

Species interactions within a fouling diatom community: Roles of nutrients, initial inoculum and competitive strategies

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Abstract

Diatoms constitute an important component of the fouling community. Although a lot of work has dealt with the fouling diatom community structure, work on the species interactions within the community is still meager. In this regard, a study was carried out by transferring the natural diatom biofilms into controlled conditions so as to understand the roles of nutrients, initial cell inoculum and the seasonal variations in species composition in structuring the fouling diatom community. The diatom community exhibited seasonal variations during the monsoon, postmonsoon and premonsoon periods. During each of these seasons, the diatom species interactions varied depending upon the species composition. It was observed that excess nutrients favored those species with comparatively higher growth rates thereby suppressing the growth of other co-existing species. This competitive trait was found to be effective at an appropriate cell density ratio of the competitive and target species. Understanding such pathways will be useful for modeling the fouling diatom species interactions in various habitats under different resource conditions.

Keywords: Fouling diatoms, nutrients, initial inoculum, competitive strategies

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Introduction

Fouling of natural and artificial structures is a serious problem in the marine environment resulting in vast economic losses. While fouling organisms are considered as a nuisance to ships, buoys and other floating structures, as well as to pilings in harbors, they may cause serious inefficiencies to sound devices, electronic units, cables and other instruments intended for underwater research. In the process of fouling, bacteria and diatoms are the initial colonizers of any substrata immersed in seawater (Cooksey et al., 1980). The source of the attached diatoms includes the water column, adjacent surfaces and benthos. When dislodged from their original substrate, any substratum in the pelagic zone serves as an "island" for immigration to these tytoplanktonic cells. Hence diatoms are found on various types of natural as well as artificial substrates where they tend to settle and develop into a biofouling community. These diatom communities are responsible for a major input of energy in the form of reduced carbon to the surface (Cooksey et al., 1984) and provide chemical cues for the settlement of higher organisms such as barnacles and polychaetes resulting in macrofouling (Harder et al., 2002; Lam et al., 2005; Patil & Anil, 2005a).

In recent times, eutrophication is another factor aggravating the problems related to marine phytoplankton (Meyer-Reil & Koster, 2000), which can also affect the fouling diatom community structure and behavior. The role of exploitative or resource competition determining species abundance in biofilms is bound to be more complex than in phytoplankton because of the extreme gradients of nutrients and light that can develop in biofilms (Steinman et al., 1989; Stevenson & Glover, 1993). The vast economic loss resulting from fouling has instigated extensive investigations of its cause and more particularly of practical methods for its prevention (Callow, 1996; Callow & Callow, 2002). However, attention has been focused mainly upon the fouling community structure and its seasonal and spatial variations on a long-term basis (Rendall & Wilkinson, 1983; Edyvean & Moss, 1986; Bhosle, 1993; Mitbavkar et al., 1997; Patil & Anil, 2005b). Surprisingly, little attention has been devoted to the initial stages of fouling, especially that of diatom species interactions which plays an important role in the process of biofouling. Strong interference interactions between biofilm forming heterotrophic bacteria (Wimpenny et al., 2000) and between bacteria and algae (Murray et al., 1986) have been proven, however for interactions between benthic microalgae knowledge is still scarce (Juttner, 1999; Van der

Grinten et al., 2004, 2005). Studies on the fouling diatom community interactions are important in order to gain understanding of the community development and behavior under changing environmental conditions. Such studies will provide important information that will further aid in the development of antifouling techniques.

We conducted a laboratory experiment by transferring natural biofilms aged between one and four days in order to study the role of nutrients and the initial inoculum of different species in regulating the fouling diatom community structure. In such *in vitro* experiments, the conditions will not mimic the natural environment. However, as Sommer (1996) suggested some of the differences are inherent necessities in experimentation because the effects of competition as the mechanism under study have to be isolated from confounding influences such as grazing and physical disturbances. In this study we made an attempt to find answers to the following questions: (1) whether the seasonal variations in the fouling diatom community structure influences the final outcome of nutrient competition, (2) are there species-specific differences in fouling diatom responses to nutrient limitation, and (3) does the initial inoculum affect the outcome of nutrient competition among the fouling diatoms?

Materials and methods

In the present study, laboratory competition experiments were performed on fouling natural diatom assemblages under enriched and non-enriched nutrient conditions. Such a study permits analysis of the influence of biogenic element ratios on mixed populations of diatoms. For obtaining the attached natural diatom assemblage, glass slides were immersed at the Dona Paula Bay ($15^{\circ} 27.5' N$, $73^{\circ} 48' E$), Goa, west coast of India. The diatom species diversity of this locality undergoes considerable variation during the three seasons i.e., post monsoon (January '99), pre monsoon (May '99) and monsoon (September '99) (Mitbavkar & Anil, 2000; Patil & Anil, 2005b). Hence, this experiment was carried out thrice, once in each season. During the post monsoon, pre monsoon and monsoon, the water temperature was 26 ± 2.9 °C; 27 ± 0.42 °C; 29 ± 1.08 °C and salinity was 31.5 ± 0.77 ; 32 ± 0.6 ; 26.6 ± 0.54 psu respectively. In the present study area, microorganisms dominate substrates for one to four days, followed by the arrival of macro organisms such as barnacles and polychaetes (personal observation). As our aim was to

understand the progression of a diatom community in the very early stages in an undisturbed condition, this study restricted the field exposures to only four days.

Natural biofilm formation

Every season, a total of 16 glass slides (7.6 x 2.2 cm), fixed on to a wooden frame were suspended at the sub-surface level (~ 1m below the low tide level). Four glass slides were retrieved on consecutive days, in order to obtain 1 day (1D), 2 days (2D), 3 days (3D) and 4 days (4D) old diatom community. The slides were removed from the wooden frame and immediately transferred to glass test tubes containing filtered seawater. This process was carried out carefully so as to minimize the disturbances to the slide community. During retrieval, passage of the attached communities through a water / air interface can remove poorly attached cells; especially bacteria and can also add bacteria which live at the interface. Although in this study, the diatom results were not compromised; such bacterial/diatom interactions in biofilms have to be considered in future studies. In the laboratory, the field recovered slides were immediately subjected to microscopic quantification and characterization of the diatom community.

Laboratory incubation of biofilm

Out of the four slides recovered every day, two were incubated in f/2 medium (Guillard & Ryther, 1962) (nutrient enriched-NE) whereas the other two were incubated in filtered seawater (unenriched-UE). The replicate slides were incubated separately in two different glass beakers. The salinity of seawater was 32 psu. The molar ratios of N: P: Si (μM) in NE and UE during post-monsoon, pre-monsoon and monsoon are shown in Table 1. The slide-containing beakers were incubated at 27 °C, which was close to the ambient temperature and illuminated at an incident irradiance of 50 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, produced by fluorescent tubes over a 12h light: 12h dark photoperiod. “[Insert Table 1 about here]”

The slides were later observed for direct diatom counts every alternate day. Microscopic observation was done by placing the slide in a petri plate containing the respective medium. The slide was placed in such a way that it did not touch the petri plate with the edges placed on two raised glass pieces. After observation, the same slide was incubated back into fresh NE or UE respectively for a period between 11 and 14 days. Care was taken so as to reduce the

disturbances that can affect the diatom community. The previous medium was preserved in Lugol's Iodine for analysis of cell loss, which was done by following the method given by Hasle (1978).

