae were only registered in the period between November and the end of July, their maximum biomass was 6 ng l⁻¹ in March. Pynnesiothyceae and Chrysophyceae were evenly distributed over the year. The maximum biomass of Pynnesiothyceae was found in summer and winter (28 and 30 ng l⁻¹, respectively), while Chrysophyceae reached their maximum biomass (32 ng l⁻¹) in early summer. One peak of Chlorophyceae was recorded, during winter period (12 ng l⁻¹). Synechococcus was found around the year with an average of 15 ng l⁻¹ and a peak in November (23 ng l⁻¹). High biomass level of
Bacillariophyceae was detected only during the beginning of winter period (8 ng l\(^{-1}\)). For the rest of the year, diatoms remained at concentrations <5 ng l\(^{-1}\).

**Discussion**

Seasonality in phytoplankton community structure and the phenomenon of phytoplankton succession among eukaryotic algae is well documented for high latitudes and upwelling regions (Smayda, 1980). A more pronounced seasonality has been reported in the Gulf of Aqaba because of seasonal change between mixing and stratification (Al-Najjar, 2000; Rasheed et al., 2003).

Changes in community structure of eukaryotic phytoplankton in relation to winter mixing have been reported for subtropical waters (Venrick, 1993; Smayda, 1980; Kimor and Golandsky, 1977). However, only few published studies discuss seasonality of the ultraphytoplankton groups in subtropical regions. Campbell and Vaulot (1993) found a stable ultraphytoplankton community year round in permanently stratified waters.

A seasonal fluctuation in ultraphytoplankton group abundance in waters where winter mixing exceeds the euphotic zone was reported by Olson et al. (1990). Lindell and Post (1995) reported the seasonal succession among the ultraphytoplankton in the Gulf of Aqaba by using flow cytometer and HPLC depending on the concentrations of combined monovinyl and divinyl chl \(a\). Their finding agrees with our finding in that prochlorophytes were dominant during the stratified summer period. During this time, nutrients become scarce and water turbulence is minimal (Rasheed et al., 2002). Smaller cells (with higher ratios of surface area to volume, s:v) are generally more able to take up low concentrations of nutrients than large cells (Eppley et al., 1969; Raven, 1986).

The observed decline in prochlorophytes concurrent with an increase in eukaryotic algae as conditions progressed from a stratified to a mixed water column is similar to the trend reported for the Sargasso Sea (Olson et al., 1990). However, in the Gulf of Aqaba, the phenomenon was much more pronounced; the prochlorophytes decreasing to their lower concentrations and the eukaryotic algae increasing to become the dominant group. Eukaryotic algae (as a group) do best in turbulent waters with high levels of nutrients. These correlative observations suggest that the extent of water column turbulence is one of the determining influences on group dominance within the phytoplankton community.

Vertical mixing is the most likely to affect other phytoplankton population indirectly by controlling other environmental factors, such as nutrient concentrations and light levels (Smayda, 1980; Lewis, 1978) but may also affect competition between groups (Kemp and Mitsch, 1979). In addition, mixing may also directly affect phytoplankton parameters such as cellular metabolism, sinking rate, and cell integrity of fragile groups (Smayda, 1980; Margalef, 1986). In summer and fall, when nitrate is more depleted than phosphate, cyanobacteria become more prominent in the biomass. The appearance of \(N_2\)-fixing cyanobacteria can supply up to 35% of summer primary production of the Gulf (Post et al., 2002).

Seasonality patterns of phytoplankton interrelation to mixing regime were observed. However, some taxa such as dinoflagellates, prymnesiophyceae and chrysophyceae did not clear seasonality. Taxa with short burst of abundance as dinoflagellates, prymnesiophyceae and chrysophyceae probably depend on short term nutrient pulses from deep mixing (Sommer, 2000). In comparison, taxa with fall-winter-spring maxima such as cryptophyceae, chlorophyceae have consistent response to the elevated nutrient conditions in winter, while taxa with summer maxima such as prochlorophyceae are well adapted to nutrient scarcity during stable stratification.

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**Figure captions:**

**Figure 1:** Sampling site at offshore station A at the northern Gulf of Aqaba.

**Figure 2:** Seasonal variations in water temperature (\(^{\circ}\)C) down to 400 m at station A (Fig. 1) at the northern Gulf of Aqaba during the year 2001.

**Figure 3:** Changes in phytoplankton community compositions in the upper 400 m water column of the Gulf of Aqaba during the year 2001. Dino: autotrophic dinoflagellates. Cryp: Cryptophyceae. Prym: Prymnesiophyceae. Chrys: Chrysophyceae. Chlor:

**Fig. 4:** Seasonal variations in absolute concentrations (ng l⁻¹) of the different phytoplankton groups during the year 2001.

**Table Caption:**

Figure 3

Percentage from chl a

Jan  | Mar  | Apr  | May  | Jun  | Jul  | Aug  | Sep  | Nov  | Dec

0%   | 20%  | 40%  | 60%  | 80%  | 100% |

Figure 4

Chlorophyta

Cyanobacteria

Cryptophytes

Chrysophyta

Diatoms

Dinoflagellates

Prochlorophytes

Prymnoophyta

Conc. (ng/l)
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