Marine Biology (2001) 138: 163–171 © Springer-Verlag 2001

Frederico P. Brandini · Eduardo T. da Silva
Franciane M. Pellizzari · Alessandra L. O. Fonseca
Luciano F. Fernandes

Production and biomass accumulation of periphytic diatoms growing on glass slides during a 1-year cycle in a subtropical estuarine environment (Bay of Paranaguá, southern Brazil)

Received: 27 April 2000 / Accepted: 16 August 2000

Abstract Production rates, chlorophyll concentrations and general composition of periphytic diatom communities growing on glass slides were studied in relation to environmental parameters during one seasonal cycle in the Bay of Paranaguá, southern Brazil. Slides were routinely submersed at 1, 2 and 3 m depth and recovered weekly for microscopic examinations, analyses of chlorophyll, cell counts and in situ photosynthetic incubations using the Winkler titration method. Water samples were also collected at surface and bottom layers for determinations of temperature, salinity, nutrients and chlorophyll in the water. The periphytic community was mainly formed by epipelic and epipsammic species, dominated by *Navicula phyllepta, Cylindrotheca closterium, Navicula* spp. and *Amphora* sp. Weekly chlorophyll *a* and cell accumulations on slides varied from <1–32 mg m^{-2} and up to 31×10^8 cells m^{-2}, respectively. Photosynthetic rates varied from <1 to 35 mg oxygen mg chlorophyll a^{-1} h^{-1}, with higher values in summer. Daily production varied from 5 to 3,600 mg oxygen m^{-2} day^{-1} (<0.01–1.4 g carbon m^{-2} day^{-1}). Multiple regression analysis revealed that vertical differences in light conditions and grazing pressure jointly affected the influence of temperature on the seasonal patterns of cell densities and chlorophyll concentrations according to depth.

Communicated by O. Kinne, Oldendorf/Luhe

F. P. Brandini (✉) · E. T. da Silva
Universidade Federal do Paraná, Centro de Estudos do Mar,
Av. Mira Mar s/n, Pontal do Sul, Pontal do Paraná,
CEP 83255-000, Paraná, Brazil

F. M. Pellizzari · A. L. O. Fonseca · L. F. Fernandes
Universidade Federal do Paraná, Departamento de Botânica,
Setor de Ciências Biológicas, Centro Politécnico,
Jardim das Américas, BR-116, Curitiba,
CEP 81531-970, Paraná, Brazil

Introduction

Benthic diatoms are important microautotrophs in marine habitats, growing on a great variety of substrates (Grøntved 1960; Amspoker and McIntire 1978; Rioux-Gobin 1987; Sullivan and Moncrieff 1988; MacIntyre and Cullen 1995). Most common are pennate epipelic diatoms that develop on intertidal mudflats along temperate and subtropical estuarine environments (Admiraal et al. 1982; Amspoker and McIntire 1986; Laird and Edgar 1992).

In addition to their importance as food for benthic invertebrates (Pace et al. 1979; Decho and Fleeger 1988; Sullivan and Moncrieff 1990) they supply organic carbon to the planktonic system during resuspension by tidal currents and wind-induced turbulence (Brown and Austin 1973; Demers et al. 1987; Wainright 1990; Delgado et al. 1991; de Jonge and van Beusekom 1992).

Epipelic diatoms are usually the dominant microautotroph on mud flats in the inner intertidal zones of the Bay of Paranaguá, a mangrove-bordered subtropical lagoon environment in southeast Brazil. A significant role in the food-web of the bay is confirmed by the dominance of diatoms in the digestive tracts of most benthic invertebrates (P. Lana, personal communication). Resuspended diatoms also contribute to the biomass of the water column over the entire bay (Brandini and Thamm 1994; Brandini and Fernandes 1996). Except for taxonomic studies (Moreira-Filho and Kutner 1962; Moreira-Filho et al. 1975), basic ecological and quantitative information on the benthic diatoms in the Bay of Paranaguá remains unknown. This investigation describes the seasonal pattern of periphytic diatom populations growing on glass slides in the Bay of Paranaguá in relation to environmental parameters. Our goal was to gain new insight into the ecological role of benthic (mostly epipelic) diatoms in a shallow subtropical mudflat habitat.

Materials and methods

Field work

The pier of Pescal Fisheries, located at the southwestern margin of Galheta Channel at the entrance of the Bay of Paranaguá, was selected as the study site. This site is hydrographically representative of the western sector of the bay, where mudflats are extensive (Fig. 1a). Sets of 12 acid-cleaned glass slides (1 × 5 cm) were arranged on three plexiglass plates (four slides on each plate). The plates were attached to an anchored line at 1, 2 and 3 m depth, respectively (Fig. 1b). Slides were kept horizontal with both sides exposed for colonization. The mean area of the 46 slides available for colonization was 10.2 cm^2. The suspension line was tied to a buoy and anchored to the bottom. To keep the line vertical and to level the plates with tidal changes in water height, the buoy was also tied to the pier columns by a combination of ropes and rubber extensors. This was done to keep the slides at the same respective depth and to avoid exposure of the 1-m-deep plate during low tide.

