

STREAM PERIPHYTON PHOTOACCLIMATION RESPONSE IN FIELD CONDITIONS: EFFECT OF COMMUNITY DEVELOPMENT AND SEASONAL CHANGES¹

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The photochemical behavior of intact stream periphyton communities in France was evaluated in response to the time course of natural light. Intact biofilms grown on glass substrata were collected at three development stages in July and November, and structural parameters of the biofilms were investigated (diatom density and taxonomy). At each season, physiological parameters based on pigment analysis (HPLC) and pulse-amplitude-modulated (PAM) chl fluorescence technique were estimated periodically during a day from dawn to zenith. Regardless of the community studied, the optimal quantum yield of PSII (F_v/F_m), the effective PSII efficiency (Φ_{PSII}), the nonphotochemical quenching (NPQ), and the relative electron transport rate (rETR) exhibited clear dynamic patterns over the morning. Moreover, microalgae responded to the light increase by developing the photoprotective xanthophyll cycle. The analysis of *P-I* parameters and pigment profiles suggests that July communities were adapted to higher light environments in comparison with November ones, which could be partly explained by a shift in the taxonomic composition. Finally, differences between development stages were significant only in July. In particular, photoinhibition was less pronounced in mature assemblages, indicating that self-shading (in relation to algal bio-

mass) could have influenced photosynthesis in older communities.

Key index words: PAM fluorescence; photoacclimation; photoadaptation; photoinhibition; photoprotection; stream periphyton; xanthophyll cycle

Abbreviations: DD, diadinoxanthin; DR, xanthophyll cycle de-epoxydation ratio; DT, diatoxanthin; F_0 , fluorescence steady-state level of a dark-adapted sample; F_m , fluorescence maximal level under saturating light in actinic irradiance; F'_m , fluorescence maximal level under saturating light of a dark-adapted sample; F_i , fluorescence steady-state level under ambient light; F_v/F_m , optimal quantum yield of PSII; I , incident irradiance; I_k , saturation onset irradiance; NPQ, nonphotochemical quenching; PAM, pulse amplitude modulated; *P-I*, photosynthesis-irradiance; rETR, relative electron transport rate; rETR_m, maximal relative electron transport rate; α_1 , initial slope of photosynthesis-irradiance curves; β_1 , photoinhibition parameter; Φ_{PSII} , effective quantum yield of PSII

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Periphyton mainly consists of photosynthetic organisms (both eukaryotes and cyanobacteria) but also bacteria and fungi, can colonize any type of substratum in the photic zone, and grows imbedded within a polysaccharidic matrix (Wetzel 2001). It has been demonstrated that the contribution of periphyton to carbon fixation and nutrient cycling can be significant, particularly in lotic systems (Wetzel 2005). It can even be greater than that of

phytoplankton or macrophytes in some clear shallow lakes (Liboriussen and Jeppesen 2003). Mature periphytic biofilms usually present a complex three-dimensional architecture, which is influenced by several abiotic (from natural or anthropogenic sources) and biotic environmental factors during the colonization period (Vermaat 2005 and references therein). Among these factors, light plays a potential major role as a prerequisite for photosynthetic processes (Hill 1996). In situ benthic light environment is highly variable at different temporal and spatial scales. The occurrence of clouds, the water turbidity, and the riparian vegetation influence the quality and quantity of PAR reaching the biofilms: in open sites, irradiance fluctuates over the day from limiting to potentially oversaturating levels, whereas in more shaded sites, light is limited and sometimes transient due to movements of the streamside vegetation (Hill 1996). Studies based on measurements of periphyton photosynthesis–irradiance (*P-I*) relationships showed that these communities respond to this heterogeneity by presenting typical “sun-shade acclimations,” but with photosynthetic characteristics substantially different from those expected and found in higher plants, macroalgae, or even phytoplankton (Boston and Hill 1991, Hill et al. 1995). When considering shorter timescales (i.e., diurnal variation), microalgae need to exhibit phenotypic plasticity (i.e., photoacclimation) not only to maximize photosynthesis but also to protect the photosynthetic apparatus from light in excess (Falkowski and Laroche 1991). By definition, light impairment of PSII is called photoinhibition when a slowly reversible decrease in the overall photosynthesis results, meaning that the mechanisms developed by microalgae are not enough to protect them from a prolonged exposition to high light (Long et al. 1994). To date, it appears that the phenomenon of photoinhibition rarely occurs at the community level. Among the different photoprotective mechanisms, the production of carotenoids, especially those of the xanthophyll cycle, can help dissipate energy excess through thermal radiation. This process, which is part of the so-called NPQ (Müller et al. 2001), has been intensively studied in diatoms, mainly in laboratory conditions using cultures (e.g., Demers et al. 1991, Arsalane et al. 1994, Olaizola et al. 1994, Lavaud et al. 2002, 2004, Dimier et al. 2007), but its functioning at the whole community level, especially in benthic diatom-dominated ones, is not well documented (Serôdio et al. 2005). Also, it has been demonstrated that this relative resistance of periphyton communities to high-light stress is clearly a function of mat thickness (Hill and Boston 1991, Hill 1996). Indeed, the light is attenuated within the matrix by cells, detritus, and inorganic particles, meaning that the underlayers of cells are exposed to lower light conditions (Dodds et al. 1999). This shading effect, which depends on the biofilm microscale vertical

organization, must be considered when examining the relationship between structure and function (i.e., photosynthesis) in natural intact biofilms. Some motile diatoms may also exhibit down micro-migration in the matrix to a depth where light is attenuated. This behavioral strategy of photoacclimation cannot be discarded as it is still understudied in periphytic mats, whereas it has been well described in microphytobenthos inhabiting intertidal flats since the work of Kromkamp et al. (1998).

However, all these results are based on laboratory incubations using artificial light sources, which are not representative of in situ light conditions in terms of spatial, temporal, and spectral short-term variability. In recent years, the PAM fluorescence technique has emerged as a useful tool to measure photosynthetic parameters in situ under ambient light conditions in a noninvasive way (Schreiber et al. 1986).

