

Effect of ageing on survival of benthic diatom propagules

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Abstract

A comparison of viable benthic diatom propagules based on the observations recorded immediately and after 5 years of ageing at 5°C is presented. The number of viable benthic diatom propagules decreased with ageing. However, they exhibited an apparently longer lag phase. Although diatoms belonging to the genera Amphora, Navicula and Thalassiosira were dominant during immediate observation, only Amphora and Navicula survived the ageing process. The non-viability of Thalassiosira indicates that ageing for five years was beyond its critical period of survival. The other diatom genera that survived the ageing process were Odontella and Grammatophora.

Keywords: Ageing; Dormancy; Germination potential; Grammatophora spp; Lag phase; Odontella spp.

1. Introduction

When environmental conditions become unfavorable for vegetative growth, many diatoms form resting stages to survive cell degradation or death (Anderson, 1975; Malone, 1980; Hargraves and French, 1983; Fryxell, 1990). Various external factors like availability of nutrients (N, P, Fe, Si), effect of temperature, salinity, light and pH induce spore formation (Fryxell, 1983). The resting stages sink to the sediment and germinate when resuspended into

surface waters (McQuoid and Hobson, 1996) or when favorable conditions return (Hollibaugh et al., 1981).

Diatoms can retain photosynthetic capacity in the dark deep sediments and thus form an important pool of potential primary producers, which can resume photosynthesis if resurfaced (Fielding et al., 1988). Viable diatoms have been recorded upto 15 cm depth in the study area (Mitbavkar and Anil, 2002, 2006), and upto 20 cm (Steele and Baird, 1968) and 40 cm (McQuoid et al., 2002) depth in other areas. However, resumption of photosynthesis is possible only if resurfacing occurs within periods much shorter than the observed maximum survival time (Peters and Thomas, 1996), usually considered the 'critical period' for dark survival (Smetacek and Passow, 1990). In addition, occasional resuspension into the euphotic zone for short periods followed by resinking at the same or distant place could provide diatom propagules with 'recharging' periods of brief photosynthesis without inducing germination (French and Hargraves, 1980). Such resuspension of seed cells into illuminated conditions influences the viability of diatom propagules and affects seeding of the euphotic zone.

The ability of diatoms to survive in the dark (Smetacek, 1985) is an important factor controlling the distribution and seasonal occurrence of various diatom species (Peters, 1996). Dark survival has been highlighted as one possible reason for the survival of diatoms during the Cretaceous period with relatively slight generic loss as compared to other planktonic organisms (Gould, 1994).

Sediment samples are usually stored at refrigeration temperatures (5°C) prior to/after analysis. To evaluate the effect of long-term ageing on microphytobenthic diatoms, intertidal sediment samples, which were stored at 5°C for approximately 5 years, were analyzed for microphytobenthic diatom propagules in a series of experiments in 2004. The data obtained

was compared with the results of earlier investigations (Mitbavkar and Anil, 2002, 2006), referred to as immediate observations.

2. Methods

2.1. Study area

Sediment sampling was carried out on a sand flat at Dias Beach (15°27' N; 73° 48'E) located near Dona Paula Bay and surrounded by the Mandovi and Zuari estuaries (Fig.1). This beach is about 200 m in length and is sheltered and protected on both sides by rocky cliffs.

2.2. Effect of ageing on germination of benthic diatom propagules

Mitbavkar and Anil (2002, 2006) studied the diatoms of the microphytobenthic community at Dias Beach, a tropical intertidal sand flat. During this study, sediment samples were collected on a monthly basis for 17 months (May 1998 – September 1999). These samples were subjected to qualitative and quantitative analysis of microphytobenthic diatoms. Following standard procedures, the sediment samples were stored at 5°C in the refrigerator.

To determine the effect of ageing on the germination potential of benthic diatoms, the incubation experiments were repeated in 2004 after approximately 5 years of ageing. We selected five sediment samples (0-5 cm section), all collected from the mid-tide level. Since the southwest monsoon strongly influences the phytoplankton community in these environments, sediment samples collected during pre-monsoon (February), monsoon (August and September), and post-monsoon (November and January) seasons were selected. During storage, the sediment samples were kept in the dark and exposed to light only when removed

for sample analysis. Loss of moisture from samples was minimal since they were stored in airtight bags. Biological activity in these samples cannot be totally ruled out.