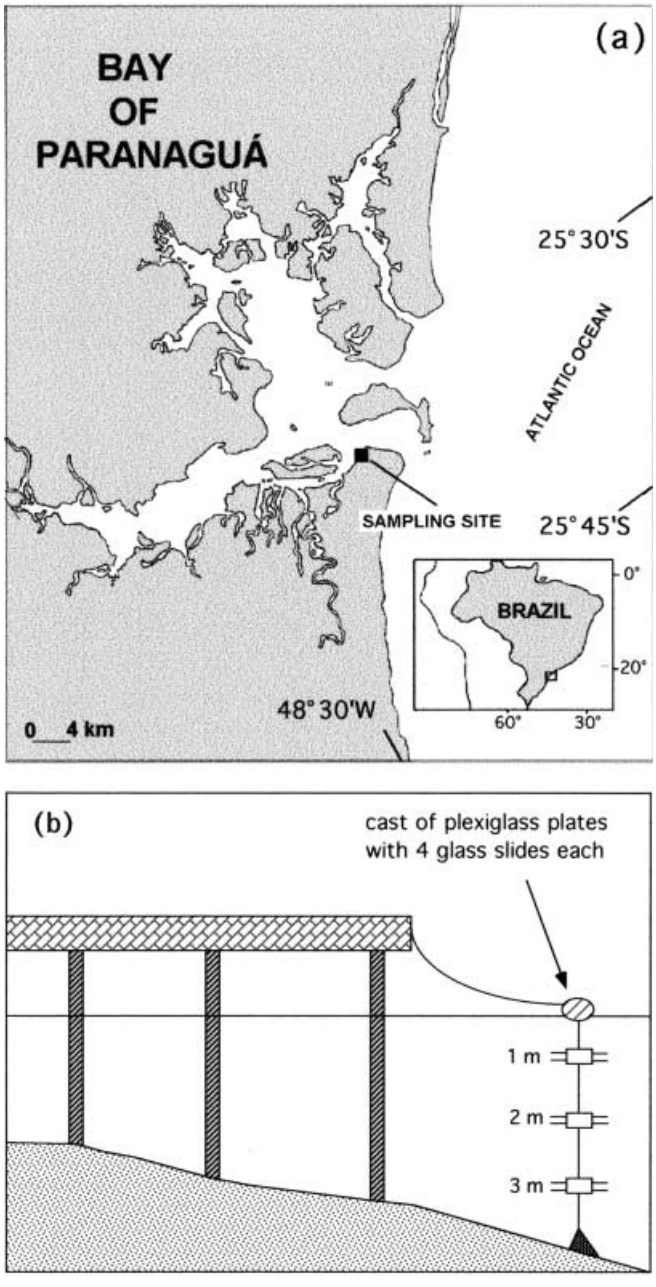

Fig. 1 Map of the Bay of Paranaguá showing the sampling site (**a**) and details of glass slides support attached to the pier (**b**)

After 7 days of submergence, the slides were collected and replaced by a new set of acid-cleaned slides. Sampling was conducted this way from 1 November 1993 to 24 October 1994, always between 0900 and 1100 hours. Slides were placed into glass production flasks and into test tubes for photosynthetic experiments ($n = 5$) and chlorophyll determinations ($n = 5$), respectively. In the field, two slides from each depth were used for in situ measurements of photosynthetic rates by the oxygen light-and-dark incubation method. After incubation, the slides were gently immersed in ambient water and transported to the laboratory inside a cool, dark container for analyses of species composition and chlorophyll accumulation.

Environmental parameters

Both at low and high tide on the sampling days, Secchi depths were determined and samples of surface and bottom waters were collected for subsequent measurements of temperature (mercury thermometer), salinity (refractometer), and concentrations of nitrate, phosphate and silicate by colorimetric techniques (Strickland and Parsons 1972). Chlorophyll *a* in surface waters was also determined by spectrophotometric readings of 90% acetone extracts (Jeffrey and Humphrey 1975) following the filtration techniques of Strickland and Parsons (1972). Daily incident photosynthetically active radiation (PAR) was monitored continuously by a Biospherical Instruments QSR-240 radiometer, and daily precipitation was supplied by the meteorological stations at Morretes, Antonina and Paranaguá cities.

Analyses of cell accumulation and species composition

For enumeration, the periphytic material was carefully scraped off one slide from each depth, diluted with pre-filtered (Whatmann GF/C filters) seawater to final volumes of 17–58 ml, and fixed with 1% formalin solution. Two procedures were adopted for analysing these samples. A 0.1-ml aliquot was placed on a microscope slide, on which the most abundant and smaller cells (< 100 μm) were counted in transects of 4.5 mm^2, under 320 × total magnification using a Zeiss inverted microscope equipped with phase contrast. Second, a 2-ml aliquot was sedimented according to the Utermöhl (1958) technique and the whole chamber bottom was examined at 80× magnification with a Zeiss inverted microscope, for enumeration of cells larger than 100 μm. Total cell densities in terms of cells per square centimetre were estimated, accounting for the substrate area (i.e. 10.2 cm^2), counting area under the microscope, and volume of filtered seawater used to dilute the periphytic material.

Two procedures were adopted for analysing the general composition of diatoms that settled during the 7 days of submersion: (1) in vivo microscopic analysis of periphytic composition over one entire slide of the 1-m set; (2) for more precise taxonomic determinations, the material stripped from the slides after the in vivo analysis was cleaned with potassium permanganate, with the addition of oxalic acid, and washed with distilled water until the appropriate pH was reached. Permanent slides were prepared according to Reid (1978), using Naphrax as the mounting medium. Light microscopy was performed with an Olympus BX40 microscope using an 100 × oil immersion objective.

Chlorophyll *a* accumulation per unit area

Slides from 1, 2 and 3 m depth and those originating from the photosynthesis experiments were transferred to test tubes and kept immersed in 6 ml of 90% acetone for 24 h under dark refrigeration (4 °C). The slides were then washed with acetone and final acetone extracts were diluted to a final volume of 8 ml. After sample centrifugation, spectrophotometric techniques were used for measuring chlorophyll concentrations in the extracts following the

equation of Jeffrey and Humphrey (1975). These were multiplied by 8 (final acetone volume) and divided by 10.2×10^{-4}, the mean substrate area in square metres for periphyton fixation, to obtain chlorophyll concentrations as an index of periphyton biomass accumulation over the 7 days of slide incubation.

Photosynthetic rates and daily production per unit area

Two slides from each depth were incubated in situ to measure photosynthetic rates of the periphytic community by the oxygen light-and-dark technique. The following procedure was adopted to estimate the amount of oxygen produced by the algal community on each glass slide.

The oxygen concentration at the onset of incubation was determined by two Winkler titrations of the pre-filtered water to be used for slide immersion. The total amount of oxygen (milligrams) in each bottle was calculated by multiplying the oxygen concentration of the pre-filtered water (milligrams per millilitre) by the total volume (millilitres) of the respective bottle (less 1 ml, the volume of Winkler reagents).

Two slides from each depth were carefully immersed in separate 100-ml glass bottles, filled with the same Whatman GF/C pre-filtered water. The bottles were tied to the line and incubated in situ at the original sampling depth. Periods of incubation varied from 30 to 105 min, depending on apparent algal concentration and water temperature (in summer, incubation periods never exceeded 30 min). After the light incubation, the same slides were transferred to dark bottles for respiration measurements at in situ temperature. After the removal of slides, Winkler reagents were added to both light and dark bottles. When precipitation was completed, a few millilitres of the supernatant inside the Winkler/production bottles was taken out with pipettes, leaving space enough for Winkler titrations directly in the bottles. The difference in oxygen concentrations between light and dark bottles was divided by the area of the slide (10.2 cm^2) and by the incubation period (hours) to represent gross photosynthesis expressed as mg oxygen m^{-2} h^{-1}. Since the photosynthetic rates depend differently on the photon fluence rates, daily production in terms of mg oxygen m^{-2} was roughly estimated by multiplying gross photosynthesis by the ratio of total daily PAR over the incident underwater PAR during the incubation period (see below). Assimilation numbers in terms of mg oxygen mg chlorophyll a^{-1} h^{-1} were calculated by dividing gross photosynthesis by the amount of chlorophyll over the area of the glass slide.