Some authors have already performed periodical field measurements of chl fluorescence to study the diurnal plasticity of photosynthesis of aquatic organisms, such as seagrasses (Ralph et al. 1998, Beer and Bjork 2000), macroalgae (Schofield et al. 1998, Ensminger et al. 2001, Gévaert et al. 2003), sponges (Beer and Ilan 1998), corals (Jones and Hoegh-Guldberg 2001), microphytobenthic communities (Perkins et al. 2001, Migné et al. 2007), and even outdoor microalgal cultures (Masojidek et al. 1999, Vonshak et al. 2001). To our knowledge, our study is the first one dealing with stream periphyton in field conditions.

In this work, semidiurnal patterns and seasonal differences of the photochemical behavior of natural stream periphytic communities were determined under outdoor natural irradiance variation by measurements of chl fluorescence and pigment composition. The potential effect of biomass accumulation occurring during colonization and their ability to develop photoprotective mechanisms such as the xanthophyll cycle were investigated.

MATERIALS AND METHODS

Study area. The study area is located in the end section of the Stream Sensée, upstream near the town of Douai (Nord-Pas de Calais, France; 50°19'32" N, 3°4'6" E). The surface water velocity is $\sim 1 \text{ m} \cdot \text{s}^{-1}$, but the stream is often subject to high turbulences due to the frequent passages of barges. The sampling site is scarcely covered by the riparian vegetation and thus exposed to direct sunlight most of the day. During the colonization periods, PAR (400–700 nm) reaching the water surface was continuously recorded (in $\mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$, average over 15 min) using a quantum sensor (RAM, Herrsching, Germany). Conductivity, temperature, pH, and dissolved oxygen were also measured weekly in situ (multiparametric probe Multi 350i WTW, Weilheim, Germany). Samples were regularly collected for nutrient analysis (nitrate, total phosphorus, and dissolved organic carbon) following French and international standard colorimetric methods (NF EN ISO 13395, NF T 90023, and NF EN 1484, respectively).

Sample collection. Natural biofilms were collected at different development stages in summer (after 2, 4, and 6 weeks

between May and July) and autumn 2006 (after 3, 5, and 7 weeks between September and November). Substrata consisted of glass slides (76 × 26 mm) that were placed in floating racks immersed vertically and parallel to the water flow. At each season, the diurnal patterns of chl fluorescence and pigment content were obtained on the same day to minimize confounding seasonal effects on periphyton development (evaluated by the diatom community composition and abundance, see below) and to work under similar environmental conditions (irradiance, temperature in particular). Therefore, the substrata were deployed sequentially and then collected simultaneously. After collection, the glass substrata were transported to the laboratory in cool-boxes filled with site water, thereby maintaining the in situ temperature. Within 1 h after collection, the samples were transferred to a dark, temperature-controlled (20°C in July and 10°C in November) chamber until the following measurements were initiated.

Periphyton structure and taxonomic composition. Three glass slides were randomly selected for each development stage. The biofilms were removed from the slide with a razor blade and pooled in a preservative 5% formalin solution (Formol 37%). The total diatom density (number of cells · cm⁻² of colonized glass slide) was estimated in three replicates using a Nageotte counting chamber (VWR, Fontenay-sous-Bois, France) at 200 magnification. The diatom taxa were identified to the species level (Krammer and Lange-Bertalot 1986–1991) by examining 400 diatom valves at 1,000 magnification on permanent slides of cleaned diatom frustules digested in boiling H₂O₂ (30%) and HCl (35%) (10/1, v/v) and mounted in a high refractive index medium (Naphrax; Northern Biological Supplies Ltd., Ipswich, UK). The contribution of species to the total biovolume was assessed based on the theoretical biovolume (μm³ · cell⁻¹) for each species taken from the literature (Wetzel and Likens 1991, Hillebrand et al. 1999).

Fluorescence measurements. The day after collection, several colonized glass slides were placed horizontally in an outdoor aquarium (100 L) filled with stream water. Water was continuously agitated with underwater pumps and regularly replaced to maintain conditions (temperature, turbulence...) close to that of the natural site (22°C in summer and 10°C in autumn). The bottoms of the slides were freed from any algae before measurements. Fluorescent signals were measured with a portable fluorometer (PAM 2000; Walz, Effeltrich, Germany) at dawn and then at several light stages during the morning on the same spot for three to six slides for each development stage.

In the early morning, three slides were transferred to complete darkness for 10 min. We used a homemade dark incubator, which allowed us to determine the fluorescence yields underwater maintaining the optic fiber modulated light probe at a standard distance (2 mm) from the intact biofilms. First, the minimum fluorescence (F_0) was determined after a weak (5 s) far-red modulated light (735 nm). Then the maximum fluorescence (F_m) was reached by exposing the biofilm to a saturating white light pulse (0.8 s). The optimal quantum yield of PSII was calculated using the Genty et al. (1989) equation:

$$F_v/F_m = (F_m - F_0)/F_m \quad (1)$$

Preliminary results indicated that a 10 min dark-acclimation period was sufficient to accurately estimate F_v/F_m at dawn and low light, but this should be extended at higher irradiance. As the same period was applied whatever the irradiance (due to methodological reason), F_v/F_m was probably underestimated at zenith.

The effective quantum yield of PSII (Φ_{PSII}) was evaluated for six other glass slides. This parameter was measured under

ambient light by means of a homemade support carrying an axis where it was possible to insert and block the tip of the optic fiber of the fluorometer at a constant distance (2 mm) from the immersed biofilm and at a 60° angle to avoid possible shading. Φ_{PSII} was calculated according to Genty et al. (1989):

$$\Phi_{PSII} = (F'_m - F_t)/F'_m \quad (2)$$

where F_t is the fluorescence steady-state level under ambient light, and F'_m is the maximum level of fluorescence measured during a saturating pulse (0.8 s).

Then the NPQ was calculated according to the modified equation of Bilger and Bjorkman (1990):

$$NPQ = (F_m^* - F'_m)/F'_m \quad (3)$$

where F_m^* is the mean ($n = 3$) maximum fluorescence yield measured on dark-adapted biofilm at the beginning of the experiment.

