

A comparison of specific growth rates of periphytic diatoms of varying cell size under laboratory and field conditions

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Abstract Diatom species grown under non-limiting nutrient availability in multispecific biofilms were sampled from glass substrates immersed in the field and in experimental freshwater microcosms, and their growth responses were determined. The major species that developed on the substrates were common to both experiments and the specific growth rates (k) ranged from 0.06 to 0.41 division day⁻¹. An inverse relationship between k and cell sizes was observed, which is in accordance with allometry results reported by several authors. Although found in lower amounts, the large, slow-growing species accounted significantly in total community biovolume, underlying their significance for ecological purposes. From growth characteristics data, life history strategies were drawn for the dominant species recorded, from small pioneer species to large taxa that are more favoured by high resource supply. Kinetic data measured in both laboratory and in situ experiments stressed that the difficulties to mimic the field in laboratory experiments may have a strong impact on growth kinetics.

Keywords Periphytic diatoms · Growth rates · Cell size · Life history strategies · Field study · Freshwater microcosms

Introduction

The structure of microalgal communities plays an important role in determining the trophic interactions within aquatic food webs (from autotrophic production to zooplankton feeding), and several models have been proposed to establish cell size/algal growth rate relationships in phytoplankton assemblages. The concept of relative specific growth rates was originally used for marine phytoplankton (Goldman, 1980) and applied by Bothwell (1985, 1989) to freshwater periphytic diatom communities. Surveys by Banse (1982), Tang (1995) and Kagami & Urabe (2001), using cell biovolume as a measure of size, found a uniform distribution of phytoplankton biomass among logarithmically increasing size classes. These allometric studies led to the commonly accepted conclusion that big diatom species have longer cell cycles than smaller ones.

Benthic diatom communities, which are important primary producers in streams, are composed of a large number of species with various ecological preferences (Lange-Bertalot, 1979; Steinberg & Schiefele, 1988; van Dam et al., 1994), which confer to these organisms a high relevance among biological

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indicators. Growth responses of freshwater diatoms are mainly used for determination of ecological optima (Baars, 1983; Suzuki & Takahashi, 1995), for algal toxicity assays (Conway & Williams, 1979; Schmitt-Jansen & Altenburger, 2005) and for the evaluation of environmental conditions (Biggs, 1990; Admiraal et al., 2000). Species-specific studies of growth responses have already been performed on monocultures of diatoms; however, little can be said about their significance for natural multispecific populations. Extrapolating responses from one species of diatom to another, or even from species-by-species studies, can therefore be misleading, due to interactive effects in the whole environment (Tilman, 1977; Tilman et al., 1981, 1986; van Donk & Kilham, 1990; Wood & Leatham, 1992; Descamps-Julien & Gonzalez, 2005). Specific growth rates, which vary among species depending on individuals' sizes, may thus also be enhanced by enriching elements or reduced by competitive exclusion between organisms or contaminations with toxicants.

This study attempts to elucidate the relationships between the integrated metabolic parameter growth

rate of many diatom species grown in multispecific cultures and their specific biovolume, with regards to life history strategies sensu Grime (1977). Here, we investigated the contributions of both laboratory and in situ relative specific growth rates.

Materials and methods

As part of a wider evaluation of periphyton in the industrial watershed of Decazeville (see Morin et al., 2008a), data for this study were collected from a site in the Riou-Mort stream, South West France (44° N/ 2° E), placed upstream of the metal discharge from a small tributary carrying seepage from a former zinc factory (Fig. 1). The growth rate experiments using artificial substrate samplers were carried out in March 2005 over a 20-day period. The survey was complemented with a laboratory assay conducted in March 2006, the experimental systems being inoculated with natural biofilm taken from the same site. Periphytic communities in both studies were composed of thin biofilms dominated by diatoms.