Incubations for photosynthesis versus irradiance curves were conducted on 14 February with periphytic material from 1- and 2-m depth. Duplicate slides from each depth were exposed to artificial cool fluorescent light for 30 min at a constant temperature of 27 °C, which was the approximate in situ temperature in February. Incubations started with respiration measurements in bottles wrapped with aluminium foil. The two slides were then removed carefully and transferred to other bottles for sequential incubations at 2, 5, 12, 35, 70 and 100% of total incident light, corresponding to 448 μmol photons m^{-2} s^{-1} as measured by the Biospherical sensor. The various light intensities were simulated by covering the production flasks with black cloth of different optical densities which were checked with the same Biospherical sensor used for measuring surface incident light. After incubations, Winkler titrations were performed directly in the bottles (see above) and the slides were placed into test tubes for chlorophyll *a* determinations as described earlier. Multiple regression analyses were used to clarify which environmental factor controls the periphyton growth on glass slides during the seasonal cycle.

Results

Environmental parameters

The local water temperature showed a well-defined seasonal pattern, ranging from 17.3 °C in July to 36.8 °C in December (Fig. 2a). There were no remarkable temperature differences between surface and bottom waters, or between high and low tides throughout the study period. Precipitation, averaged from the three meteorological stations, varied between 8.6 mm and 2.4 mm, showing a rainy period from December to April and a dry period from May to November. Salinity ranged from 20 to 34 ppt (Fig. 2b); the highest salinity was usually observed in the bottom water during high tide and the lowest in the surface water during low tide. Secchi disc readings at low and high tides showed similar seasonal patterns (Fig. 2c). Transparency was low during warm and rainy periods, ranging between 1 and 2 m between December 1993 and April 1994. Highest transparencies (3.5 m) were observed in November 1993 at high tide and May/June 1994 at low tide.

Nitrate concentrations were usually below 1 μM, with isolated peaks between 1.5 and 4 μM in late December, March and April (Fig. 3a). The pattern of seasonal variation was irregular with high and low values occurring at any time, depth or tidal condition. Phosphate concentrations also varied irregularly throughout the period ranging from 0.3 to 1 μM (Fig. 3b). Peak concentrations

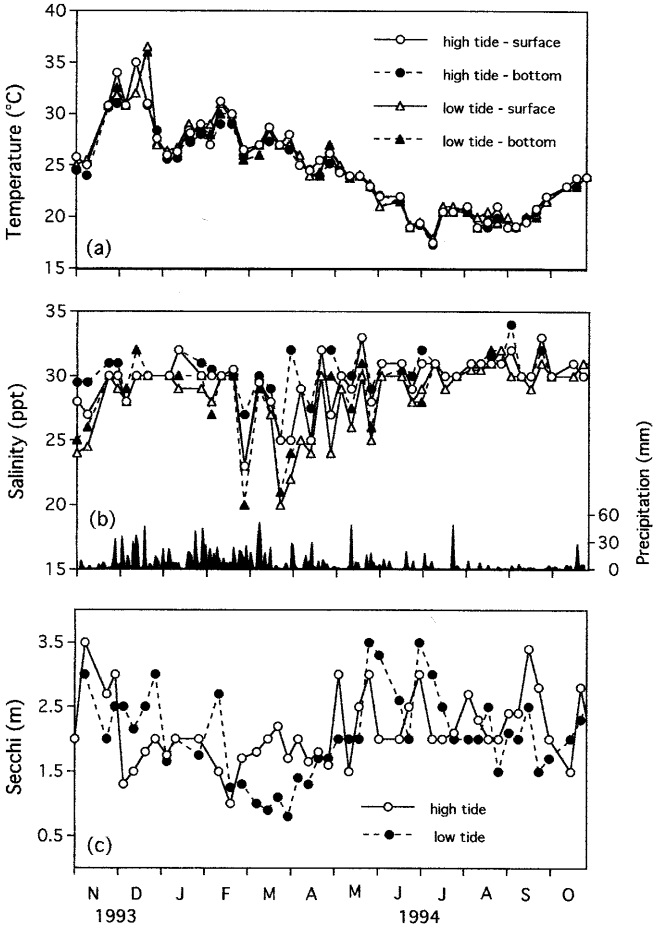

Fig. 2 Seasonal variation of temperature (**a**), salinity and precipitation (**b**) and Secchi readings (**c**), during high and low tide at surface and bottom layers in the water column of the study site, between November 1993 and October 1994

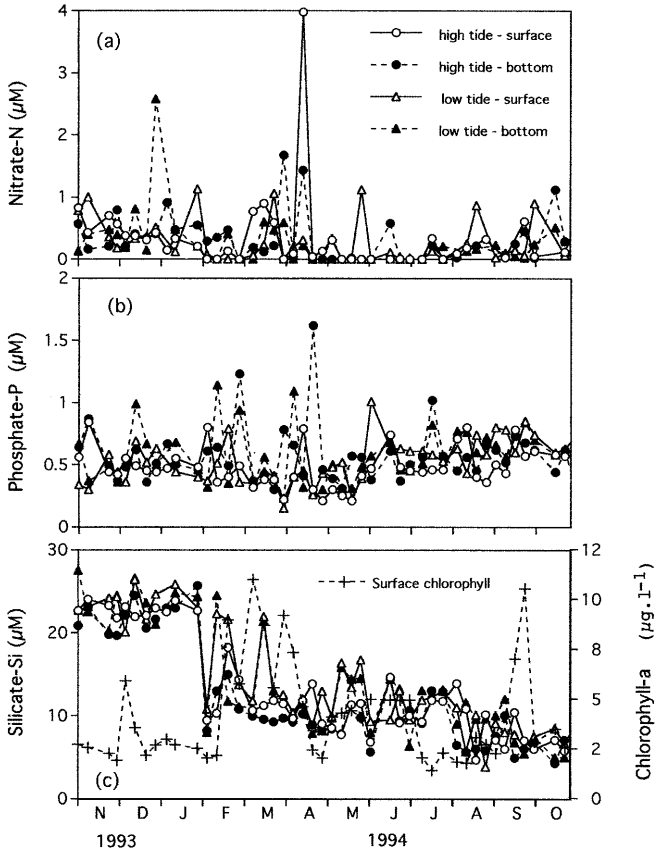

Fig. 3 Seasonal variation of nitrate-nitrogen (**a**), phosphate-phosphorus (**b**), silicate-silicon **c** and water chlorophyll *a* concentrations (**c**), during high and low tide at surface and bottom layers in the water column of the study site, between November 1993 and October 1994