Finally, the Φ_{PSII} was used to calculate the rETR according to Genty et al. (1989):

$$rETR = \Phi_{PSII} I 0.5 \quad (4)$$

where I (in μmol photons · m⁻² · s⁻¹) is the instantaneous PAR measured at the water surface using a semispherical quantum sensor (SA-190; Li-Cor, Lincoln, NE, USA) connected to a data-logger (Li-1400, Li-Cor; 1 measurement every 15 s, averaged over 1 min). The term “relative” ETR is used here because the chl a -specific absorption coefficient required to calculate the “true” ETR could not be obtained in the biofilms.

Changes in rETR were followed as a function of the variation in ambient light during the morning to establish composite $P-I$ curves. The relationship was best described by the model of Eilers and Peeters (1988) using least-squares nonlinear regression to calculate characteristic photosynthetic parameters, such as the photosynthetic efficiency at low light (α_t), the maximum photosynthetic rate (rETR_m), and the onset of light saturation (I_k) (Henley 1993). A $P-I$ curve was obtained for each replicate; therefore, the statistical analysis of α_t , I_k , and rETR_m was based on the comparison of the means of six replicates. Note that the curves illustrated further were constructed using the mean α_t , I_k , and rETR_m parameters ($n = 6$). The model of Platt et al. (1980) was also used to calculate the photoinhibition parameter (β_1), which corresponds to the slope of the curve past the point of the maximal photosynthesis, but it was less consistent than the former, and a $P-I$ curve could not be obtained for each replicate.

Pigment content. Three glass slides for each development stage were collected at dawn, around midmorning, and then at zenith. Each glass slide was scraped with a razor blade, and the suspension was gently filtered through a Whatman GF/F glass fiber filter (VWR), which was immediately frozen in liquid nitrogen and stored at -80°C until extraction. The filters were thawed, grinded, and then left overnight in dim light at +4°C in glass tubes containing 5 mL of methanol/methylene chloride (20/1, v/v). The extracts were centrifuged, and supernatants were then filtered with 0.45 μm PTFE membranes (Millipore, St Quentin en Yvelines, France). Sample volumes of 50 μL were injected into an HPLC system equipped with a P600 photodiode array detector (Thermo, Cergy Pontoise, France) and a Zorbax ODS-5μ C₁₈ reverse phase column (Agilent Technologies, Massy, France). The separation was carried out following a method modified from Wright et al. (1991). Pigments were identified by their retention time and absorption spectra versus standards and those given in the literature (Jeffrey et al. 1997) and were quantified using external standards supplied by DHI Water and Environment (Hoersholm, Denmark). The xanthophyll cycle de-epoxydation ratio (DR) was calculated as follows:

$$DR = DT/(DD + DT) \quad (5)$$

where DT and DD are the molar concentrations of diatoxanthin and diadinoxanthin, respectively.

Statistics. All the data were pooled for each season to compare both series of experiments. Then both seasons were analyzed separately in order to point out a potential effect of the development stage. If not mentioned, significant differences between mean values were determined by exact non-parametric tests using StatXact (v 8.0.0; Cytel Studio Inc., Cambridge, MA, USA). When differences were significant ($P < 0.05$), multiple comparisons were performed according to the method of Siegel and Castellan (1988). The *nlme* package (Pinheiro and Bates 2000) of the R statistical computing environment (v 2.6.1; Ihaka and Gentleman 1996) was used for nonlinear regressions on log-transformed data.

RESULTS

Physical and chemical characteristics during colonization.

Physicochemical characteristics demonstrated opposite trends throughout the two colonization periods, resulting in contrasted patterns when comparing July and November data (Table 1). Water temperature and incident irradiance gradually increased from May to July, the opposite taking place from September to November. In particular, the study site was exposed to an average PAR nearly three times higher in July ($31 \pm 1 \text{ mol photons} \cdot \text{m}^{-2} \cdot \text{d}^{-1}$, mean \pm CI 95%) than in November ($11 \pm 1 \text{ mol photons} \cdot \text{m}^{-2} \cdot \text{d}^{-1}$). The pH (7–8) and nutrient (NO_3^- and total phosphorus) and dissolved organic carbon concentrations were similar in both seasons.

Algal community composition and structure. Pigment content: In addition to chl *a* and β -carotene, the detected pigments were representative of diatom antennary complexes, with large amounts of fucoxanthin and chl *c* and also the xanthophylls diadinoxanthin and diatoxanthin. The amount of chl degradation products (e.g., chlorophyllides, phaeophytins, and phaeophorbides) was limited whatever the season studied. In summer, some traces of lutein and chl *b* indicated the presence of some chlorophytes (data not shown).

TABLE 1. Mean values ($n = 6-7$, start–end) of the physicochemical characteristics of the study site during the two colonization periods: incident light (PAR), water temperature (T), pH, conductivity (C), dissolved oxygen (O_2), nitrate (NO_3^-), total phosphorus (Total P), and dissolved organic carbon (DOC).

	Colonization period	
	May–July	Sept.–Nov.
PAR ($\text{mol photons} \cdot \text{m}^{-2} \cdot \text{d}^{-1}$)	31 (29–40)	11 (27–9)
T ($^{\circ}\text{C}$)	20 (12–24)	14 (20–13)
pH	7.9 (8.2–7.9)	7.9 (7.7–8.2)
C ($\mu\text{S} \cdot \text{cm}^{-1}$)	569 (577–475)	611 (492–643)
O_2 (% sat)	110 (104–137)	84 (91–65)
NO_3^- ($\text{mg} \cdot \text{L}^{-1}$)	7 (20–3)	10 (7–12)
Total P ($\text{mg} \cdot \text{L}^{-1}$)	0.1 (–)	0.1 (–)
DOC ($\text{mg} \cdot \text{L}^{-1}$)	3.4 (3.7–3.4)	2.9 (3.6–2.3)

Among all the photosynthetic pigments identified, chl *a* was the most abundant, and its amount per cell was around 2.5 times higher in the communities collected in November than in those collected in July (Table 2). Furthermore, β -carotene and pigments related to the xanthophyll cycle (DD + DT) were significantly more concentrated (in moles per 100 moles of chl *a*) in July than in November as opposed to fucoxanthin.

In July, the biofilms collected after 2 weeks of colonization (2w-biofilms) presented lower chl *a* cell content and fucoxanthin concentrations than the 6w-biofilms, whereas the opposite was true for β -carotene (Table 3). DD and DT concentrations did not vary significantly. In November, all the studied pigment proportions did not vary significantly between development stages.