2.3. *Sample analysis*

Since this study was carried out on a sandy beach and included the observation of attached forms, direct microscopic observation and quantification of diatoms posed a problem. Even if diatoms in their attached state could be observed directly, there was always a chance of missing cells attached to the other side of the grain. Therefore, the extinction dilution method (Most Probable Number method -MPN) (Imai et al., 1984; Yamochi, 1989; Imai et al., 1990; Ishikawa and Tamaguchi, 1994; Itakura et al., 1997) was employed for quantification of diatom flora.

An appropriate amount (1 g wet weight) of sediment was suspended in *f/2* medium prepared in autoclaved, filtered, aged seawater (Guillard and Ryther, 1962) at a concentration of 0.1 g wet weight ml⁻¹. This stock was subjected to serial ten-fold dilution (10⁻¹ to 10⁻⁵) with the culture medium. 1 ml aliquots of diluted suspensions were inoculated into five replicate culture wells. Incubation was carried out at 27±1°C with an incident irradiance of 50 μmol photons m⁻² s⁻¹, produced by fluorescent tubes over a 12 h light: 12 h dark photocycle. The growth of diatoms in each culture well was examined microscopically using an inverted microscope (Olympus IX71) after an incubation period of 6-8 days. The wells in which growth was observed were scored as positive. The MPN (for a series of 3 ten-fold dilutions) of diatoms in the sediment sample (MPN g⁻¹ wet sediment) was calculated according to a statistical table (Thronsen, 1978). This table covers a range of five dilution steps and a set of three consecutive dilutions have to be chosen out of the five cultured to get the MPN number.

The diatom density per cubic centimeter wet sediment was obtained by multiplying the MPN value with the apparent specific gravity of the wet sediment (Imai and Itakura, 1999). Diatoms were identified based on the keys provided by Heurck (1896), Subrahmanyam (1946), Desikachary (1987), Tomas (1997) and Horner (2002).

3. Results

The viable benthic diatoms observed during 1998-1999 are expressed as 'immediate' observation and the propagules recorded after 5 years ageing of sediment are represented as 'aged' observation. The viable diatom propagules recorded during the respective observations are shown in Table 1.

In the immediate observation, a total of 7 centric and 12 pennate genera were recorded and divided into 3 groups based on their abundance in the study area. Amphora, Navicula (pennate) and Thalassiosira (centric), which were present throughout the period of investigation, formed the dominant group. The second group comprised forms that were frequently encountered in sediment and included 4 centric diatoms (Odontella, Coscinodiscus, Cyclotella and Melosira) and 6 pennate diatoms (Cocconeis, Nitzschia, Pleurosigma, Thalassiothrix, Cymbella and Tabellaria). The diatoms that appeared rarely formed the third group and included Bellerocha, Streptotheca, Fragilariopsis, Grammatophora and Raphoneis.

After ageing for 5 years at 5°C, only 1 centric (Odontella) and 3 pennate (Amphora, Navicula and Grammatophora) diatoms were observed (Table 1) at much lower levels compared to their abundance in the immediate observation (Fig. 2). In the immediate observation, Odontella, Amphora and Navicula were recorded during all the 5 sampling months wherein Amphora and Navicula were reported as consistent genera in the sediment. In

the aged observation, substantial reduction in abundance of Amphora (99.84%) and Navicula (87.22-98.55%) was observed. In addition, Odontella, Amphora and Navicula were recorded only during certain months as shown in Fig. 2.

Grammatophora, described as a rare genus in the immediate observation, survived ageing in samples collected during November 1998 and January 1999 (Fig. 2). In the immediate observation, it was recorded only once, i.e., during January 1999.

Thalassiosira, an abundant centric diatom in the overlying water column, was encountered abundantly in the sediment during all the sampling months during immediate observation. However, it failed to germinate following ageing.

Cyclotella and Surirella survived the ageing process in 10^{-1} dilution. However, for comparison with the immediate observation, only the diatoms that emerged in 10^{-2} , 10^{-3} and 10^{-4} dilutions are considered in this paper.

4. Discussion

The appearance of diatoms on incubation of fresh sediment can be attributed to two sources: either they are the products of multiplication of vegetative cells or germination of resting stages. The diatoms that appeared on incubation of aged sediment are most likely the ones that have germinated from their resting or hibernating mode of life, as prolonged survival of cells in the vegetative state is limited.

In this observation, when aged sediment samples (5 years) were placed in nutrient-rich, illuminated conditions, only a few diatom genera were able to resume vegetative growth. The reduction in germination levels as compared to that in the immediate observation was not only in terms of number of genera but also the cell density representing each genus (Fig. 2). The

diatoms that arose even after 5 years of ageing are probably the sturdy ones and are ecologically important considering their survival, even after prolonged burial in sediments.

