

# MICROPHYTOBENTHIC SPECIES COMPOSITION, PIGMENT CONCENTRATION, AND PRIMARY PRODUCTION IN SUBLITTORAL SEDIMENTS OF THE TRONDHEIMSFJORD (NORWAY)<sup>1</sup>

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In spring 2005, monthly sampling was carried out at a sublittoral site near Tautra Island. Microphytobenthic identification, abundance (ABU), and biomass (BIOM), were performed by microscopic analyses. Bacillariophyceae accounted for 67% of the total ABU, and phytoflagellates constituted 30%. The diatom floristic list consisted of 38 genera and 94 species. Intact light-harvesting pigments chl *a*, chl *c*, and fucoxanthin and their derivatives were identified and quantified by HPLC. Photoprotective carotenoids were also observed (only as diadinoxanthin; no diatoxanthin was detected). Average fucoxanthin content was  $4.57 \pm 0.45 \mu\text{g fucoxanthin} \cdot \text{g sediment dry mass}^{-1}$ , while the mean chl *a* concentration was  $2.48 \pm 0.15 \mu\text{g} \cdot \text{g}^{-1}$  dry mass. Both the high fucoxanthin:chl *a* ratio (considering nondegraded forms) and low amounts of photoprotective carotenoids indicated that the benthic microalgal community was adapted to low light. Microphytobenthic primary production was estimated in situ (MPPs, from  $0.15$  to  $1.28 \text{ mg C} \cdot \text{m}^{-2} \cdot \text{h}^{-1}$ ) and in the laboratory (MPPp, from  $6.79$  to  $34.70 \text{ mg C} \cdot \text{m}^{-2} \cdot \text{h}^{-1}$  under light saturation) as <sup>14</sup>C assimilation; in April it was additionally estimated from O<sub>2</sub>-microelectrode studies (MPP<sub>O<sub>2</sub></sub>) along with the community respiration. MPP<sub>O<sub>2</sub></sub> and the community respiration equaled  $22.9 \pm 7.0$  and  $7.4 \pm 1.8 \text{ mg C} \cdot \text{m}^{-2} \cdot \text{h}^{-1}$ , respectively. A doubling of BIOM from April to June in parallel with a decreasing photosynthetic activity per unit chl *a* led us to suggest that the microphytobenthic community was sustained by heterotrophic metabolism during this period.

**Key index words:** benthic diatoms; microphytobenthos; pigments; primary production; sublittoral sediments; Trondheimsfjord

**Abbreviations:** ABU, microphytobenthic abundance; BIOM, microphytobenthic biomass; cDOM, colored dissolved organic matter; *d*, richness index; DPM, disintegrations per minute; *H'*, diversity index; *J'*, equitability index;  $\lambda'$ , dominance index; MPP<sub>O<sub>2</sub></sub>, gross primary production estimated from the O<sub>2</sub> microelectrode; MPPp, potential primary production; MPPs, primary production in situ; NH<sub>4</sub><sup>+</sup>, ammonium; NO<sub>2</sub><sup>-</sup>, nitrite; NO<sub>3</sub><sup>-</sup>, nitrate; % PAR, benthic PAR as percentage of surface irradiance; PO<sub>4</sub><sup>3-</sup>, phosphate; RA, relative abundance; RB, relative biomass; *S*, observed number of species; SiO<sub>2</sub>, silicate

The importance of diatoms and other microalgae in marine intertidal and shallow-water benthic habitats has long been recognized. Comprehensive reviews of the biology and ecology of these organisms have been published by Round (1971), McIntire and Moore (1977), MacIntyre et al. (1996), and Cahoon (1999). A recent compilation, based on 85 worldwide studies, underscored the relevance of these microalgae for ecosystem primary production in a number of estuarine, intertidal, and shallow-water littoral environments (Cahoon 1999). Considering the primary production in the water column and in the sediment together, benthic diatoms may contribute up to 50% of the total primary production (Perissinotto et al. 2002, Montani et al. 2003). In shallow water ecosystems, the most important limiting factor for benthic photosynthesis is light availability at the sediment surface, which, besides water depth, strongly depends on the optical properties of the water (Kirk 2000). Suspended matter and phytoplankton diminish water transparency. Concentrations of both commonly increase toward the coast, and shallow, coastal waters may sustain rather poor benthic primary production, since the light availability with decreasing water depth may be

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counteracted by higher particle concentrations (Meyercordt and Meyer-Reil 1999). In sublittoral sediments, microphytobenthos communities have to adapt to a series of stress factors, such as sediment transport, gradients in light, and variations in temperature, nutrient concentrations, and salinity gradient (Sdrigotti and Welker 2002, Blasutto et al. 2005).

There is no standard method for measuring microphytobenthic primary production. Most studies quantifying benthic microalgal production have used some variant of the light-dark chamber method and measurements of either dissolved oxygen flux or uptake of  $^{14}\text{C}$ -labeled substratum (Cahoon 1999). Nondestructive methods such as different oxygen-sensing techniques (Glud et al. 2000) and variable fluorescence techniques (pulse-amplitude-modulated [PAM] fluorescence; Schreiber et al. 1986) are now widely used to determine high-resolution distribution of microphytobenthic photosynthesis in time and space. However, microelectrodes are fragile and require delicate instrumentation that complicates in situ studies, while fluorescence techniques still offer only relative photosynthetic rate measurements. Extensive replications are needed to quantify the spatial variability when estimates are scaled up to larger areas (Migné et al. 2004).

Benthic diatom assemblages are heterogeneously distributed in space and time, and analysis of taxonomic composition and relative abundance at a large scale is difficult. The problem of evaluating the taxonomic composition of microphytobenthic communities is now simplified by the utilization of HPLC pigment analysis. In phytoplankton studies, chl *a* concentrations give overall information about the distribution of microalgal biomass. However, many investigations show that chl *a* in itself is insufficient to trace fluctuations in benthic diatom standing stock (de Jonge 1980 and references therein). Other pigments characterizing benthic diatoms, such as the light-harvesting pigments chl  $c_1$ ,  $c_2$ , and fucoxanthin and its derivatives—the photoprotective carotenoids diadino- and diatoxanthin—are involved in the photosynthetic process (Stauber and Jeffrey 1988).

While the importance of intertidal benthic microalgae in nearshore food webs has been recognized and studied extensively over the past 20 years, the role of subtidal benthic microalgae has received vastly less attention. To date, few data from shallow-water benthic habitats are available, even less in subarctic regions. Very little research on nonplanktonic, marine diatoms has been carried out in Norwegian waters. To our knowledge, the only published taxonomic records are those of Gemeinhardt (1935), Castenholz (1967), and Taasen and Høisæter (1981). Therefore, the aim of this study was to investigate a sublittoral site in the Trondheimsfjord (Norway) to (i) examine the sediment-associated microphytobenthic community and its species composition, (ii) investigate microphytobenthic pigment composition by

the HPLC technique, (iii) estimate the in situ microphytobenthic photosynthetic activity (MPPs) by the  $^{14}\text{C}$ -tracer method, and (iv) test whether the  $^{14}\text{C}$ -tracer and  $\text{O}_2$ -microelectrode methods give comparable estimates of primary production.