Biomass indicators: At each season, both biomass indicators (chl *a* and cell density) increased with

TABLE 2. Mean values (\pm CI 95%, $n = 9$) of chl *a* cell content ($10^{-6} \mu\text{g chl } a \cdot \text{cell}^{-1}$) and pigment ratio (moles per 100 mol of chl *a*) at dawn for pooled communities collected in July and November.

Season	July	November
Chl <i>a</i> **	4.4 ± 0.9	8.2 ± 2.3
Chl <i>c</i>	na	22.1 ± 1.6
Fucoxanthin***	59.0 ± 7.8	86.6 ± 5.1
β -carotene**	7.3 ± 2.3	3.5 ± 0.4
DD+DT***	9.5 ± 0.7	5.4 ± 0.4

DD, diadinoxanthin; DT, diatoxanthin; na, data not available.

For each ratio, a significant seasonal difference is indicated as ** ($P < 0.01$) or *** ($P < 0.001$).

TABLE 3. Mean values (\pm CI 95%, $n = 3$) of chl *a* cell content ($10^{-6} \mu\text{g chl } a \cdot \text{cell}^{-1}$) and pigment ratio (moles per 100 mol of chl *a*) for communities collected at different development stages at dawn in July and November.

Development stage (w) (July)	Development stage (w) (November)		
	2	4	6
Chl <i>a</i> *	3.1 ± 0.4	5.3 ± 1.9	4.7 ± 0.9
Chl <i>c</i>	na	na	na
Fucoxanthin*	44.2 ± 4.7	67.0 ± 8.1	65.7 ± 3.5
β -carotene**	11.0 ± 1.6	6.4 ± 1.4	4.4 ± 3.9
DD+DT	9.6 ± 0.4	9.7 ± 1.3	9.1 ± 1.8

Development stage (w) (November)	Development stage (w) (November)		
	3	5	7
Chl <i>a</i>	9.7 ± 4.8	9.4 ± 3.4	5.4 ± 2.3
Chl <i>c</i>	24.1 ± 3.1	21.7 ± 0.6	20.6 ± 3.0
Fucoxanthin	85.8 ± 14.0	90.3 ± 5.6	83.6 ± 5.7
β -carotene	3.7 ± 0.9	3.0 ± 0.1	3.8 ± 1.0
DD+DT	5.9 ± 0.3	5.0 ± 0.7	5.2 ± 0.3

DD, diadinoxanthin; DT, diatoxanthin; na, data not available.

For each ratio, significant differences between development stages are indicated as * ($P < 0.05$) or ** ($P < 0.01$).

community age (Fig. 1). There was a significant seasonal pattern as the diatom density increased faster in July: biofilms collected after 6 weeks in July were more dense ($1.42 \times 10^6 \pm 0.07 \times 10^6$ cells \cdot cm $^{-2}$) than after 7 weeks in November ($1.13 \times 10^6 \pm 0.05 \times 10^6$ cells \cdot cm $^{-2}$). In the mean time, the amount of chl *a* per surface unit reached comparable values in July (5.6 ± 0.8 μ g chl *a* \cdot cm $^{-2}$) and November (5.4 ± 1.1 μ g chl *a* \cdot cm $^{-2}$) at the end of the colonization period.

Taxonomic composition: Taxonomic composition of the diatom communities collected was assessed by calculating the relative abundances of the eight main species (i.e., the species reaching a mean relative abundance >10% in at least one of the sampled communities) (Fig. 2). These results underline a clear seasonal pattern in the species composition. It appears that the July samples were dominated by *Pseudostaurosira brevistriata* (Grunow) D. M. Williams et Round (PSBR) and *Staurosira construens* var. *binodis* (Ehrenb.) P. B. Ham. (SCBI). Conversely, the samples collected in November were discriminated by the dominant *Achnantheidium minutissimum* (Kütz.) Czarn. (ADMI) and *Gomphonema parvulum* (Kütz.) Van Heurck (GPAR), but also *Nitzschia dissipata* (Kütz.) Grunow (NDIS) and the large species *Cocconeis placentula* Ehrenb. (CPLA). At each season, the communities can be separated according to their development stage. In July, 2w-biofilms were characterized by the dominance of *Nitzschia fonticola* (Grunow) Grunow (NFON, 31%), whereas 6w-biofilms presented the highest proportions of *P. brevistriata* and *S. construens* var. *binodis* (15%, $P < 0.05$). In November, the significant increase in *N. fonticola* proportions observed throughout colonization was compensated by a significant decrease in *N. dissipata* and *G. parvulum* ($P < 0.05$). This shift in the taxonomic composition during the colonization period led to a significant increase of the less abundant (<10%) but larger species (i.e., species whose theoretical biovolume >500 μ m 3), such as *C. placentula*,

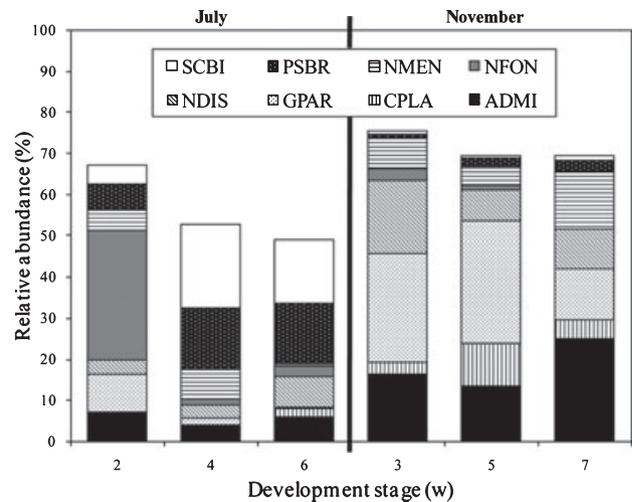


FIG. 2. Mean relative abundances ($n = 3$) of eight predominant species (>10% in at least one replicate) within diatom communities collected in July (after 2, 4, and 6 weeks of colonization) and in November (after 3, 5, and 7 weeks). ADMI, *Achnantheidium minutissimum*; CPLA, *Cocconeis placentula*; GPAR, *Gomphonema parvulum*; NDIS, *Nitzschia dissipata*; NFON, *Nitzschia fonticola*; NMEN, *Navicula menisculus*; PSBR, *Pseudostaurosira brevistriata*; SCBI, *Staurosira construens* var. *binodis*.

