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Contribution of epilithic diatoms to benthic–pelagic coupling in a temperate river

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ABSTRACT: Water residence time in the middle course of rivers is often too short to allow substantial phytoplankton development, and primary production is essentially provided by benthic phototrophic biofilms. However, cells occurring in the water column might derive from biofilm microalgae, and, reciprocally, sedimenting microalgae could represent a continuous source of colonizers for benthic biofilms. A comparative study of biofilm and pelagic microphytic communities (with special focus on diatoms) was carried out over 15 mo in the Garonne River, France. Diatoms dominated both biofilm and pelagic microphytic communities. Typically benthic diatoms were found in high abundance in the water column, and their biomass in the water was correlated with their biomass in the biofilm, indicating the benthic origin of these cells. Variations in river discharge and temperature drove the temporal distribution of benthic and pelagic communities: under high flow mixing (winter) communities showed the greatest similarity, and during low flow (summer) they differed the most. Even during low flow, typical benthic species were observed in the water column, indicating that benthic–pelagic exchanges were not exclusively due to high water flow. Moreover, during low flow periods, planktonic diatoms typically settled within biofilms, presumably because of higher water residence times, and/or upstream reservoir flushing.

KEY WORDS: Periphyton · Distribution · Biofilm · Microphytobenthos · Phytoplankton · Algal ecology · Community structure · HPLC

INTRODUCTION

Rivers carry drifting and *in situ*-produced organic matter and organisms as a result of physical-chemical conditions and their corresponding biological responses. Planktonic organisms are produced *in situ*, but their occurrence is mostly restricted to large rivers or sheltered areas (Vannote et al. 1980, Basu & Pick 1996). In headwaters, heavy canopy cover and high water velocity are incompatible with substantial phytoplankton development (e.g. Reynolds et al. 1994). In estuarine reaches the water residence time is higher and can favour suspended primary producers, though surface turbidity can still limit phytoplankton production (Phlips et al. 2000) and constrain phytobenthos to a limited area (e.g. Soetaert & Herman 1995). In the middle course of large rivers, the water residence time is critical to allow substantial phytoplankton development. Phytoplankton develops mostly in slowly moving waters (Sabater et al. 2008), but when the channel is shallow and waters are moving fast, light penetration favours the development of phytobenthic organisms. Hence, in these stretches, phytobenthic production is essential for sustaining the riverine food web (Lawrence et al. 2002, Liess & Hillebrand 2004, Dodds 2006), as well as the key biogeochemical processes occurring in the river (Battin et al. 2003, Teissier et al. 2007).

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Benthic algae on the river bed are generally associated with heterotrophic microorganisms (bacteria, flagellates and ciliates) embedded in a mucous matrix composed of exopolymeric exudates (EPS) and trapped detritic and mineral particles, to form biofilms (Lock et al. 1984, Romaní et al. 2004). These biofilms are shaped by abiotic and biotic influences (e.g. light, flow, nutrients, grazing, allelopathy) that affect their structure and functions (e.g. Hillebrand 2002, Sabater et al. 2002, Lyautey et al. 2005a, Boulêtreau et al. 2006, Leflaive & Ten-Hage 2007, Mathieu et al. 2007). The dynamics of epilithic biofilms include a growth phase, corresponding to an ecological succession of microbial colonizers onto the substratum (e.g. Korte & Blinn 1983, Lyautey et al. 2005a), and a detachment phase. Detachment of components of the biofilm can occur either through flow abrasion and/or through self-detachment processes (Biggs & Close 1989, Boulêtreau et al. 2006). Also meio- and macrofauna drilling and grazing the biofilm influence its architecture and growth dynamics (Lawrence et al. 2002, Gaudes et al. 2006, Kathol et al. 2011). In the middle course of fast-moving rivers, cells occurring in the water column are derived essentially from the detachment of phototrophic biofilms (Roeder 1977, Ameziane et al. 2003). Conversely, drifting microalgae could represent a continuous source of colonizers for benthic biofilms, implying a certain upstream–downstream connectivity of microphytobenthic communities (Pusch et al. 1998).

The potential couplings between biofilm and pelagic microalgae in the middle course of the Garonne River (France) were analysed according to their respective community structure. Emphasis was placed on diatoms since they dominate the biofilm microphytobenthic community in the middle course of the Garonne River (Leflaive et al. 2008, Majdi et al. 2011). We specifically aimed to (1) determine whether biofilm export to the water column was continuous or whether it mainly occurred after floods and (2) determine the key environmental factors influencing benthic–pelagic coupling and diatom community structure and similarity between benthic and pelagic communities.

MATERIALS AND METHODS

Study site

The Garonne River is the largest river of southwestern France (647 km length, 57 000 km^2 catchment area). It rises in the Pyrenees Mountains, and displays a flow regime characterized by an intense spring-flood period after snowmelt, followed by a long low flow water period for the rest of the year. This low flow period is interrupted only by flash floods caused by heavy rainfall. The Garonne's river bed consists mainly of shallow alternating cobble bars. A thick diatom-dominated biofilm colonizes the upper surface of cobbles, especially during low flow periods (Majdi et al. 2012a). Sampling was conducted at a cobble bar in a sixth-order stretch of the Garonne River, 36 km upstream of the city of Toulouse (Fig. 1; longitude: 43° 23′ 45″ N; latitude: 01° 17′ 53″ E; elevation: 175 m above sea level [a.s.l.]). The epilithic microbial and invertebrate communities at this site are described elsewhere (Lyautey et al. 2005b, Leflaive et al. 2008, Majdi et al. 2011, 2012a). The canopy is open, but the residence time is too short for substantial phytoplankton development, and benthic biofilms provide most of the primary production (Ameziane et al. 2002, 2003).