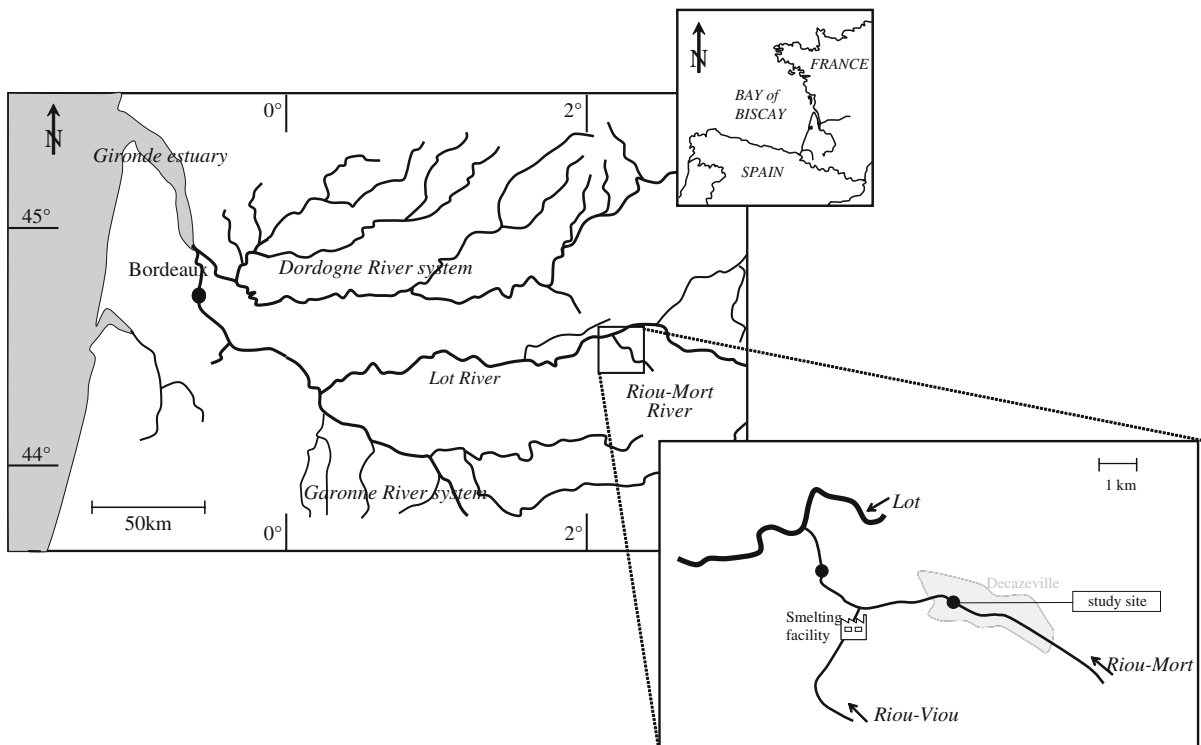


Fig. 1 Location of the study site

Field sampling

Two caged, artificial samplers loaded with six vertically aligned glass slides (see Morin et al., 2007) were installed at each site for algal collection, at a depth of about 10 cm below the surface. Water velocity and light irradiance were standardized as closely as possible because of their potential influence on growth rates.

The samples (three replicate slides per sampling date) were removed after 4, 7, 14 and 20 days for processing. On each sampling occasion, temperature, pH, conductivity and dissolved oxygen were measured in the stream (WTW, Weilheim, Germany), and stream water samples were collected simultaneously and brought back to the laboratory for nutrient measurements according to French and international standards (NF T90-023, NF EN ISO 11732 and NF EN ISO 13395).

Laboratory assays

Diatoms sampled on glass slides immersed for 4 weeks in the Riou-Mort river (March 2006) were inoculated into freshwater microcosms (Gold et al., 2003; Morin et al., 2008b). Among the 66 species (representing 27 genera), the dominant taxa were *Navicula lanceolata* (Agardh) Ehrenberg (35.8%), *N. gregaria* Donkin (11.1%), *Planothidium lanceolatum* (Brébisson ex Kützing) Lange-Bertalot (9.3%), *Surirella brebissonii* Krammer & Lange-Bertalot (5.2%) and a certain number of *Gomphonema* spp. (*G. micropus* Kützing, *G. parvulum* [Kützing] Kützing, *G. olivaceum* [Hornemann] Brébisson; 9.0%) and *Nitzschia* spp. (*N. dissipata* [Kützing] Grunow, *N. palea* [Kützing] W. Smith, *N. linearis* var. *linearis* [Agardh] W.M. Smith; 5.3%).

In the experimental microcosms, the periphytic algae were allowed to grow on similar glass slides as those used in the field to maintain their benthic growth features and collected after a colonization duration of 1, 2, 4 and 6 weeks. The culture medium used for the experiment was a fourfold diluted WC medium (Guillard & Lorenzen, 1972), without EDTA and supplemented with silica as proposed by Gold et al. (2003). This ensured nutrient (C, H, N, O, P, S) as well as trace element (Mn, B, Cu, Fe, Cl, Co, Mo, Zn) and vitamin (thiamine, biotin, cobalamin) supply. Cultures were grown in triplicate and allowed to

settle on the slides in the experimental units placed in an air-conditioned room where temperatures were kept constant, illuminated from above by fluorescent tubes (Claude Standard 18 W 10/54/154, Sylvania Lighting International, France) giving an average photon flux density of $70 \mu\text{mol s}^{-1} \text{m}^{-2}$ (Quantum sensor LI-COR LI-192SA, Lincoln, NE, USA) with a 10:14 light–dark cycle. The systems were under continuous water movement (approximate speed: 0.4 cm s^{-1}), induced by the load difference between the height of the intermediate column feeding the systems and the three artificial streams.

At each sampling date, physical and chemical parameters in the microcosms were determined according to the procedures used for field assays. Depending on the results of water analyses, culture medium was added as required to compensate for the decrease of nutrient concentrations due to algal uptake.

Diatom counts and identification

Periphyton film was removed from the artificial substrates by scraping with a cutter blade and diluted to a standard volume of 100 ml to form three replicate periphyton samples per site and per sampling date. Suspensions of the material were subsampled for various analyses. Diatom cell density was estimated for each replicate sample by counting the total number of cells exhibiting chloroplasts in 10 fields ($1.25 \mu\text{l}$ each, 0.5 mm depth) of a Nageotte counting chamber, using light microscopy at $400\times$ magnification (photomicroscope Leica DMRB, Wetzlar, Germany). Data were recorded as cells per unit area of sampled substrate (number of cells cm^{-2}).