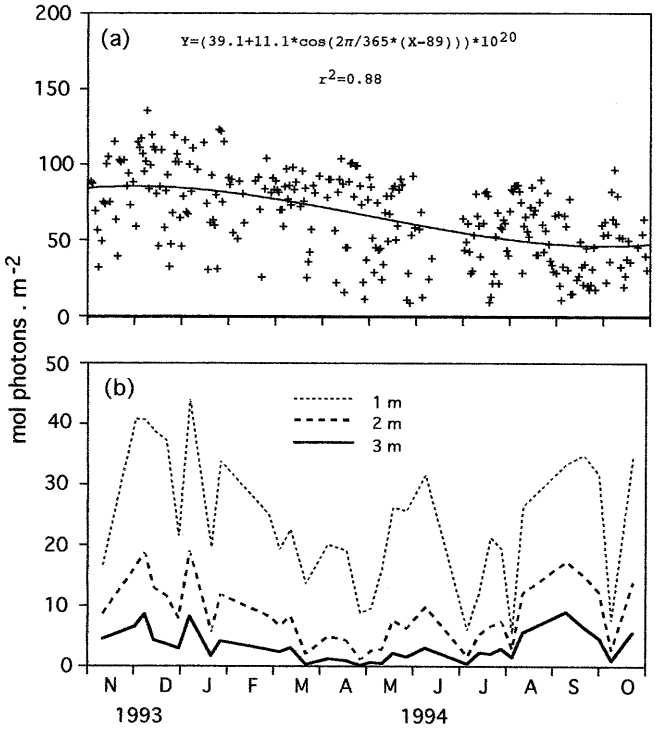

Fig. 4 Seasonal variation of photosynthetically active radiation incident on the surface water of the Bay of Paranaguá (**a**) and available light at each depth (**b**) between November 1993 and October 1994

were found in the bottom water during high and low tides. Higher silicate concentrations were found at the beginning of the study cycle, fluctuating between 20 and 30 μM (Fig. 3c). In early February 1994 a sharp decrease down to less than 10 μM was observed, and despite a few isolated peaks at low tide, mean silicate concentrations in the water thereafter ranged from 5 to 15 μM until late October 1994. The chlorophyll *a* concentration in the surface water varied from 1.4 to 11 μg l^{-1}.

Total daily incident PAR varied from 8 to 132 mol photons cm^{-2} day^{-1} throughout the season (Fig. 4a). The seasonal curve of the daily data points was fitted to a periodical model. Monthly averages were higher from November to January, decreasing gradually toward the winter periods of July/August. Cloud cover accounted for differences between maxima and minima in all seasons. PAR seasonal regimes at 1, 2 and 3 m (Fig. 4b) were obtained though an exponential decay model, using the extinction coefficient derived from the mean of the two daily Secchi readings.

Periphytic composition

A total of 58 diatom species, mostly raphe-bearing pennate forms, was found during the annual cycle (Table 1). Centric forms were less frequent, with the exception of *Melosira* sp., which was abundant in November 1993 and March 1994. Most diatoms were benthic or thycoplanktonic. The assemblage was generally composed of highly motile species (e.g. *Navicula phyllepta*, *Cylindrotheca closterium*) and colonial species of *Navicula* and *Amphora*. *Navicula phyllepta*, *Navicula pargemina* and *Navicula platyventris* almost always dominated followed by *Cylindrotheca closterium*, *Nitzschia longissima* and *Nitzschia sigma*. The genera *Pleurosigma*, *Melosira*, *Licmophora*, *Thalassiosira* and *Coscinodiscus* were frequently represented but at lower densities. *Amphora* sp. (*Hallamphora* section) occurred at relatively high densities in summer (December–March). On occasions, large species such as *Licmophora* aff. *ehenbergii*, *Nitzschia sigma*, *Nitzschia ventricosa* and *Pleurosigma* spp., colonized the slides in great numbers.

In vivo analysis

The in vivo analysis of slides from 1 m depth revealed that they were usually entirely covered by a layer of diatom cells, invertebrates and fine grains of silt. Motile species occurred in large dense patches, wherein no other diatoms could affix, although other motile species crossed back and forth over those patches. Hydroid stolons provided additional substrate for many cells of *Pleurosigma* sp. and *Nitzschia sigma*. By number, diatoms

Table 1 List of diatom species colonizing glass slides in the Bay of Paranaguá, southern Brazil, between November 1993 and October 1994

Achnanthes sp.
Actinocyclus ehenbergii var. *crassa* (Wm. Smith) Hustedt
Actinocyclus ehenbergii var. *tenella* (Brebisson) Hustedt
Actinoptychus campanulifer A. Schmidt
Actinoptychus undulatus (Bailey) Ralfs
Amphora angusta var. *ventricosa* (Gregory) Cleve
Amphora ostrearia var. *lineata* Cleve
Amphora aff. *Ovalis* Kützing
Amphora sp. (section *Hallamphora*)

Bacillaria paradoxa Gmelin

Caloneis westii (Wm. Smith) Hendey
Cocconeis dirupta Gregory
Coscinodiscus sp.
Cyclotella litoralis Lange and Syvertsen
Cyclotella striata (Kützing) Grunow
Cyclotella stylorum Brigtwell
Cylindrotheca closterium (Ehenberg) Reimer
Cymathodiscus planetophorus (Meister) Hendey
Cymatotheca weissflogii Hendey

Delphineis surirella var. *australis* (Petit) Navarro
Diploneis bombus Ehenberg
Diploneis weissflogii (A. Schmidt) Cleve

Fallacia numularia (Greville) D.G. Mann
Fallacia sp.
Fryxelliella floridana A.K. S.K. Prasad

Gyrosigma scalproides (Rabenhorst) Cleve

Licmophora aff. ehenbergii (Kutzing) Grunow
Licmophora gracilis (Ehenberg) Grunow
Licmophora sp.
Lyrella sp.

Margaritum terebro (Leuduger-Fortmorel) H. Moreira
Mastoglia apiculata Wm. Smith
Melosira moniliformis (Müller) Agardh
Minidiscus chilensis Rivera and Koch
Minidiscus comicus Takano

Navicula aff. *Pargemina* Underwood and Yallop
Navicula phyllepta Kützing
Navicula platyventris Meister
Navicula sp.
Nitzschia lanceolata Wm. Smith
Nitzschia longissima (Brebisson) Grunow
Nitzschia sigma (Kützing) Wm. Smith
Nitzschia ventricosa Kitton

Opephora marina (Gregory) Petit

Paralia sulcata (Ehenberg) Cleve
Parlibellus delognei (Van Heurck) E.J. Cox
Parlibellus hagelsteinii (Hustedt) E.J. Cox
Parlibellus tubulosus (Brun) E.J. Cox
Pleurosigma angulatum (Qüeckett) Wm. Smith
Pleurosigma elongatum Wm. Smith
Psamodycton panduriforme (Grunow) D.G. Mann

Thalassionema nitzschioides (Gregory) Van Heurck
Thalassiosira eccentrica (Ehenberg) Cleve
Thalassiosira nanolineata (Mann) Fryxell
Thalassiosira oestrupii (Ostenfeld) Cleve
Thalassiosira sp.
Tryblionella coarctata (Grunow) D.G. Mann
Tryblionella granulata (Grunow) D.G. Mann

largely dominated the microphytes during periods of high accumulation in summer, covering the entire slide, scattered or grouped in rather conspicuous patches. Strings of dichotomic cyanophyte colonies and solitary euglenophytes were sometimes observed, but always at lower densities than diatoms. Amphipods and sessile ciliates were common mainly in samples from 2 and 3 m.