Sample collection

Epilithic biofilms were sampled weekly from December 2008 to March 2010 (n = 48), when the river remained wadeable (discharge < 175 m^3 s^{-1}). On each sampling occasion, we randomly collected 12 cobbles (mean diameter = 10 cm) by sliding them into a plastic bag underwater (depth = 30 to 50 cm). The biofilm

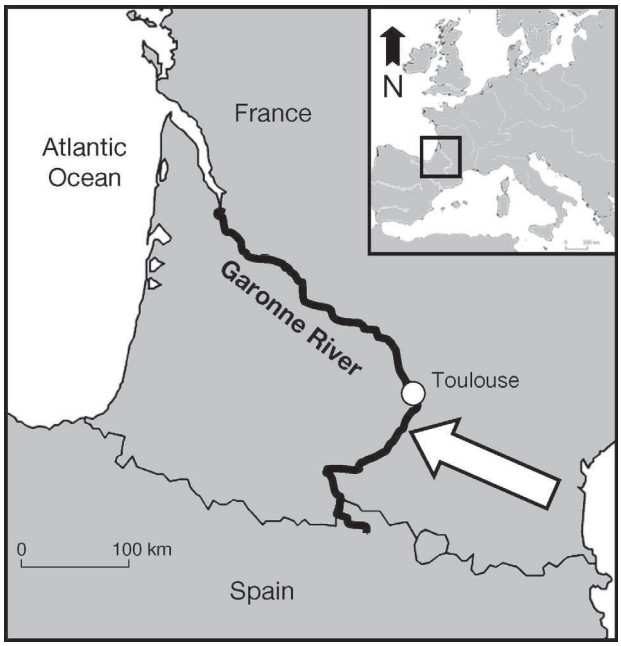

Fig. 1. Location of the study site at the Garonne River, France

was gathered by scraping the total upper surface of each cobble with a scalpel and toothbrush. Then biofilm samples were suspended in ultrapure water (Milli-Q filtration; Millipore) to obtain the respective biofilm suspensions (25 ml each). These suspensions were divided into 3 groups of 4 replicates to be used for meiofaunal counts, algal pigment analyses and estimation of epilithic ash-free dry mass (AFDM). The procedures are detailed in Majdi et al. (2011, 2012a).

One large cobble (mean diameter = 30 cm) was additionally collected from October 2008 to March 2010 (n = 19), and the biofilm was scraped off from its entire upper surface with a scalpel and toothbrush. This biofilm sample was preserved with formaldehyde (final concentration = 4%) for the identification of benthic (biofilm) diatoms.

Simultaneously, river water in the upper 10 cm of the water column was collected for (1) pelagic diatom identification (4 replicate 125 ml water samples preserved with Lugol's solution, Sigma-Aldrich) and (2) pelagic phytopigment measurement. For the latter, 3 replicate 500 ml water samples were filtered onto 1.2 µm glass fibre filters (GF/C, Whatman), which were immediately folded in aluminium and then preserved in liquid N_2, and stored in the laboratory at –80°C until analysed.

Physical and chemical characteristics

The mean daily discharge (MDD) was supplied by a gauging station of the French water management authority (DIREN Midi-Pyrénées, Marquefave station) located 10 km upstream of the study site. No tributaries or dams occur between the gauging station and the study site. Water height and streambed flow velocity were measured (mean of 3 measurements flanking the sampling area) on each sampling occasion with a flowmeter (Flo-Mate 2000, Flow-Tronic). Days after flood were estimated as the number of days between a given sampling occasion and the last critical flood (MDD > 300 $m^3 s^{-1}$; Majdi et al. 2012a). Water temperature, conductivity, pH and dissolved O_2 were measured with an automated multiparameter probe (YSI 6000, Yellow Springs Instruments), which was permanently set 5 cm above the streambed.

Phytopigment analysis

Four biofilm suspensions were centrifuged (3220 × *g*, 20 min), and the obtained pellets were freeze-dried. We removed 250 mg subsamples from each pellet and extracted biofilm algal pigments in 3 steps. A total of 25 ml (10, 10 and 5 ml) mixture of 98% cold-buffered methanol and 2% of 1 M ammonium acetate was used for the extraction. Each subsequent step consisted of 15 min extraction at – 20°C. For pelagic phytopigment extraction, frozen GF/C filters were cut into small pieces in centrifuge tubes containing 5 ml of 98% cold-buffered methanol (with 2% of 1 M ammonium acetate). Pigment extraction was promoted by 250 W ultrasonication for 30 s (Sonifier 250A, Branson Ultrasonics). Then, pigment extracts were incubated for 15 min at –20°C before centrifugation. The extracts were finally centrifuged (3220 × *g*, 3 min) to settle filter pieces.

For both biofilm and pelagic phytopigment analyses, 1 ml of the pigment extract was filtered through a 0.2 µm polytetrafluoroethylene syringe filter, and the filtrate was analysed with a high-performance liquid chromatograph consisting of a 100 µl loop autosampler and a quaternary solvent delivery system coupled to a diode array spectrophotometer (LC1200 series, Agilent Technologies) through a C8 column (4.6 × 100 mm, 3 µm, Thermo). The mobile phase was set after Barlow et al. (1997). Microphytic pigments were determined and quantified by comparing their retention time and absorption spectra with those of reference pure standards (DHI LAB products; see Majdi et al. 2011 for further details). Biofilm and pelagic phytopigment concentrations were both expressed per surface units (e.g. mg m^{-2}), by considering the sampled surface for the biofilm, or by considering the water height for the water column.

We performed chemotaxonomic analysis with the CHEMTAX software (Version 1.95; Mackey et al. 1996) to derive the biomass of biofilm and pelagic microphythic groups from their contribution to total chlorophyll *a* (chl *a*). Biomarker pigment ratios were used from Majdi et al. (2011) to supply the initial matrix required to run the chemotaxonomic analysis.

Diatom identification

The 4 water sample replicates were pooled and thoroughly homogenized before a 20 to 50 ml (depending on diatom concentration) subsample was placed in a settling chamber. Diatoms were allowed to settle for 20 to 50 h depending on subsample volume. Diatom cell contents were digested with HCl (37%) and subsequently heated at 100°C for 2 h with H_2O_2 (Biggs & Kilroy 2000). The heating step was repeated twice. Resulting cleaned frustules were

rinsed with ultrapure water on a 0.2 µm pore filter and finally suspended in 1 to 3 ml ultrapure water. A subsample of 200 µl was pipetted onto a coverslip and permanently preserved in Naphrax® mounting medium (Northern Biological Supplies). The same procedures were applied to biofilm samples, except for the settlement step. At least 400 diatoms were counted under a light microscope at 1000× magnification, identified to species level and attributed to benthic or pelagic habitats, according to their distribution in inland waters after Krammer & Lange-Bertalot (1991). Pennate diatoms were mostly classified as benthic, whereas centric diatoms were mostly classified as planktonic.