Aliquots assigned to taxonomic analysis of diatom assemblages were prepared according to the procedure described in ANSP protocols (Charles et al., 2002). After digestion of the formalin-fixed sample in boiling hydrogen peroxide (30% H_2O_2) and hydrochloric acid (35%), permanent slides were prepared by mounting the cleaned diatom frustules on a glass microscope slide in Naphrax[®] (Brunel Microscopes Ltd, UK), a high refractive index (1.74) medium. At least 400 frustules were counted per replicate slide at $1,000\times$ magnification and identified following the Süßwasserflora (Krammer & Lange-Bertalot, 1986–1991) classification and recent nomenclature updates. Specific cell volumes were calculated using a set of

geometric shapes and mathematical equations for biovolume calculations (Hillebrand et al., 1999) from literature linear dimensions.

Absolute abundances of each species, in individuals cm^{-2} , were calculated after total diatom densities and relative abundances of each species identified during taxonomic determinations. These data also allowed the quantitative characterization of diatom communities in terms of biovolume, separating three classes of cell sizes (Morin et al., 2007), and evolution through the duration of the study.

Calculation of specific growth rates

For each experimental condition, growth rates (k , expressed as divisions day^{-1}) and generation—doubling—times (t_d) of the most abundant diatoms from the three replicates were calculated according to Guillard (1973). Growth rate differences between the diatoms collected over the two experiments were investigated among the species found in all the samples and representing at least 2% of the relative abundances.

The number of divisions per day was calculated from the slope of the linear portion of a curve showing log cell number as a function of time: $k = \frac{\ln(x_2) - \ln(x_1)}{d_2 - d_1}$, where x_1 is the cell number at day d_1 (cell cm^{-2}) and x_2 the cell number at day d_2 (cell cm^{-2}). In this study, instead of the two countings needed, three or four dates (depending on the results obtained for k) were sampled during the exponential growth phase, and regression coefficients

were calculated by regression analysis of the logarithm of the cell counts against the time in days, in order to increase the precision of the results. From k , generation times (in days) were computed by $t_d = \frac{\ln(2)}{k}$.

Correlations between growth rate and cell sizes were analyzed using linear regressions (MS-Excel) performed on \log_{10} -transformed biovolume data. T -statistics were used to test the regression coefficient (R^2) for significance, at the 0.01 α -level.

Results

Laboratory and field colonization conditions

The physical and chemical parameters measured in the water samples for both experiments are shown in Table 1.

pH (7.7 ± 0.1) and oxygen saturation ($>75\%$) were very similar between treatments. The temperature imposed in the microcosms was quite stable ($18.8 \pm 0.0^\circ\text{C}$) but fluctuated in the field from 2.7 to 13.9°C during the experimental period. Lower nutrient concentrations were generally measured in the field but were still high enough not to be limiting for diatom development. The conductivities were very different between experiments. In laboratory microcosms, values were around $170 \mu\text{S cm}^{-1}$; whereas mean values exceeding $2,000 \mu\text{S cm}^{-1}$ were recorded in the field study, indicating greater overall mineral content in the stream attributed to wastewater

Table 1 Summary of physical and chemical data of the water for the microcosm and field studies (SE: standard errors)

		Laboratory experiment		Field study	
pH	Mean \pm SE	7.6	± 0.0	7.7	± 0.1
Temperature ($^\circ\text{C}$)	Mean \pm SE	18.8	± 0.0	7.7	± 2.2
Oxygen saturation (%)	Mean \pm SE	94.5	± 0.5	76.5	± 4.9
Conductivity ($\mu\text{S cm}^{-1}$)	Mean \pm SE	173.2	± 2.8	2546.0	± 533.1
Nitrate (mg l^{-1})	Mean \pm SE	25.0	± 1.9	4.8	± 0.3
Nitrite (mg l^{-1})	Mean \pm SE	0.8	± 0.5	0.3	± 0.1
Ammonium (mg l^{-1})	Mean \pm SE	0.1	± 0.0	1.2	± 0.3
Kjeldahl nitrogen (mg l^{-1})	Mean \pm SE	5.9	± 0.6	2.2	± 0.1
Orthophosphate (mg l^{-1})	Mean \pm SE	1.9	± 0.3	0.3	± 0.0
Irradiance ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	Mean	70		640	
Water velocity (cm s^{-1})	Mean	0.4		~ 1.5 (15) ^a	

^a In situ current velocity is given inside the caged samplers and, in brackets, that of the water surface

treatment (e.g. pH adjustment) on the mining site (Audry et al., 2006). Light irradiance reaching the stream surface during the colonization periods was of about $640 \mu\text{mol m}^{-2} \text{s}^{-1}$ in the field in March 2005 and approximately $590 \mu\text{mol m}^{-2} \text{s}^{-1}$ in March 2006 (<http://www.meteofrance.com>).

Growth experiments in indoor microcosms

Global qualitative characterization of diatom communities revealed very similar taxonomic composition between the diatoms inoculated in the microcosms and those sampled at week 1 in the experimental unit, with a Jaccard similarity index of 0.95. Species richness in the systems was of 58 species representing 21 genera, but growth rates were assessed for the 13 main species (i.e. the species reaching a relative abundance $>2\%$ in the communities considered). The communities were characterized by an association of *Navicula gregaria* (21.1%), *N. lanceolata* (15.1%), *Nitzschia palea* (13.3%), *N. dissipata* (9.2%) and *Surirella brebissonii* (6.8%) at week 1, and then most of these species

suffered a significant decrease ($P < 0.05$) in relative abundances, the communities being represented by higher amounts of *Nitzschia palea* (35.6%) and *Navicula(dicta) seminulum* (Grunow) Lange Bertalot (33.6%).