#### MATERIALS AND METHODS

*Study site.* Trondheimsfjord is one of the largest fjords in Norway. It stretches westward from Ørland and northward to Steinkjer, with a length of 126 km and a surface of 1420 km<sup>2</sup>. The average depth is ~400 m, while the maximum is 620 m. The fjord is ice free all year. Its drainage area is ~20,000 km<sup>2</sup>, causing marked seasonal variations in freshwater input from six major river systems. All runoff from the different rivers in the Trondheimsfjord is rich in yellow humic matter (colored dissolved organic matter [cDOM]; Sakshaug 2000). Tidal amplitude is ~1.6 m. In the surface, the salinity varies between 18 (in spring) and 34 psu. The study site was located at 8 m depth on soft bottom substrate within the sublittoral area of Tautra Island (63°34.83' N, 10°37.86' E; Fig. 1). Macroalgae were totally absent at the sampling station.

*Sampling.* Monthly samplings were carried out in April, May, and June 2005. Each month, seven virtually undisturbed sediment cores were taken by a Niemistö corer (Yleismuovi Oy, Helsinki, Finland) using a polycarbonate sample tube (50 mm internal diameter). In April 2005, three sediment cores were collected for  $\text{O}_2$ -microelectrode measurements. The top 5 mm of each core was subsampled to obtain a homogenized pool, which was used for the following analyses: granulometry, ABU, MPPs, and potential primary production (MPPp). For pigment determination, 60 cm<sup>3</sup> of the homogenized pool was immediately frozen. Samplings were performed at the same time of day each month. At the moment of sampling, temperature, salinity, and irradiance were recorded in situ (SAIV/CTD model SD204, Bergen, Norway).

*Granulometry.* Sediment samples of ~10–15 g were taken for grain-size analysis. Small pebbles and shell fragments (>2 mm) were separated from the sand and mud fractions by sieving at 2 mm. Since a few grams of sediment are not representative of the gravel grain size, the gravel % is to be considered only a rough estimate. Each sample was disaggregated, and the organic matter oxidized using hydrogen peroxide 10% (Carlo Erba, Rodano, Italy) at 60°C for 24 h and then wet-sieved at 62.5 μm to separate sand from mud. The sand fraction (62.5–2000 μm) was analyzed in a settling tube (Macrogranometer with tube length of 180 cm and internal diameter of 20 cm), and the mud fraction on a Micromeritics ET5000 Sedigraph (Micromeritics Instrument Corporation, Norcross, GA, USA), using a 0.5% sodium hexametaphosphate dispersing solution (Carlo Erba). Grain-size data were represented at ½ Phi intervals. Since there were no direct riverine inputs nearby the sampling station, we assumed that the sediment granulometry did not change significantly during the study period. For this reason, the grain-size analysis was performed only once.

*Nutrient analyses.* Overlying water for the determination of dissolved inorganic nutrient concentrations (nitrite,  $\text{NO}_2^-$ ; nitrate,  $\text{NO}_3^-$ ; ammonium,  $\text{NH}_4^+$ ; phosphate,  $\text{PO}_4^{3-}$ ; and silicate,  $\text{SiO}_2$ ) was sampled and filtered on Millipore HA filters, 0.45 μm pore size (Millipore Corporation, Billerica, MA, USA). Analyses were performed at room temperature on a five-channel Bran+Luebbe Autoanalyzer 3 Continuous Flow Analyzer (Bran+Luebbe, Norderstedt, Germany), using standard procedures (Bran+Luebbe 2003a,b,c,d,e and references therein). Performance of the system was checked before and after sample analysis by running replicates of internal standards.

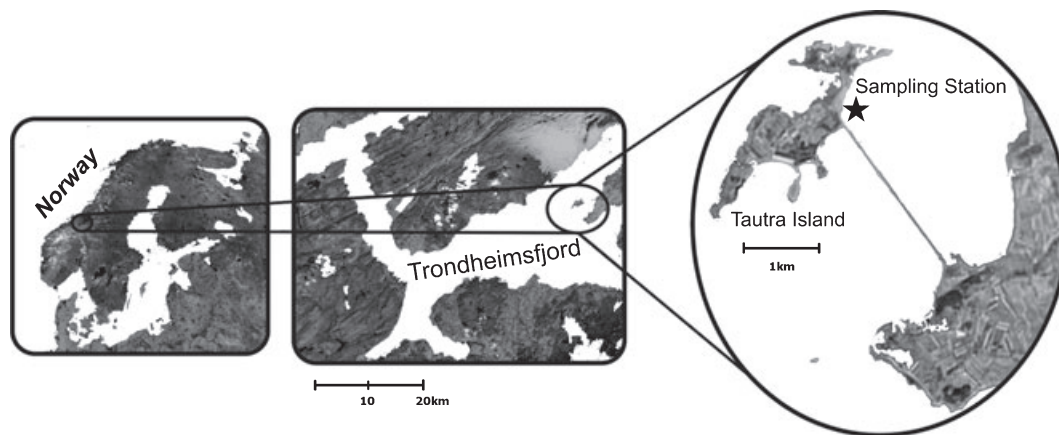


FIG. 1. Location of the sampling station nearby Tautra Island in Trondheimsfjord.

**Microphytobenthic abundance.** Subsamples of  $2 \text{ cm}^3$  were sucked up with a syringe and directly fixed with 10 mL 4% final concentration of formaldehyde (Carlo Erba) buffered solution  $[\text{CaMg}(\text{CO}_3)_2]$  in prefiltered seawater ( $0.2 \mu\text{m}$  filter; Schleicher and Schuell, Dassel, Germany). After manual stirring,  $20 \mu\text{L}$  aliquots of the sediment suspension were drawn off from the slurries and placed in a counting chamber (Thalassia, Trieste, Italy). Only cells containing pigments and not empty frustules were taken into consideration for ABU and BIOM estimates. Empty frustules were considered only to compile the floristic list. For each sampling, three replicate subsamples were counted under a Leitz inverted light microscope (Fluovert FS; Leica Microsystems AG, Wetzlar, Germany) (Utermöhl 1958) using a  $\times 32$  objective ( $\times 320$  total magnification). The qualitative determination of microphytobenthic assemblages was carried out to the genus and, when possible, to the species level using floras of Van Heurck (1899), Hendey (1976), Germain (1981), Dexing et al. (1985), Ricard (1987), Round et al. (1992), and Tomas (1997).

**Biomass estimation.** We calculated the biovolume of algal cells to assess carbon content of algae. The microscopically measured microalgal shapes were used to estimate biovolume using mathematical equations for the volume of cells of different geometrical shapes (Hillebrand et al. 1999). The carbon content ( $\mu\text{g C}$ ) of microalgae was calculated from the transformation of cell volume to plasma volume, including an estimate of the vacuole volume, and the calculation of cell carbon was in turn based on the plasma volume multiplied by a factor of 0.11 for diatom cells and phytoflagellates without a theca (Strathmann 1967). Biomass expressed as  $\mu\text{g C} \cdot \text{cm}^{-3}$  was obtained by multiplying abundance ( $\text{cell} \cdot \text{cm}^{-3}$ ) by the carbon content of each counted cell.