Diatoms were also categorized by their morphology and growth-form (solitary, prostrated, erected, or chained) and mobility (mobile or immobile), based on Hudon & Legendre (1987), DeNicola et al. (2006) and Passy (2007). Data were expressed as the relative contributions of every taxon to total diatom abundance.

Data analysis

Spearman rank correlation analyses were used to explore the couplings between biofilm and pelagic phytopigments, using STATISTICA software (Version 8.0, Statsoft). The influence of biotic and abiotic factors on diatom species distribution in the biofilm and in the water column was examined by means of multivariate analysis, using CANOCO software (Version 4.5, Biometris). Rare species (contribution < 1%) were not considered. The relative species abundances were square-root transformed prior to the analysis. Diatom species distribution was first analysed by a detrended correspondence analysis. As the total inertia observed was <2.6, a predominance of linear species response was expected (Ter Braak 1987, 1994). Hence, a redundancy analysis (RDA) was applied, in which the ordination axes were constrained to be linear combinations of provided environmental factors. Factors were listed according to the variance (eigenvalues: λ) explained by each factor when added to the model, discarding possible co-variability with other factors (conditional effects; Ter Braak & Smilauer 2002). More specific details about this method are given by Borcard et al. (1992). The statistical significance was checked using a Monte Carlo permutation test (499 unrestricted permutations, $\alpha = 0.05$).

An analysis of similarity (ANOSIM) using non-metric multidimensional scaling (nMDS) was performed to explore the similarity between biofilm and pelagic diatom communities regarding discharge and temperature constraints, with the PRIMER software (Version 5, PRIMER-E). The ANOSIM was based on Bray-Curtis similarity calculated from non-transformed species relative abundances. R has an absolute interpretation of its value that is potentially more meaningful than its statistical significance: large R-values, close to 1, indicate a clear separation of the communities, whereas small values, close to 0, imply little or no separation (Clarke & Warwick 2001). Samples were then presented on a nMDS biplot according to their diatom community similarities with other sam-

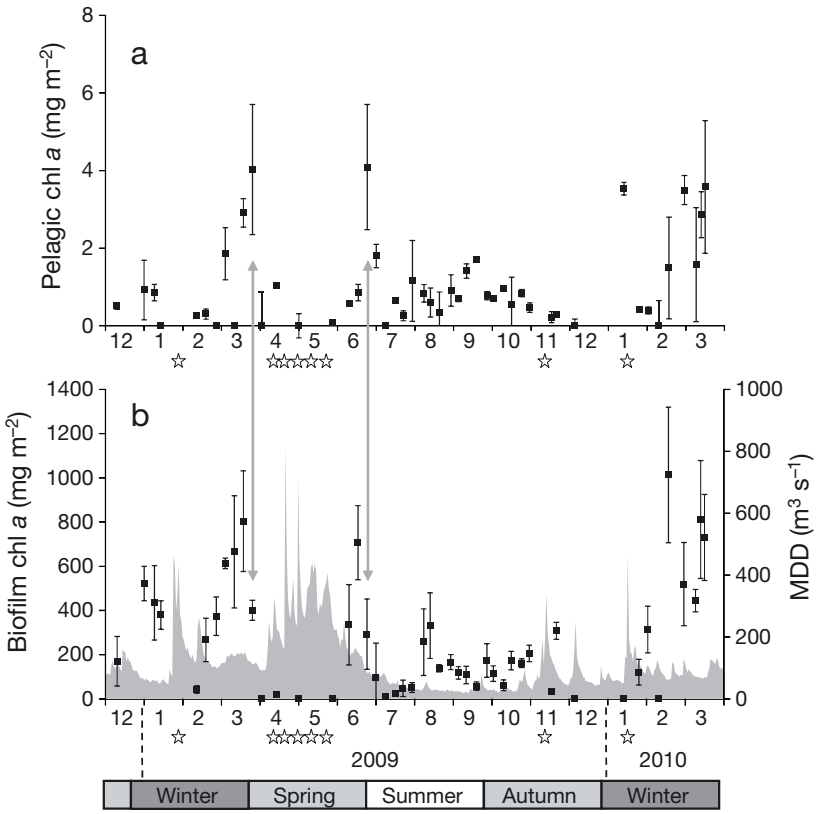

Fig. 2. Temporal dynamics of (a) chlorophyll *a* (chl *a*) in the water column (mean ± SE, n = 3) and (b) mean daily discharge (MDD) and chl *a* in the epilithic biofilm (mean ± SE, n = 4). Black squares: chl *a*; shaded area: MDD. Months (where 1: January, 2: February, etc.), years, seasons and critical floods during which MDD > 300 $m^3 s^{-1}$ (represented by stars) are indicated on the *x*-axis. Grey arrows represent the 1 wk lag observed between biofilm and pelagic chl *a* maxima