Of the diatoms that germinated in the aged observations (Amphora, Cyclotella, Grammatophora, Navicula, Odontella and Surirella spp.), Surirella spp. were not recorded in the immediate observations. Since Cyclotella and Surirella spp. were recorded in the 10^{-1} dilution; it is also not included for quantitative comparison with immediate observations.

The abundance of Odontella and Grammatophora spp. that emerged in some samples in the aged observations was similar to that in the immediate observation indicating that prolonged storage under the conditions mentioned above did not affect its germination potential significantly. Odontella is a centric diatom but exhibits a benthic mode of life. It possibly possesses a higher germination potential as compared to other centric genera. Grammatophora, a pennate diatom, emerged twice during the aged observation as compared to once during the immediate observation. Its absence during November 1998 may possibly be linked to the high abundance of Cocconeis. During January 1999, when Grammatophora was encountered in the immediate observation, Cocconeis was recorded but in much lower abundance. Allelopathic interactions between the two genera may have played a role in determining their germination capability. Detailed experiments are required to determine the allelopathic interactions between these diatoms.

It is interesting to note that Odontella (Group II) and Grammatophora (Group III) survived better compared to Amphora and Navicula spp. (both Group I species). Amphora and Navicula spp. are classified as Group I species in the immediate observation along with Thalassiosira spp., i.e., species occurring throughout the period of investigation. Amphora and Navicula spp. germinated after 5 years of ageing (in 1 and 3 samples respectively) but at

reduced levels compared to the immediate observations (Fig. 2). Non-emergence of Thalassiosira spp. indicated that it could not survive storage for prolonged periods (5 years here) at 5°C in the absence of light. However, other studies indicate different survival periods for Thalassiosira spp. Imai et al., (1990) have reported survival of Thalassiosira spp. in sediment for 3 years. Thalassiosira tumida, isolated from plankton of the Weddell Sea, survived up to 302 days (Peters and Thomas, 1996). Results of the current study cannot be directly compared with the studies mentioned above since there are several differences in experimental conditions between these studies. Our results indicate that the critical survival period for Thalassiosira spp. is less than 5 years, under storage conditions similar to those in these experiments (5°C; dark conditions).

Overall, Group I species had lower tolerance to the storage conditions mentioned here. The 'sturdy' diatoms that survived prolonged ageing belonged to Groups II (frequently encountered species) and III (rarely observed species) in the immediate observation.

Diatoms, an important component of the microphytobenthic community in intertidal areas (Meadows and Anderson, 1968; Round, 1979), are subjected to cold, dark conditions when they are displaced from surface sediment to deeper layers through bioturbation, wave action, migration, etc. In such a situation, diatoms may remain buried deep within the sediment for long periods. Under such conditions, diatoms are subjected to prolonged absence of light, lowered temperature and decrease in oxygen levels available for vegetative cells that affect the physiological processes of diatoms during ageing (Lewis et al., 1999). Diatoms adapt to such conditions by lowering their metabolic rates, with negligible degradation of pigments (Steele and Baird, 1968; French and Hargraves, 1980) and uptake of dissolved organic compounds (Admiraal and Peletier, 1979).

Oxygen-deficient sediments are considered excellent for conservation of diatom and dinoflagellate resting stages in Koljö Fjord (McQuoid et al., 2002). However, the present study area is an intertidal sandflat with minimum chances of anoxic zones occurring in sediment. Thus a minimum amount of metabolic activity may be possible under storage conditions thereby affecting the dormancy period of diatom propagules.

A major difference between experimental and environmental conditions is the temperature at which the sediments were stored. Temperature during ageing (5°C) is lower than that likely *in situ* (21-30°C); this temperature difference may have served as one of the factors controlling reduction in metabolic rate of diatoms, which in turn, determines the length of the encystment period of diatom resting stages.

Diatom propagules, when observed following an incubation period of 6-8 days, were observed to be viable. However, they had still not multiplied implying that they were still physiologically dormant. Lengthened lag phases allow diatoms to assimilate nutrients before they start proliferating (Peter and Thomas, 1996). After spending long periods under dormancy during which the cells are in a metabolically weak state, perhaps a recovery period is necessary following their exposure to favourable conditions so as to rebuild and reorganize the cell contents before they divide. During this period, competition for nutrients along with allelopathic interactions may act as important factors controlling the germination process resulting in genera-specific selection leading to survival of the fittest. Extended lag phases of diatoms, observed after prolonged periods of darkness, hinder the germination success of diatom resting stages, thus influencing seeding of the overlying water column. This has important repercussions on the annual recurrence and characteristic species succession observed in diatoms blooms (Guillard and Kilham, 1977; Smayda, 1980).

