Dynamics of benthic diatom colonization in a cadmium/zinc-polluted river (Riou-Mort, France)

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Abstract

Periphytic diatom communities were sampled from glass substrates immersed along a gradient of organic and metallic pollution. We investigated the influence of nutrients and a combination of nutrients and metals on biofilms and diatom communities settling on the glass over three weeks. Biofilm was characterized through organic biomass, chlorophyll *a* concentrations and metal content; structure of diatom assemblages was assessed by studying densities, mean biovolumes and taxonomic composition. Exposure to organic pollutants resulted in an increase of biomass (dry weight, chlorophyll *a* concentrations and diatom densities) and diatom community structure was similar to that at an unpolluted site relative to nutrient concentrations. *Cyclotella meneghiniana* was dominant and the species *Nitzschia palea, Navicula gregaria* and *Melosira varians* were well-represented. Downstream of the metal-contamination source, biofilm biomass, as well as chlorophyll *a* concentrations, decreased as cadmium and zinc content got higher (up to 60µgCd/g dry weight and 1400µgZn/g dry weight). Concurrently, the size distribution of diatoms, changing from larger to smaller individuals, reflected changes in the taxonomic composition of the assemblages where *Eolimna minima* was found in high proportions. Statistically significant amounts of abnormal frustules were also enumerated in the metal-polluted environment ($p<0.05$).

Keywords

Biomonitoring; Periphyton; Diatom assemblages; Streams; Cadmium; Zinc; Heavy metals effects; Morphological abnormalities

Introduction

Diatom community structure can be affected by high levels of micropollutants, and in particular, metals which are often found in rivers. With the implementation of the European Water Framework Directive (WFD, 2000/60/CE), there is a need for data to allow the characterization of the effects of pollutants on aquatic environments based on selected biological key compartments, notably diatom communities.

Several studies have indicated a change in the structure of biofilms when exposed to metal pollution: PERES *et al.* (1997) and GOLD (2002) reported lower cell densities in contaminated sites, diatom assemblages were also found to be very different in reference compared to polluted sites (IVORRA 2000). Cell deformities affecting general cell shape and/or valve ornamentations were observed under metal stress, and are suspected to be an indicator of such pollution (DICKMAN 1998, TORRES et al. 2000, GOMEZ and LICURSI 2003).

Our study aimed at identifying *in situ* descriptors of metal-pollution effects, with the aim to improve diatom bio-indication tools. In a zinc-mining impacted site generating cadmium and zinc pollution, the development of benthic communities was monitored from bare surfaces to mature biofilms. After a three-week development, diatom communities were characterized and we describe here the effects attributed to the pollution studied.

Material and methods

Field sampling sites

Biofilms were left to colonize glass substrates in the Riou-Mort stream, a small tributary of the river Lot located in the industrial basin of Decazeville (South West France, 44°N / 2°E). Three sites presenting different types and levels of pollutions were selected (figure 1):

- the reference site (Firmi), located about three kilometers upstream from the anthropized area of Decazeville, with very low metal concentrations, and non-limiting nutrient content;

- the organically polluted site (Decazeville), located in the urban zone, highly nutrient-polluted and showing no metal concentration;

- the metal polluted site (Joanis), located just after the confluence of the Riou-Mort with the Riou-Viou, a stream carrying seepage from a former zinc factory, presenting high levels of dissolved cadmium and zinc (SAY 1978). In the year 2000, AUDRY *et al.* (2004) measured concentrations of 16mg/L dissolved cadmium and 1300mg/L dissolved zinc (average values).

Physical and chemical characterization of sites and metal concentrations in the water

Temperature, pH, conductivity and dissolved oxygen were measured at each site and sampling date in the stream (WTW, Weilheim, Germany), during the 20-day experimental period (July 2004).

Stream water samples were simultaneously collected and brought back to the laboratory for nutrient measurements. Phosphate, silica, ammonium, nitrite and nitrate concentrations were determined according to French and international standards (NF T90-023, NF T90-007, NF EN ISO 11732 and NF EN ISO 13395, respectively).

Metal concentrations at Joanis were analysed by the TGM laboratory, University Bordeaux1.

Periphyton sampling method

At each of the three sites, two plastic racks, perforated and equipped with glass slides (so-called, diatometers), were left in the water column for 4 to 20 days. They were immersed 5 centimeters below the water surface under similar light penetration conditions, perpendicular to the current (figure 2). The diatometers (300 cm^2 area for both sides) were used as artificial substrates for algal attachment. Periphyton samples were taken from early stages of development to mature biofilms (PETERSON and STEVENSON 1990, BARRANGUET et al. 2005) after 4, 7, 14 and 20 days. Each time, one glass substrate was removed from each of the

diatometers at the three sites, and scraped with a cutter blade to form two replicate periphyton samples per site and per sampling date.