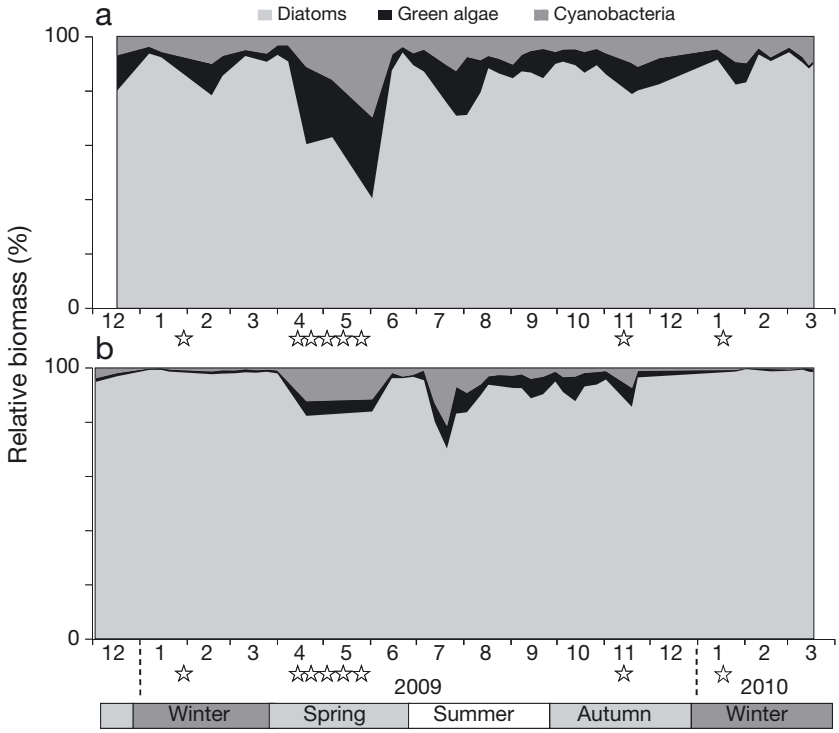

Fig. 3. Relative biomass of microphytic groups (a) in the water column and (b) in the epilithic biofilm. Months, years, seasons and critical floods during which MDD > 300 $m^3 s^{-1}$ (represented by stars) are indicated on the *x*-axis

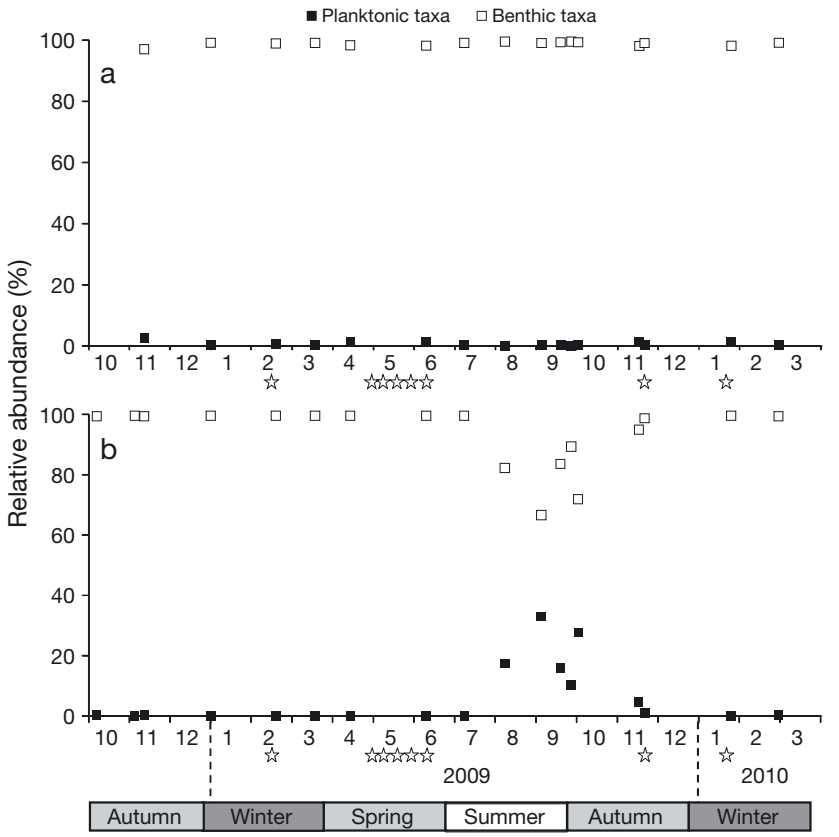

Fig. 4. Relative abundance of typically planktonic and typically benthic diatom species after Krammer & Lange-Bertalot (1991) found (a) in the water column and (b) in the epilithic biofilm. Months, years, seasons and critical floods during which MDD > 300 $m^3 s^{-1}$ (represented by stars) are indicated on the *x*-axis

ples. On the same plot, samples were sorted into groups according to discharge constraints (low: 0 to 50, medium: 51 to 100, and high: >100 $m^3 s^{-1}$) and according to temperature constraints (low: 0 to 9, medium: 9 to 17, and high: >17°C).

RESULTS

Microalgal dynamics and benthic–pelagic couplings

Chl *a* averaged 287 mg m^{-2} (ranging from 3 to 1012 mg m^{-2}) in the biofilm and 0.8 mg m^{-2} (0.1 to 4.1 mg m^{-2}) in the water column (Fig. 2). Hence, the algal biomass in the water was negligible compared to that in the biofilm.

Biofilm and pelagic chl *a* showed considerable variations throughout the study period: they both tended to increase during periods of low flow, whereas they sharply decreased after floods (Fig. 2). The biofilm and pelagic chl *a* dynamics were correlated (Spearman rank correlation, n = 38, r = 0.53, $p < 0.001$). Some of the declines in chl *a* in the biofilm (e.g. late March and June 2009) corresponded to a lagged pelagic chl *a* decline (Fig. 2). From August to October 2009, biofilm and pelagic chl *a* dynamics followed opposite patterns.

The CHEMTAX analysis showed that diatoms dominated the microphytic biomass both in the biofilm and in the water column (Fig. 3). Biofilm and pelagic fucoxanthin (i.e. diatom biomarker pigment) were strongly correlated (Spearman, n = 38, r = 0.61, $p < 0.001$). Cyanobacteria and green algae contributed to the

Table 1. Code, occurrence in the present study, type after Krammer & Lange-Bertalot (1991), mobility and growth form after Hudon & Legendre (1987), DeNicola et al. (2006) and Passy (2007), and mean contribution of the most common diatoms that were considered in the redundancy analysis (i.e. with a community contribution >1% in at least 1 sample). Species affinities (+, –) with discharge conditions were computed from the redundancy analysis. B: biofilm; P: pelagos; i: immobile; m: mobile; p: prostrated; e: erected; s: solitary centric; c: chained