Most of the diatoms reached their exponential phase of growth in the first 3 weeks of the experiment. The specific growth rates (Table 2) ranged from 0.06 to $0.27 \text{ div day}^{-1}$, and generation times were below 3 days for the fastest growing species (*Navicula(dicta) seminulum*) and increased to over 2 weeks for the species *Surirella brebissonii*. Specific growth rates were also strongly correlated to cell biovolume (Fig. 2; $R^2 = 0.53$, $P < 0.01$), expressing size dependence of the growth rates for the diatom species recorded.

The increase in total community biovolume ($1,800\text{--}45,500 \mu\text{m}^3$) calculated at each date was concomitant with an increase in the number of cells (Fig. 3). However, differences in the distribution of species sizes were noticed: small to medium species accounted for the greatest part of the community

Table 2 Individual biovolumes (in μm^3) and growth characteristics (k in div day^{-1} , t_d in days) for species common to both laboratory and field assays

Species	Biovolume	Laboratory experiment			Field study		
		Mean $k \pm \text{SE}$	Mean $t_d \pm \text{SE}$	R^2	Mean $k \pm \text{SE}$	Mean $t_d \pm \text{SE}$	R^2
<i>Navicula(dicta) seminulum</i>	69	0.27 ± 0.01^a	2.57 ± 0.10	0.93	–	–	–
<i>Eolimna minima</i>	88	0.17 ± 0.03^a	4.38 ± 0.74	0.90	–	–	–
<i>Craticula molestiformis</i>	119	0.12 ± 0.00^a	5.97 ± 0.14	0.86	–	–	–
<i>Nitzschia pusilla</i>	185	0.16 ± 0.03^a	4.59 ± 0.96	0.77	0.32 ± 0.04^c	2.21 ± 0.31	0.86
<i>Planothidium frequentissimum</i>	219	0.09 ± 0.02^b	8.00 ± 1.37	0.75	0.31 ± 0.03^b	2.24 ± 0.22	0.98
<i>Navicula veneta</i>	279	0.11 ± 0.00^a	6.59 ± 0.17	0.71	0.35 ± 0.09^c	2.12 ± 0.54	0.94
<i>Nitzschia acicularis</i>	289	–	–	–	0.41 ± 0.06^a	1.74 ± 0.27	0.85
<i>Gomphonema parvulum</i>	326	0.07 ± 0.00^b	9.93 ± 0.37	0.73	0.41 ± 0.04^c	1.71 ± 0.18	0.94
<i>Nitzschia palea</i>	391	0.10 ± 0.02^b	6.96 ± 1.19	0.81	0.33 ± 0.02^a	2.14 ± 0.14	0.97
<i>Planothidium lanceolatum</i>	475	0.11 ± 0.00^a	6.39 ± 0.12	0.96	0.32 ± 0.08^c	2.30 ± 0.59	0.88
<i>Navicula gregaria</i>	485	–	–	–	0.33 ± 0.02^c	2.14 ± 0.14	0.94
<i>Nitzschia capitellata</i>	549	0.10 ± 0.01^a	7.24 ± 1.17	0.85	0.34 ± 0.02^a	2.02 ± 0.10	0.97
<i>Nitzschia dissipata</i>	625	0.06 ± 0.00^a	11.00 ± 0.33	0.85	–	–	–
<i>Navicula lanceolata</i>	1,227	–	–	–	0.26 ± 0.03^b	2.75 ± 0.40	0.84
<i>Surirella angusta</i>	1,315	0.08 ± 0.01^b	8.59 ± 1.31	0.81	–	–	–
<i>Nitzschia linearis</i> var. <i>linearis</i>	1,624	–	–	–	0.30 ± 0.01^b	2.31 ± 0.11	0.77
<i>Nitzschia linearis</i> var. <i>tenuis</i>	1,785	–	–	–	0.36 ± 0.16^a	2.80 ± 1.61	0.74
<i>Surirella brebissonii</i>	2,654	0.06 ± 0.03^a	15.51 ± 8.89	0.72	0.17 ± 0.02^b	4.16 ± 0.56	0.66
<i>Ulnaria ulna</i>	4,724	–	–	–	0.10 ± 0.01^b	7.20 ± 1.11	0.85

Calculations were based upon: ^athe three-first dates, ^bthe four dates, ^cthe last three dates

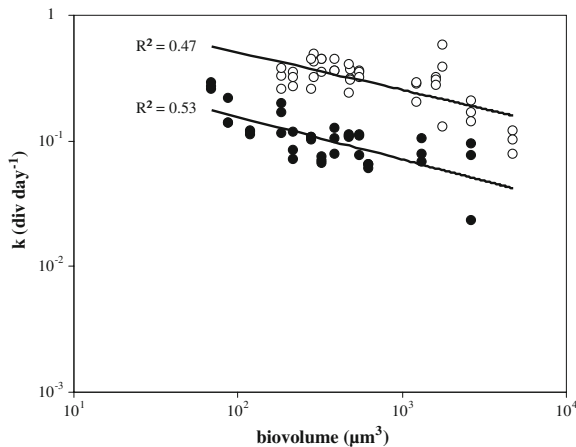


Fig. 2 Relationships between growth rate and cell biovolume (logarithmic sizes) of the diatoms inventoried in the laboratory (●, $k = 0.74\text{vol}^{-0.34}$) and field (○, $k = 2.05\text{vol}^{-0.31}$) experiments

(from more than 60% to almost 95%) in terms of cell numbers, but at least 75% of the total biovolume, of which medium-sized ($100\text{--}500\ \mu\text{m}^3$) accounted for 65%.