Diatom densities

The accumulation of cells at all depths was mostly dominated by *Navicula* species, followed by *Cylindrotheca closterium*, *Nitzschia longissima* and *N. sigma*. Accumulated biomass, quantified as chlorophyll *a,* showed a well-defined seasonal pattern. Two bloom seasons were distinguished, one from November 1993 though March 1994 and the other at the onset of the following summer in October 1994 (Fig. 5).

The number of diatoms accumulated on the glass slides during submersion was usually greater at 1 m depth, varying from 0.05×10^8 cells m^{-2} to a maximum of 31×10^8 cells m^{-2} on 15 March. Earlier peak densities, between 10 and 25×10^8 cells m^{-2}, were observed on 29 November 1993 at 1 and 3 m; on 20 December 1993 at 2 m and on 10 October 1994 at all three depths. The accumulation of cells was always low at 3 m except for two short bloom periods at the beginning of observations (November/December 1993) and at the end of the study period (October 1994). In early April, the growth of diatoms at 1 and 2 m decreased markedly, with densities as low as those usually observed at 3 m. Low densities persisted throughout the cold season, until late September.

With the onset of the next bloom season, in October, diatom densities increased sharply, up to 25×10^8 cells m^{-2} at all three depths with equal contributions by *Nitzschia* spp., *Navicula* spp. and *Cylindrotheca closterium*.

Photosynthetic rate and biomass accumulation

Chlorophyll *a* concentrations varied from 0.6 to 32 mg m^{-2} at 1 m, from 0.5 to 20.4 mg m^{-2} at 2 m and from 0.3 to 23.9 mg m^{-2} at 3 m (Fig. 6a). In early November 1993, concentrations were similar at all three depths but at 1 m they exceeded those at 2 and 3 m during the warm periods. The seasonal pattern at all three depths was similar to that observed for cell densities.

Photosynthetic efficiency (PE) varied from 0.6 to 47 at the surface, 1.3 to 30 at 2 m and <0.5 to 35 mg oxygen mg chlorophyll a^{-1} h^{-1} at 3 m (Fig. 6b). Although the pattern of seasonal variation was irregular, maximum PE was usually observed at 1 m throughout the year except between 2 February and 15 March (the most productive period), on 5 April, 7 July and 19 September, when PE was maximum at sub-surface levels.

Daily production varied from 15 to 3,600 at 1 m, 11 to 500 at 2 m and 5 to 400 mg oxygen m^{-2} at 3 m (Fig. 6c). Seasonal variations followed the same trends as those of chlorophyll and PE as shown in Fig. 6a, c.

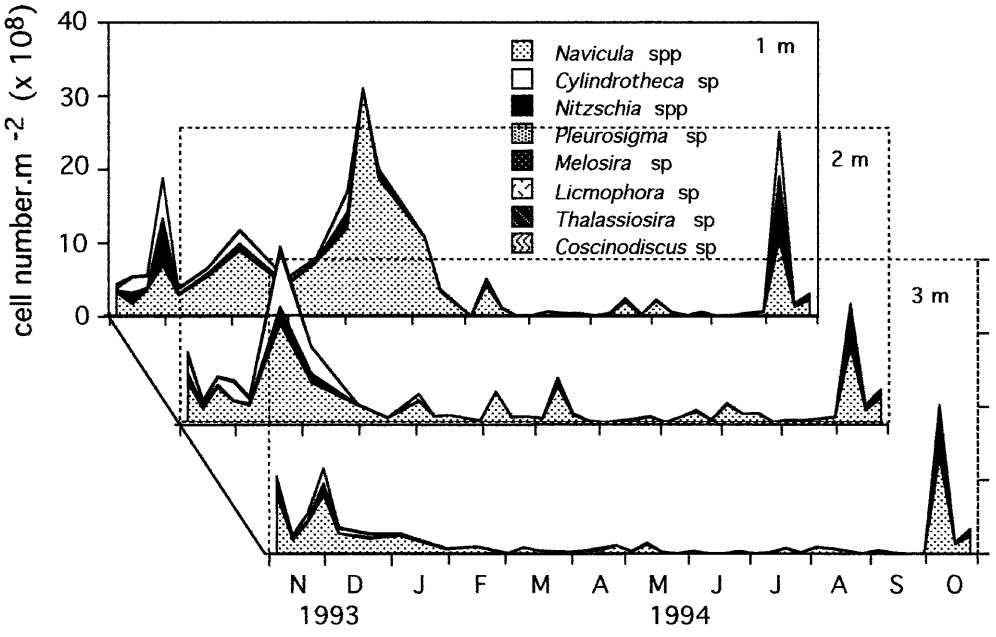

Fig. 5 Seasonal variation of diatom cell densities colonizing glass slides after 7 days of immersion in the Bay of Paranaguá, between November 1993 and October 1994

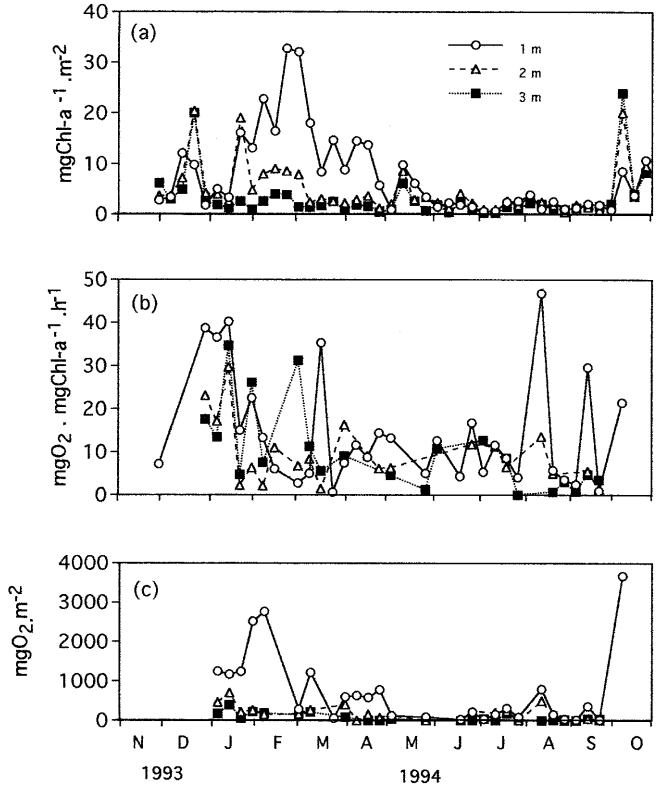

Fig. 6 Seasonal variation of chlorophyll *a* (**a**), assimilation number (**b**) and daily production (**c**) of periphytic community accumulated on glass slides after 7 days of immersion in the Bay of Paranaguá, between November 1993 and October 1994

