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ESSAY ON THE CHARACTERIZATION OF ENVIRONMENTAL FACTORS STRUCTURING COMMUNITIES OF EPILITHIC DIATOMS IN THE MAJOR RIVERS OF THE CANTON OF GENEVA, SWITZERLAND

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EPILITHIC DIATOMS ENVIRONMENTAL FACTORS RIVERS GENEVA SWITZERLAND GEOTYPES ABSTRACT. - The goal of this study is to evaluate the influence of four Geneva geotypes (characterized by the composition of trace elements in water), of the season and of water quality on the distribution of epilithic diatoms in the main rivers in the canton of Geneva (Switzerland). To achieve this, we used 72 samples of diatoms as well as some physical-chemical and bacteriological analyses. The sampling was conducted in the framework of monitoring the surface water quality between 1998 and 2004. For each of these 72 samples, the season (winter-spring or summer) as well as one of the four geotypes ("Jura", "Plaine", "Alpes", "Marais") was accounted for. Nine sample groups based upon the distribution of 120 diatom taxa were defined by statistical analyses. The flora of these nine groups is mainly composed of common and frequentlyoccurring species in the surface waters of the Swiss Plateau that represents good to average water quality. Nonetheless, some of the rarest species confined to poorly-nourished calcareous regions, were sampled only in certain groups. Our results show that in addition to the water quality, the geochemical signature influences the floral composition of Geneva's diatoms. It would be interesting to expand this type of study to the Swiss hydrographical network, to define its ecoregions, to measure its influence on the assemblage of diatoms and to ultimately adapt it to our objectives of biological quality.

INTRODUCTION

Since 1998, when the federal ordinance on water protection ("Ordonnance sur la Protection des Eaux", or "OEaux") came into effect, the Canton of Geneva (Switzerland) has implemented the Modular Stepwise Procedure (MSP) proposed by the Federal Office for the Environment (Conseil fédéral suisse 1998, Office fédéral de l'environnement 1998). This procedure suggests different methods and indices for monitoring the state of surface waters. For example, the Swiss Diatom Index (DI-CH), which is based on epilithic diatoms, diagnoses the quality of water (Hürlimann & Niederhauser 2007).

The current study attempts to evaluate the influence of Geneva geotypes, of season and of water quality on the distribution of epilithic diatoms in the major rivers. On a European scale, this type of study has already enabled the definition of several ecoregions and their diatomic reference assemblages (Cantonati *et al.* 2001, Ministère de l'Écologie et du Développement durable 2005, 2009, Rimet *et al* 2004, 2007, Tison *et al* 2005). This step has not yet been undertaken by the Swiss hydrographic system.

MATERIALS AND METHODS

The canton of Geneva, with an area of 282 km², is located in southwestern Switzerland, along the French border. It forms a

basin that catches the water draining from the Jura and the Alps. The hydrographic catchment area count 315 km of watercourses whose two main receptacles are Lake Geneva and Rhône River (Fig. 1). Lake Geneva provides 80 % of the potable water of canton Geneva and its water quality, as well as the Rhône's, is good. The agricultural surface represents 39.5 percent of the territory while that of housing and infrastructure represents 33.3 percent. The majority of precipitation occurs in winter.

Diatoms: Between 1998 and 2004, 72 samples of benthic diatoms were collected in the main rivers of the Canton of Geneva (Table I).

The benthic diatoms were sampled, cleaned and mounted in Naphrax in accordance with the calculation protocol from the Swiss Diatom Index (Hürlimann & Niederhauser 2007). For microscopic assessments, an Olympus light microscope using Nomarski differential interference contrast optic at a magnification of 1000x was used. The species identification was essentially made with the help of the flora of the diatoms from Krammer and Lange-Bertalot (1986, 1988, 1991a,b), completed by those of Diatoms of Europe (Krammer 2002, 2003, Lange-Bertalot 2001) and *Iconographia Diatomologica* (Lange-Bertalot & Metzeltin 1996, Reichardt 1999). For each of these samples, the Swiss Diatom Index (DI-CH) was calculated. It characterizes in biological terms the quality of water based on the concentrations in ammoniacal nitrogen, nitrite, inorganic nitrogen, total phosphorus, chloride and dissolved organic carbon. It then defines