All the periphyton samples were diluted to a standard volume of 100mL and divided into four aliquots assigned to various analyses. 5mL were preserved with 1mL of formalin solution (37% formol) for analysis of diatom assemblages. 20mL were filtered through a GF/C filter; the filters were kept refrigerated (4°C) in the dark for less than 24 hours, for chlorophyll *a* measurements in accordance to French standard NF T90-117. A further 20mL were used for particulate matter analysis: biofilm dry weights were determined following the European standard NF EN 872; after drying and weighing, samples were ashed at 500°C for 1 hour in a muffle furnace and results were reported as ash-free dry matter (AFDM). 50mL were put in Teflon© jars for metal concentration measurements: after filtration and mineralization, cadmium concentrations were measured by atomic absorption spectrophotometry (Varian AA400 – Zeeman correction, Victoria, Australia) and by autosampler, and zinc concentrations by flame atomic absorption spectrometry (Varian AA20). Detection limits were 0.1µg/L for cadmium and 10µg/l for zinc.

Quantitative and qualitative analysis of diatom assemblages

Diatom density was estimated in each untreated sample using a Nageotte counting chamber by enumeration of the total number of cells exhibiting chloroplasts in 10 fields (1.25µL each, 0.5mm depth) using light microscopy at 400x magnification (photomicroscope Leica DMRB, Wetzlar, Germany). Data were recorded as cells per unit area of sampled substrate (number of diatom cells/cm²).

Samples assigned to taxonomic analysis of diatom assemblages were prepared according to the procedure described in ANSP protocols (CHARLES et al. 2002). After digestion of 3mL 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© (Northern Biological Supplies Ltd, UK), a high refractive index (1.74) medium. A minimum of 400 diatom valves were identified on each slide at 1,000x magnification, following the Süβwasserflora (KRAMMER and LANGE-BERTALOT 1986 - 1991) classification. Relative abundances of each species and species richness were estimated, and diatom diversity was calculated using the Shannon index. Community biovolume was estimated after the theoretical biovolume (given in the literature) of each species found in the samples. The total biovolume of the community (B) was calculated as follows: $B = \sum_{i=1}^{n} (RA_{sp\_i} \times B_{sp\_i})$, where n: species richness of the sample, RA_{sp_i}: relative abundance of species i, B_{sp_i}: theoretical volume of species i. Individual deformities (cells with abnormal general shape and / or diatoms with deformed valve wall ornamentation) were observed and their frequency determined.

Statistical analyses

Data were checked for normality and variance equality using STATISTICA software (v. 5.1, StatSoft, 1998) before launching the tests. Analysis of variance (ANOVA) methods were performed to study the effect of site (Firmi, Decazeville and Joanis) on biofilm characteristics (dry weights, AFDM, pigments and cadmium content) and diatom densities. If the null hypothesis was disproven, we used Tukey HSD post-hoc test to detect significant differences between groups. We considered all statistical results significant that had their probability (noted *p*) less than 0.05.

A principal component analysis (PCA) was performed with PC-ORD software (MCCUNE and MEFFORD 1999) on data related to relative abundances of diatom species, in order to visualise taxonomic differences between diatoms communities collected in the different sites. Only the species having cumulative relative abundances representing more than 1% (of the whole dataset) were considered.

To test the assumption that diatom abnormalities reflect a high level of metal contamination, we calculated the correlation coefficients between the frequency of deformed valves and cadmium concentrations in the biofilm. We then calculated a one-tailed *p*-value indicating the probability of obtaining a mean correlation value larger than the observed value given the null hypothesis of randomly occurring abnormalities.

Results

Field colonization conditions

The physical and chemical parameters measured in the sampled waters are shown in table 1.

At all sites, pH was around 7.5 and water temperatures were about 20°C during this study. Comparable silica levels were found in the three sites, and at concentrations sufficient for diatom development. Maximum nutrient values were observed in Decazeville and Joanis. Decazeville is impacted by quite strong organic and domestic contaminations: orthophosphate pollution as well as high levels of reduced forms of nitrogen like ammonia which nitrify downstream (the highest nitrate and nitrite concentrations were found at Joanis). Conductivity values were quite high (about 1500µS/cm) and a peak was observed on the 5^{th} of July in Decazeville and Joanis.

Biofilm settlement at Joanis took place under much higher cadmium (more than 20 fold) and zinc (more than 200 fold) water concentrations than in Firmi and Decazeville.