**Pigment extraction and analyses.** In a test tube,  $60 \text{ cm}^3$  of the homogenized pool of sediment was frozen to ensure that the epoxidation/de-epoxidation state in the cells reflected the in situ light condition. Extraction was performed under a darkened fume hood to eliminate any possibility of photooxidative pigment breakdown during analysis. Prior to extraction, ice-cold methanol 100% (Merck, Darmstadt, Germany) was bubbled with  $\text{N}_2$  gas to avoid pigment oxidation. Slices of frozen sediment ( $20 \text{ cm}^3$ ) were cut from the test tube, mixed with 50 mL of methanol, and ground with a glass rod until the mixture appeared homogeneous. The mixture was transferred to a 50 mL methanol resistant falcon tube, bubbled with  $\text{N}_2$  for 1–2 min, stirred, and extracted at  $4^\circ\text{C}$  in a refrigerator for 2 h. After incubation, 5 mL of extract were poured into a glass filtration funnel using a Whatman GF/F glass fiber filter ( $0.54 \mu\text{m}$ ; Biomap, Milano, Italy). The extract was collected in

glass vials and refiltered through a disposable sterile syringe with a Minisart RC 25 filter ( $0.2 \mu\text{m}$ ; Sartorius, Göttingen, Germany) into a 2 mL HPLC amber vial before analysis. Until analysis, the extract was stored at  $-20^\circ\text{C}$  (freezer). Pigment concentrations were normalized to sediment dry mass. To estimate the pigment composition, a Hewlett Packard 1100 Series HPLC (Palo Alto, CA, USA) system was used according to the procedure of Rodríguez et al. (2006). It was equipped with a quaternary pump system, injector, autosampler, Waters Symmetry  $\text{C}_8$  column ( $150 \times 4.6 \text{ mm}$ ,  $3.5 \mu\text{m}$  particle size,  $100 \text{ \AA}$  pore size; Waters, Milford, MA, USA), diode array absorbance detector ( $400\text{--}700 \text{ nm}$ ), and data system (Agilent ChemStation 1990–2002). The autosampler drew  $77 \mu\text{L}$  from each sample vial and  $23 \mu\text{L}$  water (total volume of  $100 \mu\text{L}$ ), which were mixed automatically five times before injection to improve the sharpness of peaks. Water was added to increase the polarity to improve the separation of chl *c*. Mobile phases: solvent C was a mixture of methanol:acetonitrile:aqueous pyridine (0.25 M pyridine) in the ratio 50:25:25 (v/v); solvent D was acetonitrile:acetone in the ratio 80:20 (v/v). Analytical separation was performed using the method of Zapata et al. (2000). Conversion from  $\mu\text{g pigment} \cdot \text{g}^{-1}$  dry sediment to  $\text{mg pigment} \cdot \text{m}^{-2}$  was performed by multiplication with 20.6, based on an average bulk density of  $2.06 \text{ g} \cdot \text{cm}^{-3}$  (de Jong and de Jonge 1995).

**Primary production using  $^{14}\text{C}$ .** Microphytobenthic primary production was estimated both in situ and in the laboratory as  $^{14}\text{C}$  uptake. Nine milliliters of homogenized surficial sediment was sucked up with a syringe and resuspended in 180 mL of overlying filtered seawater ( $0.2 \mu\text{m}$  filter; Sartorius);  $50 \mu\text{L}$  of  $^{14}\text{C}$  ( $\text{NaH}^{14}\text{CO}_3$ ; DHI, Hørsholm, Denmark) was added to reach a final activity of  $2.5 \mu\text{Ci} \cdot \text{mL}^{-1}$  ( $92.5 \text{ kBq} \cdot \text{mL}^{-1}$ ; Steemann-Nielsen 1952). To avoid the loss of labeled organic matter during analyses, 4 mL of CARBO-SORB<sup>®</sup> (Perkin-Elmer, Boston, MA, USA) was added. After stirring, the slurry was transferred into 20 glass vials containing 9 mL. Six vials were used for the in situ production, while the remaining 14 were divided in two series of seven for the MPPp. Three light and three dark samples were fixed on a rosette, lowered to the bottom, and incubated for 2 h, from 1200 to 1400 h. Carbon incorporation was stopped with 1% final concentration of glutaraldehyde (Merck). Samples were brought to the laboratory and transferred to glass scintillation vials; unincorporated inorganic  $^{14}\text{C}$  was removed by adding 0.25 mL of HCl (Merck) 5N and leaving the samples in a fume hood overnight. In a thermostatic chamber, MPPp was measured as the rate of  $^{14}\text{C}$ -bicarbonate uptake at in situ temperature under a gradient of light intensities between 10 and  $500 \mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$

(10–20–50–100–200–500) obtained using a slide projector (Leica Camera AG, Solms, Germany). Different irradiances were obtained by varying the distance between light source and samples. Carbon incorporation was stopped after 30 min by adding 0.25 mL of HCl. Ten milliliters of the Scintillation cocktail Ultima Gold XR (Perkin-Elmer) was added to the samples, which were placed in 50 mL plastic conical vials. They were centrifuged at 3500g for 10 min, and the supernatant was removed and analyzed on a TRI-CARB 1900 TR Liquid Scintillation Analyzer (Packard; Perkin-Elmer) including quenching correction, obtained using internal standards. The pellet was suspended in 10 mL of scintillation liquid, vortexed until the solution was homogeneous, and again centrifuged. This procedure was repeated three times. Disintegrations per minute (DPM), resulting from the three extractions, were calculated; DPM obtained from dark samples were subtracted from DPM derived from light samples. To convert DPM into primary production, expressed as  $\text{mg C} \cdot \text{m}^{-2} \cdot \text{h}^{-1}$ , a formula (Saggiomo et al. 1990) was modified for sediments (from  $\text{mg C} \cdot \text{m}^{-3}$  to  $\text{mg C} \cdot \text{m}^{-2}$ ) and applied. To check the protocol's efficiency, a fourth extraction was performed obtaining DPM of the same order of magnitude of the scintillation analyzer's background noise. All MPPs and MPPp measurements were made within 2 d of sampling. No correction factor was applied to adjust for disturbed sediment samples.

**Primary production using  $\text{O}_2$  microelectrodes.** Steady-state  $\text{O}_2$  microprofiles were measured in intact sediment cores installed in a water bath in the laboratory at in situ temperature. Prior to measurements, the core was exposed to a 12:12 h light:dark (L:D) cycle ( $180 \mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ , respectively) by a halogen lamp (Schott KL 1500; Schott AG, Mainz, Germany). A stable water flow above the sediment surface was established by an internal rotating magnet, which maintained a diffusive boundary layer of 300–500  $\mu\text{m}$ . Flushing by an air pump kept the water of the surrounding water bath at atmospheric oxygen saturation at all times. Microprofiles were carried out using Clark-type  $\text{O}_2$  microelectrodes with a guard cathode (Revsbech 1989), having external tip diameter  $<50 \mu\text{m}$ , stirring sensitivity  $<1\%$ , and a 90% response time  $<2 \text{ s}$ . The electrode was calibrated by a two-point calibration in anoxic and air-saturated samples, respectively (Glud et al. 2000). The sensor was positioned manually by a micromanipulator, and the sensor current was measured using a picoammeter (Uni-sense, Aarhus, Denmark) connected to a strip chart recorder (Revsbech and Jørgensen 1986). Areal rates of oxygen respiration and production were measured in the dark and

in the light ( $180 \mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ ), respectively, as the diffusive  $\text{O}_2$ -flux rate across the diffusive boundary layer ( $J$ ), using Fick's first law of diffusion,  $J = D_0 \cdot [dC(z) \cdot (dz)^{-1}]$  (Jørgensen and Revsbech 1985, Crank 1989). The molecular diffusion coefficient of oxygen ( $D_0$ ) was corrected for temperature and salinity (Li and Gregory 1974). The sediment dark respiration was calculated as the flux of  $\text{O}_2$  into the sediment from the overlying water, whereas the net  $\text{O}_2$  production in light equaled the flux of  $\text{O}_2$  out of the sediment. The gross microphytobenthic primary production as estimated from the  $\text{O}_2$  microelectrode (MPP $_{\text{O}_2}$ ) was calculated from adding the dark respiration to the net  $\text{O}_2$  production. This method will generally underestimate the gross photosynthetic oxygen production (by 5%–30%) due to enhanced  $\text{O}_2$  respiration in the light, associated with photosynthetic production (Epping and Jørgensen 1996). The standard deviation of the  $\text{O}_2$ -flux rate is not induced by the microsensors technique (which has a much smaller error) but reflects the natural patchiness of the benthic microalgal distribution in the sediment, and it is in accordance with previous studies (Barranguet et al. 1998, Cahoon 1999, Hancke and Glud 2004).