Amphora copulata (Kütz.) Schoeman et R. E. M. Archibald, and *Gyrosigma sciotense* (Sullivan et Wormley) Cleve over the smaller ones, whatever the season ($P < 0.05$, data not shown).

Changes in photochemical behavior over the morning. *Fluorescence parameters* (F_v/F_m , Φ_{PSII} and NPQ): The fluorescence parameters F_v/F_m , Φ_{PSII} , and NPQ regularly measured during the morning from dawn to zenith for the different communities studied varied significantly with ambient irradiance, whatever the season. Communities were exposed to a daily average PAR of 40 mol photons \cdot m $^{-2}$ in July as opposed to 6 mol photons \cdot m $^{-2}$ in November; each day of experimental measurements was thus representative of the studied season (Fig. 3). For both seasons, the irradiance gradually increased over the

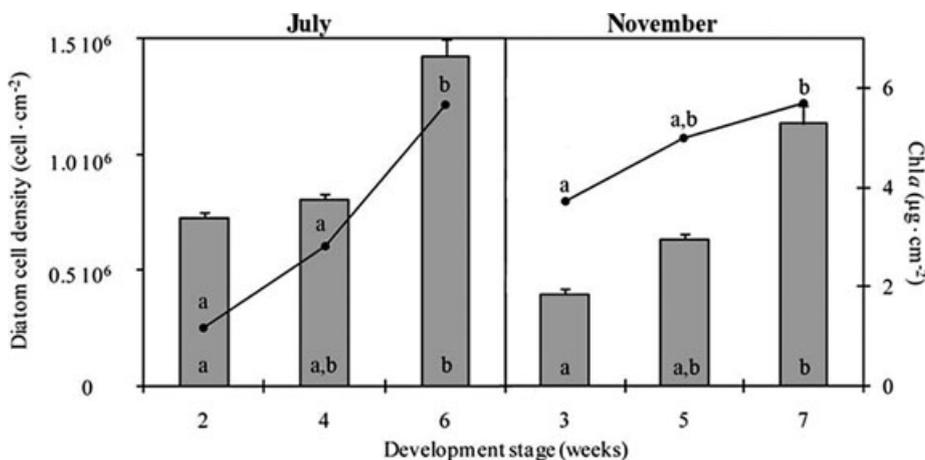


FIG. 1. Mean values ($n = 3$) of the diatom cell density (bars + 95% CI, left axis) and the amount of chl *a* per surface unit (solid line, CI not represented for clarity, right axis) during the colonization period in July and November. Letters indicate homogeneous statistical groups ($P > 0.05$).

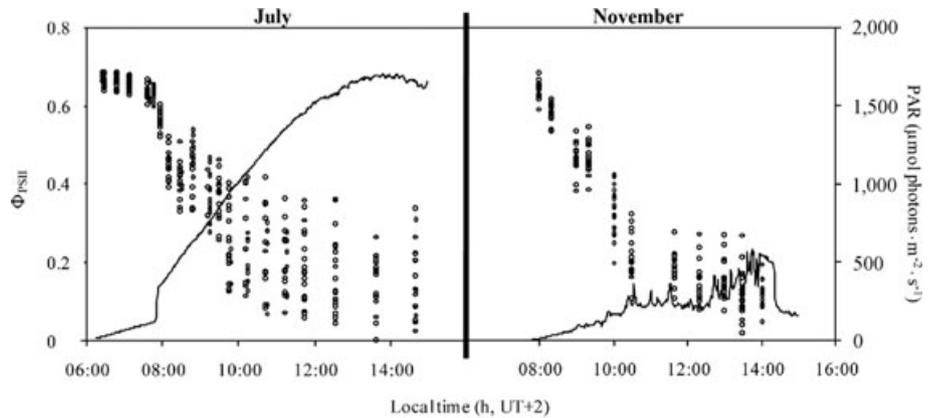


FIG. 3. Time course of Φ_{PSII} values (data pooled, open circles) and incident light (PAR, solid line) measured during the morning in July and November.

morning; the experiments were, however, performed mostly under clear skies in July, whereas the weather was cloudier in November, and therefore the maximum values were $\sim 1,700 \mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ at 14:00 h (UT + 2) and only $600 \mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ at 14:00 h, respectively). Despite the quite different range of light intensities encountered by the communities, the variation in Φ_{PSII} was comparable between seasons (Fig. 3). It dropped from 0.67 ± 0.01 at dawn to 0.14 ± 0.04 at the end of the experiment in July and from 0.64 ± 0.01 to 0.11 ± 0.02 in November (or 79% and 83% of inhibition, respectively, Fig. 4). However, the decrease in F_v/F_m was more pronounced in July (from 0.65 ± 0.05 to 0.20 ± 0.10 or 69% of inhibition) than in November (from 0.70 ± 0.01 to 0.38 ± 0.03 or 46% of inhibition, Fig. 4). NPQ showed an opposite pattern between dawn and zenith with a similar increase between seasons from 0.3 ± 0.1 to 4.0 ± 0.9 in July and from 0.2 ± 0.1 to 2.2 ± 0.4 in November (both ~ 11 -fold, Fig. 4).

In summer, some differences in the community response to the light variation were observed between development stages (2, 4, and 6 weeks), indicating that the 2w-biofilms were more affected by high-light stress (i.e., higher decrease in F_v/F_m and Φ_{PSII} and higher increase of NPQ, Table 4).

Furthermore, the absolute values of F_v/F_m , Φ_{PSII} (and NPQ) estimated at dawn were lower (and higher, respectively) in young (2w) than in old (6w) biofilms. In November, there were no significant differences between samples collected after 3, 5, and 7 weeks of colonization.

Composite P-I curves. The I_k and $rETR_m$ values were significantly higher in July than in November, whereas the α_I values show an opposite pattern (Table 5). In July, I_k and $rETR_m$ increased with development stage, whereas differences between α_I values were not significant (Table 6). In comparison, the photosynthetic parameters calculated in November were relatively constant in all the studied communities (Table 6). According to β_I values, photo-inhibition was significant only in the 2w-biofilms in July (Fig. 5 and Table 6).