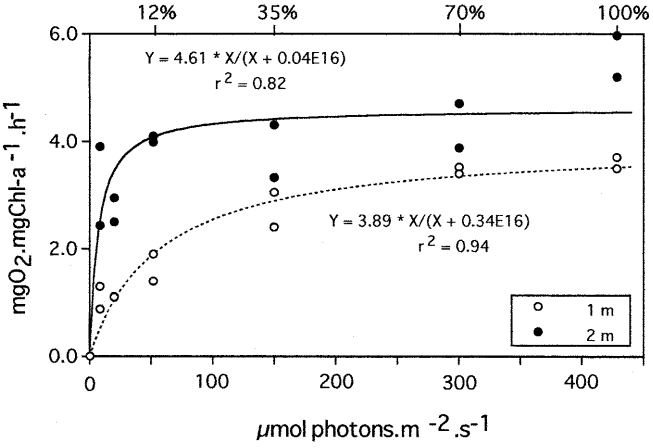

Fig. 7 Summer photosynthesis vs. irradiance curves of 1- and 2-m depth periphytic communities accumulated on glass slides after 7 days of immersion in the Bay of Paranaguá, in 14 February 1995

The photosynthesis versus irradiance curves obtained at 1 and 2 m on 14 February (Fig. 7) were adjusted according to the Michaelis-Menten model ($R^2 = 0.94$ at 1 m and $R^2 = 0.82$ at 2 m). They showed an extremely shade-adapted periphyton at 2 m, where only 80 µmol photons m^{-2} s^{-1} was sufficient to saturate the photosynthetic rate. Although light intensities used for incubations were not as high as in natural conditions, photosynthetic rates were kept at maximum levels in a broad range of saturating light intensity. At 1 m, saturation was reached at much higher irradiances, about 320 µmol photons m^{-2} s^{-1}.

Discussion

Hydrography and nutrients

The hydrographic regime exhibited seasonal patterns similar to those observed in adjacent waters (Brandini

et al. 1988; Rebello and Brandini 1990). During summer, salinity stratifications in the water column, as indicated by the larger differences between surface and bottom values in Fig. 2b, was enhanced by the high freshwater input, typical of rainy summer periods (Brandini et al. 1988). During the dry winter season, more homogeneous vertical salinity and temperature distributions can be expected due to less freshwater input and more wind-induced mixing of the water column.

The gap in silicate concentrations between the beginning and the end of the study period was due to a higher input from freshwater drainage in summer, which increased silicate concentrations from November 1993 to January 1994. This was followed by silicate uptake by the diatom-dominated summer phytoplankton community of the bay (Brandini and Thamm 1994). An opposite temporal variation between silicate and phytoplankton chlorophyll concentrations at the surface between November 1993 and April 1994 can be clearly seen in Fig. 3c. After April, besides the uptake by planktonic diatoms, less continental drainage kept silicate concentrations lower than at the beginning of the period.

Species composition

Although productivity and cumulative biomass changed with season, community structure remained stable during the study period. Few species (*Navicula phyllepta*, *N. platyventris*, *C. closterium*, *Nitzschia longissima* and *N. sigma*) predominated. The same species, with similar relative contributions to total biomass, were present during the highly productive periods relative to the low productive period. Therefore, changes in total community biomass mainly reflected quantitative changes among few dominant species (e.g. *Navicula* spp. or *Nitschia* spp).

Species composition, biomass formation and accumulation during 7 days were affected by composition and quantity of the first colonizers of the slides. For instance, patch-forming species seemed to preclude the growth of others within the patch, promoting selectivity among periphyton species. Consequently, cells arriving later had less space to attach to and multiply.

Biomass accumulation and production rates

The capacity of benthic diatoms to reach maximum photosynthetic rates at low saturating irradiances and to keep them over a broad range of irradiance, as observed in our photosynthesis versus irradiance curves of February (Fig. 7), are important advantages in the turbid estuarine waters of the Bay of Paranaguá. Diatoms can thus occupy deeper sectors of the bay by retaining maximum rates at low irradiances. This increases the spatial distribution of benthic diatoms and augments the autotrophic biomass contribution to the shallow bay ecosystem. Moreover, similar photosynthetic rates over the broad range of saturating irradiance shows that the daily production rates can be roughly estimated from total daily underwater irradiance, as performed in this investigation.

Light penetration usually accounts for depth differences in chlorophyll accumulation and production rates, as noted in a variety of environments (Wulff and McIntire 1972; Admiraal and Peletier 1980; Hudon and Bourget 1983; MacIntyre and Cullen 1995). During the rainy season (February–April) water turbidity increased due to higher inputs of suspended particles and dissolved humic compounds drained from land and adjacent mangroves, decreasing water transparency (Fig. 2c). Optimum light conditions were thus restricted to the uppermost layer of the euphotic zone, favouring biomass accumulation on the 1-m slides. We speculate that this intensified depth differences in chlorophyll accumulation during this period. By contrast, as water transparency increased from May through August, and optimal light conditions extended down into the water column, decreasing depth differences in chlorophyll accumulation were observed. Seasonal trends of chlorophyll, cell densities and production rates also paralleled the seasonal temperature trend. Members of the genus *Amphora*, common in our samples, were reported by Wulff and McIntire (1972) to develop more rapidly at higher temperatures.

Besides temperature and irradiance, grazing pressure by amphipods and sessile ciliates may have contributed to the vertical differences in chlorophyll biomass accumulation. These invertebrates grazed mainly on the deeper slides where periphytic biomass accumulation was always less than on those at 1 m depth. Fluctuations of environmental conditions in the uppermost layers are probably unfavourable for these invertebrates. Moreover, changes in community structure or total biomass accumulation on deeper slides are certainly the consequence of grazing pressure on a few dominant species (e.g. *Navicula* sp.). Kawamura and Hirano (1992) reported that seasonal changes in benthic diatoms densities in a Japanese bay were more closely related to light and grazing by amphipods than to temperature.

In order to identify what environmental factors control photosynthetic rates and biomass build-up on the slides, we conducted a multiple regression analysis comparing biological against environmental data. The mean daily water parameters obtained at low and high tides from surface and bottom layers were used for the 1- and 3-m-depth data set, respectively. We used mean water column values for the 2-m-depth data set. The analysis showed that seasonal changes of chlorophyll concentrations at 1 m were mainly controlled by temperature ($r^2 = 0.731$; $P = 0.001$) followed by PAR ($P = 0.003$); at 2 m by PAR ($r^2 = -0.450$; $P = 0.018$), and none of the environmental parameters were able to explain the seasonal changes in periphyton growth at 3 m (Table 2).