**Diversity analysis.** Univariate diversity analysis was performed on diatom ABU using PRIMER-5 software (PRIMER-E Ltd., Plymouth, UK), considering richness ( $d$ , Margalef), equitability ( $J$ , Pielou), diversity ( $H'$ , Shannon), and dominance ( $\lambda'$ , Simpson) (Shannon and Weaver 1949, Simpson 1949, Pielou 1966, Margalef 1986).

**Statistical analysis.** The Shapiro–Wilk test of normality and the Bartlett test of homogeneity of variances were applied to abundance data. However, neither normality nor homoscedasticity conditions were met. Therefore, a Friedman test (non-parametric two-way analysis of variance [ANOVA] by ranks) was performed. Subsequently, a pair-wise one-tail Wilcoxon test was applied to detect significant differences between pairs of monthly abundances.

## RESULTS

**Abiotic and biotic parameters.** The granulometric analysis revealed sandy silt sediment (61.9% silt, 30.5% sand, and 7.6% clay). Main physical variables, nutrient concentrations, ABU and BIOM of diatoms, and total microphytobenthos (MPB) are shown in Table 1. Bacillariophyceae accounted for

TABLE 1. Abiotic parameters: temperature, benthic PAR, % PAR (benthic PAR as percentage of surface irradiance), and salinity measured in situ during sampling; nutrient concentrations in the overlying water (NA, not available).

Abiotic parameters									
	Temperature (°C)	PAR ( $\mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ )	% PAR	Salinity (psu)	$\text{NH}_4^+$ ( $\mu\text{M}$ )	$\text{NO}_2^-$ ( $\mu\text{M}$ )	$\text{NO}_3^-$ ( $\mu\text{M}$ )	$\text{PO}_4^{3-}$ ( $\mu\text{M}$ )	$\text{SiO}_2$ ( $\mu\text{M}$ )
April 2005	7.9	33	2.4	33.7	0.58	0.06	0.67	0.28	1.06
May 2005	8.5	27	1.6	32.3	0.90	0.09	4.48	0.47	1.85
June 2005	9.2	33	1.7	30.8	NA	0.10	1.80	0.01	0.40
Biotic parameters									
	Total ABU		Diatom ABU		Total BIOM		Diatom BIOM		
	$10^4 \text{ cells} \cdot \text{cm}^{-3}$	$10^4 \text{ cells} \cdot \text{g}^{-1}$	$10^4 \text{ cells} \cdot \text{cm}^{-3}$	$10^4 \text{ cells} \cdot \text{g}^{-1}$	$\mu\text{g C} \cdot \text{cm}^{-3}$	$\mu\text{g C} \cdot \text{g}^{-1}$	$\mu\text{g C} \cdot \text{cm}^{-3}$	$\mu\text{g C} \cdot \text{g}^{-1}$	
April 2005	$13.62 \pm 4.01$	$16.31 \pm 4.80$	$7.42 \pm 2.49$	$8.89 \pm 2.98$	$33.2 \pm 6.8$	$39.7 \pm 8.13$	$29.5 \pm 9.9$	$35.3 \pm 11.85$	
May 2005	$12.38 \pm 4.17$	$15.38 \pm 5.18$	$8.68 \pm 4.30$	$10.78 \pm 5.34$	$36.8 \pm 15.7$	$45.7 \pm 19.50$	$33.9 \pm 15.9$	$42.1 \pm 19.75$	
June 2005	$14.78 \pm 3.23$	$17.81 \pm 3.89$	$11.28 \pm 3.14$	$13.59 \pm 3.78$	$61.3 \pm 18.2$	$73.9 \pm 21.94$	$59.0 \pm 18.4$	$71.1 \pm 22.17$	

Biotic parameters: abundance and biomass of diatoms and total microphytobenthos are expressed both per  $\text{cm}^3$  and per gram of sediment dry mass. Data are reported as mean values of three replicate subsamples  $\pm$  SD.

67% of the total ABU, while phytoflagellates represented 30%. Resting cells were a minor component, accounting for 3%. Diatoms comprised 87% of the total BIOM, while resting cells and phytoflagellates accounted for 7% and 6%, respectively. *Navicula* and *Nitzschia* were the most abundant Bacillariophyceae genera (41.4% and 13.9%, respectively), followed by *Fragilariopsis* and *Amphora* (6.6% and 5.5%, respectively). Considering the Bacillariophyceae biomass, *Nitzschia* spp. and *Navicula* spp. accounted for 16.2% and 14.2%, respectively, followed by *Pleurosigma* spp. and *Amphora* spp. (10.4% and 9.8%, respectively; Table 2). The floristic list consisted of 38 genera and 94 species (see Appendix S1 in the supplementary material). Among these species, we recognized 12 freshwater species; all others were marine diatoms. Ten planktonic species were distinguished, while the majority of diatoms were benthic. The latter were further differentiated into 14 epipsammic and 46 epipelagic species.

**Diversity analysis.** The highest ABU measured in June 2005 led to the highest values of  $S$  (65),  $d$  (5.55), and  $\lambda'$  (0.10) in correspondence with the minimum value of  $J'$  (0.72). The lowest value of  $d$  (4.78) was calculated in correspondence with the lowest observed number of species ( $S = 54$ ) in April 2005 (Table 3).

**Statistical analysis.** Analysis of variance indicated that significant differences ( $P = 0.01$ ) were present among the three monthly abundances. In particular, a significant difference was observed between the abundance in April and June, the median of April being significantly less than the median of June ( $P = 0.03$ ).

**Pigment determination.** Pigment concentrations, ratios between pigments, and ratios between pigments and the microphytobenthic carbon are listed in Table 4. Average values for fucoxanthin and its

TABLE 2. Microphytobenthic community composition and main diatom genera, expressed as relative abundance (ABU %) and relative biomass (BIOM %).

	ABU %	BIOM %
Taxa		
Bacillariophyceae	67	87
Undetermined phytoflagellates	30	6
Resting cells	3	7
Total MPB	100	100
Diatom genera		
<i>Navicula</i>	41.4	14.2
<i>Nitzschia</i>	13.9	16.2
<i>Fragilariopsis</i>	6.6	1.3
<i>Amphora</i>	5.5	9.8
<i>Gyrosigma</i>	4.2	6.8
<i>Pleurosigma</i>	3.9	10.4
<i>Pinnularia</i>	2.5	4.0
<i>Mastogloia</i>	1.9	3.5
<i>Entomoneis</i>	1.2	6.8
Other	19.2	27.0
Total diatoms	100	100

MPB, microphytobenthos.