Enumeration of diatoms

In this study, since the attached diatom community had to be observed in the live state and later re-incubated, it would have been difficult to identify some of the smaller sized species. Hence, prior to this study, we identified and prepared a list of diatom species that were found in the present study area. Identification of the smaller diatoms was done by SEM (eg. *Cocconeis*, *Amphora*, *Navicula*, *Cylindrotheca closterium*), while the bigger sized diatoms (eg. *Nitzschia longissima*, *Achnanthes longipes*, *Licmophora*, *Odontella*, *Melosira*) were identified with the help of light microscopy (200X). For SEM, the diatom community from the slides was scraped into distilled water and washed several times through centrifugation. Later, the sample was treated with HCl to remove organic material. After several washings the sample was treated with HNO₃. Finally, the sample was washed and spread on glass cover slips for SEM observation (Tomas 1997). Diatoms were identified based on the keys provided by Heurck (1896), Subrahmanyam (1946), Desikachary (1987) and Tomas (1997). The diatom flora on either surface of the slide was analyzed and is expressed in terms of their variation per dm². Specific growth rates (μ) of the dominant diatom species were calculated with the following equation and subjected to linear regression analysis.

$$\mu = (\ln N_t - \ln N_0) / t$$

Where N_t = Cell concentration for every second day

N_0 = Cell concentration at $t = 0$

t = time interval (days) of exponential growth

Competition experiment between Navicula delicatula and Amphora coffeaeformis in culture

Isolation and culture of benthic diatoms. Glass slides were suspended for 4 days at the sub-surface level (~ 1m below low tide level) at Dona Paula Bay. After retrieval, the slides were rinsed with filtered seawater and the biofilm was brushed off with sterile paintbrushes into 50 ml Corning tubes containing filtered seawater. A dilution series of the biofilm suspension was inoculated onto 1% agar prepared with f/2 nutrient medium. Agar plates were incubated at 27 ± 1 °C under a 12 h light: 12 h dark photocycle. Distinguishable diatom colonies of *A. coffeaeformis* and *N. delicatula* formed within 1 week of inoculation, which were isolated and successively sub-cultured until monospecific diatoms were obtained. Pure cultures were then maintained in f/2 nutrient medium enriched seawater.

Experimental protocol. Pure diatom suspensions of each of the two species were prepared by brushing the culture flasks with sterile paintbrushes. Subsequently, aliquots of the two diatom suspensions were transferred into polystyrene multiwells (Corning-430343; 3 replicates). The corning well is 10 ml in capacity and the volume of culture medium was 5 ml and the multiwells were covered to avoid evaporation. The initial inoculum density ratios of *N. delicatula* and *A. coffeaeformis* were (a) 99:1 (b) 95:5 (c) 85:15 (d) 75:25 (e) 50:50 (f) 25:75 (g) 15:85 (h) 5:95 and (i) 1:99. The final concentration was 5×10^4 cells ml⁻¹. These multiwells were incubated at 27 ± 1 °C under 12h light: 12h dark condition and were observed microscopically at regular intervals for quantification. Results are presented in terms of percentage cells mm⁻².

RESULTS

Field incubated slides

A progressive increase in fouling diatom abundance on the field-incubated slides from Day 1 to Day 4, was a common feature observed during the three seasons. The diatom cell abundance was highest during post monsoon followed by monsoon and pre monsoon (Fig. 1 and Fig. 2). Diatoms encountered during the three seasons are presented in Table 2. Pennate genera were dominant throughout the study period. Amongst these, *N. delicatula* was the most dominant diatom. “[Insert Table 2 about here]”

Laboratory incubation of the field retrieved 1D to 4D-old diatom communities

Subsequent to laboratory incubation, the diatom abundance increased with time, the increase being higher in NE than UE incubations in the monsoon and pre monsoon seasons. However, in the post monsoon season, no significant difference was observed between the NE and UE incubations (Fig. 1 and Fig. 2). This observation reveals that the cells in the monsoon and pre monsoon were nutrient limited whereas those in post monsoon were not. This difference could probably arise due to the seasonal variations in species composition and also the supply of nutrients.

Post monsoon. *N. delicatula*, *A. coffeaeformis*, *Thalassionema frauenfeldii*, *Pleurosigma angulatum* and *C. closterium* were the dominant species of the 1D to 4D-old field diatom community.

NE incubation. *N. delicatula*, the most dominant diatom in the 1D-old field community, continued to be so subsequent to laboratory incubation, even after a period of 11 days (Fig. 3). *A. coffeaeformis* and *N. delicatula* exhibited similar growth rates (0.26 and 0.2 d⁻¹ respectively) after incubation of 2D to 3D-old field communities. Incubation of the 4D-old field community, revealed an increased abundance of *A. coffeaeformis* eventually surpassing *N. delicatula* with a longer incubation period. The dislodged community from the slide had a meager population of *A. coffeaeformis* as compared to *N. delicatula*. The rest of the subordinate species in the diatom community were also found in this sloughed off community (data not shown). The *A. coffeaeformis* to *N. delicatula* abundance ratio in the initial field inoculum of the 4D-old community was ~ 0.15:0.85 and linear regression analysis revealed a negative correlation ($r^2 = 0.94$) between their growth rates (1.3 to 0.31 and -0.08 to -0.18 d⁻¹ respectively) with longer incubation period.

UE incubation. *N. delicatula* exhibited a higher abundance (Fig. 4) due to the higher growth rate (0.2 d⁻¹) than the other species on incubation of the 1D to 3D-old field communities. On incubation of the 4D-old field community, the two major competitors, *A. coffeaeformis* and *N. delicatula* were growing at almost the same rate (0.26 and 0.08 d⁻¹ respectively). The two competitors did not contribute much to the dislodged community while the other minor diatom

species did. Linear regression analysis revealed a positive correlation between their growth rates with longer incubation period ($r^2 = 0.55$).

Pre monsoon. The diatom community from the field incubations changed over time from their initial composition. The communities in laboratory incubations also diverged over time from those in field incubations. The dominant species were *N. delicatula*, *Navicula subinflata*, *Amphora turgida*, *Amphora hyalina*, *A. coffeaeformis* and *Odontella rhombus*.