Current velocity values (http://hydro.rnde.tm.fr/) were quite constant during the experimental period (0.13±0.01m^3/s), except for day 19 when the flow increased two-fold (0.24 m^3/s).

Biofilm description (see table 2 and fig. 3)

At each site, the dry weight and organic content increased as the biofilm settled, except for the Decazeville site, where dry weight decreased at the last sampling date. In general, dry weight

and organic content of Decazeville biofilms from day 4 to day 14 were significantly higher (p<0.05) than those measured at Firmi and Joanis.

Total chlorophyll *a*, as an estimate of algal biomass, showed the same trends, except for at Joanis after 20 days, where a large increase of pigment concentration was observed.

Diatom densities on artificial substrates allowed the quantitative characterization of communities. At Firmi, a mean density of 3200 cells/cm² (standard error = 125 cells/cm²) was found after three weeks colonization. 10300 cells/cm² were found at Decazeville, whereas 11500 cells/cm² were recorded at Joanis. Standard errors respectively represented 250 and 980 cells/cm². A site effect was observed (p<0.05) and Tukey tests separated Firmi from the sites downstream.

Metal accumulation (see table 2)

Accumulation of cadmium in Joanis biofilms increased in the first week of glass slide colonization and then stabilized around 1400µg/gDW. Cadmium concentrations in biofilms were significantly lower (p<0.05) in Firmi and Decazeville sites.

Zinc levels were analyzed in Decazeville and Joanis biofilms sampled at day 20 and bioaccumulation was much higher at Joanis: around 4200µg/gDW, whereas 2800µg/gDW was measured at Decazeville.

Characterization of diatom assemblages

Although species richness and diversity were similar between sites (42 taxa were identified per sample and Shannon index values averaged 3), taxonomic composition and relative abundances of diatom communities differed. Firmi and Decazeville assemblages were dominated by *Cyclotella meneghiniana* KÜTZING, and the species *Nitzschia palea* (KÜTZING) W.SMITH, *Navicula gregaria* DONKIN and *Melosira varians* AGARDH were well represented.

In the Joanis site, the dominant taxa, *Eolimna minima* (GRUNOW) LANGE-BERTALOT, was found in large amounts (around 50% of the total community).

Result of PCA based on relative abundances of diatom species (fig.4) revealed great differences between the three sites, and showed strong homogeneity within each site. Axes 1 and 2 accounted for around 37% and 27% respectively of the total variability. Axis 1 separated diatom communities developed up- and downstream of the metal pollution source. Firmi and Decazeville stations were found on the left-half plane (negative values) and the Joanis site on the right-half plane (positive values). Separation along Axis 2 expressed the gradient of organic pollution.

Biovolume calculated for each site represented about 1400μm^3 (average for a diatom cell) at Firmi and 1200μm^3 at Decazeville, and decreased to 700μm^3 at Joanis. Differences in species sizes were also noticed: species measuring less than 100 μm^3 represented half of the taxa identified at Joanis, whereas they were found in low percentages (about 10%) at Firmi and Decazeville. More than 50% of big species (> 500μm^3 biovolume) were enumerated at Firmi and Decazeville, and only counted for 20% of the relative abundance at Joanis (fig. 5).

The frequency of abnormal frustules (fig. 6), which were almost only found at Joanis, increased significantly as biofilm settled. Most of them were araphids: *Fragilaria spp.* (average abundance in the cadmium-polluted site after a 20-day exposure: 3.01‰), *Ulnaria ulna* (NITZSCH.) LANGE-BERTALOT (36.17‰). Deformities were also observed among raphids like *Achnanthidium saprophila* (KOBAYASI & MAYAMA) ROUND & BUKHTIYAROVA (1.00‰), *Eolimna minima* (2.01‰), *Gomphonema parvulum* (KÜTZING) KÜTZING (4.52‰) and *Nitzschia palea* (1.00‰). A few abnormal frustules of centric diatoms (*Cyclotella meneghiniana* (0.50‰)) and monoraphids (*Cocconeis pediculus* EHRENBERG (0.50‰), *Planothidium frequentissimum* (LANGE-BERTALOT) ROUND & BUKHTIYAROVA (1.00‰), *P. lanceolatum* (BREBISSON ex KÜTZING) LANGE-BERTALOT (1.00‰)) were found as well.