TABLE 3. Univariate diversity indices: richness ( $d$ , Margalef), equitability ( $J'$ , Pielou), diversity ( $H'$ , Shannon), and dominance ( $\lambda'$ , Simpson).<sup>a</sup>

Sample	Number of taxa ( $S$ )	Richness ( $d$ )	Equitability ( $J'$ )	Diversity ( $H'$ )	Dominance ( $\lambda'$ )
April 2005	54	4.78	0.73	1.27	0.10
May 2005	63	5.51	0.73	1.32	0.09
June 2005	65	5.55	0.72	1.31	0.10

<sup>a</sup>Shannon and Weaver (1949), Simpson (1949), Pielou (1966), Margalef (1986).

derivatives were  $4.57 \pm 0.45$  and  $1.92 \pm 0.19 \mu\text{g} \cdot \text{g}^{-1}$ , respectively. The mean chl  $a$  value was  $2.48 \pm 0.15 \mu\text{g} \cdot \text{g}^{-1}$ , while the mean diadinoxanthin value was  $0.26 \pm 0.02 \mu\text{g} \cdot \text{g}^{-1}$ . Chl  $a$  derivatives and chl  $c_{1+2}$  were not distinguished and have been presented together. Shade-adapted MPB contained 42%–45% of fucoxanthin, 22%–29% of chl  $a$ , and 2.5%–2.8% of photoprotective carotenoid diadinoxanthin relative to total pigments (m:m).

**Primary production.** Values of MPPs as well as MPPp under light saturation were higher in April than in June 2004. From April to June, MPPp values were recorded at increasingly higher saturating irradiances (from 100 to 500  $\mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ ; Table 5). Oxygen microprofiles across the benthic boundary layer were obtained with a vertical resolution of 100  $\mu\text{m}$ . In the dark, an average of three profiles showed an  $\text{O}_2$  concentration decrease with increasing depth from the air-saturated concentration of the water ( $\sim 300 \mu\text{mol O}_2 \cdot \text{L}^{-1}$ ) to the anoxic part of the sediment at 0.30 cm depth ( $[\text{O}_2] < 0.1 \mu\text{mol} \cdot \text{L}^{-1}$ , Fig. 2a). The shape of the profile showed a moderate  $\text{O}_2$  consumption equally distributed across the oxygenic part of the sediment. In the light, the  $\text{O}_2$  concentration increased from the overlying water toward the sediment surface, reaching a maximum concentration ( $446 \pm 53 \mu\text{mol O}_2 \cdot \text{L}^{-1}$ ) just beneath the sediment surface (0.01 cm depth). Lower into the sediment, the  $\text{O}_2$  concentration decreased with depth, until all the  $\text{O}_2$  was consumed at 0.4 cm depth (Fig. 2b). The sediment community respiration in the dark and the net production in the light ( $180 \mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ ) is shown in Figure 3. The estimated  $\text{MPP}_{\text{O}_2}$  equaled  $30.4 \text{ mg C} \cdot \text{m}^{-2} \cdot \text{h}^{-1}$ .

## DISCUSSION

**Abiotic parameters.** PAR is the most important factor influencing the development of the microalgal community (Sundbäck 1986, Miles and Sundbäck 2000). At 8 m depth, no PAR increase was observed from April to June, and the percentage of incident PAR at the bottom (% PAR) varied from 1.6% to 2.4%. In this period, despite increasing irradiance in air, high water turbidity was caused by phytoplankton blooms or high concentration of cDOM from freshwater runoff (Sakshaug 2000). For this

TABLE 4. Pigment concentrations from surface sediments and ratios between pigments (m:m), and between pigments and the microphytobenthic carbon (m:m), estimated as biomass.

	Fuco		Fuco der		Chl <i>a</i>				
	$\mu\text{g} \cdot \text{g}^{-1}$	$\text{mg} \cdot \text{m}^{-2}$	$\mu\text{g} \cdot \text{g}^{-1}$	$\text{mg} \cdot \text{m}^{-2}$	$\mu\text{g} \cdot \text{g}^{-1}$	$\text{mg} \cdot \text{m}^{-2}$			
April 2005	4.14 ± 0.32	85.28 ± 6.59	1.71 ± 0.15	35.23 ± 3.09	2.87 ± 0.02	59.12 ± 0.41			
May 2005	3.98 ± 0.57	81.99 ± 11.74	1.65 ± 0.18	33.99 ± 3.71	2.19 ± 0.21	45.11 ± 4.33			
June 2005	4.88 ± 0.46	100.53 ± 9.48	2.38 ± 0.25	49.03 ± 5.15	2.39 ± 0.21	49.23 ± 4.33			
	Chl <i>c</i> <sub>1+2</sub> + chl <i>a</i> der		Diadino		Total				
April 2005	0.83 ± 0.06	17.10 ± 1.24	0.25 ± 0.03	5.15 ± 0.62	9.80	201.88			
June 2005	0.72 ± 0.09	14.83 ± 1.85	0.25 ± 0.01	5.15 ± 0.21	8.79	181.07			
May 2005	1.13 ± 0.10	23.28 ± 2.06	0.29 ± 0.02	5.98 ± 0.41	11.07	228.04			
	Fuco:chl <i>a</i>	Diadino:chl <i>a</i>	Fuco:total	Chl <i>a</i> :total	Diadino:total	Fuco der:fuco	Chl <i>a</i> der:chl <i>a</i>	Chl <i>a</i> :C	Total:C
April 2005	1.44	0.09	0.42	0.29	0.025	0.41	0.29	0.07	0.25
May 2005	1.82	0.11	0.45	0.25	0.028	0.41	0.33	0.05	0.19
June 2005	2.04	0.12	0.44	0.21	0.026	0.49	0.47	0.03	0.15

Data are reported as mean values ( $\mu\text{g} \cdot \text{g}^{-1}$  and  $\text{mg} \cdot \text{m}^{-2}$ ) of three replicate subsamples ± SD. Conversion from  $\mu\text{g}$  pigment  $\cdot \text{g}^{-1}$  dry sediment to  $\text{mg}$  pigment  $\cdot \text{m}^{-2}$  was performed by multiplication with 20.6, based on an average bulk density of  $2.06 \text{ g} \cdot \text{cm}^{-3}$  (de Jong and de Jonge 1995). Fuco, fucoxanthin; fuco der, fucoxanthin derivatives; chl *a* der, chl *a* derivatives (together with chl *c*<sub>1+2</sub>); diadino, diadinoxanthin; total, total pigments; C, microphytobenthic carbon.

TABLE 5. Primary production measured in situ (MPPs), in the laboratory (MPPp, at 10–500  $\mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ ) and by means of microelectrodes (MPP<sub>O<sub>2</sub></sub>, at 180  $\mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ ).

	MPPs ( $\text{mg C} \cdot \text{m}^{-2} \cdot \text{h}^{-1}$ )	MPPp <sup>a</sup> ( $\text{mg C} \cdot \text{m}^{-2} \cdot \text{h}^{-1}$ )	Saturating light ( $\mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ )	MPP <sub>O<sub>2</sub></sub> <sup>b</sup> ( $\text{mg C} \cdot \text{m}^{-2} \cdot \text{h}^{-1}$ )	MPPs $\cdot$ (chl <i>a</i> ) <sup>-1</sup> ( $\text{mg C} \cdot (\text{mg chl } a)^{-1} \cdot \text{h}^{-1}$ )	MPPp $\cdot$ (chl <i>a</i> ) <sup>-1</sup> ( $\text{mg C} \cdot (\text{mg chl } a)^{-1} \cdot \text{h}^{-1}$ )
April 2005	1.28 ± 0.08	34.70 ± 19.24	100	30.37	10.41	282.11
May 2005	0.28 ± 0.12	10.88 ± 5.32	200		2.53	98.37
June 2005	0.15 ± 0.07	6.79 ± 6.11	500		1.15	52.03

<sup>a</sup>Measured from slurred samples at the saturating irradiance.

<sup>b</sup>Measured in intact sediment cores. Data are reported as mean values of three replicates ± SD for MPPs and two replicates ± SD for MPPp.

reason, low irradiance at the bottom was recorded in May and June.