NE incubation. Incubation of the 1D and 2D-old field community resulted in continued dominance of *N. delicatula* and *A. hyalina* (Fig. 3). The 3D-old field community had a higher initial field inoculum of *A. turgida* whose growth rate was found to be higher than *A. hyalina* (0.52 and 0.26 d^{-1} respectively). The 4D-old field community was represented by *A. coffeaeformis* along with the other two species of *Amphora* and *N. delicatula*. With increased incubation period, *A. turgida* and *N. delicatula* dominated the community. Diatoms such as *O. rhombus* and *N. subinflata* which also had a higher initial field inoculum, could not hold on to the substrata and were found in the sloughed off community. While *O. rhombus* was lost soon after incubation, high numbers of *N. subinflata* were encountered with longer incubation period. Linear regression analysis revealed a positive correlation between the growth rates of *A. turgida* (0.86 to 0.56 d^{-1}) and *N. delicatula* (0.9 to 0.50 d^{-1}) with increase in the incubation period ($r^2 = 0.86$).

UE incubation. The 1D to 4D-old field community behaved in a similar pattern as that in NE, even though the initial inoculum of *A. turgida* was low as compared to *A. hyalina* (Fig. 4). Although *A. turgida* and *N. delicatula* dominated the community with increased incubation period, the other species of *Amphora*, viz. *A. hyalina* and *A. coffeaeformis* were observed in the sloughed off community in contrast to NE incubation where they were not totally eliminated. Linear regression analysis revealed a positive correlation between the growth rates of *A. turgida* (0.66 to 0.35 d^{-1}) and *N. delicatula* (0.68 to 0.49 d^{-1}) with longer incubation period ($r^2 = 0.85$).

Monsoon. Dominance of diatoms differed with the age of the biofilm. In addition to *N. delicatula*, *N. subinflata*, *C. closterium*, *A. turgida*, *A. coffeaeformis* and *A. hyalina*, encountered in post and pre monsoon, this community included other diatom species such as *T. nitzschoides* and *N. longissima*.

NE incubation. In the 1D-old field community, *N. delicatula* and *N. subinflata* continued their dominance after incubation (Fig. 3). In addition to these diatoms, the community showed the presence of *C. closterium*. The 2D-old field community comprised major species of 1D-old field community with an addition of *A. turgida*. The initial inoculum of the *Amphora* species was low as compared to others. In the 3D-old field community, *N. delicatula* was closely followed by *N. subinflata*, *N. longissima* and *A. coffeaeformis* (Fig. 3). *N. longissima* was a dominant component of the 4D-old field community (73%) and this species maintained its dominance over *N. delicatula* throughout the incubation period. *C. closterium* and *T. frauenfeldii* with a high initial field inoculum were not found to establish on the original substrata and were found in the sloughed off community. This community also included *N. delicatula*, *N. subinflata* and *A. coffeaeformis*. Linear regression analysis revealed a negative correlation between the growth rates of *N. delicatula* (-0.04 to -0.11 d^{-1}) and *N. longissima* (0.4 to 0.25 d^{-1}) with increase in the incubation period ($r^2 = 0.98$).

UE incubation. The 1D to 2D-old field community revealed similar patterns as that of NE (Fig. 4). The 3D-old field community had a high initial inoculum of *N. delicatula*, *N. subinflata*, *T. nitzschioides*, *N. longissima* and *C. closterium*. In the 4D-old community, *N. longissima*, even though with a high initial inoculum, failed to establish itself and gave way to other diatoms such as *N. delicatula*, *C. closterium*, *N. subinflata*, *A. turgida*, *Amphora hyalina* and *A. coffeaeformis*. In this case, difference between the original substrate and sloughed off community was minimal.

Competition between N. delicatula and A. coffeaeformis in culture

The outcome of the competition experiment carried out with pure cultures of *A. coffeaeformis* and *N. delicatula*, grown at various initial density ratios is given in Fig. 5 (data for only three of the possible nine combinations are shown). At an initial density ratio of *N. delicatula* and *A. coffeaeformis* ranging from 99:1 to 85:15, *A. coffeaeformis* showed an increasing trend with time. With an increase in initial density from 1 to 50% of *A. coffeaeformis*, the time required to overtake *N. delicatula* reduced from 7 to 3 days. At densities ranging from 75% to 99%, the initial inoculum density of *A. coffeaeformis* being higher, dominated over *N. delicatula* throughout the incubation period (data not shown). So, the time taken for *A. coffeaeformis* to overtake *N. delicatula* was inoculum size dependent.

DISCUSSION

Biofilms developed in situ

As reported in earlier literature, pennate diatoms were more abundant in the fouling film than centric diatoms (Characklis and Cooksey 1983; Cooksey et al. 1984). *N. delicatula* and *A. coffeaeformis* were encountered during all the seasons in the one to four day old biofilms developed in the natural conditions. However, compared to all three seasons, during post monsoon the abundance of the two species was considerably higher. *A. coffeaeformis* abundance was relatively lower during the pre monsoon and monsoon. In general, the increase in the abundance of the different species with age of the biofilm reveals that the effect of cell immigration and cell reproduction overrides that of the loss factors such as grazing and cell death.

Laboratory incubation of the one to four-day-old natural biofilms

In order to discuss the results, we will take into account only those species, which made a contribution of more than 5% to the entire community and played an important role in deciding the community structure throughout the experimental duration. Incubation of the one to four days old mixed natural diatom community in the NE and UE conditions revealed differences in the growth rates of the abundant species with the increase in the experimental duration. A schematic representation of the community interactions in NE and UE during the three seasons is given in Fig. 6. During the post monsoon season, *A. coffeaeformis* abundance was increasing in both the nutrient conditions, but the growth rate was slower in UE condition. In the NE condition, the abundance of *A. coffeaeformis* increased from a relatively minor early cell density to dominance of the final diatom community. Other diatom species concurrently declined in importance. Buzzelli et al. (1997) observed that the best growth rate of *A. coffeaeformis* cultures occurred at 900 μM nitrate and 36 μM orthophosphate, which is close to N: P ratio in the NE condition. Such a behavior in diatom succession can be explained by non-interactive models wherein the fast immigrants are replaced by fast reproducers (Stevenson, 1986) under nutrient enrichment. This effect of elimination of *N. delicatula* (fast immigrants) by *A. coffeaeformis* (fast reproducers) was found to be density dependent, which was determined by the initial *in situ* inoculum of *A. coffeaeformis*.

In the pre monsoon season, nutrient availability did not play an important role for *N. delicatula* and *A. turgida*. Since the growth rate of *A. turgida* was similar to that of *N. delicatula* in NE and UE conditions, both species increasingly dominated the community during incubation. However, nutrient availability did affect the distribution of *A. coffeaeformis* and *A. hyalina* wherein they were completely eliminated from the community in UE as compared to NE, where they made a minor contribution to the community. *A. coffeaeformis* and *A. hyalina* survived in NE due to higher nutrient concentrations. However, in UE low concentrations of nutrients along with low initial inoculum resulted in their total elimination. Hence, *A. coffeaeformis*, which eliminated *N. delicatula* in the post monsoon community in NE, was not successful in the pre monsoon because its initial inoculum was too low to overcome competition with *N. delicatula* and *A. turgida*, both in NE and UE conditions. The results obtained during pre monsoon also highlight the importance of initial inoculum over nutrient availability.