Higher photosynthetic efficiencies were usually observed in summer (Fig. 6b), but on a seasonal time scale

Table 2 Multiple regression analysis applied to environmental parameters against accumulated biomass at 1, 2 and 3 m depth. *PAR* Photosynthetically active radiation

Depth environmental parameters	1 m		2 m		3 m	
	r^2	P	r^2	P	r^2	P
Water chlorophyll	−0.070	0.668	−0.370	0.053	−0.274	0.174
PAR	−0.563	0.003	−0.450	0.018	−0.162	0.397
Salinity	−0.044	0.690	0.019	0.888	0.105	0.426
Temperature	0.731	0.001	0.215	0.370	0.130	0.609
Phosphate	−0.174	0.184	0.074	0.617	−0.037	0.826
Nitrate	−0.043	0.735	−0.024	0.893	0.268	0.203
Silicate	−0.114	0.610	0.343	0.186	−0.111	0.678
Regression	0.749	< 0.001	0.646	< 0.001	0.416	0.004

the environmental parameters did not explain (multiple regression analysis, $P > 0.20$) vertical or temporal variations. The *t*-test among the 1-, 2- and 3-m-depth data sets showed a significant difference only between 1 and 2 m ($P = 0.01$); differences between 1 and 3 m ($P = 0.12$) and 2 and 3 m ($P = 0.20$) were not significant. Although 1-, 2- and 3-m-depth slides were submitted to different light regimes, their periphytic populations showed similar photosynthetic rates, reflecting an extremely shade-adapted periphyton growing at 2 and 3 m. This was confirmed by the photosynthesis versus irradiance curves (Fig. 7) in which only 12% of available PAR was sufficient to saturate photosynthetic rates at 2 m. Moreover, the chlorophyll-to-cell ratio increased with depth from 7.07×10^{-9} to 11.6×10^{-6} (Fig. 8), suggesting changes in chlorophyll contents per cell according to light conditions. We speculate that despite comparable PEs at all three depths, biomass accumulation of periphytic populations at 3 m may have been depressed due to grazing pressure, masking the environmental controls (e.g. temperature and PAR) of diatoms growing on deeper slides.

Bloom seasons last approximately 4 months at 1 m, 3 months at 2 m and only 1 month at 3 m. In other words, the duration of the growing season decreases from surface light-saturated populations to deeper light-limited and grazing-affected populations.

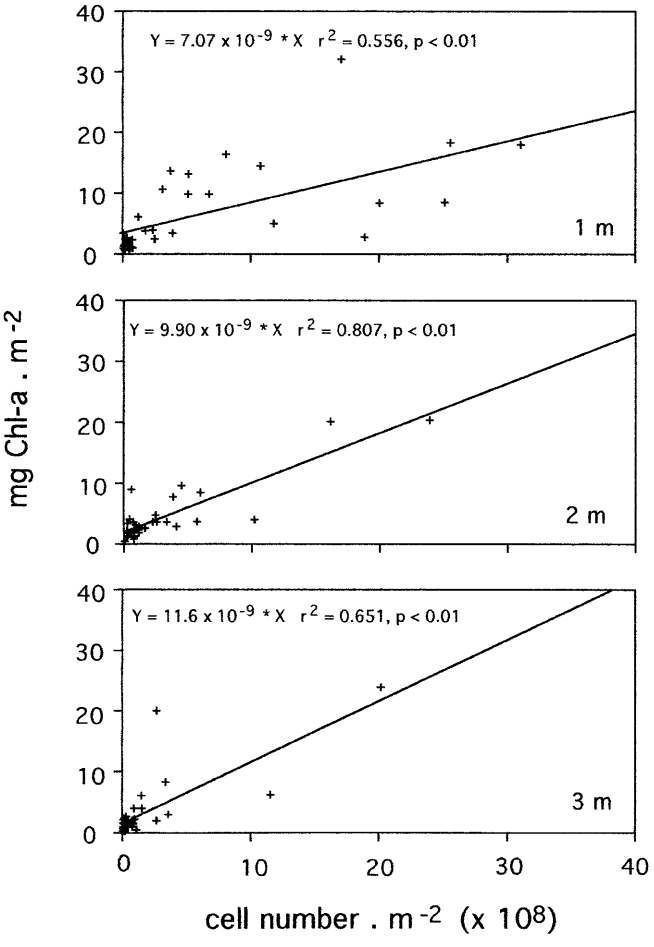

Fig. 8 Linear regressions between total data of cell number and chlorophyll *a* contents at each depth

Conclusions

We found the daily production of periphytic diatoms at saturating light intensities as high as 3,600 mg oxygen m^{-2} (= 1.4 g carbon m^{-2}). Grøntved (1960) reported microalgal daily production on mudflats of Danish fjords ranging seasonally from 0.3 to 1.5 g carbon m^{-2}. Cadeé and Hegeman (1974) estimated 0.27 g carbon m^{-2} in the Wadden Sea. Pomeroy (1959) measured 0.54 g carbon m^{-2} in coastal areas of Georgia. Magni and Montani (1997) reported values between 0.46 and 1.7 g carbon m^{-2} on intertidal flats in Japan, and Souza (1983) found a maximum of 3.6 g carbon m^{-2} on estuarine intertidal flats of southeastern Brazil. In tidal flats of the Bay of Paranaguá, near our experimental site, Fonseca (1998) measured daily benthic microalgal production up to 7.3 g carbon m^{-2}. The range of daily production by the periphytic community measured in this investigation (ca. 0.2–1.4 g carbon m^{-2}) is close to these figures. This indicates that our production rates measured in artificial substrates (i.e. periphyton) may be comparable to those of natural benthic microautotrophs on mudflats or on hard substrates of the Bay of Paranaguá.

Brandini (1990) estimated the maximum daily production of phytoplankton in coastal areas of the southeastern Brazilian shelf to be about 0.3 g carbon m^{-2}. Thus, benthic diatom production can exceed

by up to 4 times the phytoplankton production in the euphotic zone in shallow subtropical waters off southeastern Brazil.

The production of periphytic diatoms on glass slides in the Bay of Paranaguá seems to be controlled by temperature, whenever light is not limiting and grazing pressure is not strong enough to halt the accumulation of cells. Low temperatures in winter maintain low photosynthetic rates and less biomass accumulation. In summer, although optimum temperatures may enhance the growth of diatoms, biomass may not accumulate due to intense grazing pressure or less light availability.

We may conclude that vertical differences in light conditions and grazing pressure jointly affected the influence of temperature on the seasonal patterns of cell densities and chlorophyll concentrations according to depth.