Xanthophyll cycle. Whereas the amount of chl *a*, chl *c*, fucoxanthin, and β -carotene remained fixed during the morning (data not shown), the amounts of DD and DT fluctuated. Figure 6 illustrates the time course of the de-epoxydation ratio (DR) of diadinoxanthin into diatoxanthin measured at dawn, around midmorning, and at zenith. It increased significantly with irradiance ($P < 0.01$) for each development stage, and this effect was more pronounced in young biofilms. Nevertheless, differences between

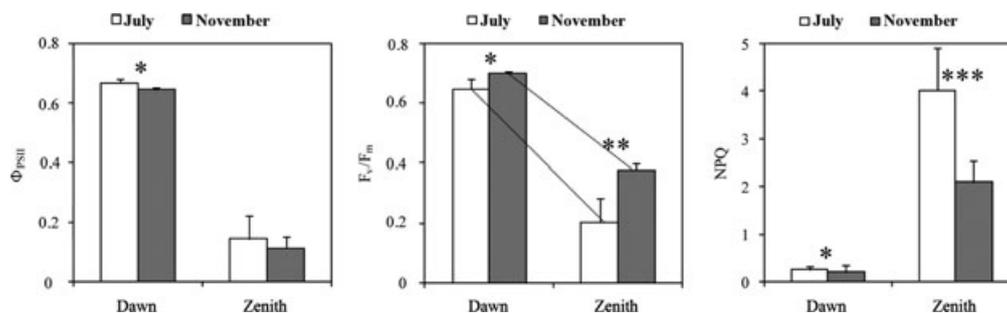


FIG. 4. Mean values (+ 95% CI) of F_v/F_m ($n = 9$), Φ_{PSII} ($n = 18$), and NPQ ($n = 18$) measured at dawn and zenith on communities collected in July (open bars) and November (black bars). At each time of measurement, a significant seasonal difference is indicated as * ($P < 0.05$) or ** ($P < 0.01$). Dashed lines indicate a significant seasonal difference in the variation of values between dawn and zenith ($P < 0.01$). NPQ, nonphotochemical quenching.

TABLE 4. Mean values (\pm 95% CI, $n = 3-6$) of the fluorescence parameters estimated at dawn and at zenith and corresponding variations (%) for communities collected at different development stages in July and November.

Development stage (w) (July)	2	4	6
F_v/F_m			
Dawn*	0.63 \pm 0.04	0.62 \pm 0.09	0.69 \pm 0.01
Zenith*	0.11 \pm 0.04	0.21 \pm 0.13	0.29 \pm 0.10
%*	83 \pm 4	69 \pm 14	58 \pm 11
Φ_{PSII}			
Dawn*	0.66 \pm 0.01	0.66 \pm 0.01	0.68 \pm 0.01
Zenith***	0.05 \pm 0.04	0.17 \pm 0.05	0.20 \pm 0.01
%***	92 \pm 6	74 \pm 7	70 \pm 2
NPQ			
Dawn	0.3 \pm 0.1	0.2 \pm 0.1	0.3 \pm 0.0
Zenith***	6.2 \pm 0.9	3.8 \pm 0.6	2.1 \pm 0.3
%**	95 \pm 2	95 \pm 4	86 \pm 3
Development stage (w) (November)	3	5	7
F_v/F_m			
Dawn*	0.68 \pm 0.01	0.70 \pm 0.02	0.71 \pm 0.02
Zenith	0.36 \pm 0.02	0.33 \pm 0.15	0.37 \pm 0.05
%	47 \pm 2	53 \pm 22	47 \pm 3
Φ_{PSII}			
Dawn	0.65 \pm 0.02	0.64 \pm 0.01	0.65 \pm 0.02
Zenith	0.11 \pm 0.04	0.11 \pm 0.02	0.11 \pm 0.02
%	82 \pm 6	83 \pm 3	83 \pm 4
NPQ			
Dawn	0.5 \pm 0.4	0.1 \pm 0.0	0.1 \pm 0.1
Zenith	2.6 \pm 1.1	2.2 \pm 0.4	1.9 \pm 0.6
%	89 \pm 7	95 \pm 3	95 \pm 4

At each season and for each parameter, significant differences between development stages are indicated as * ($P < 0.05$), ** ($P < 0.01$), or *** ($P < 0.001$).

biofilms were significant only at midmorning in July ($P < 0.05$) and at zenith in November ($P < 0.01$).

DISCUSSION

Photoacclimation. The course of the monitored fluorescence parameters was correlated with ambient irradiance. Therefore, the communities demonstrated relative plasticity in their photobiology in response to the large variation in light encountered by the communities during the experiment (up to

TABLE 5. Mean values (\pm CI 95%, $n = 18$) of the characteristic photosynthetic parameters α_I , rETR_m, and I_k ($\mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$) derived from the model of Eilers and Peeters (1988) for pooled communities collected in July and November.

Season	July	November
α_I **	0.31 \pm 0.03	0.42 \pm 0.06
I_k ***	520 \pm 103	75 \pm 13
rETR _m ***	149 \pm 24	29 \pm 3

For each parameter, a significant seasonal difference is indicated as ** ($P < 0.01$) or *** ($P < 0.001$).

TABLE 6. Mean values (\pm 95% CI, $n = 6$) of the characteristic photosynthetic parameters α_I , rETR_m and I_k ($\mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$) derived from the model of Eilers and Peeters (1988) and β_I ($n = 3-6$) derived from the model of Platt et al. (1980) for communities collected at different development stages in July and November.

Development stage (w) (July)	2	4	6
α_I	0.31 \pm 0.01	0.30 \pm 0.06	0.29 \pm 0.04
I_k *	352 \pm 17	453 \pm 221	670 \pm 153
rETR _m **	108 \pm 5	147 \pm 44	191 \pm 30
β_I *	0.08 \pm 0.03	0.06 \pm 0.10	-0.03 \pm 0.02
Development stage (w) (November)	3	5	7
α_I	0.38 \pm 0.08	0.48 \pm 0.13	0.38 \pm 0.05
I_k	75 \pm 12	60 \pm 2	92 \pm 28
rETR _m	28 \pm 2	26 \pm 3	35 \pm 9
β_I	0.02 \pm 0.04	0.01 \pm 0.03	0.02 \pm 0.03

For each parameter, significant differences between development stages are indicated as * ($P < 0.05$) or ** ($P < 0.01$).