Discussion

The results obtained for days 4, 7 and 14 demonstrated that *in situ* combined high levels of organic contaminants and heavy metals were detrimental to biofilm settlement, but also that the organic matrix under metal exposure develops adaptive strategies. Biofilm growth was followed at the three sites from substrate immersion to a 20-day colonization. Quantitative differences were observed between the three stations. At each site, primary production (chlorophyll *a*) and biofilm dry weight increased with immersion duration; moreover biofilm settlement and development depended on nutrient availability and on the presence or not of dissolved cadmium and zinc: at Firmi, biofilm was not as dense as at Decazeville, where increases in nutrient availability favored biofilm development (LAWRENCE et al. 2004). Despite a high nutrient potential, metal pollution in Joanis waters did not allow biofilm to grow as thick and dense as in Decazeville, as described by IVORRA *et al.* (2002) in a polluted Dutch river. In Joanis, polluted by combined nutrient and metals, biofilm thickness was reduced compared to the Decazeville site. Colonization rates (as measured by accumulating total AFDM per day) also supported the notion that the nutrient-enriched site Decazeville (mean colonization rate: 0.02gAFDM/day) was a significantly more productive station than upstream site Firmi (0.002gAFDM/day) or even downstream Joanis (0.006gAFDM/day). In contrast, diatom densities were quite similar between Decazeville and Joanis sites, but most of the diatoms found at Joanis were small and attached taxa, enveloped in a thinner organic layer protecting individuals from strong exposure (LEHMANN et al. 1999).

On the 20th July (one day before the fourth sampling), the river Riou-Mort was in spate at the Decazeville and Joanis sites; the flow increased from about 0.13 to 0.24m^3/s. This moderate hydrological event probably led to different rates of erosion of the biofilms on the glass substrates. GHOSH and GAUR (1998) showed that species relatively weakly attached to the substrate, which are often associated with particulate matter, are likely to disappear when exposed to higher velocity currents. At Decazeville, the very thick and dense organic matrix

may have undergone extensive loss of organic matter, of suspended particles, of weakly attached algae (for instance, filamentous algae which were well represented), whereas at Joanis the thin and strongly attached biofilms were less affected by the increase of flow.

Qualitative modifications were also revealed. Chlorophyll *a* and diatom densities revealed a strong effect of organic pollution; the high nutrient levels seemed to set the degree of diatomic biomass development, that was quite similar at Decazeville and Joanis. However, although the actual quantity of biomass was similar, metal pollution dramatically changed the algae it was composed of. Community biovolume calculations and ratios of small to big taxa were found to reveal differences between metal-free and contaminated sites. Average biovolume for a single cell at Firmi and Decazeville was larger than 1000μm^3 and was drastically reduced at Joanis (700μm^3). The contribution to the total community biovolume of small species (<100μm^3) and big ones (>500μm^3) was also relevant for distinguishing between up- and downstream of the metal pollution source, but needed more calculations than relative abundances of species from each size category. These results are in accordance with reductions in cell sizes reported for metal-stressed environments (CATTANEO et al. 1998) and also with studies demonstrating that the uptake of metal ions from solution into the cell cytoplasm is directly linked to the specific surface area of the algae (KHOSHMANESH et al. 1997). As intracellular cadmium is suspected of perturbing algal metabolism (PERREIN-ETTAJANI et al. 1999, PAULSSON et al. 2000, BERTRAND et al. 2001, PINTO et al. 2003), some kind of selection may happen. When exposed to metal contaminations, taxa presenting lower cell surface areas would be less disfavored than big species which are able to remove higher amounts of poisonous matter (JOUX-ARAB et al. 2000). To our knowledge, very few studies have attempted to correlate cell size to metal contamination. The dominance of small, adnate species under cadmium and zinc pollution was however described by MEDLEY and CLEMENTS (1998) and CATTANEO et al. (2004). Several authors have also observed shorter valves during

contamination events. GENSEMER (1990) examined the reduction in cell size of *Asterionella ralfsii* var. *americana* KÖRN in response to additions of aluminium to cultures, and CATTANEO et al. (2004) reported striking size changes in the dominant diatom taxa during a period of cadmium, zinc and iron contamination in Lac Dufault (Canada). Size reduction of individuals as well as of the global community appeared to be an excellent specific indicator of metal contamination. However, it involves an additional counting effort and thus increased cost which is hardly compatible with routine biomonitoring.