In the monsoon season, *N. longissima* was the key controller of the community structure. Through direct interference competition in the NE condition, *N. longissima* completely eliminated *N. delicatula*, *N. subinflata* and *C. closterium* from the shared environment. The high densities achieved by the opportunistic species probably denied access to the key nutrients to its competitors, thereby suppressing their growth and resulting in lowered diversity. In UE, *N. longissima* could not prosper, even though its initial field inoculum was high, probably because it could not sequester the key nutrients present in low concentrations. So, the exclusion of the dominant species due to resource limitation proved to be advantageous to the other species which could grow efficiently even in NE, each probably having a different resource requirement, thereby resulting in a diverse community which led to co-existence.

There are two aspects of diatom population dynamics, which will influence the eventual outcome of competition, one is the ability to grow quickly and efficiently, and the other is to achieve a high final biomass when local nutrient exhaustion occurs. However, species with high initial growth rates may not always have a high carrying capacity and vice versa. Forster & Jezequel-Martin (2005) for various species of marine benthic diatoms showed that maximum growth rates were similar in early stages of batch growth, but the achieved biomass in the stationary growth phase was very variable between species. Underwood & Provot (2000) also reported on differences in growth rates of benthic diatom species over a 7 d period depending on

the nutrient concentration and salinity. Van Der Grinten (2004) reported dominance of diatoms over cyanobacterial species in nitrogen-limited biofilms thereby suggesting that species growth rates also depend on the nutrient concentrations. The growth rate of a particular species also varies in mixed cultures as compared to that in unialgal culture. Van Der Grinten et al. (2005) showed that a cyanobacteria could out compete a diatom in mixed culture biofilms, even though the diatom had a superior performance in monoculture. In the present study, the range of diatom species growth rates was in agreement with that reported in literature for benthic diatoms (Underwood & Provot, 2000; Forster & Jezequel-Martin, 2005; Van Der Grinten et al., 2005).

Hudon & Bourget (1981) and Hoagland et al. (1982) have reported similar benthic diatom succession through density-dependent competitive interactions for nutrient and light. Stevenson et al. (1991) demonstrated that enhanced nutrient supply decreased micro algal diversity with increase in colonization time (12 weeks). Our study which was restricted to 4 days due to the appearance of macro organisms, revealed an increasing trend in species diversity. This difference could be due to the spatial variations or due to the time factor. Subsequent incubation of the communities obtained in this study, in controlled conditions revealed differences in species diversity depending on nutrient availability. While in NE, the species diversity decreased in post monsoon and monsoon and remained stable in pre monsoon; in UE species diversity remained stable in post monsoon and monsoon and decreased in pre monsoon. Species interactions in post monsoon, pre monsoon and monsoon might have resulted in the variations in species diversity.

Although, *N. delicatula* dominated in the UE condition with nutrient conditions similar to natural condition, the change in community structure in the NE condition shows that certain species, which were nutrient limited, dominated once excess nutrients were supplied. On the contrary, the near exclusion of *N. delicatula* from the biofilm community may also be associated with the familiar concept of differing relationships between growth rates and nutrient concentrations among competing species (Mickelson et al., 1979). Under this hypothesis, *N. delicatula* would have had higher growth rates than other competing diatoms at low micro environmental nutrient concentrations. Addition of nutrients to the system would then increase the growth rates of other diatom species more rapidly than for *N. delicatula*. Such changes in community structure reflect the processes, which probably occur in the natural eutrophicated environments. Also, since competition is influenced by the light intensity, in the present study,

the intensity of $50 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ which was lower to that observed in the natural environment could have influenced the results to some extent.

Cell to cell interactions, such as the bacteria to diatom and the diatom-to-diatom interactions are also known to play a role in structuring the diatom communities. The presence of certain bacterial species or some entity produced by them and secreted into the medium is known inhibit or reduce the attached diatom component considerably (Cooksey & Cooksey, 2005) either due to the result of less matrix polymer synthesis, or its hydrolysis by the bacteria (Cooksey et al., 2001). Among the marine phytoplankton species, allelopathic interactions play an important role in ecology. Pratt (1966) found a temporary inhibition of *Skeletonema costatum* by filtrates taken from a culture of *Olithodiscus luteus* only when the *Olithodiscus* concentration exceeded 10^8 cells liter⁻¹. *A. coffeaeformis*, which is known for domoic acid production, a water-soluble toxin (Maranda et al., 1989), is reported to have certain toxic effects (Buzzelli et al., 1997). *A. coffeaeformis* was also capable of highest biomass production in the comparative study of microphytobenthic diatoms under different culture conditions probably due to its more efficient inorganic carbon uptake mechanism (Forster & Jezequel-Martin, 2005). However, since the toxicity is known to depend on the concentration of the particular species, its effect will vary depending on the initial inoculum. These bacteria/diatom and diatom/diatom interactions need to be addressed in future studies.

Although in mixed pure cultures, *A. coffeaeformis* overtook *N. delicatula* at all initial concentrations ranging from 1 to 99%, the period within which it could overtake *N. delicatula* reduced with the increase in the percentage of initial inoculum. The growth inhibition of the latter occurred within three days at a similar initial inoculum as that observed in the mixed natural community (*A. coffeaeformis*: *N. delicatula*; 0.15:0.85) (Fig. 5c). While emphasizing the importance of the initial inocula in determining algal community composition, Talling (1962) also stressed that differences in intrinsic growth rates are of major importance in determining community dominants. There can be some disadvantages in conducting the competition experiments in multiwells without renewal of the culture medium. However, since these are attached forms and had to be observed directly under the microscope, we carried out this experiment in multiwells. Obviously, this differs from the natural diatom community exposed to large volumes of flowing water, and could allow experimental populations to experience

unusually low nutrient concentrations or unusually high concentrations of excreted substances. This may not be a fatal problem, but possibly made competition more intense than it would be in the natural environment.

In conclusion, seasonal variations were observed in the biofilm diatom community structure. Interspecific competition between species was controlled by the nutrient conditions, the initial cell inoculum and the community composition. It was observed that the competitive traits of a particular species become effective in producing dominance over a given time period at an appropriate initial cell density ratio of the competitor and the target species. Understanding of such pathways will be useful for modeling fouling diatom species interactions within biofilms in various habitats under different resource conditions. Our results may also suggest which diatom species are important for use in bioassays for antifouling treatments.

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Table 1. The molar ratios of N:P:Si (μM) in nutrient enriched (NE) and unenriched (UE) medium during the post-monsoon, pre-monsoon and monsoon seasons.

Seasons	NE	UE
Post-monsoon	882:42:123	1.3:1.8:25
Pre-monsoon	882:42:123	1.5:3.2:37
Monsoon	882:42:123	0.45:1.2:50

Table 2. Diatoms encountered in post monsoon, pre monsoon and monsoon season.