Code	Species and authority	Occurrence	Type	Mobility	Growth form	Contribution (%) Biofilm	Pelagos	
abia	*Achnanthidium biasolettianum* (Grun.) Round & Bukhtiyarova	B & P	Benthic	i	p	7.51	16.09	+
aina	*Amphora inariensis* Krammer	B & P	Benthic	m	p	0.11	1.84	
alan	*Planothidium lanceolatum* (Brebisson *ex* Kütz.) Lange-Bertalot	P	Benthic	i	p		0.01	
amin	*Achnanthidium minutissimum* (Kütz.) Czarnecki	B & P	Benthic	i	p	13.20	16.71	–
amma	*Achnanthidium macrocephalum* (Hust.) Round & Bukhtiyarova	B	Benthic	i	p	1.93		–
aped	*Amphora pediculus* (Kütz.) Grun.	B & P	Benthic	m	p	0.67	2.02	–
caff	*Cymbella affinis* Kütz.	B & P	Benthic	i	e	0.44	2.04	–
cato	*Cyclotella atomus* Hust.	B	Planktonic	i	s	5.48		–
ccae	*Encyonema caespitosum* Kütz.	B	Benthic	i	e	0.47		
cccp	*Cyclotella cyclopuncta* Hakansson & Carter	B & P	Planktonic	i	s	0.02	0.21	
ccis	*Cymbella cistula* (Ehr.) Kirchner	B	Benthic	i	e	1.31		
chel	*Cymbella helvetica* Kütz.	B & P	Benthic	i	e	0.69	0.81	+
clep	*Cymbella leptoceros* (Ehr.) Kütz.	B	Benthic	i	e	0.12		
cmen	*Cyclotella meneghiniana* Kütz.	B & P	Planktonic	i	s	0.26	0.05	–
cmin	*Encyonema minutum* (Hilse) Mann	B & P	Benthic	i	e	4.25	1.55	
cped	*Cocconeis pediculus* Ehr.	B & P	Benthic	i	p	1.61	1.87	–
cpla	*Cocconeis placentula* Ehr.	B & P	Benthic	i	p	2.11	4.11	–
cple	*Cocconeis placentula* var. *euglypta* (Ehr.) Grun.	B & P	Benthic	i	p	1.80	0.42	–
cpli	*Cocconeis placentula* var. *lineata* (Ehr.) Van Heurck	B & P	Benthic	i	p	1.64	8.52	–
cppl	*Cocconeis placentula* var. *pseudolineata* Geitler	B & P	Benthic	i	p	0.35	0.17	
csle	*Encyonema silesiacum* (Bleisch) Mann	B & P	Benthic	i	e	2.36	8.89	+
ctum	*Cymbella tumida* (Brebisson) Van Heurck	B & P	Benthic	i	e	0.29	1.98	–
dehr	*Diatoma Ehrenbergii* Kütz.	B & P	Benthic	i	c	2.00	0.78	+
dmon	*Diatoma moniliformis* Kütz.	B & P	Benthic	i	c	1.34	1.09	+
dvul	*Diatoma vulgaris* Bory	B & P	Benthic	i	c	4.77	1.22	
farc	*Fragilaria arcus* var. *arcus* (Ehr.) Cleve	B & P	Benthic	i	e	0.21	0.30	+
fcap	*Fragilaria capucina* var. *capucina*	B & P	Benthic	i	c	0.15	0.03	
fcva	*Fragilaria capucina* var. *vaucheriae* (Kütz.) Lange-Bertalot	B & P	Benthic	i	c	1.01	0.51	
glat	*Gomphonema lateripunctatum* Reichardt & Lange-Bertalot	B	Benthic	i	e	0.26		
gmin	*Gomphonema minutum* (Ag.) Agardh	B & P	Benthic	i	e	0.53	1.83	
gnod	*Gyrosigma nodiferum* (Grun.) Reimer	B & P	Benthic	m	e	0.08	0.19	
goli	*Gomphonema olivaceum* (Horn.) Brébisson	B & P	Benthic	i	e	3.41	2.05	+
gpum	*Gomphonema pumilum* (Grun.) Reichardt & Lange-Bertalot	B & P	Benthic	i	e	0.01	0.85	
gter	*Gomphonema tergestinum* Fricke	P	Benthic	i	e		0.26	
mvar	*Melosira varians* Agardh	B & P	Benthic	i	c	5.61	0.05	
nape	*Mayamaea atomus* var. *permitis* (Hust.) Lange-Bertalot	B	Benthic	m	p	0.08		
nbac	*Sellaphora bacillum* (Ehr.) Mann	B & P	Benthic	m	p	0.05	0.30	–
ncpl	*Nitzschia capitellata* Hust. in Schmidt et al.	B & P	Benthic	m	e	0.08	0.01	
ncpr	*Navicula capitatoradiata* Germain	B & P	Benthic	m	p	1.77	0.76	–
ncte	*Navicula cryptotenella* Lange-Bertalot	B & P	Benthic	m	p	5.41	3.29	+
ndis	*Nitzschia dissipata* (Kütz.) Grun.	B & P	Benthic	m	e	8.68	0.96	+
nfon	*Nitzschia fonticola* Grun. in Cleve & Möller	B & P	Benthic	m	e	5.23	0.69	+
nheu	*Nitzschia heufleriana* Grun.	B & P	Benthic	m	e	0.20	0.01	
nlan	*Navicula lanceolata* (Ag.) Ehr.	B & P	Benthic	m	e	0.33	0.18	
nmeg	*Navicula antonii* Lange-Bertalot	B & P	Benthic	m	p	0.08	0.91	
nmin	*Eolimna minima* (Grun.) Lange-Bertalot	B & P	Benthic	m	p	0.01	1.20	
npal	*Nitzschia palea* (Kütz.) Smith	B & P	Benthic	m	e	0.93	0.45	+
nphy	*Navicula phyllepta* Kütz.	B & P	Benthic	m	p	0.04	0.53	+
nrch	*Navicula reichardtiana* Lange-Bertalot	B & P	Benthic	m	p	0.94	0.35	+
nsem	*Sellaphora seminulum* (Grun.) Mann	B	Benthic	m	p	0.33		
nsit	*Nitzschia sinuata* var. *tabellaria* Grun.	B & P	Benthic	m	e	0.24	0.20	
ntpt	*Navicula tripunctata* (Müller) Bory	B & P	Benthic	m	p	4.43	4.13	+
ntrv	*Navicula trivalis* Lange-Bertalot	P	Benthic	m	p		0.22	
nzsu	*Nitzschia supralitorea* Lange-Bertalot	P	Benthic	m	e		0.22	
rabb	*Rhoicosphenia abbreviata* (Ag.) Lange-Bertalot	B & P	Benthic	i	e	0.47	0.67	–
rsin	*Reimeria sinuata* (Gregory) Kociolek & Stoermer	B & P	Benthic	m	e	0.40	2.96	–
sbku	*Surirella brebissonii* var. *kuetzingii* Krammer et Lange-Bertalot	B	Benthic	m	e	0.64		
sbre	*Surirella brebissonii* Krammer & Lange-Bertalot	B & P	Benthic	m	e	0.16	0.11	