Growth experiments in field conditions

Altogether 106 species distributed over 38 genera were recorded in March 2005; however, only 14 taxa were found in relative abundances $>2\%$ of the community and allowed growth rate calculations. In the first dates, diatom assemblages were dominated by *Ulnaria ulna* (Nitzsch) Lange-Bertalot (39.1%), *Nitzschia palea* (15.1%) and *Navicula gregaria* (8.3%), and communities developed after 20 days were characterized by an association of *Nitzschia palea* (30.5%), *Navicula gregaria* (16.7%) *Gomphonema parvulum* (15.8%), *Nitzschia linearis* var. *linearis* (6.0%) and *Ulnaria ulna* (5%).

Comparing the growth of the species from both sites showed a large spectrum of k values varying from 0.10 (*Ulnaria ulna*) to $0.41\ \text{div day}^{-1}$ (*Gomphonema parvulum* and *Nitzschia acicularis* (Kützing) W.M.Smith), that were significantly correlated with the logarithm of the cell size (Fig. 2; $R^2 = 0.47$; $P < 0.01$).

As the number of cells increased (from 700 to almost $23,000\ \text{cells cm}^{-2}$), total community biovolume followed the dynamics of biofilm growth, reaching $16,500\ \mu\text{m}^3$ on the last date (Fig. 3).

Although dominated by medium-sized diatoms, biomass accrual was greatly influenced by large ($>500\ \mu\text{m}^3$) diatoms, representing 20% relative abundances and 60% of the total biomass at the end of the study.

Comparison of field growth rate data with laboratory measurements

Despite the fact that the indoor microcosms and field studies had been conducted in March of two different years, both experiments revealed comparable specific composition of diatom communities, with Jaccard similarity indices (performed on a dataset using the main species) between all the samples reaching at least 80%.

Table 2 illustrates the variability in growth rates for experiments done in the field and in the laboratory: for a given species, field growth rates were two- to sixfold those found in controlled conditions, and the highest variations were recorded for *Gomphonema parvulum* and *Nitzschia palea*. Despite less controlled environmental conditions, the allometric relationship was quite as meaningful in field samples as in the laboratory experiments, growth rates significantly correlating with the logarithm of cell size (Fig. 2).

Discussion

Growth responses of several diatom species under artificial and natural conditions

Our experiments using natural communities grown under field and laboratory conditions revealed consistent responses of the periphytic communities; diatoms were predominant in all the biofilm samples, which is in accordance with the theory proposed by Tilman et al. (1986) that diatoms have high resource competitive ability at low water temperatures. The main species recorded are often predominant in the nutrient-impacted benthic algal assemblages found in the study region (Gold et al., 2002; Tison et al., 2005), and moreover, many of them are fairly common in winter (Eulin & Le Cohu, 1998). According to generally accepted knowledge concerning species tolerance and sensitivity to pollution (van Dam et al., 1994), dominant diatoms were α meso- to