1,700 or 500 $\mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ in July and November, respectively).

The F_v/F_m ratio can be used to evaluate the effect of environmental factors (e.g., light) on the physiological state of plants and algae (Maxwell and Johnson 2000). The values obtained at the beginning of our experiments were ~ 0.70 , indicating that the algae were photochemically efficient. Except for the 2w-biofilms in July, our results show that microalgae were able to cope with ambient light and support the hypothesis that photoinhibition was limited at the community level. First, although the reduction in Φ_{PSII} was pronounced (up to 92% in November) under saturating light, a decrease in photosynthetic capacity (rETR) was not observed, even at maximal irradiance. This reduction was essentially due to the drop in F_m' , which could be the result of the concomitant NPQ increase. Second, the analysis of the pigment content did not reveal the presence of chl degradation products in relation to excessive photooxidative damages (Long et al. 1994).

The increase in NPQ over the morning could be correlated with the de-epoxydation of diadinoxanthin into diatoxanthin, similar to what has been described in higher plants and some algae with the violaxanthin-zeaxanthin cycle (Demmig-Adams and Adams 1992, Lohr and Wilhelm 1999, Ruban et al. 2004). This photoprotective mechanism might help diatoms to respond within minutes to an increase in light by dissipating energy in excess as thermal radiations (Olaizola et al. 1994, Kashino and Kudoh 2003, Lavaud et al. 2004).

Along with this physiological process but operating on a different timescale (from seconds to minutes), some diatoms (e.g., *Navicula* and *Nitzschia* species) may have exhibited down micromigration

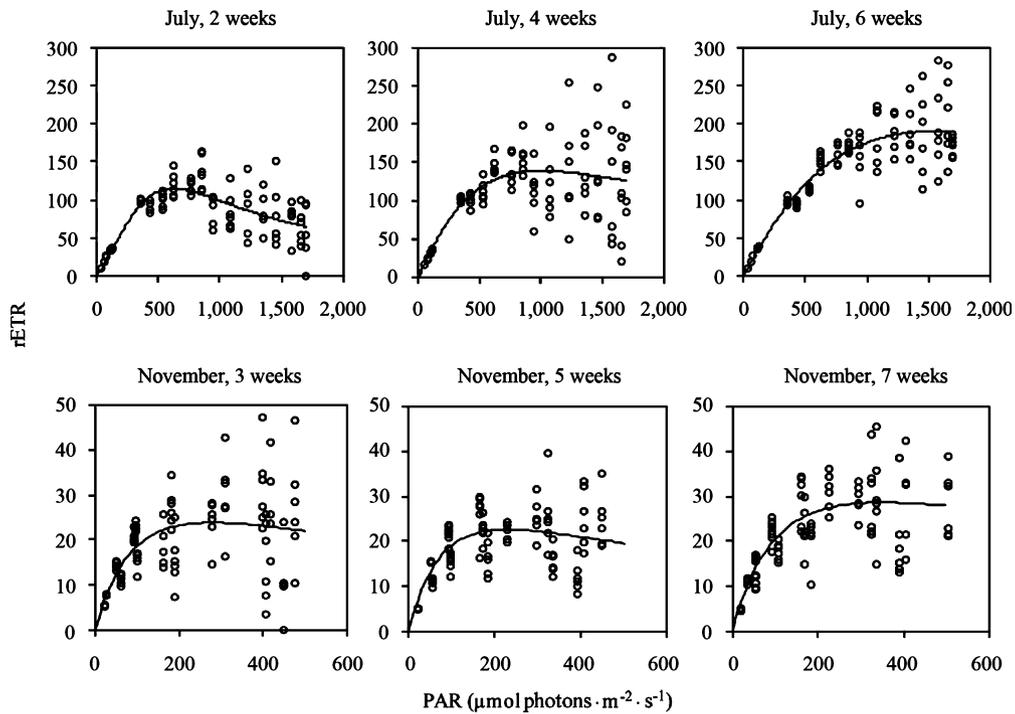
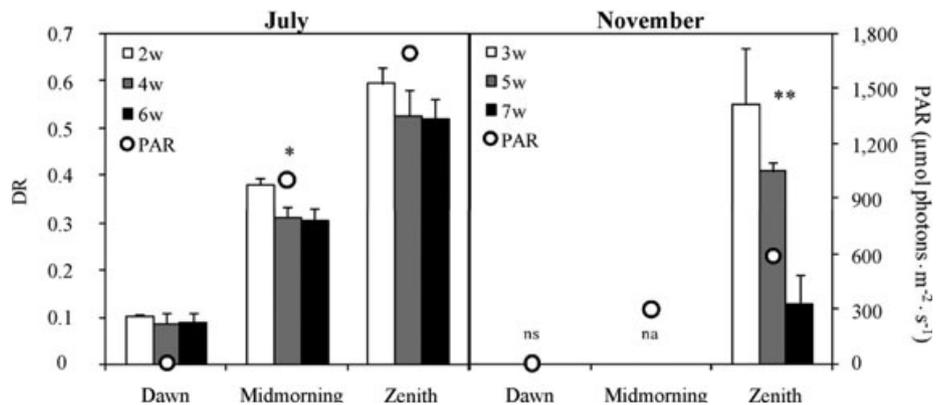


FIG. 5. Mean composite *P-I* curves (solid lines) obtained by fitting the model of Eilers and Peeters (1988) to each replicate of rETR measurements (open circles) carried out simultaneously with increasing incident irradiance for the different communities collected in July and November. rETR, relative electron transport rate.

FIG. 6. Time course of ambient light (PAR, $\mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$) and the de-epoxydation ratio (mean DR + 95% CI, $n = 3$) of diadinoxanthin into diatoxanthin for communities collected in July and November. At each time of measurement, significant differences between development stages are indicated as * ($P < 0.05$) or ** ($P < 0.01$). na: data not available.



in the matrix to a depth where light is attenuated (see the following section).