Taxonomic differences between diatom assemblages were investigated using principal component analysis. PCA discriminated communities according to their pollution characteristics. Separation along Axis 1 (in accordance with the gradient of cadmium and zinc) allowed us to characterize metal sensitivity of some species. High relative abundances of *Cyclotella meneghiniana* (at the end at the experiment, this species represented around 50% of the total community at both Firmi and Decazeville sites) and *Melosira varians* (which represented more than 10% of those assemblages) were correlated to negative values on Axis 1, i.e. with high sensitivity to cadmium and zinc. Species from the genera *Cyclotella* were already described as metal-sensitive. RUGGIU *et al.* (1998) described it for *C. bodanica*, VAN DAM *et al.* (1990) for *C. comensis* GRUNOW in VAN HEURCK, SHEHATA *et al.* (1999) for *C. comta* (EHR.) KÜTZING or GOLD (2002) for *C. stelligera* CLEVE and GRUN (in VAN HEURCK). Although its status is still discussed by BARRANGUET *et al.* (2002) and BLANCK *et al.* (2003) who found it quite tolerant, many authors found *Melosira varians* sensitive to heavy metals (PERES et al. 1997, MEDLEY and CLEMENTS 1998, IVORRA 2000, FEURTET-MAZEL et al. 2003, GOLD et al. 2003a, b). This study also revealed a sensitivity of *Navicula gregaria* exposed to metal contamination. Representative of Joanis communities (corresponding to positive Axis 1 values), *Eolimna minima* was found to be very tolerant (according to several indications found in the literature: PERES *et al.* 1997, GOLD 2002, FEURTET-MAZEL *et al.* 2003), as well

as *Mayamaea atomus* var. *permitis*. Higher Axis 2 values characterized communities from the Firmi site, which were strongly opposed to the Decazeville communities grown under high levels of organic matter. *Surirella brebissonii* KRAMMER & LANGE-BERTALOT settled quickly and stabilized in abundances close to 10% at Firmi, when Decazeville assemblages were associated with higher proportions of saprophile species such as *Gomphonema parvulum, Navicula lanceolata* (AGARDH) EHRENBERG and *Nitzschia palea.*

In agreement with DICKMAN (1998), who suspected deformed individuals to be a valid indicator of metal pollution, significant frequencies of diatom deformities were associated with metal-contaminated waters in our study ($p<0.05$). A significant correlation ($p<0.05$, $r^2=0.92$) was shown between cadmium concentrations in biofilm and the frequency of abnormal cell morphology of diatoms. Anomalous *Fragilaria* species have already been observed by several authors in metal-polluted conditions (ANDRESEN and TUCHMAN 1991, MCFARLAND et al. 1997, DICKMAN 1998). Samples collected previously on the Riou-Mort river (during experiments conducted in 2003 and 2004) also exhibited deformed frustules of the following species: *Eolimna minima, Gomphonema parvulum*, *Nitzschia palea*, which were also found in this study. Although the frequency of abnormalities was statistically meaningful in the metal-contaminated site, there is a need to improve this tool, in order to be used for river quality assessment. We suggest more time be devoted to enumerating deformed valves on slides, by increasing the counting effort.

Summary

1. Biofilm development, as well as diatom community settlement, are affected by combined organic and heavy metal pollution.
2. Under the conditions of this study, the diatoms *Eolimna minima* and *Mayamaea atomus* var. *permitis* showed high resistance to cadmium/zinc contamination.

3. The results suggest that calculating the total biovolume of the diatom community could serve as an additional criterion to assess metal pollution.
4. The frequency of abnormal forms, as a tool for monitoring heavy metal-pollution, also seems to be a relevant indicator.

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	Firmi				Decazeville				Joanis			
	4 days	7 days	14 days	20 days	4 days	7 days	14 days	20 days	4 days	7 days	14 days	20 days
pH	7.4	7.4	7.4	7.3	7.9	7.7	7.7	7.6	8.0	7.7	7.7	7.7
conductivity (µS/cm)	1377	1223	1410	1427	3340	1104	1052	961	2350	1426	1290	1257
temperature	17.6	16.4	19.4	24.0	19.6	17.4	17.0	23.1	19.7	17.6	16.7	24.8
O_2 (mg/L)	7.0	8.0	9.3		4.6	4.5	4.0		7.2	7.3	6.4	
NH_4 (mg/L)	0.545	0.718	1.33	2.34	0.888	7.42	8.06	4.9	0.773	1.95	2.5	3.43
NO_3 (mg/L)	2.96	3.61	2.46	2.89	3.08	1.53	<dl	1.12	23.71	52.41	38.36	36
NO_2 (mg/L)	0.184	0.184	0.236	0.24	0.711	0.1	0.302	0.12	0.932	0.968	0.913	1.32
PO_4 (mg/L)	0.01	0.10	0.10	0.04	0.17	0.99	1.16	0.95	0.58	0.87	0.82	2.86
Si (mg/L)	10.5	13.5	12	14.5	14	11	10	11	12	11.5	12	12.5
Cd (µg/L)	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	26	26	27	24
Zn (µg/L)	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	2104	2170	2305	1617

Table 1. Values corresponding to physical and chemical characteristics of stream water determined during the experimental period (dl: detection limit: 0.3mg/L for NO_3, 1µg/L for cadmium and 10µg/L for zinc).