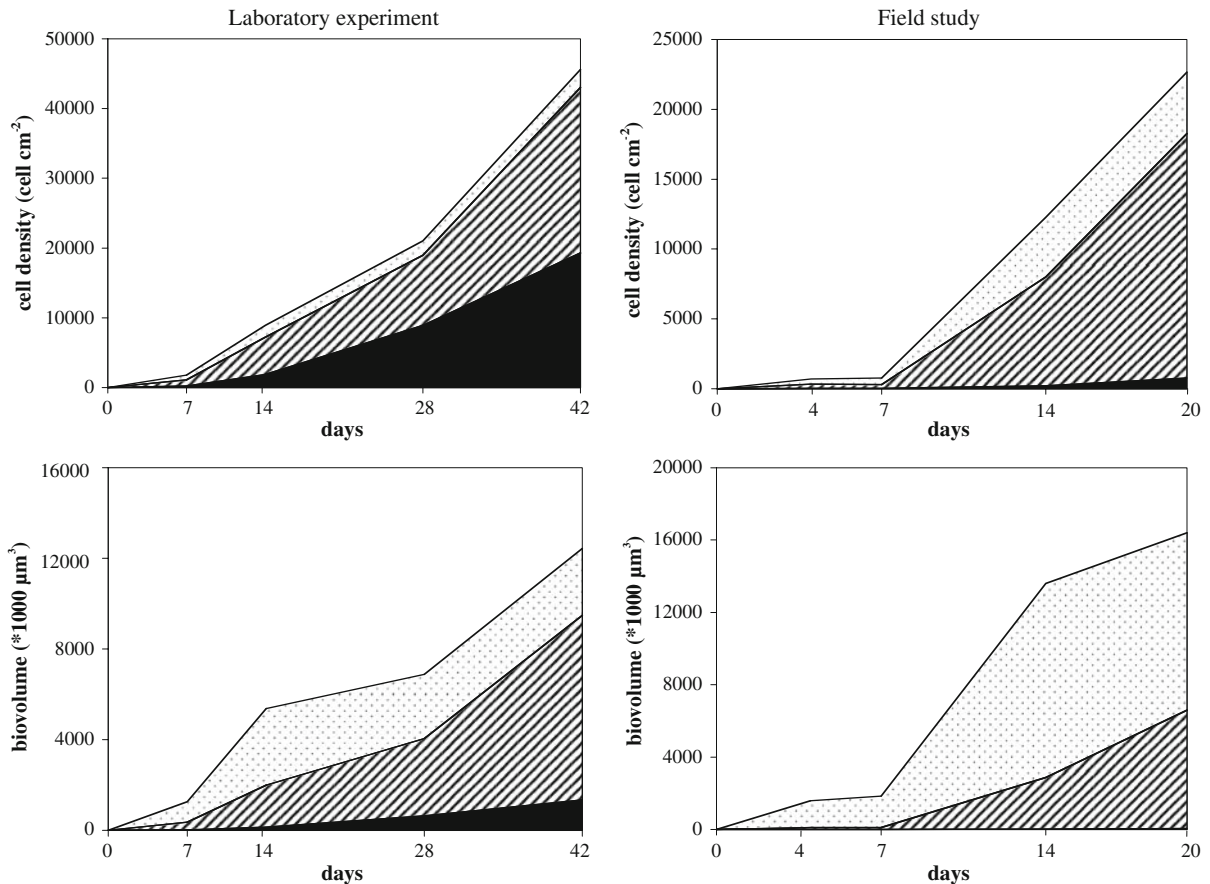


Fig. 3 Cell densities of small species (less than 100 μm³, in black), medium species (100–500 μm³, crosshatched) and large species (more than 500 μm³, in white with black points), and their contribution to the total biovolume of the community

polysaprobic species usually found in eutrophic to hypereutrophic environments. The persistence of diatoms in disturbed environments has been widely documented (for a review, see Peterson, 1996), due to small cell size and rapid immigration/growth which were also observed in this study through the dates used for k calculations. A literature survey of kinetic data for varied diatom species showed that the growth responses of the 19 diatom species studied [in both field (0.10–0.41 div day⁻¹) and laboratory (0.06–0.27 div day⁻¹)] were somewhat lower than growth rates established in unialgal cultures of diatoms (Kilham, 1975; Tilman & Kilham, 1976; Baars, 1983; Suzuki & Takahashi, 1995) or the values provided in several studies using various taxonomic groups (Tang, 1995). Indeed, the scaling of size-dependant growth rates has been shown to differ among taxonomic groups in phytoplankton (Banse,

1982) or among growth forms (Nielsen, 2006). As mentioned in the above allometric studies, the highest growth rates throughout both experiments were still lower for diatom species with larger cell sizes.

Dominance or co-dominance of small to medium species was observed throughout the duration of both studies; however, their contributions to the total biovolume (and hence, total biomass) of the community, in particular in the latest stages of biofilm development, were drastically reduced: in field samples after a 20-day colonization, species having higher biovolumes (>500 μm³) represented 20% relative abundances but 60% of the total biovolume (Fig. 3). Patterns in the size structure of phytoplankton communities have been shown to reflect seasonal variations, resource availability or perturbations (Kamenir et al., 2007; Naselli-Flores et al., 2007; Salmaso & Padisák, 2007). Although the calculation

of diatom community biovolumes may be too time-consuming for routine biomonitoring purposes (Lavoie et al., 2006), Cattaneo et al. (1997) demonstrated a significant relationship between size distribution of periphytic assemblages and trophic status of the streams. Since large species are usually found at low abundances in benthic diatom communities, typology works often focus on these underrepresented species and manage to overweight them using log-transformed abundance values (ter Braak & Verdonschot, 1995; Griffith et al., 2002; Tison et al., 2005). It has been underlined that these taxa have a high ecological significance (Snoeijs et al., 2002), and here, we show that their importance is also noticeable through the part they hold in the biovolume of the community. These observations thus confirm the ecological relevance of the use of log transformation of periphytic diatom relative abundance for the assessment of ecological conditions of stream water. This transformation also increases the accuracy of biotypologies (Tison et al., 2005; Park et al., 2006).