phytoplankton throughout the study period. Their contribution to biofilm microphytobenthos was less important, especially during winter. However, biofilm and pelagic zeaxanthin (i.e. cyanobacterial biomarker pigment) were correlated (Spearman, n = 38, r = 0.41, p < 0.01), whereas biofilm and pelagic chl *b* (i.e. green algal biomarker pigment) were not (Spearman, n = 38, r = 0.07, p = 0.7).

A total of 190 diatom species were identified during the study period (Table 1 and Table S1 in the supplement at www.int-res.com/articles/suppl/a069p047_supp.pdf). Typically benthic species (e.g. *Achnantidium* spp., *Nitzschia* spp., *Navicula* spp.) dominated both in the biofilm and in the water column (Fig. 4). Benthic diatoms were overwhelmingly dominant in the water column, accounting for 99% of the community throughout the study period. The proportion of typically planktonic species in the biofilm increased up to 34% during the late summer low water period. This was especially due to the occurrence of *Cyclotella atomus*, which represented up to 32% of biofilm diatoms during this period.

Factors influencing diatom species distribution

Axes 1 and 2 of the RDA performed with the biofilm variables explained 31.5 and 13.8% of diatom species distribution variance, respectively (Fig. 5a). Biofilm diatom distribution was significantly influenced by temperature, discharge and pH (Table 2). Axis 1 summarized the opposite patterns of discharge and temperature that were characteristic of the hydrological periods. Diatom taxa associated with low discharge values and high temperatures (mainly *Amphora* spp., *Cocconeis* spp., *Cyclotella* spp., *Melosira* spp.) scored towards the right side of the biplot, whereas diatom species associated with high discharge values and low temperatures (mainly *Achnantes* spp., *Cymbella* spp., *Fragilaria* spp., *Gomphonema* spp., *Navicula* spp., *Nitzschia* spp.) scored towards the left side of the biplot. A clear trend was also observed when considering diatom structural and mobility properties. Immobile, prostrated and/or solitary forms were associated with higher temperatures, whereas mobile and erected forms were associated with lower temperatures.

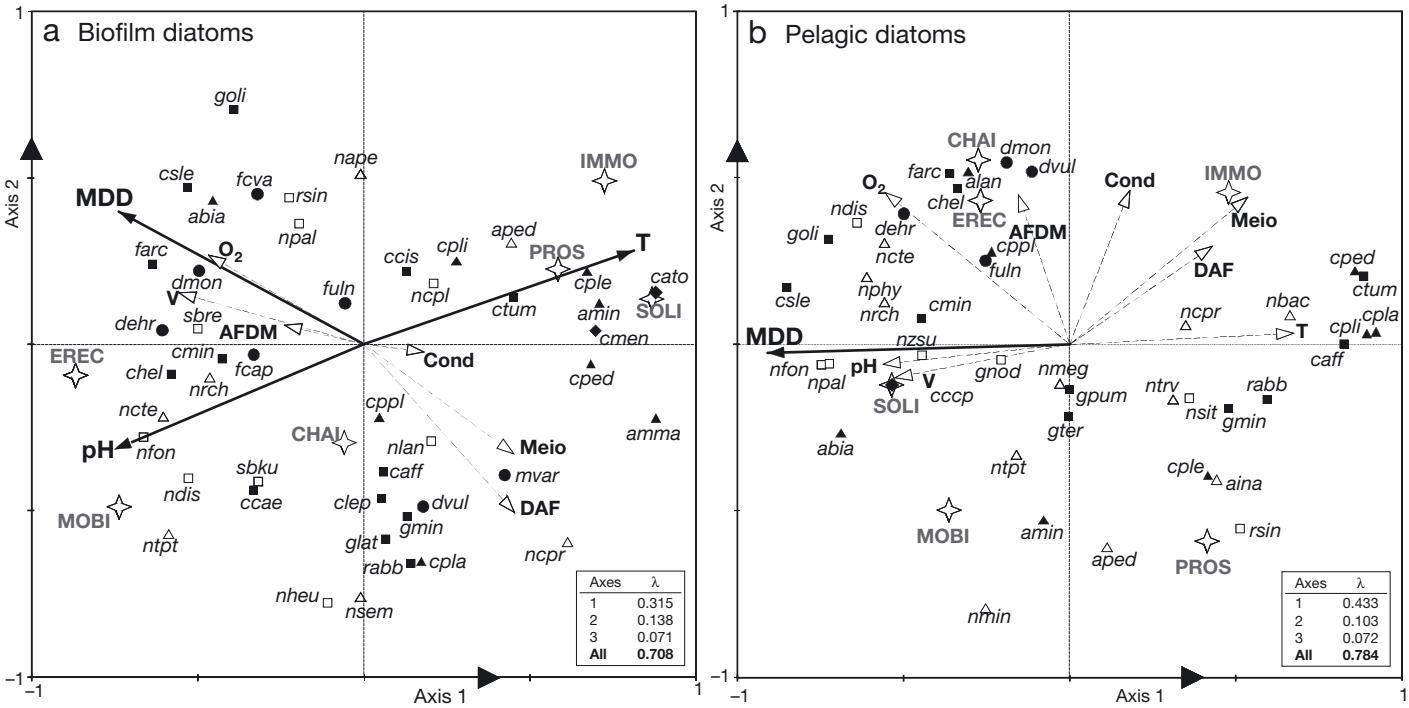

Fig. 5. Redundancy analysis showing the distribution of (a) biofilm and (b) pelagic diatom species' (see Table 1 for abbreviations of species names) relative abundances according to environmental factors. Ordination axes were rescaled from −1 to 1. Eigenvalues (λ) are indicated for main ordination axes. Dashed arrows represent non-significant factors. Bold arrows represent significant factors (Monte Carlo permutation test, p < 0.05). Solid symbols: immobile species; open symbols: mobile species; growth forms — triangles: prostrated; squares: erected; circles: chained; diamonds: solitary centrics. Star symbols represent the summed abundance of species according to their mobility and posture, represented by the groups (IMMO: immobile; MOBI: mobile) and growth forms (PROS: prostrated; EREC: erected; CHAI: chained; SOLI: solitary centrics) (see Table 1). Environmental factor abbreviations — MDD: mean daily discharge; DAF: days after flood; T: temperature; O_2: dissolved oxygen; V: flow velocity; Cond: conductivity; AFDM: biofilm ash-free dry mass; Meio: biofilm-dwelling meiofauna density