	Firmi				Decazeville				Joanis			
	4 days	7 days	14 days	20 days	4 days	7 days	14 days	20 days	4 days	7 days	14 days	20 days
DW (mg/cm²)	0.029	0.081	0.176	0.350	0.311	0.358	0.984	0.584	0.026	0.094	0.319	0.407
AFDM (mg/cm²)	0.011	0.026	0.049	0.197	0.112	0.149	0.450	0.224	0.015	0.047	0.135	0.168
Chl.*a* (µg/cm²)	0.22	0.31	0.81	0.84	0.79	1.02	1.96	2.21	0.19	0.39	2.00	5.58
[Cd] (µg/g DW)	<dl	<dl	<dl	2	67	59	47	39	1081	1868	1427	1327
[Zn] (µg/g DW)	nm	nm	nm	nm	nm	nm	nm	2766	nm	nm	nm	4171

Table 2. Mean dry weight values (DW), ash free dry matter values (AFDM), chlorophyll *a* concentrations (Chl.*a*) and cadmium ([Cd]) and zinc ([Zn]) concentrations in biofilm grown on artificial glass substrates (nm: not measured).

Figure captions

figure 1: Location of the study sites.

figure 2: Schematic representation of one rack of artificial substrates, as moored at the three experimental sites.

figure 3: Mean ash-free dry mass values (plain lines) and colonization rates (dotted lines) and chlorophyll *a* concentrations in the biofilms of the different sites during the experiment (•: Firmi; ■: Decazeville; ▲: Joanis). Error bars: standard deviations.

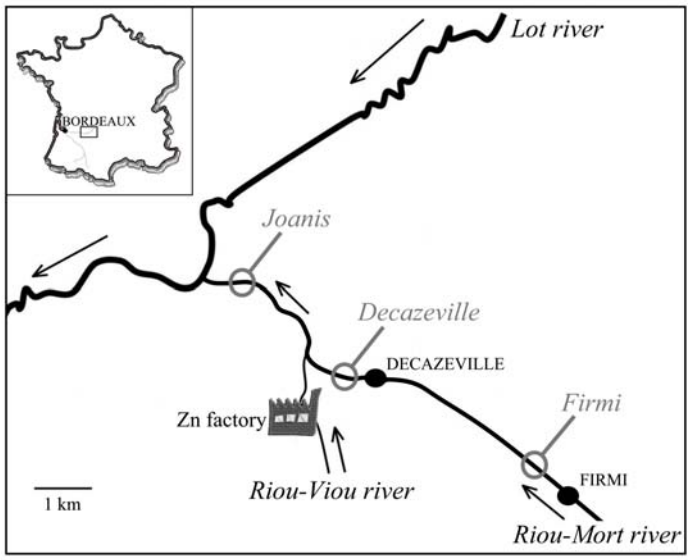
figure 4: Principal Component Analysis (PCA), with regions of data points identified by sample site (•: Firmi; ■: Decazeville; ▲: Joanis). Vectors plotted indicate the correlation scores for significant correlations between species relative abundances and the two principal components axes. (ADMI: *Achnanthidium minutissimum,* ADSA: *Achnanthidium saprophila,* CMEN: *Cyclotella meneghiniana,* EOMI: *Eolimna minima,* GPAR: *Gomphonema parvulum,* MAPE: *Mayamaea atomus* var. *permitis,* MVAR: *Melosira varians,* NGRE: *Navicula gregaria,* NLAN: *Navicula lanceolata,* NPAL: *Nitzschia palea*; PLFR: *Planothidium frequentissimum,* SBRE: *Surirella brebissonii,* UULN: *Ulnaria ulna*)

figure 5: Deformed individuals of *Achnanthidium minutissimum* (ADMT), *Cyclotella meneghiniana* (CMTG), *Encyonema minutum* (ENMT), *Fragilaria capucina* (FCAT), *F. capucina* var. *capitellata* (FCCT), *Gomphonema parvulum* (GPAT), *Planothidium frequentissimum* (PLFT) and *Ulnaria ulna* (UULT) collected from Joanis site.