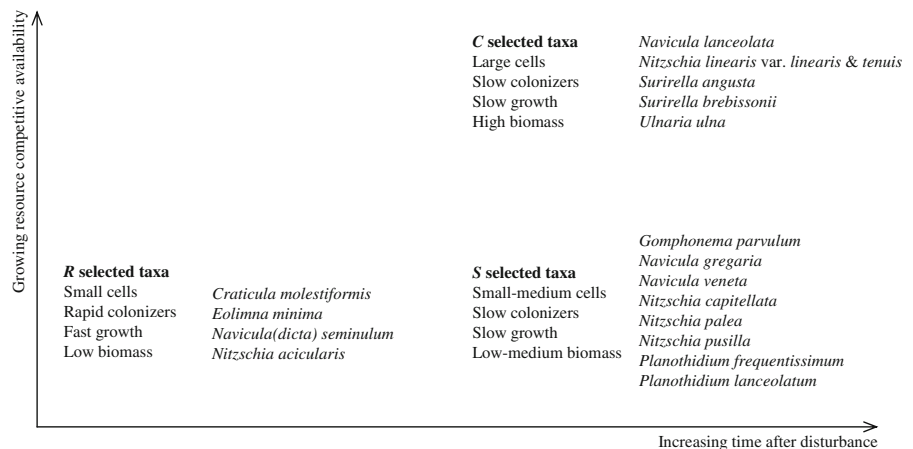
Hypothesized life history strategies

The relative abundances of small, medium and large species over time (Fig. 3) reflect the process of algal community development, and quantification of the community was quite different when considering cell numbers or total biovolume for each size. The C–S–R selection strategies (Grime, 1977) are perhaps too general to account for most possible responses to different combinations of stresses and disturbances;

however, based upon k calculations and growth curves of the 19 species used in this study, a matrix defined by disturbance and stress can be drawn (Fig. 4). In the present study, disturbance frequency is quantified in physical terms (as recommended by Poff, 1992) and assessed through the immersion of blank substrates in the water. Stress here does not refer to nutrient supply, which is not a growth-limiting parameter here, but rather to canopy inducing light competition.

The *R* (ruderal) selected taxa group incorporates early colonists of denuded or unstable habitats or opportunistic species (Hoagland et al., 1982). These small solitary Naviculaceae and Nitzschiaceae, loosely attached to the substrate (Kelly et al., 2005), are reported to be highly motile (Hudon & Legendre, 1987; Tuji, 2000; Kelly et al., 2005), which enhances their immigration rates. Moreover, these cells have rapid growth characteristics (see Table 2) and generally high disturbance resistance. Once established as dominant in the earliest stages of biofilm development, those species that are attached to the substrate may form algal mats favouring the implantation of many stalked and filamentous species that are favoured by high nutrient supply (Burkholder et al., 1990). Although their resistance to a wide range of disturbance or stress enables them to persist within mats of *C* or *S* selected taxa during the latest stages of biofilm development, these species are little represented in “peak biomass” communities grown under non-limiting nutrient supply, due to species replacement (by *C* or *S* selected species) and to their small size little influencing total biovolume (see Fig. 3).

Fig. 4 Hypothesized C–S–R grouping from growth characteristics determined in this study



Taxa of the *S* (stress tolerant) selection group are slower colonizers adapted to succeed in stressed habitats. Many of the group are saprophilous species (van Dam et al., 1994); they appear to have intermediate growth rates, between the fast rates of *R* selected taxa and the slow rates of the *C* selected taxa, and their abundances (varying between 40% and 70% relative abundances in the field experiment) are only slightly influenced by successional development of the biofilm. Most taxa within this group have small- to medium-sized cells with various holdfast mechanisms ranging from simple prostrate adhesion (*Planothidium* sp.; Cox 1996) to stalked structures (*Gomphonema parvulum*; Cox 1996) sometimes forming ramified arborescent associations (Hudon & Legendre, 1987) or small rosettes (Hoagland et al., 1982). Together with the large *C* selected taxa, these low-profile diatoms form thickened mats of prostrate, stalked and filamentous cells embedded in mucilage, an assembly that contributes to community development entrapping other algae and serving as a mechanism for attachment to the substrate.

In this study, almost none of the *C* (competitive) selected taxa were numerous at the first dates of sampling. These large, mid-late successional species, which probably utilize the mat of species firmly attached to the substratum to settle, are then highly competitive with abundant nutrient supply (Stevenson et al., 1991; Biggs et al., 1998). Traits enabling dominance of *C* selected species are related to large cell size and to attachment features: stalked or short filamentous structures (*Ulnaria ulna*; Biggs et al. 1998) mixed with mucilage-forming diatoms (*Navicula* and *Nitzschia* spp.; Hoagland et al. 1982; Biggs et al. 1998). Some stellate colonies of diatoms such as *Ulnaria* sp. have been reported to have the ability to attach not only to the substrates, but also to diatom stalks (Hoagland et al., 1982; Roemer et al., 1984). This would imply that these rosette-forming diatoms would create tall structures that overtop basal-layer pioneer taxa (i.e. *R* selected) and thus have an advantage over other vertically positioned diatoms in competition for space, and hence resources (light and nutrients). This way, they may also limit or even suppress the growth of other populations. These coherent mucilaginous communities could also enable fixation of non-attached (floating or freely motile) cells like *Surirella* sp. or *Navicula lanceolata* (Kelly et al., 2005).