Table 2. Conditional effects from the redundancy analysis (RDA). Each environmental factor is ordered according to its eigenvalue (λ), indicating the importance of its own contribution to explain the distribution variance of pelagic and biofilm diatom species. Significant factors at $p < 0.05$ are highlighted in bold. MDD: mean daily discharge; DAF: days after flood; T: water temperature; O_2: dissolved oxygen; V: flow velocity; Cond: water conductivity; AFDM: biofilm ash-free dry mass; Meio: biofilm-dwelling meiofauna density

Factor	λ	p
RDA pelagic		
MDD	**0.36**	**0.002**
O_2	0.08	0.068
T	0.06	0.100
Cond	0.06	0.158
pH	0.05	0.218
Meio	0.05	0.248
DAF	0.04	0.278
AFDM	0.04	0.352
V	0.04	0.390
RDA biofilm		
T	**0.24**	**0.004**
MDD	**0.11**	**0.004**
pH	**0.09**	**0.004**
DAF	0.05	0.242
Cond	0.05	0.192
AFDM	0.05	0.070
Meio	0.05	0.210
O_2	0.04	0.362
V	0.03	0.508

Axes 1 and 2 of the RDA performed with the water column variables explained 43.3 and 10.3% of the distribution variance of diatom species, respectively (Fig. 5b). Pelagic diatom distribution was largely and significantly influenced by discharge (Table 2). The diatom species' affinities with discharge (seasonality) clearly matched with the distribution patterns observed in the biofilm.

Comparison of biofilm and pelagic diatom communities

The results of the ANOSIM showed that biofilm and pelagic diatom communities differed most during periods of low flow and high temperatures (Table 3, Fig. 6). Biofilm and pelagic communities became more similar under increasing discharge and decreasing temperatures (i.e. winter conditions). Intra-habitat comparisons showed maximum community differences between low and high discharges (ANOSIM: pelagic, R = 0.905; biofilm, R = 0.656) and between low and high temperatures (ANOSIM: pelagic, R = 0.676; biofilm, R = 0.899). The R-values

Table 3. Test for diatom species composition similarities (ANOSIM) between the water column and the epilithic biofilm, according to discharge and temperature constraints. The R-value and the significance level (*, ** and *** for $p < 0.05$, 0.01 and 0.001, respectively) are provided. Mean daily discharge (MDD) constraints (in $m^3 s^{-1}$): Low, 0 to 50; Med, 51 to 100; High, >100. Temperature constraints (in °C): Low, 0 to 9; Med, 9 to 17; High, >17

Biofilm	Water column		
	Low	Med	High
MDD			
Low	1**		
Med		0.66**	
High			0.832***
Temperature			
Low	0.872**		
Med		0.865**	
High			0.931**

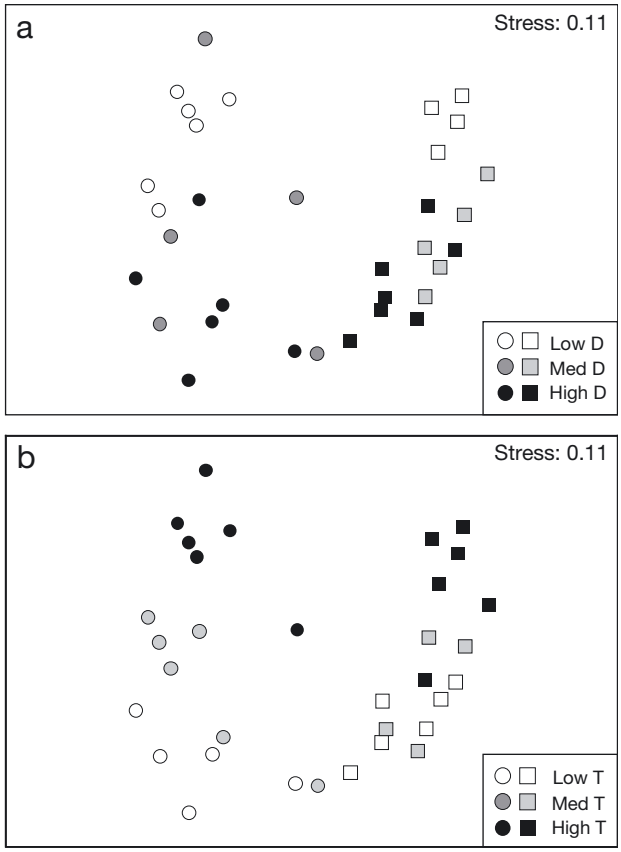

Fig. 6. Non-metric multidimensional scaling plots showing diatom assemblage similarities according to (a) discharge (D) and (b) temperature (T) constraints. Circles: biofilm assemblages; squares: pelagic assemblages. Mean daily discharge constraints (in $m^3 s^{-1}$): Low D, 0 to 50; Med D, 51 to 100; High D, >100. Temperature constraints (in °C): Low T, 0 to 9; Med T, 9 to 17; High T, >17

showed that pelagic communities were more influenced by discharge, while biofilm communities were more influenced by temperature.