Pennate diatoms	Post monsoon	Pre monsoon	Monsoon
<i>Achnanthes longipes</i> Agardh	+	+	+
<i>Achnanthes subsessilis</i> Kutzing	+	+	+
<i>Amphora coffeaeformis</i> (Agardh) Kutzing	+	+	+
<i>Amphora hyalina</i> Kutzing	+	+	+
<i>Amphora rostrata</i>	+		+
<i>Amphora turgida</i> Gregory	+	+	+
<i>Cocconeis scutellum</i> Ehrenberg		+	+
<i>Cylindrotheca closterium</i> (Ehrenberg) W. Smith	+	+	+
<i>Diploneis smithii</i> (Breb – ex W. Sm.) Cl.			+
<i>Fragilariopsis oceanica</i> (Cleve) Hasle			+
<i>Grammatophora marina</i> (Lyngbye) Kutzing		+	
<i>Grammatophora serpentina</i> Ehrenberg			+
<i>Licmophora flabellata</i> (Greville) Agardh	+		+
<i>Licmophora juergensii</i> Agardh	+		+
<i>Licmophora paradoxa</i> (Lyngbye) Agardh		+	+
<i>Meuniera membranacea</i> (Cleve) P. C. Silva comb.	+		+
<i>Navicula transitans</i> var. <i>derasa</i> f. <i>delicatula</i>	+	+	+
<i>Navicula septentrionalis</i> (Grunow) Gran	+	+	+
<i>Navicula subinflata</i> Grun.	+	+	+
<i>Nitzschia angularis</i> Wm. Smith	+		+
<i>Nitzschia longissima</i> (Brebisson) Grunow			+
<i>Nitzschia sigma</i> (Kutzing) Wm. Smith var. <i>sigma</i>	+		+
<i>Pleurosigma angulatum</i> Queckett Wm. Smith		+	+
<i>Pseudo-nitzschia seriata</i> (Cleve) H. Peragallo		+	+
<i>Synedropsis gaillonii</i> (Bory) Ehrenberg	+		+
<i>Thalassionema frauenfeldii</i> (Grunow) Hallegraeff	+		
<i>Thalassionema nitzschioides</i> (Grunow)			+
Centric diatoms			
<i>Biddulphia mobiliensis</i> (Bailey) Grunow	+		
<i>Biddulphia obtusa</i> (Kutzing) Ralfs in Pritchard		+	
<i>Chaetoceros</i> sp1	+		
<i>Coscinodiscus marginatus</i> Ehrenberg		+	
<i>Hyalodiscus stelliger</i> (Bailey) Mann		+	
<i>Melosira nummuloides</i> (Dilwyn) C.A. Agardh		+	+
<i>Odontella rhombus</i> (Ehrenberg) W. Smith		+	+
<i>Thalassiosira eccentrica</i> (Ehrenberg) Cleve			+

Legends to figures

Fig. 1. Total abundance (mm^{-2}) of the fouling diatoms on the glass slides incubated in nutrient enriched (NE) condition during the post-monsoon, pre-monsoon and monsoon (vertical lines above bars indicate standard deviation).

Fig. 2. Total abundance (mm^{-2}) of the fouling diatoms on the glass slides incubated in unenriched (UE) condition during the post-monsoon, pre-monsoon and monsoon (vertical lines above bars indicate standard deviation).

Fig. 3. Species composition (%) of the fouling diatom community on the glass slides incubated in nutrient enriched (NE) condition during the post-monsoon, pre-monsoon and monsoon.

Fig. 4. Species composition (%) of the fouling diatom community on the glass slides incubated in unenriched (UE) condition during the post-monsoon, pre-monsoon and monsoon.

Fig. 5. *Navicula delicatula* (●) and *Amphora coffeaeformis* (○). Percentage cell abundance (mm^{-2}) in mixed culture at different initial cell density ratios of (a) 99:1 (b) 95:5 and (c) 85:15.

Fig. 6. Schematic representation of the fouling diatom community interactions encountered during the laboratory incubations in nutrient enriched (NE) and unenriched (UE) conditions during the (a) post monsoon, (b) pre monsoon and (c) monsoon. (ND: *Navicula delicatula*; AC: *Amphora coffeaeformis*; AH: *Amphora hyalina*; AT: *Amphora turgida*; NL: *Nitzschia longissima*; Numbers represent the abundance ratios for the dominant species).