code	river	site	year	month	season	Geotype
1	Allondon	Flies	1998	may	hp	"Jura"
2	Allondon	Flies	1998	nov.	hp	"Jura"
3	Allondon	Fabry	1998	nov.	hp	"Jura"
4	Allondon	embouchure	1998	nov.	hp	"Jura"
5	Eaux Froides	Les Iles	1998	nov.	hp	"Jura"
6	Eaux Froides	Les Iles	1998	may	hp	"Jura"
7	Eaux Chaudes	passerelle aval	1998	may	hp	"Jura"
8	Eaux Chaudes	passerelle aval	1998	nov.	hp	"Jura"
9	Roulave	embouchure	1998	may	hp	"Jura"
10	Roulave	embouchure	1998	nov.	hp	"Jura"
11	Lion	St-Genis	1998	may	hp	"Jura"
12	Lion	St-Genis	1998	nov.	hp	"Jura"
13	Lion	Vézegnin	1998	may	hp	"Jura"
14	Lion	Vézegnin	1998	nov.	hp	"Jura"
15	Allemogne	Les Martinets	1998	may	hp	"Jura"
16	Allemogne	Les Martinets	1998	nov.	hp	"Jura"
17	Arve	Vessy	2000	march	hp	"Alpes"
18	Arve	Vessy	2000	june	ea	"Alpes"
20	Arve	Vessy	2000	nov.	hp	"Alpes"
21	Chamburaz	embouchure	2001	feb.	hp	"Marais"
22	Chamburaz	embouchure	2001	aug.	ea	"Marais"
33	Marnot	embouchure	2001	feb.	hp	"Marais"
34	Marnot	embouchure	2001	aug.	ea	"Marais"
35	Châtelet	Pont du Nt du Chât.	2002	feb.	hp	"Plaine"
36	Châtelet	Pont du Nt du Chât.	2002	may	hp	"Plaine"
37	Châtelet	Pont du Nt du Chât.	2002	aug.	ea	"Plaine"
38	Couchefatte	Moulin Roget	2002	feb.	hp	"Plaine"
39	Couchefatte	Moulin Roget	2002	may	hp	"Plaine"
40	Couchefatte	Moulin Roget	2002	aug.	ea	"Plaine"
41	Rhône	amt Allondon	2002	feb.	hp	"Alpes"
42	Rhône	amt Allondon	2002	july	ea	"Alpes"
43	Rhône	amt Allondon	2002	nov.	hp	"Alpes"
44	Rhône	amt STEP Aïre	2002	feb.	hp	"Alpes"
45	Rhône	amt STEP Aïre	2002	july	ea	"Alpes"
46	Rhône	amt STEP Aïre	2002	nov.	hp	"Alpes"
47	Rhône	aval STEP Aïre	2002	feb.	hp	"Alpes"
48	Rhône	aval STEP Aïre	2002	july	ea	"Alpes"
49	Rhône	aval STEP Aïre	2002	nov.	hp	"Alpes"
50	Rhône	BFM Rive Gauche	2002	july	ea	"Alpes"
51	Rhône	BFM Rive Gauche	2002	nov.	hp	"Alpes"
52	Rhône	Chèvres Rive Droite	2002	feb.	hp	"Alpes"
53	Rhône	Chèvres Rive Droite	2002	july	ea	"Alpes"
54	Rhône	Chèvres Rive Droite	2002	nov.	hp	"Alpes"
57	Versoix	Sauverny	2003	feb.	hp	"Jura"
58	Versoix	Sauverny	2003	sept.	ea	"Jura"
59	Versoix	amt Divonne	2003	feb.	hp	"Jura"
60	Versoix	amt Divonne	2003	sept.	ea	"Jura"
61	Crève-C□ur	embouchure	2003	feb.	hp	"Marais"
62	Versoix	embouchure	2003	feb.	hp	"Jura"
63	Versoix	embouchure	2003	sept.	ea	"Jura"
64	Versoix	pont de Bossy	2003	feb.	hp	"Jura"
65	Versoix	pont de Bossy	2003	sept.	ea	"Jura"
66	Brassu	amt route de Suisse	2003	feb.	hp	"Jura"
67	Brassu	amt route de Suisse	2003	sept.	ea	"Jura"
68	Braille	amt route de Suisse	2003	feb.	hp	"Jura"

Table I. – The sampling dates, season and geotype corresponding to the 72 samples.

code	river	site	year	month	season	Geotype
69	Braille	amt route de Suisse	2003	sept.	ea	"Jura"
70	Creuson	embouchure	2003	feb.	hp	"Plaine"
71	Pry	amt route de Suisse	2003	feb.	hp	"Jura"
72	Pry	amt route de Suisse	2003	sept.	ea	"Jura"
73	Aire	pont de Certoux	2004	feb.	hp	"Plaine"
74	Aire	pont de Certoux	2004	sept.	ea	"Plaine"
75	Aire	pont des Marais	2004	fév.	hp	"Plaine"
76	Aire	pont des Marais	2004	sept.	ea	"Plaine"
77	Drize	Granges-Collomb	2004	feb.	hp	"Plaine"
78	Drize	Granges-Collomb	2004	sept.	ea	"Plaine"
79	Drize	Evordes	2004	feb.	hp	"Plaine"
80	Drize	Evordes	2004	sept.	ea	"Plaine"
81	Drize	Rivolette	2004	feb.	hp	"Plaine"
82	Marais	Bellevista	2004	feb.	hp	"Marais"
83	Clef	amont Tate	2004	feb.	hp	"Plaine"
84	Clef	amont Tate	2004	sept.	ea	"Plaine"
85	Tate	amont Clef	2004	feb.	hp	"Plaine"



Fig. 1 - Geographic location of the studied rivers and the sampling sites.

5 quality classes of water: very good, good, average, poor and bad.