DISCUSSION

The present study showed that phytoplanktonic and phytobenthic communities were generally closely coupled in the middle course of the Garonne River, and decoupling only occurred occasionally. Biofilm and pelagic chl *a* maxima showed lags that accounted for biofilm self-detachment events which occurred independently of high flow conditions. Furthermore, biofilm chl *a* was also reduced by flood events, but massive drift of microphytic cells was not detected. Diatoms strongly dominated both phytoplanktonic and phytobenthic communities, as reported by Eulin & Le Cohu (1998) and Leflaive et al. (2008). Throughout the year, almost all diatom species (99%) found in the water column were benthic species, sensu Krammer & Lange-Bertalot (1991). This finding confirms the benthic (biofilm) origin of the diatom cells occurring in the water column. However, planktonic diatom species (mostly *Cyclotella atomus*) contributed up to 34% of diatom assemblages in the biofilm during the summer to autumn low water flow period. These planktonic forms were probably derived from upstream lentic areas, including an incipient oxbow lake located 1.3 km upstream and the Mancies dam located 20 km upstream of the study site. These reservoirs supply the Garonne with a large number of phytoplankton cells (Ameziane et al. 2003). Planktonic (centric) diatoms are less mobile than those with a raphe (pennate diatoms), and this low mobility could favour their entrapment within the biofilm matrix during low flow conditions in rivers (Roeder 1977).

The pelagic and biofilm diatom communities differed most under periods of low water flow and were more alike under high flow conditions. In our study, the distribution of the diatom species in the 2 compartments and, hence, their benthic–pelagic exchanges were primarily shaped by discharge. This has also been observed by Biggs & Close (1989) and Uehlinger et al. (1996), who showed that >60% of the periphyton biomass variance in streams could be explained by discharge variations. Hudon & Legendre (1987) determined that the epilithic biofilm architecture was due to a combination of morphology, growth-form and mobility of diatom species, and that the mobile taxa were the most prone to become dislodged by flow velocity. This phenomenon apparently occurred in the Garonne, since the presence of mobile taxa in the water column increased with greater discharge (Fig. 5b). However, also within the biofilm, mobile diatoms were more abundant under high flow conditions (Fig. 5a), and therefore the observation could result from the close correspondence between the high occurrence of mobile diatoms in the biofilm matrix and in the water column. The observed species distribution patterns in our study may also be partially explained by the fact that mobile diatoms can display migration behaviour within biofilms (Consalvey et al. 2004) and, thus, can reduce their exposition to flow erosion. Our results also showed that temperature was a significant predictor for diatom distribution in the biofilm. We especially found that erected growth forms occurred during cold months, corroborating the findings of Eulin & Le Cohu (1998). Solitary centric diatoms and prostrated growth forms, however, mostly occurred in summer, as was observed by Tornés & Sabater (2010). In the Garonne River, temperature and discharge showed opposite seasonal patterns, and their combination defines a characteristic seasonal gradient which accounts for most of our observed diatom distribution.

During September 2009, the water flow in the Garonne was low and constant, and pelagic chl *a* increased, whereas biofilm chl *a* decreased. This unusual uncoupling suggests that factors other than flow or temperature could be involved in benthic–pelagic exchanges. Self-detachment of the biofilm is known to occur during low flow periods, which, in the Garonne River, commonly coincide with periods of high temperatures. During these periods, the maturing biofilm shifts towards more heterotrophic assemblages (Peterson et al. 1990, Boulêtreau et al. 2006) and is able to host relevant consumer densities. During September 2009, 90 to 140 meio-invertebrates (mostly nematodes, rotifers and chironomids) dwelled in each square centimetre of biofilm (Majdi et al. 2012a). Although we did not detect meiofaunal density as a significant factor shaping biofilm diatom composition, it is possible that meiofaunal activity could disrupt mat cohesion and could further favour self-detachment processes (e.g. Pinckney et al. 2003, Sabater et al. 2003). Macro-invertebrate grazers can strongly influence biofilm biomass and architecture (Lawrence et al. 2002, Hillebrand 2009). In particular, selective grazing on certain species and avoidance of others can shape microphytic assemblages (e.g. Steinman et al. 1987). In the Garonne biofilms, meiofauna grazes on epilithic diatoms, but the grazing pres-

sure remains relatively low (Majdi et al. 2012b,c). Moreover, macro-invertebrate grazers (mostly Trichoptera and Ephemeroptera larvae) were abundant on cobbles in early summer and could be responsible for the dramatic reduction in biofilm chl *a* (Majdi et al. 2012a). Eulin & Le Cohu (1998) also showed that prostrated diatoms were less often consumed by macro-invertebrates than erected and chained growth forms. Hence, the macro-invertebrate summer grazing pressure could partly explain why erected (*Gomphonema* spp., *Cymbella* spp., *Nitzschia* spp., *Surirella* spp.) and chained (*Fragilaria* spp., *Diatoma* spp., *Melosira varians*) diatoms dominated during winter, while prostrated diatoms (*Cocconeis* spp., *Amphora* spp., *Navicula* spp.) dominated during summer. However, this suggestion must be considered with caution, since the ecological preferences of diatoms might also influence their distribution. For example, *Cocconeis* spp. are epiphytes and their summer abundance could also be explained by the increasing availability of macrophytes in Garonne pools and/or of filamentous green algae in the biofilm during summer (Eulin & Le Cohu 1998).

Our results showed that the epilithic biofilm was an essential and constant provider of diatoms for the water column in the Garonne River, thus corroborating the pioneering work of Roeder (1977). However, during the summer low flow period, susbstantial numbers of planktonic diatoms can settle on the biofilm. While hydrodynamics and temperature (seasonality) primarily shaped these exchanges, biotic processes such as biofilm self-detachment and grazing are suspected to play a role, especially during the summer low flow period. The observed habitat exchanges of both 'benthic' and 'planktonic' diatoms raise the question of the adequacy of the 'benthic' versus 'planktonic' type categorisation. Out of 190 taxa observed in our study, 78 were observed both in the biofilm and in the water column. So apparently, several diatom taxa were not restricted to one or the other habitat, but occurred both on benthic substrata and in the water column. The occurrence of typical 'biofilm' diatoms in the water column during high flow periods shows that at least a part of the diatom community lives in the biofilm and finds itself in the water column at a given moment. Our data did not allow us to discern whether benthic diatoms gave rise to the planktonic population or, if instead, they were transiently exported to the water column. More frequent (e.g. daily) observations under controlled conditions would confirm the possible use of the 2 habitats in the dynamics of diatom species.

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