Representativeness of microcosm experiments

Ideally, the experimental systems should be as similar as possible to the environment they will be compared to. Although our microcosm experiments were supposed to mimic the field conditions we observed in March 2005, some technical limitations restricted the harmonization of certain environmental parameters (increasing temperatures, conductivity, irradiance). Moreover, our results indicate that many “uncontrolled” field factors still interact and affect the k and t_d values of the 13 diatoms studied. It would have been expected that the communities in the field would not be especially fast-growing in comparison to laboratory cultures. However, among the species we studied, most grew better in situ than in microcosms, which appears to be in conflict with Kagami and Urabe (2001), who found lower growth rates, for a given cell size, in field experiments than in laboratory cultures. The exponents of the power function for field (0.31) and laboratory (0.34) data were higher than the ranges of values estimated by Tang (1995). This however suggests that the slopes and determination coefficients could be comparable between field and laboratory experiments, when using a similar species inoculum. The growth rates of algal species would thus not exclusively be determined by cell size, but also by specific “culture” conditions and by the algal classes considered (data from the literature take into account various microalgal groups).

The differences in growth rates for a given species observed between laboratory and field results would be explained by various determinants alone or in interaction, which were unclearly identified in this study but are likely to be species-specific. The relationship between growth characteristics of algae and resource (N, P, Si) competitive ability have been described by a number of authors (Kilham, 1975; Tilman et al., 1981; Tilman, 1990; van Donk & Kilham, 1990), and although it is obviously not the only parameter involved, nutrient supply was quite different between field (continuous inputs) and laboratory (sporadic additions of culture medium to compensate nutrient depletion) conditions. Under these nutrient-unlimited conditions, the impact of many physical parameters is not to be neglected. Indeed, most of the diatom species used for the calculation of growth constants are able to develop over a wide range of conductivities, as shown in

Europe by van Dam et al. (1994) and in US rivers by Potapova and Charles (2003). Concerning the species that developed in the artificial streams but were not found in the field, it can be argued that their growth abilities would have been altered by the one order magnitude difference in conductivities with the natural waters; some of them (like *Navicula(dicta) seminulum*, *Eolimna minima*, *Ulnaria ulna*) are described to display a rather limited range of conductivity affinities (Potapova & Charles, 2003). However, the species *Eolimna minima* and *Ulnaria ulna* have been found in considerable amounts in summer at Decazeville (Morin et al., 2007), suggesting that temperature and light irradiance may be more important factors. The diatoms recorded here were cold-water species or taxa able to withstand cold water; however, differences in temperature regimes (i.e. stable temperatures versus daily and seasonal fluctuations) probably affected diatom settlement and species growth responses (Patrick, 1977; Montagnes & Franklin, 2001). It has been demonstrated by Nicklisch (1998) that natural fluctuation in light intensity (increases/decreases in light irradiances due to the regular course of sunlight, to clouding, etc.) is a determining factor influencing diatom growth rates, generally interacting with temperature (Baars, 1983; El-Sabaawi & Harrison, 2006). The light levels imposed in the laboratory microcosms were approximately 10-fold lower than natural saturating light levels in this area; however, the real difference between light conditions is probably smaller than appears from measurements because neither refraction nor attenuation occurred between surface and the 10-cm depth (Padisák, 2005) where the slides were exposed. We assumed that photoacclimation of the diatoms in response to changes between field and laboratory conditions occurred on a short time scale and we did not apply any preincubation period even though there was a decrease in light irradiance of the new environment. However, Falkowski and LaRoche (1991) describe that the process necessitates about one cell's generation; with regards to the generation times found in the laboratory experiment (15 days for the slowest growing species), the shift from higher to lower irradiance may have seriously delayed growth kinetics, depending on the species' photoacclimation strategies. Flow velocity is also known to significantly condition the structure of the community; Ghosh and Gaur (1998) described a stimulatory effect

of intermediate current velocities on the growth of many species, which may also partly explain the differences in cell densities between laboratory and field studies. Finally, biological factors may be involved too. We assumed that sloughing, grazing activities or the establishment of other periphytic algae, which were not observed in either of the treatments, were not here significantly to the detriment of diatoms. However, field biofilms may have benefited from continuous inputs of diatoms from the upstream pool. As demonstrated by Stevenson and Peterson (1991), immigration rates have a major impact on diatom community settlement and development. Indeed, the total number of species recorded in the field study was twice as high as in the laboratory experiment, for a comparable counting effort. It can be hypothesized that some species, although they were present in the inoculum (e.g. *Navicula lanceolata*—35.8% relative abundances in the sample inoculated—or *N. gregaria*—11.1%), were slow colonizers that did not find suitable conditions for settlement at the beginning of the microcosm experiment, and thus were not able to grow afterwards and did not survive. However, Stevenson and Peterson (1989) also demonstrated that immigration rates are species-specific and vary among habitats but, for a given site, are quite constant in time. Hence continuous immigration would not have a marked effect on diatom growth constants calculated in the exponential phase.

Growing interest is being devoted to laboratory-based experiments, since in nature attached communities are often difficult to investigate (McCormick & Cairns, 1994). Unlike a study of Roeselers et al. (2006) that demonstrated poor reproducibility of microcosm experiments in terms of species richness and community composition, the present experiment displayed low variability between replicates. The conditions within the experimental units, however, did not exactly resemble environmental conditions, which affected diatom growth kinetics as discussed above. While the nutrient levels were certainly adequate to saturate specific cellular growth rates of diatoms, they were not sufficient to saturate sustainable biomass (as discussed by Bothwell, 1989). Environmental parameters probably set k for the species studied; indeed, light and temperature conditions may explain the two- to sixfold lower growth in the laboratory. Despite this physical limitation of k , it

is worthy to underline that allometric relationships were still intact.

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