Nutrient Enriched (NE) incubation

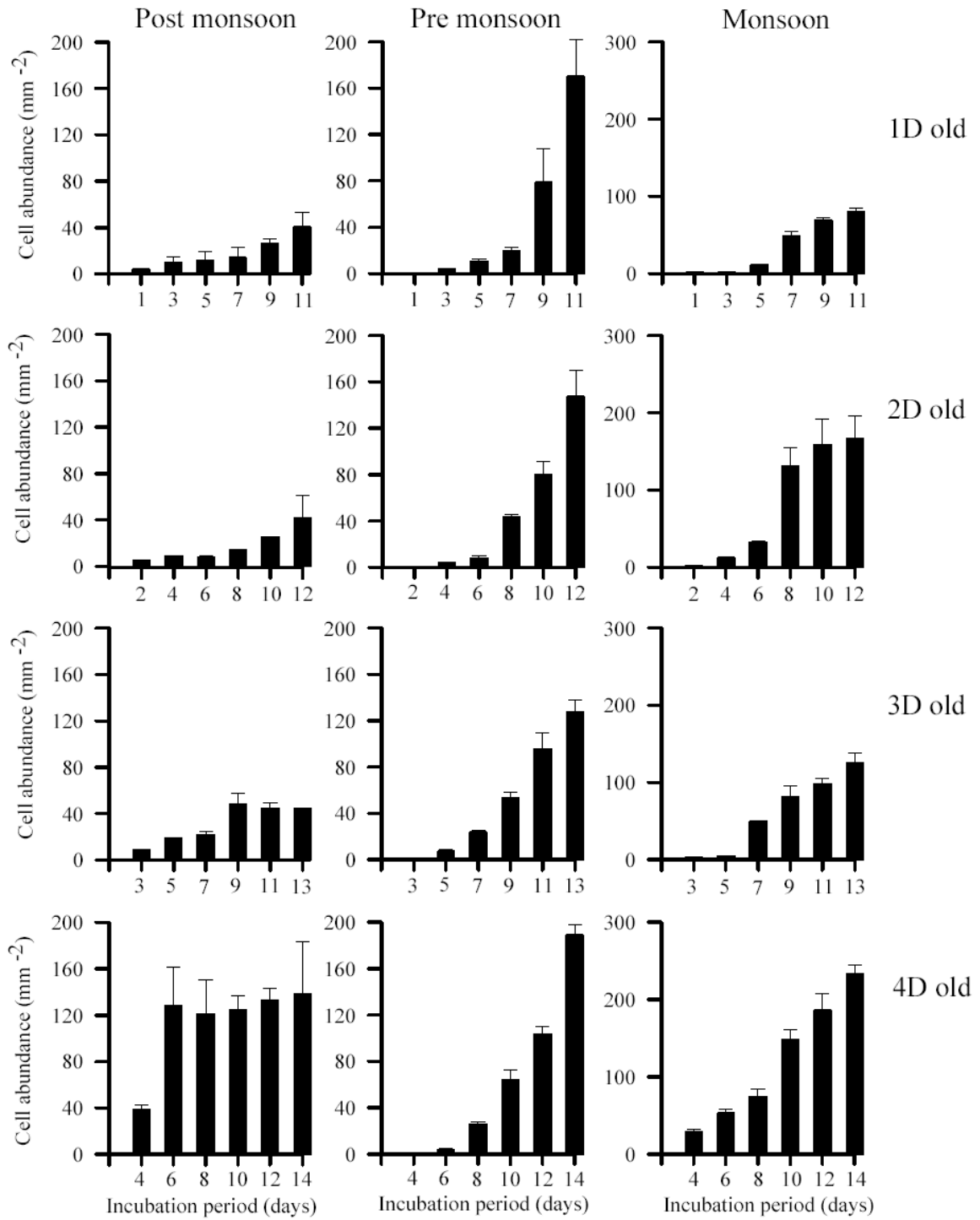


Fig. 1

Unenriched (UE) incubation

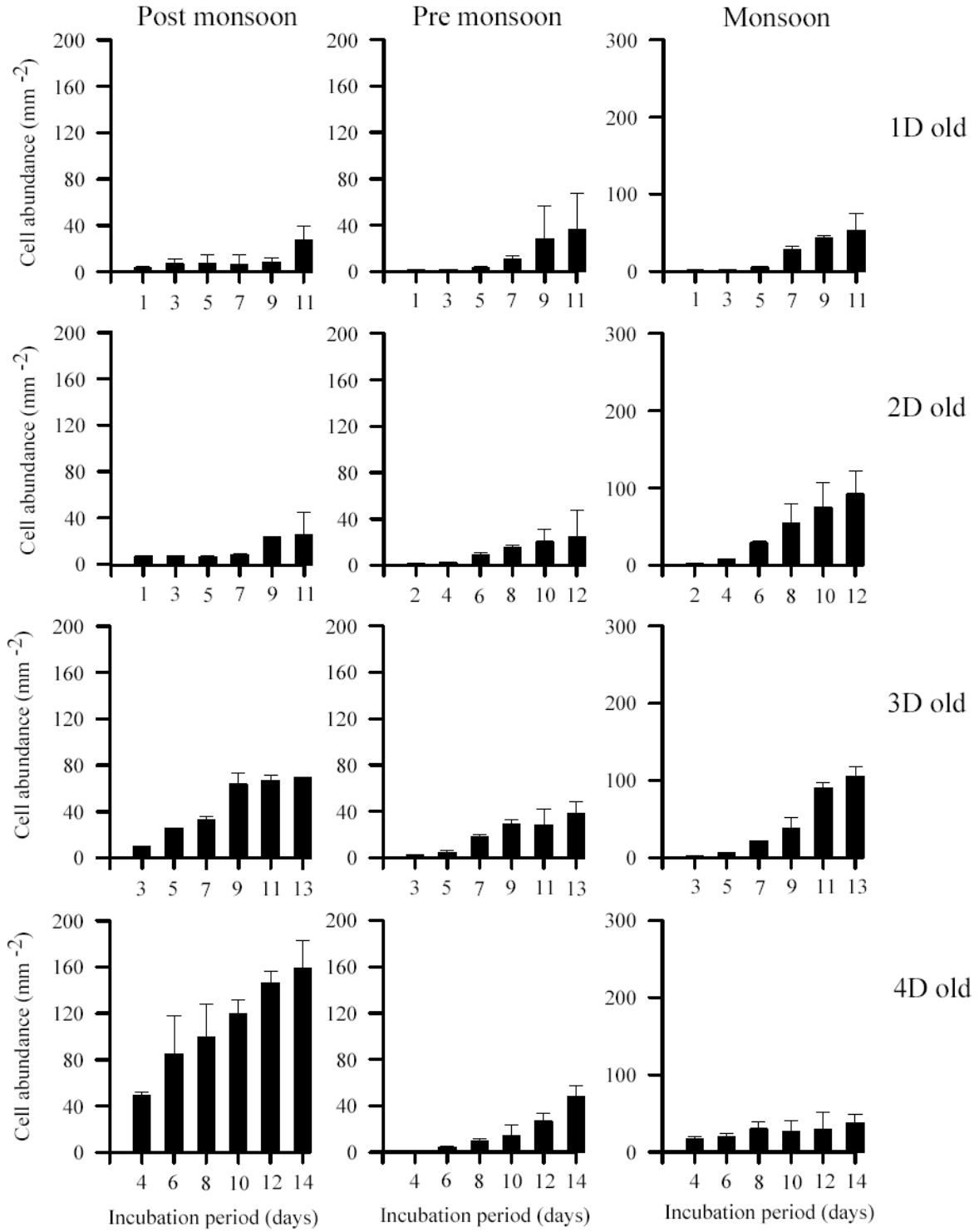


Fig. 2

Nutrient Enriched (NE) incubation

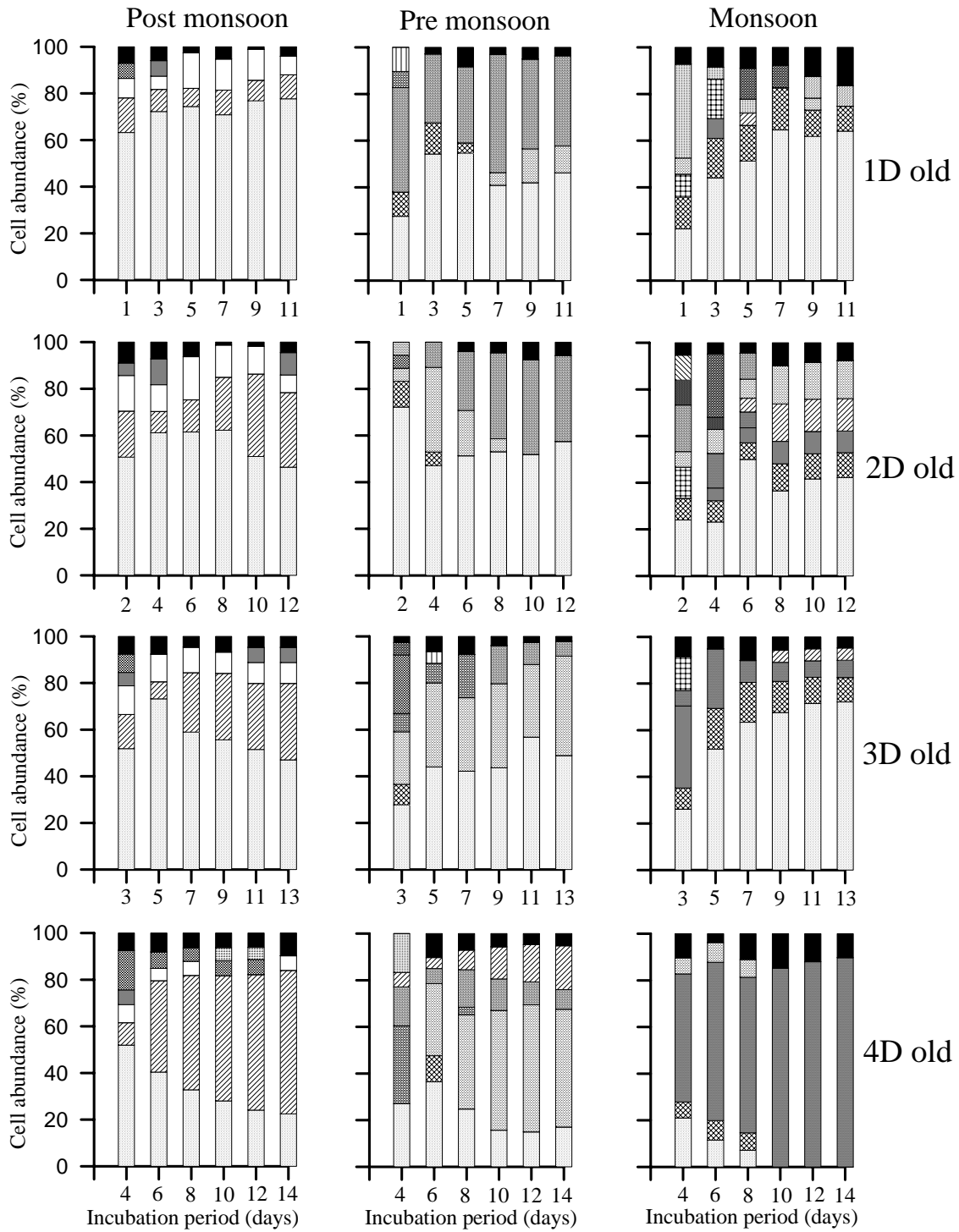


Fig. 3

Unenriched (UE) incubation

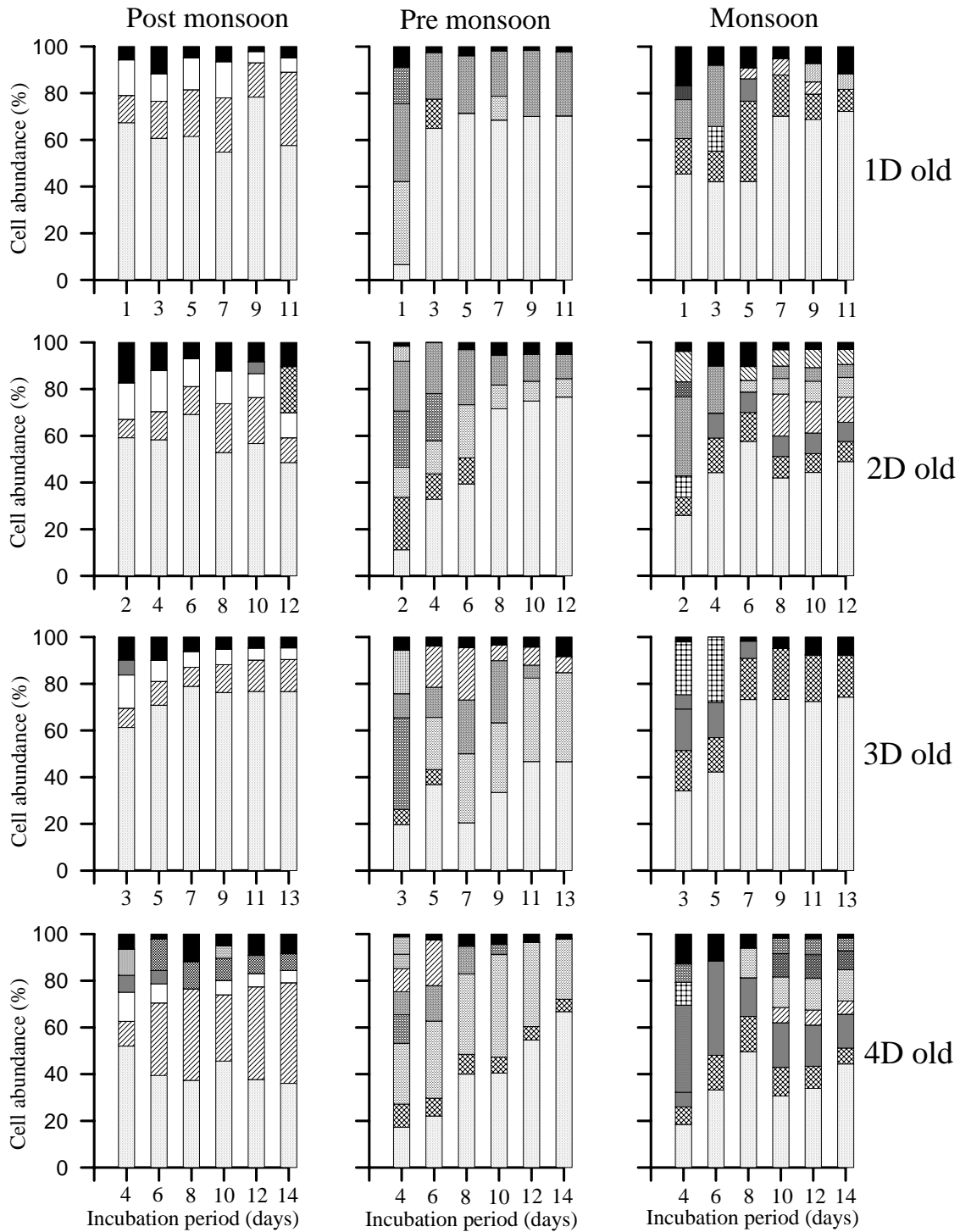























Fig. 4

Legends for Fig.3 and Fig.4

<i>Achnanthes longipes</i>	
<i>Amphora coffeaeformis</i>	
<i>Amphora turgida</i>	
<i>Amphora rostrata</i>	
<i>Amphora hyalina</i>	
<i>Biddulphia obtusa</i>	
<i>Cocconeis scutellum</i>	
<i>Coscinodiscus marginatus</i>	
<i>Fragilariopsis oceanica</i>	
<i>Grammatophora marina</i>	
<i>Melosira nummuloides</i>	
<i>Navicula delicatula</i>	
<i>Navicula subinflata</i>	
<i>Nitzschia angularis</i>	
<i>Nitzschia closterium</i>	
<i>Nitzschia longissima</i>	
<i>Odontella rhombus</i>	
<i>Pleurosigma angulatum</i>	
<i>Thalassionema frauenfeldii</i>	
<i>Thalassionema nitzschioides</i>	
Others	