*Microphytobenthic community.* The abundance of diatoms and total MPB was higher in June than in April. This increase was more evident when considering biomass instead of abundance. During this period, a change in the microphytobenthic community was observed. Smaller genera and species were replaced by larger ones with higher carbon content. Moreover, the relative biomass (RB) of some of these large species increased from April to June: the genus *Mastogloia* (RB = 0.6, 1.6, and 8.3% in April, May, and June, respectively), *Nitzschia angularis* (RB = 0.9, 1.5, and 3.5% in April, May, and June, respectively; for taxonomic authors, see Appendix S1), *Navicula directa* (RB = 1.0, 0.5, and 4.7% in April, May, and June, respectively), and *Nitzschia constricta* (RB = 1.4, 0.4, and 4.1% in April, May, and June, respectively). Finally, other large-size species were observed only in June: *Gyrosigma strigile* (RB = 3.7%) and *Surirella fastuosa* (RB = 1.7%).

A remarkable difference in the genus *Navicula*, when expressed as relative abundance (RA) or RB, was observed due to the presence of small-sized species (*Navicula* spp. 1, *N. mollis*). The genus *Navicula* was well represented with high species variability. The dimensional range was wide, and species identification using the light microscope was possible only in a few cases. Therefore, we decided to arbitrarily group specimens of this genus into three size classes: *N.* spp. 1 (up to 20  $\mu\text{m}$ ), *N.* spp. 2 (20 to 40  $\mu\text{m}$ ), and *N.* spp. 3 (>40  $\mu\text{m}$ ; Blasutto et al. 2003).

Benthic and planktonic diatoms were distinguished. Benthic species were further divided into epipsammic (living on sand) or epiphytic (attached to macroalgae or other surfaces) and epipellic (living freely on soft sediments) (Round 1971). Sometimes the distinction between epipsammic and epiphytic forms becomes arbitrary, because the same species can adhere to different substrata—macroalgae, rocks, or sand grains by means

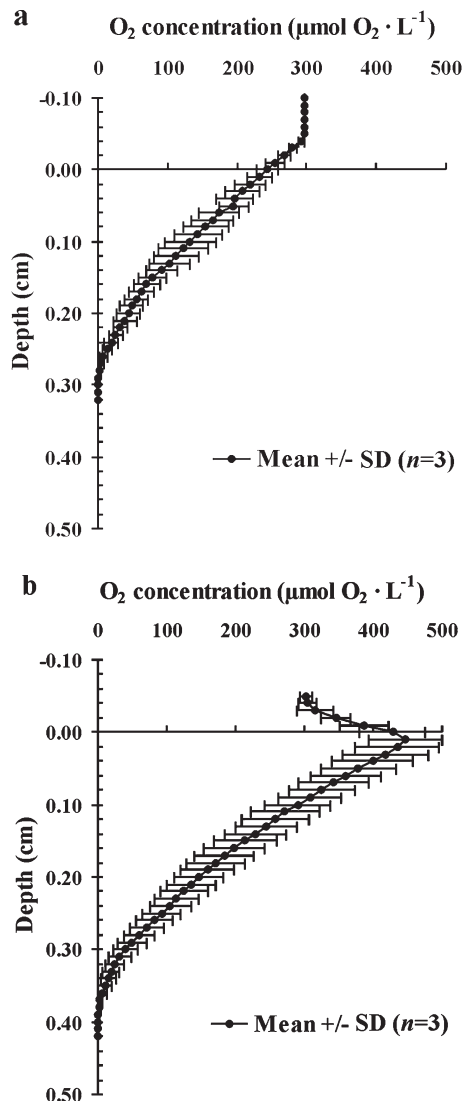


FIG. 2. O<sub>2</sub> profiles in the dark (a) and in illuminated (b, 180 µmol photons · m<sup>-2</sup> · s<sup>-1</sup>) sediment cores from St. Taura.

of stalks and apical pads. The same genera (e.g., *Amphora* and *Achnanthes*) usually occur both in epiphytic and in epipsammic assemblages (McIntire and Moore 1977). On the contrary, species forming mucilage tubes (e.g., *Navicula mollis* and *N. parva*) are typically epiphytic (Dexing et al. 1985).

The presence of 14% of epipsammic species versus 50% of epipelagic ones was attributable to the sediment texture. Cohesive sediments enable the development of epipelagic forms capable of moving through the sediment. However, sand supports the growth of epipsammic species, which adhere to the grains by means of apical pads, stalks, and tubes (Miles and Sundbäck 2000). The major epipelagic genera were *Navicula*, *Nitzschia*, *Gyrosigma*, and *Pleurosigma*, while *Amphora* together with *Mastogloia* were the most abundant epipsammic genera. Among the epipelagic genera, the RA did not vary considerably

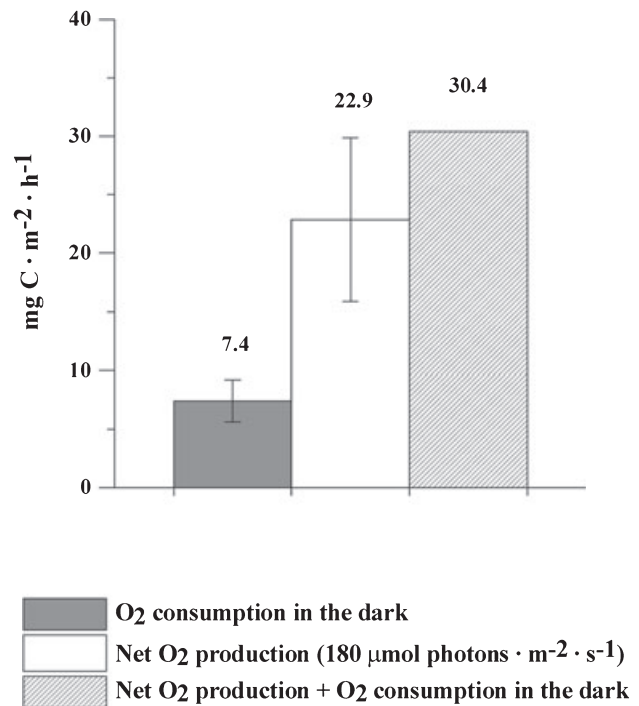


FIG. 3. Dark O<sub>2</sub>-consumption rate (= community respiration, dark bar) and net O<sub>2</sub>-production rate (= net primary production, white bar) in sediment cores from St. Taura. Rates are calculated from the diffusive flux of O<sub>2</sub> across the sediment surface (mg C · m<sup>-2</sup> · h<sup>-1</sup>). Estimated gross production (checked bar) was calculated as net O<sub>2</sub> production + dark O<sub>2</sub> consumption. The average rate is given above the bars; the error bars represent the SD ( $n = 3$ ).

for *Navicula* and *Nitzschia*, while the RA almost doubled for *Gyrosigma*, from 3.8% in April to 6.7% in June. Specifically, in June the RA increased for *G. spencerii*, *G. delicatulum*, *G. macrum*, and *G. fasciola*. In the genus *Amphora*, *A. ovalis* and *A. exigua* persisted, while *A. commutata* was well-represented in April only.

Diatom species, which are classified as freshwater by many authors (Patrick 1977, Germain 1981, Round et al. 1992), were collected at St. Taura, with salinities ranging from 30 to 33 psu. These species were *Fragilaria intermedia*, *Frustulia rhomboides*, *Nitzschia dubia*, *Pinnularia gibba*, *P. lata*, *Synedra acus*, *S. ulna*, and *Tabellaria flocculosa*. The high RA of the genus *Mastogloia* in June was also the result of the increase of freshwater input. Similarly, the freshwater taxon *Achnanthes lanceolata* (McIntire and Moore 1977) occurred in the sediment with a salinity of 33 psu.