Acknowledgements We thank the Cia Pescal for allowing the use of its harbour facilities in order to conduct field work. The CNPq provided 2 years of undergraduate grants for F. Pellizzari through the PIBIC Program (Banpesq 93003256) and a 1-year grant for K. Gutseit and A. Fonseca (CNPq-800162/86-2). We also thank Robert Owen, Guy Martel and one anonymous referee for improvements in our English.

References

Admiraal W, Peletier H (1980) Influence of seasonal variations of temperature and light on the growth rate of cultures and natural populations of intertidal diatoms. Mar Ecol Prog Ser 2: 35–43

Admiraal W, Peletier H, Zomer H (1982) Observations and experiments on the population dynamics of epipelic diatoms from an estuarine mudflat. Estuarine Coastal Shelf Sci 14: 471–487

Amspoker MC, McIntire CD (1978) Distribution of intertidal diatoms associated with sediments in Yaquina estuary, Oregon. J Phycol 14: 387–395

Amspoker MC, McIntire CD (1986) Effects of sedimentary processes and salinity on the diatom flora of the Columbia river estuary. Bot Mar 29: 391–399

Brandini FP (1990) Hydrography and characteristics of the phytoplankton in shelf and oceanic waters off southeastern Brazil during winter (July/August 1982) and summer (February/March 1984). Hydrobiologia 196: 111–148

Brandini FP, Fernandes LF (1996) Microalgae of the continental shelf off Paraná State, southeastern Brazil: a review of studies. Rev Bras Oceanogr 44: 69–80

Brandini FP, Thamm CAC (1994) Variações diárias e sazonais do fitoplâncton e parâmetros ambientais na Baía de Paranaguá. Nerítica 8: 55–72

Brandini FP, Thamm CAC, Ventura I (1988) Ecological studies in the Bay of Paranagua. III. Seasonal and spatial variations of nutrients and chlorophyll-a. Nerítica 3: 1–30

Brown SD, Austin AP (1973) Diatom succession and interaction in littoral periphyton and plankton. Hydrobiologia 43: 333–356

Cadée GC, Hegeman J (1974) Primary production of the benthic macroflora living on tidal flats in the Dutch Wadden Sea. Neth J Sea Res 8: 260–291

Decho AW, Fleeger JW (1988) Microscale dispersion of meiobenthic copepods in response to food-resource patchiness. J Exp Mar Biol Ecol 118: 229–243

Delgado M, Jonge VN de, Peletier H (1991) Experiments on resuspension of natural microphytobenthos populations. Mar Biol 108: 321–328

Demers S, Therriault JC, Bourget E, Bah A (1987) Resuspension in the shallow sublittoral zone of a macrotidal estuarine environment: wind influence. Limnol Oceanogr 32: 327–339

Fonseca ALO (1998) Composição, distribuição, variabilidade sazonal e produção primária do microfitobentos entremarés na Baía de Paranaguá (Paraná, Brasil). Masters thesis. Departamento de Botânica, Universidade Federal do Paraná, Paraná, Brasil

Grøntved J (1960) On the productivity of microbenthos and phytoplankton in some Danish fjords. Medd Komm Dan Fisk Havunders 3: 55–92

Hudon C, Bourget E (1983) The effect of light on the vertical structure of epibenthic diatoms communities. Bot Mar 26: 317–330

Jeffrey SM, Humphrey GF (1975) New spectrophotometric equations for determining chlorophylls *a, b, c* and *c*2 in higher plants, algae and natural phytoplankton. Biochem Physiol Pflanz 167: 191–194

Jonge VN de, Beusekom JEE van (1992) Contribution of ressuspended microphytobenthos to total phytoplankton in the EMS estuary and its possible role for grazers. Neth J Sea Res 30: 91–105

Kawamura T, Hirano R (1992) Seasonal changes in benthic diatom communities colonizing glass slides in Aburatsubo Bay, Japan. Diatom Res 7: 227–239

Laird K, Edgar RK (1992) Spatial distribution of diatoms in the superficial sediments of a New England salt marsh. Diatom Res 7: 267–279

MacIntyre HL, Cullen JJ (1995) Fine-scale vertical resolution of chlorophyll and photosynthetic parameters in a shallow-water benthos. Mar Ecol Prog Ser 122: 227–237

Magni P, Montani S (1997) Development of benthic macroalgal assemblages on an intertidal flat in the Seto Inland Sea, Japan: effects of environmental variability. Mer 35: 137–148

Moreira-Filho H, Kutner MB (1962) Contribuição para o conhecimento das diatomáceas do Manguezal de Alexandra. Bolm Univ Fed Parana Bot 4: 1–24

Moreira-Filho H, Valente-Moreira IM, Cecy I (1975) Diatomáceas da Baía de Paranaguá. Bol Mus Bot Munic Curitiba, 20: 1–23

Pace ML, Shimmel S, Darley WM (1979) The effect of grazing by a gastropod, *Nassarius obsoletus*, on the benthic microbial community of a salt marsh mudflat. Estuarine Coastal Mar Sci 9: 121–134

Pomeroy LR (1959) Algal productivity in salt-marshes of Georgia. Limnol Oceanogr 4: 386–397

Rebello J, Brandini FP (1990) Variação temporal de parâmetros hidrográficos e material particulado em suspenção em dois pontos fixos da Baia de Paranaguá, Paraná (junho/87-fevereiro/88). Nerítica 5: 95–111

Reid FMH (1978) Permanent slides. In: A Sournia (ed) Phytoplankton manual. UNESCO, Paris, pp 115–116

Rioux-Gobin C (1987) Phytoplankton, tripton and microphytobenthos: exchanges during the tidal cycle in a north Finistere (Brittany) estuary. Cah Biol Mar 28: 159–184

Souza ECPM (1983) Primary production of the benthic microflora living on intertidal flats in the Santos estuarine system (24°S, 46°W), São Paulo, Brazil. Bolm Inst Oceanogr 32: 177–186

Strickland JDH, Parsons T (1972) A practical handbook of sea water analysis, 2nd edn. Bull Fish Res Board Can 167: 1–310

Sullivan MJ, Moncrieff CA (1988) Primary production of edaphic algal communities in a Mississippi salt marsh. J Phycol 24: 49–58

Sullivan MJ, Moncrieff CA (1990) Edaphic algae are an important component of salt marsh food webs: evidence from multiple stable isotope analysis. Mar Ecol Prog Ser 62: 149–159

Utermöhl H (1958) Zur Vervollkommnung der quantitativen Phytoplankton-Methodik. Mitt Int Ver Theor Angew Limonol 9: 1–38

Wainright SC (1990) Sediment-to-water fluxes of particulate material and microbe by resuspension and their contribution to the planktonic food web. Mar Ecol Prog Ser 62: 271–281

Wulff BL, McIntire CB (1972) Laboratory studies of assemblages of attached estuarine diatoms. Limnol Oceanogr 17: 200–214