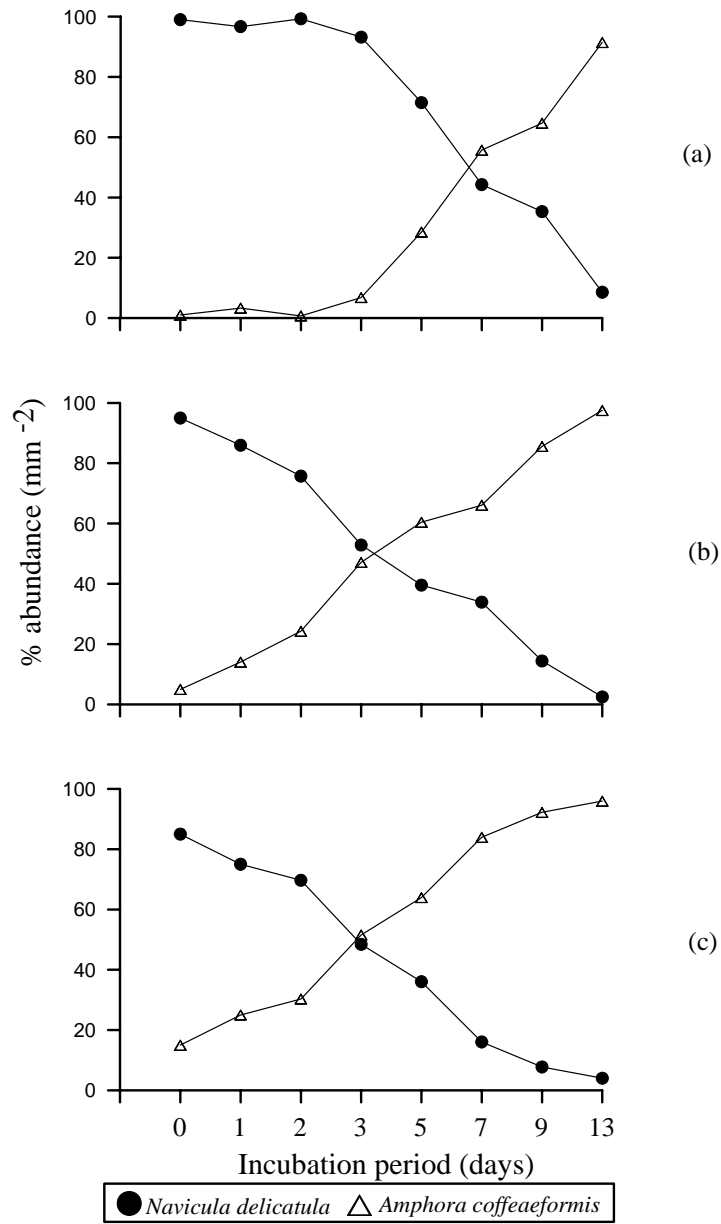


Fig. 5

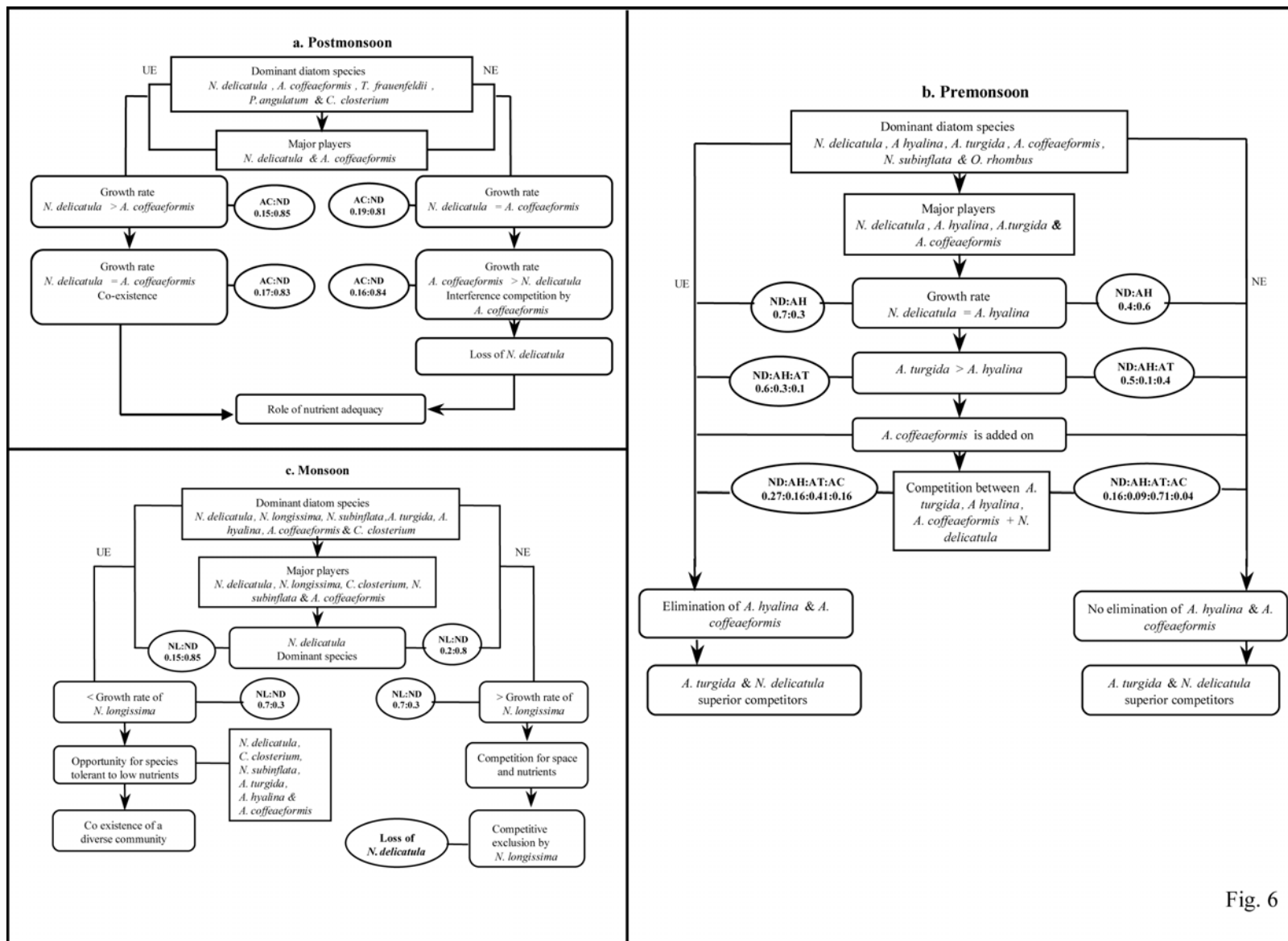


Fig. 6