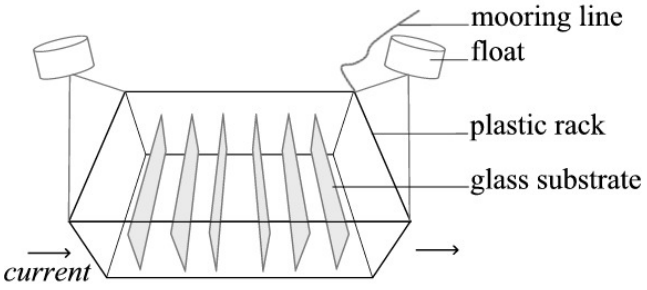
Scale bar = 10 μm

figure 6: Relative abundances of small species (less than 100μm^3, in black) and large species (more than 500μm^3, in white) and their contribution to the total biovolume of the community. Error bars: standard deviations.

Figures



Morin *et al*_fig. 1



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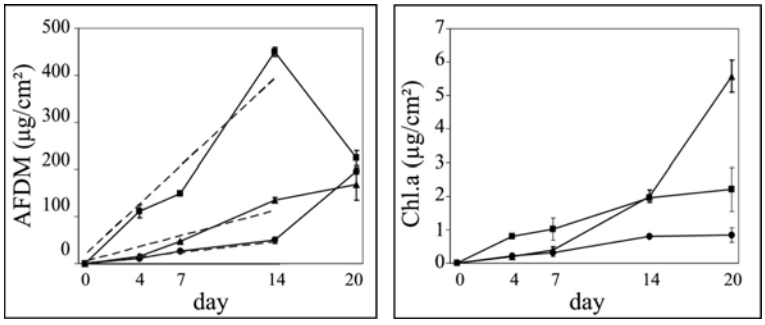