The survival periods observed in these experiments provide a basis for understanding the survival of diatom propagules in monsoon-driven coastal sediments. Since diatoms have annual recurrence patterns of bloom formation, most diatom species are expected to be dormant till the next period favourable for bloom formation. Length of the dormancy period determines which diatom propagules will seed the new bloom. If dormancy for most diatoms can last only a few years, it is likely that the new bloom is overwhelmingly seeded by the resting stages of the previous bloom, whereas if the resting stages remain viable for a much longer period, then the bloom can be reseeded from cohorts from different years. This has important implications in that one unfavourable year does not have a dramatic effect on the long-term survival of the population.

In summary, the results obtained in this study indicated that diatoms that were frequently (*Odontella* spp.) and rarely (*Grammatophora* spp.) observed survived better than the diatoms that were observed throughout the study period (*Amphora*, *Navicula*, *Thalassiosira* spp.). The ‘sturdy’ species, capable of remaining dormant for approximately 5 years, have the potential to seed the overlying water column during favourable cycles that occur within the critical ‘survival period’. They also help in overwintering and tiding over a moderately extended (again within the ‘survival period’) unfavourable period. The length of their dormancy period, however, depends on the environmental conditions (temperature shock, light availability, oxygen availability) during storage.

Extrapolation of the results of the present study to *in situ* sub-tropical conditions, where temperature never reaches 5°C, is not possible. Thus, the results of this study need to be corroborated with experiments to determine the effect of other processes that a benthic cell would experience *in situ*, i.e., mixing, resuspension, etc. Evaluating the influence of such

factors through experiments will be a step ahead in understanding the seeding mechanisms of overlying waters in monsoon-driven coastal environments.

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Legends to figures:

Fig. 1. Location of study area.

Fig. 2. Comparison of viable diatom cell abundance (cm^{-3} wet sediment) between immediate and aged observations.

Table 1 Viable diatom genera recorded on immediate and aged observations

Centric diatoms	Pennate diatoms
<i>Bellerochea</i>	<i>Amphora</i> *
<i>Coscinodiscus</i>	<i>Cocconeis</i>
<i>Cyclotella</i> *	<i>Cymbella</i>
<i>Melosira</i>	<i>Fragilariopsis</i>
<i>Odontella</i> *	<i>Grammatophora</i> *
<i>Streptotheca</i>	<i>Navicula</i> *
<i>Thalassiosira</i>	<i>Nitzschia</i>
	<i>Pleurosigma</i>
	<i>Raphoneis</i>
	<i>Surirella</i> #
	<i>Tabellaria</i>
	<i>Thalassiothrix</i>

* species recorded in the aged observations

species recorded in the aged but not immediate observations



Fig. 1

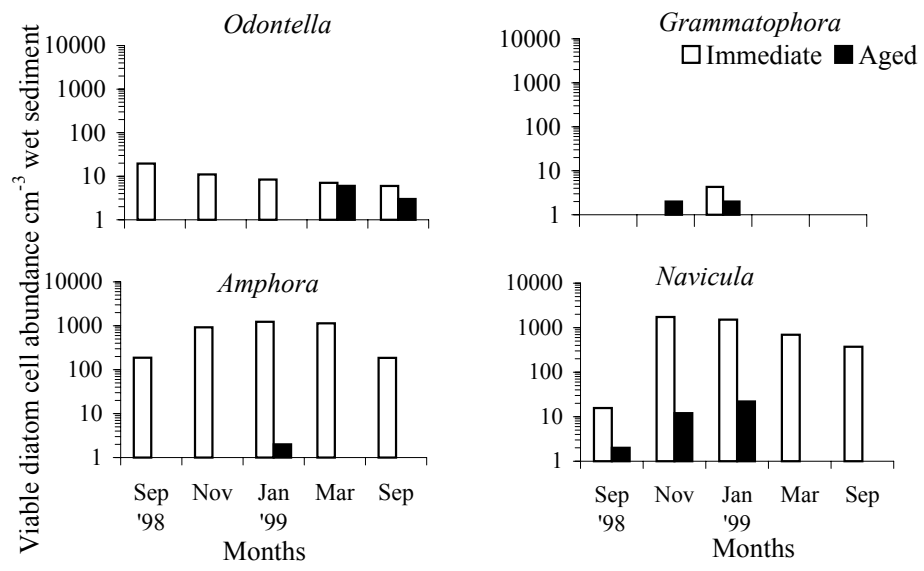


Fig. 2