Microscopic analyses of specimens collected at Taura Island revealed that some genera and species appear to be ~30% larger than those observed at a site in the Gulf of Trieste (Italy) at 17 m depth (T. Cibic, unpublished results). These species were *Navicula directa*, *Nitzschia angularis*, *N. lorenziana*, *N. sigma*, and the genera *Gyrosigma* and *Pleurosigma*.

TABLE 6. Summary of different microphytobenthic abundance (ABU) estimates.

ABU	Thickness (mm)	Region	Depth (m)	References
$4.0 \times 10^4$ – $1.5 \times 10^5$ cells · cm <sup>-3</sup>	5	Ems-Dollard Estuary, Netherlands	Intertidal site	Admiraal et al. (1982)
$0.8 \times 10^4$ – $7.9 \times 10^4$ cells · cm <sup>-3</sup> <sup>b</sup>	10	Grado and Marano Lagoon, Italy	1.5	Blasutto et al. (2005)
$1 \times 10^6$ – $17 \times 10^6$ cells · cm <sup>-2</sup> <sup>a</sup>	10	Long Island, New York, USA	Intertidal site	Cheng et al. (1993)
$1.3 \times 10^6$ – $4.3 \times 10^6$ cells · cm <sup>-3</sup>	3–4	Venice Lagoon, Italy	0.8–0.9	Facca et al. (2002)
$4.7 \times 10^6$ – $7.5 \times 10^6$ cells · cm <sup>-2</sup>	5	Gulf of Finland	1.5	Snoeijis et al. (1990)
$0.25 \times 10^6$ – $2.1 \times 10^6$ cells · cm <sup>-2</sup>	5	Laholm Bay, Sweden	14–16	Sundbäck and Jönsson (1988)
750.30 cells · mm <sup>-3</sup>	5	Lindåspollene, Norway	10	Taasen and Høisæter (1981)
720–9278 cells · cm <sup>-3</sup>	10	Adriatic Sea, Italy	13.5–66	Totti (2003)
$0.1 \times 10^4$ – $5.7 \times 10^4$ cells · g <sup>-1</sup>	10	Gulf of Trieste, Italy	20	Welker et al. (2002)
$12.4 \times 10^4$ – $14.8 \times 10^4$ cells · cm <sup>-2</sup>	5	Trondheimsfjord, Norway	8	This study

<sup>a</sup>Estimated from a figure.

<sup>b</sup>Values converted from cell · g<sup>-1</sup> to cell · cm<sup>-3</sup> for data comparison.

Like all organisms, diatoms are influenced by ambient temperatures: the organism size increases with decreasing temperature (Montagnes and Franklin 2001). Larger diatoms are often elongated in one dimension and have relatively larger nutrient storage vacuoles and lower carbon content on a volume basis. Superior storage may allow larger diatoms to outcompete smaller diatom species with faster intrinsic growth rates by achieving a slow but steady growth rate in pulsed-nutrient environments, where a small cell can grow rapidly but for only a small proportion of the time (Finkel et al. 2005).

A few reports of the specific composition of the microphytobenthic community and cell abundances are available. However, the lack of a standard unit for ABU makes comparison difficult (Table 6).

**Diversity analysis.** The univariate diversity analysis showed a microphytobenthic community characterized by a relatively high number of species and an even distribution of the different species. Overall, diversity indices revealed that the microphytobenthic community was well adapted to these environ-

mental conditions, despite the limited light availability at the bottom. The number of new species observed by means of microscopic analyses increased at each sampling (Fig. 4). Further samplings, performed at regular monthly intervals, would certainly lead to an increase in the richness of benthic diatoms.

**Pigment determination.** HPLC-isolated pigments confirmed the dominance of benthic diatoms within the microphytobenthic community since the carotenoid fucoxanthin was the major pigment throughout the study period. Presence of 19'-acyloxy derivatives of fucoxanthin or chl *c*<sub>3</sub>, indicating haptophytes, was not detected. Moreover, the absence of chl *b* excludes the possibility that Chlorophyceae, Prasinophyceae, and/or Euglenophyceae contributed to the microphytobenthic community, confirming our microscopic observations. Dinophyceae were not observed by microscopy, nor was their class-specific pigment marker, peridinin.

During the study period, total pigment concentration only slightly increased, while BIOM nearly doubled in the same period (i.e., the cell volume became larger, while the pigment content remained almost unchanged). This result is in accordance with the findings of Montagnes and Franklin (2001), who reported that diatom C and N content per unit volume decreases with increasing size. The increase of BIOM corresponded with the drop of PO<sub>4</sub><sup>3-</sup> and SiO<sub>2</sub>. Benthic microalgae have an absolute requirement for PO<sub>4</sub><sup>3-</sup> (Maestrini et al. 1997) and SiO<sub>2</sub> (Egge and Aksnes 1992, Rousseau et al. 2002), and these nutrients are the first ones to be consumed.

Chl *a* concentration alone is not a sufficient indicator of microphytobenthic biomass since its variations could be endogenous and linked to the ecophysiological status of benthic microalgae, like photoacclimation to low irradiance or cell senescence (de Jonge 1980, Méléder et al. 2005). In this study, the use of chl *a* would have underestimated the microalgal biomass, if the calculated cell carbon content had not been considered.

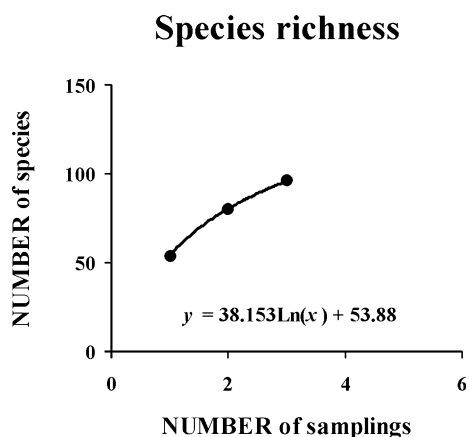


FIG. 4. Species richness related to number of samplings, showing that only three samplings were not enough to reach the plateau.



The absence of diatoxanthin and the low diadinoxanthin to chl *a* ratio indicated that the microphytobenthic community in the study site was shade adapted. During sampling (in shade) and extraction (in dark), most of the diatoxanthin in the cell will be de-epoxidated to diadinoxanthin (Demers et al. 1991, Klein and Riaux-Gobin 1991, Johnsen and Sakshaug 1993). Our diadinoxanthin:chl *a* ratio varied from 0.09 in April to 0.12 in June and was comparable with the same ratio for low- and medium-light-acclimated phytoplankton reported by Johnsen and Sakshaug (1993) and Rodríguez et al. (2006).