Effect of biofilm thickness. In July, the collected communities evolved from homogeneous and thin (<1 mm) 2w-biofilms to complex three-dimensional structured 6w-biofilms. This colonization pattern involved biomass accumulation and species succession (as already shown, e.g., Hoagland et al. 1982, McCormick and Stevenson 1991, Tuji 2000). This change in the vertical structure was linked to variation in the photochemical behavior at the whole community level. Even if the shift in the taxonomic composition may be involved, an additional hypothesis can be put forward to explain why light was a more stressful factor for the 2w- than for the 6w-bio-

films. This finding implies that our measurements integrate the contribution of several layers of cells, each layer displaying physiological as well as morphological adaptations (or acclimations) to their light microenvironment like a terrestrial plant community in a forest habitat, but within vertical distances of only a few millimeters (Hudon et al. 1987). Indeed, light can be attenuated very quickly deeper within the matrix (Meulemans 1987, Dodds 1992, Vermaat and Hootsmans 1994), particularly in dense mats such as the biofilms studied ($>10^6$ cells $\cdot \text{cm}^{-2}$). Assuming that microalgal cells at the biofilm surface and in the understory can be characterized as either sun or shade organisms, it has been suggested that photoinhibition may have occurred

at the surface of the biofilm (especially at maximal irradiance in summer) and be hidden by shaded subsurface cells (Sand-Jensen and Revsbech 1987, Dodds et al. 1999). As mat thickness increased with community age, the contribution of the shaded subsurface layers to the overall response should have been more important in older communities. Moreover, the development of larger species in late succession phases, associated with a probable change in the shape and orientation of cells, may have played a role (Hoagland et al. 1982). The observed increase in cellular chl illustrates how the underlayer cells have compensated for lower light intensities (Hudon et al. 1987, Falkowski and Laroche 1991). This trend is confirmed when considering the pigment:chl *a* ratio evolution, with the increase in fucoxanthin, an accessory light-harvesting pigment, and the decline of the photoprotective β -carotene (Siefermann-Harms 1987, MacIntyre et al. 2002) in the 6w-biofilms.

The pattern in the *P-I* parameters is consistent with this shading-effect hypothesis. Note that the three types of biofilm were collected simultaneously; therefore, cells that had developed at the top of the biofilm experienced the same light history for the last 2 weeks of the colonization period. Since only the uppermost layers of cells were stimulated by light at low irradiances (i.e., shortly after dawn), the fact that the values of α_I were comparable among development stages is not surprising. Later in the morning (i.e., at higher irradiance), light penetrated further within the matrix, and the deeper layers of cells became photosynthetically active. As the community aged, increasing numbers of shaded layers may have contributed to the observed increase in the I_k and $rETR_m$ values, which resulted in an apparent decrease in photoinhibition at the community level (Hill and Boston 1991, Roberts et al. 2004). Except for α_I (due to methodological reasons), these findings are in agreement with other studies (Hill and Boston 1991, Roberts et al. 2004).

The communities were exposed to a light range three times lower in November than in July, thereby limiting the shading effect, even at zenith, since no difference was observed in the photosynthetic parameters between development stages. When considering pigment analysis, which concerns the whole community, the contribution of this reduced euphotic zone may have been hidden by the other layers. Whereas the *P-I* curves integrate all the measurements carried out over the morning, the samples collected for pigment analysis give an instantaneous picture of the biofilm photochemistry. As mentioned above, diatoms can very quickly activate the diadinoxanthin de-epoxydation (e.g., Arsalane et al. 1994). Associated with the fact that ambient irradiance was highly variable due to the occurrence of clouds, this can explain why the DR values obtained at zenith were different between development stages. The cloudiness is also reflected in the heter-

ogeneity observed in the fluorescence measurements (e.g., $rETR$).

Photoadaptation. The higher irradiance observed in July could explain why the values of F_v/F_m were lower in summer, whatever the time of measurement. This trend is reinforced by the analysis of *P-I* parameters (lower values of α_I ; higher I_k and $rETR_m$ in July) and pigment profiles (lower cellular chl *a*; higher pool size of the photoprotective pigments β -carotene, DD and DT in July), suggesting that communities collected in July were adapted to a higher light environment in comparison to those studied in November. This is in agreement with previous studies linking contrast in light history to the variation in *P-I* relationships of communities originated from open and shaded sites (Boston and Hill 1991, Hill et al. 1995, Guasch and Sabater 1998, Hill and Dimick 2002, Roberts et al. 2004). But the role of temperature should not be discarded as its seasonal variation is correlated with light history. In particular, the low temperature in November (10°C as opposed to 22°C in July) could slow down the rate of metabolic reactions, therefore contributing to lower the I_k (<100 $\mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ as opposed to values >300 $\mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ in July), the limit of light saturation (Davison 1991). Besides, this seasonal pattern in photosynthetic capacity coincides with a shift in the species composition (Guasch et al. 1995). The independent effects of light and temperature on photosynthesis, population growth, or community structure of periphyton have been described by many authors (DeNicola 1996, Hill 1996, Kiffney et al. 2004, Rier et al. 2006), whereas very few experimental studies have focused on their interactive influence (Bothwell 1988, Vermaat and Hootsmans 1994, Vermaat 2005). Higher light and temperature conditions prevailing during colonization could have selected for both photophilic and thermophilic taxa in the communities collected in July (DeNicola 1996, Hill 1996). In addition, some traces of lutein and chl *b* detected in the July communities indicate the presence of filamentous chlorophytes or sedimented phytoplanktonic cells. Green algae, which are known to be more efficient in light utilization in summer conditions (DeNicola 1996, Hill 1996), could have influenced the photochemical behavior at the whole community level, even if it probably should not account for much.

CONCLUSION

In this work, photosynthetic performances of intact natural periphyton communities were assessed, taking into account the natural irradiance variation. Differences between biofilms were observed and attributed to adaptation to light history experienced throughout colonization. At each season, selected microalgae were able to acclimate to temporary changes in photon flux density by

developing photoprotective mechanisms, such as an effective xanthophyll cycle. Finally, this work confirms with measurements in field conditions that photoinhibition rarely occurs in periphyton at the community level and that thickness is relevant to this resistance to high-light stress.

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