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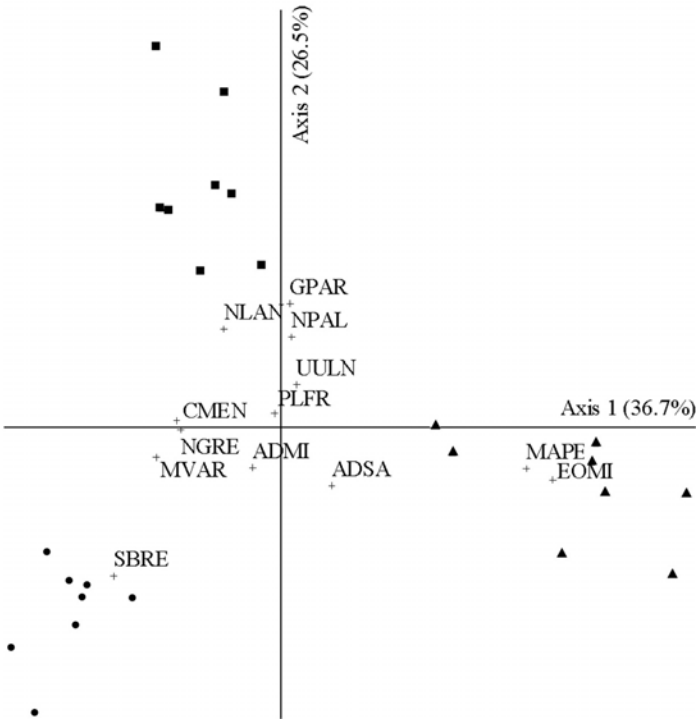
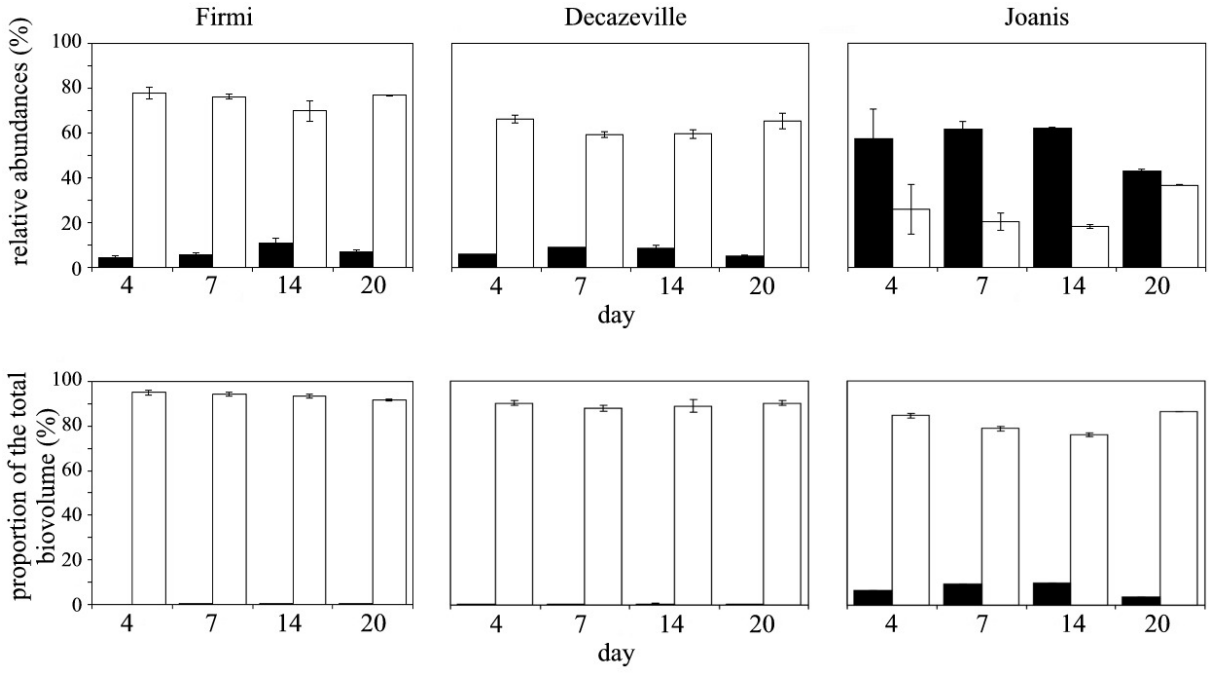
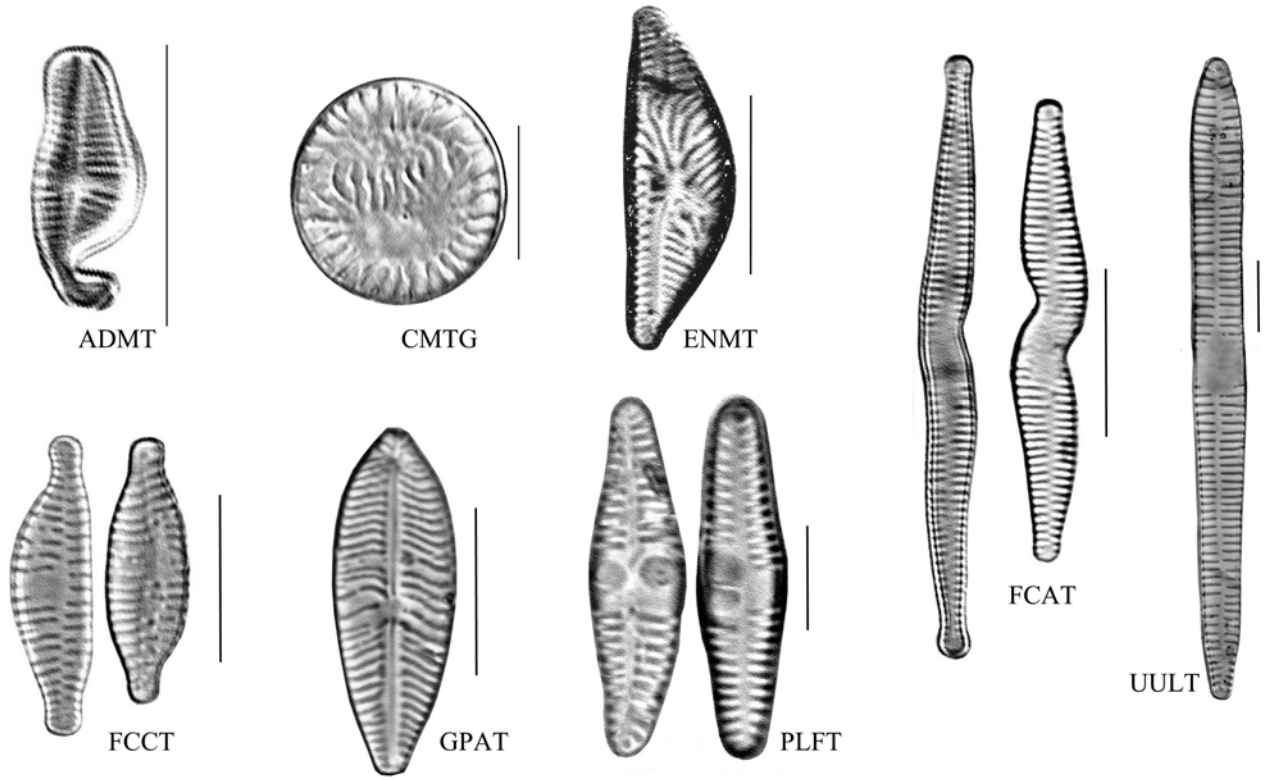


Morin *et al*_fig. 4



Morin *et al*_fig. 5



Morin *et al*_fig. 6