An increase in daily insolation from the first sampling day (26 April, 16 h and 12 min) to the last one (6 June, 20 h and 10 min) occurred at this latitude. Thus, MPB were exposed to longer day length from April to June. According to Johnsen and Sakshaug (1993), phytoplankton acclimate their pigment composition qualitatively and quantitatively as a response to variations in the light regime (irradiance, spectral composition, and day length). Cells adapted to low light should exhibit high concentrations of chl *a* and light-harvesting pigments to enhance adsorption of available light (de Jonge 1980, Sakshaug and Johnsen 2005). In our samples, the chl *a* concentration was slightly lower in April than in June, while the fucoxanthin concentration was higher over the same period, leading to a higher fucoxanthin:chl *a* ratio. These ratios were comparable to or higher than those reported by other authors (Table 7) and indicate that the microphytobenthic community is adapted to low % PAR (1%–2%, 30  $\mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ ). In the present study, the total pigments:C (microphytobenthic carbon estimated as biomass) ratio varied from 0.25 in April to 0.15 in June. While an increase of BIOM was measured, the total pigment concentration did not vary much during this period. Our total pigments:C ratio was comparable to or slightly lower than those reported by Johnsen and Sakshaug (1993) and Rodríguez et al. (2006). In addition, our chl *a*:C ratios (0.03–0.07) were very similar to those observed by Johnsen and Sakshaug (1993) under comparable irradiance (30  $\mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ ). de Jonge and Colijn (1994) reported a chl *a*:C ratio varying from 0.1 to 0.007, determined for

well-growing natural algae populations. Even though our chl *a*:C values were obtained considering the benthic microalgal carbon and not the total organic carbon, they came within the range reported by de Jonge and Colijn (1994).

The determination of sedimentary phaeopigment composition is generally needed for ecological studies of benthic communities (e.g., chl *a*:phaeopigments *a* ratio is an indicator of the physiological state of MPB; Buffan-Dubau and Carman 2000). Our fucoxanthin derivatives:fucoxanthin ratio ranged from 0.41 to 0.49, while the chl *a* derivatives:chl *a* ratio varied from 0.29 to 0.47. In the latter ratio, the numerator chl *a* derivatives also contain the contribution of chl  $c_{1+2}$ , leading to an overestimate of the same ratio. Nevertheless, these values were still low. The use of pigment ratios, however, must be considered with a degree of caution because of the large number of environmental and physiological factors affecting them (Lucas and Holligan 1999). Shallow water sediments generally contain more phaeopigments than chl *a*. The relatively swift currents and lack of macrobenthic vegetation allow the flow of suspended matter over the surface of the sediment. Consequently, the major fraction of sedimentary chl is probably in the actively growing benthic microalgae (Tietjen 1968, Lucas and Holligan 1999). The concomitant absence of chl *b*, phaeopigments *b*, and lutein further indicates that our sampling site was not an accumulation zone for green algae or terrestrial plant detritus, at least during the sampling period (Cariou-Le Gall and Blanchard 1995). Brotas and Plante-Cuny (1998) affirm that the percentage of phaeopigments is generally greater in low-tidal mudflats than in sandier sites. This is in accordance with our results, since the sediment collected at our site is classified as sandy silt.

*Primary production.* Different techniques are commonly used to determine benthic primary production: oxygen exchange,  $^{14}\text{C}$  method,  $\text{O}_2$  microelectrodes, and PAM fluorescence. The main advantage of the  $^{14}\text{C}$  method applied to resuspended sediment is that primary production can be measured with high spatial resolution. The major drawback of the slurry technique is that microalgae from deeper layers are exposed to the same light

TABLE 7. Summary of fucoxanthin:chl *a* ratios in sediments reported in literature.

Fuco:chl <i>a</i>	Region	Depth (m)	References
0.50–1.39	Gulf of Fos, France	0.5–13	Barranguet et al. (1996)
0.38–1.00	Tagus Estuary, Portugal	5	Brotas and Plante-Cuny (1998)
0.67–0.77	Cocodrie, Louisiana, USA	Mudflat	Buffan-Dubau and Carman (2000)
2.86	Gulf of Carpentaria, Australia	0–60	Burford et al. (1994)
0.38	Marenes-Oléron Bay, France	Intertidal site	Cariou-Le Gall and Blanchard (1995)
0.47	North Perry Reef, Bahamas	17–20	Decho et al. (2003)
0.64	Eden Estuary, Scotland, UK	Not reported	Defew et al. (2002)
0.33–1.65	Westerschelde Estuary, Netherlands	Tidal flat	Lucas and Holligan (1999)
0.52–0.73	St. Lawrence Estuary, Quebec, Canada	Intertidal site	Roux et al. (2002)
1.44–2.04	Trondheimsfjord, Norway	8	This study

regime as microalgae from the sediment surface. Only 1% of PAR at the surface occur at 1–3 mm depth in sandy sediments and at 0.5–0.6 mm in silty sediments; as a consequence, production can be overestimated (Jönsson 1991). For this reason, Sundbäck et al. 1990 suggested that a correction factor to intact core should be applied. In our study, no correction factor was applied to adjust for disturbed sediment samples. Nevertheless, we obtained comparable primary production values from the two different techniques (i.e., O<sub>2</sub> microprofile in intact sediment core and <sup>14</sup>C in suspended sediment) during the experiment performed in April. Although the <sup>14</sup>C method in slurry sediment has been used in the last two decades and ecological relevance has been given to the data (Sundbäck and Jönsson 1988, Barranguet et al. 1998), we believe that caution is necessary when interpreting primary production values measured in disturbed sediment samples.

During the study period, MPPs values never equaled MPPp values (i.e., the microphytobenthic community in situ was not light saturated). These results are comparable to those Sundbäck and Jönsson (1988) obtained when considering the similar benthic % PAR. In our study, the MPPp value (34.70 mg C · m<sup>-2</sup> · h<sup>-1</sup>), obtained in correspondence with the highest saturating light, and the MPP<sub>O<sub>2</sub></sub> (30.37 mg C · m<sup>-2</sup> · h<sup>-1</sup>) value were very similar. Our results were comparable to those obtained by Hancke and Glud (2004), who reported dark respiration and net production of 11.5 ± 1.7 and 17.6 ± 8.2 mg C · m<sup>-2</sup> · h<sup>-1</sup> in the dark and in the light (180 μmol photons · m<sup>-2</sup> · s<sup>-1</sup>), respectively.

In the laboratory, the microphytobenthic community was capable of photosynthesizing at progressively higher saturating light from April to June. In our study, we defined the saturating light as the irradiance that yields the highest production as measured in the light gradient. This is in agreement with Miles and Sundbäck (2000), who determined that even at high irradiances, photoinhibition of the algal community is unlikely, as the motile species are able to reposition themselves. In addition, we infer that the highest saturating light recorded in June was also due to the presence of a few still photosynthetically active planktonic species in the sediment—namely, *Fragilariopsis* spp., *F. oceanica*, and *Thalassionema* spp. These species were able to photosynthesize under high irradiance (500 μmol photons · m<sup>-2</sup> · s<sup>-1</sup>). This finding is in accordance with Sundbäck and Jönsson (1988), who suggest that under adequate light, planktonic microalgae may contribute to benthic primary production.

Values of MPPs and MPPp normalized to chl *a* followed the same decreasing pattern as MPPs and MPPp, indicating that the microphytobenthic photosynthetic capability per unit chl *a* diminished from April to June. In contrast, BIOM almost doubled during the same period. We infer that there was a

shift from the autotrophic to heterotrophic metabolism in the microphytobenthic community. The heterotrophic utilization of organic substances by diatoms is likely to be an important survival strategy when light levels are too low for photosynthesis (Sundbäck and Granéli 1988, Tuchman et al. 2006). Many of these diatoms, capable of heterotrophic metabolism, are pennate and frequently occur in shallow-water benthic environments (Hellebust and Lewin 1977).

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### Supplementary Material

The following supplementary material is available for this article:

**Appendix S1.** Floristic list with the mean (with standard deviation) and the maximum abundance of the study period.

This material is available as part of the online article from: <http://www.blackwell-synergy.com/doi/abs/10.1111/j.1529-8817.2007.00405.x>.

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