Physical-chemical analyses: The physical-chemical and bacteriological samples were taken the same month as the diatoms, in the same stations. Among the measured parameters, the following were selected: temperature, conductivity, pH, oxygen, biological oxygen demand (BOD), dissolved organic carbon (DOC), total mineral nitrogen (Nmin total, sum of nitric, nitrous compounds, and ammonium), chloride, sulfate and the number of *Escherichia coli*. BOD, DOC, Nmin total, chloride and sulfate were log-transformed prior to use, because of the non-normal distribution of their values. These parameters are indicators of anthropogenic pollution derived from agriculture, household consumption or urbanization.

The methods used for the physical-chemical quality assessment of the Geneva surface waters adhere to the different Swiss recommendations and directives, notably the chemistry module of the MSP (Liechti *et al.* 2004).

Geotypes: For each of the 72 samples, the season, linked with the flow rate (winter-spring: elevated flow rates, summer: weak flow rates), as well as one of the four geotypes: "Jura", "Plaine", "Alpes", "Marais" was considered. The geotypes were defined according to Pomian and Nirel's (2006) work on the geochemistry of Geneva's rivers. The statistical treatment (Principal component analysis and Discriminant analysis) from 2088 samples allowed for the typological determination of water based on trace elements with purely geologic origins (Uranium and Barium) and led to the distinction between four geotypes: "Jura", "Plaine", "Alpes", and "Marais" (Pomian & Nirel 2006).

Statistical analysis: Multivariate analyses (Agglomerative hierarchical clustering) were performed using the *cluster* (Maechler *et al.* 2005) and *statistical* (R Development Core Team 2009) packages based on the R software, with the aim of grouping different samples with similar species' composition. The species matrix was transformed into a dissimilarity matrix using the Chord distance prior to applying the Ward clustering method. The environmental characteristics of the resulting groups were subsequently studied with help of the box-plot representation computed with the *graphics* package (R Development Core Team 2009) under R software.

RESULTS

The cluster highlighted nine groups of samples based on the distribution of 120 species of epilithic diatoms (Fig. 2). Statistical classification that makes sense led us to choose nine groups. The dominant species of diatoms (the average percentage of the species in each of the nine groups being $\geq 2 \%$), the water quality, the geotype and the season are characterized for each of the nine groups below. The different groups are distributed in relation to the quality of the water as expressed by the DI-CH. The assessment of the water quality for each group is based on the distribution of DI-CH results in each group (Fig. 3).

Very good to good water quality

Group 1 is dominated by *Achnanthidium minutissimum* (Kützing) Czarnecki (40 %) (Table II). It is composed of stations from the catchment area of the river Allondon, where the waters are clean and originate from the Jura (Fig. 4). The samples are all taken during winter (Fig. 5).

Group 2 is dominated by the species Achnanthidium pyrenaicum (Hustedt) Kobayasi (35 %), a species linked to very clean and calcareous waters (Rimet 2009). There one finds the stations of the heads of the catchment area of the Versoix and the Allondon rivers, at the foot of the Jura. These are reference stations for these two hydro-





Fig. 2 – The nine groups of samples based upon the community composition of epilithic diatoms.

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Groups from species composition

Fig. 3 – Swiss Diatom Index (DI-CH), altitude and physical-chemical parameters in the nine sample groups. *Classes of water quality as DI-CH:* Very good to good (DI-CH \leq 4.49); Good to average quality of water (3.5 \leq DI-CH \leq 5.49); Average to poor water quality (4.5 \leq DI-CH \leq 6.49).

graphic entities, that emerge from karstic resurgences.

Good to average water quality

Group 3 is almost solely composed of *Amphora pedic-ulus* (Kützing) Grunow (47 %), an ubiquitous species with an ecological optimum in average quality waters (Hürlimann & Niederhauser 2007). The samples from group 3 are very heterogeneous as regards the geotype, the season and the conductivity.

Group 4 mainly consists of *Amphora pediculus* (23.1 %) and *Nitzschia dissipata* (Kützing) Grunow (11.4 %). The majority of the samples were taken in winter and belong to different geotypes. The conductivity is variable. It distinguishes itself from group 3 by a more important number of dominant diatoms.

The species with the highest percentage, in Group 6, is *Nitzschia dissipata* (30 %), a common species that prefers good water quality. The samples from group six were taken in February, when the quality of water is generally best, in all of the catchment areas of the Canton. These samples, however, contain more chloride than the samples from the other groups (Fig. 3, Chloride). This phenomenon is linked to the salting of roads during snow periods. In fact, samples belonging to this group were located close to roads with highly congested traffic (Drize: Highway A40; Brassus: Highway A1 and the ''Route Suisse''). The dominant geotype is that of the "Plaine".

Group 7 contains stations that are principally situated on the Rhône River, where the "Alpes" geotype is dominant. It is the largest river of the study area, deeply influenced by Lake Geneva (Service de l'écologie de l'eau 2009). The stations were sampled in winter. The water quality is at the lower limit compared to the class average. Its temperature is also lower than the other groups (Fig. 3, DI-CH, Temperature). The most abundant species is Navicula lanceolata (Agardh) Ehrenberg (23.6 %), a species that is resistant until the β -alpha-mesosaprobic class and prefers cold waters where it can develop in mass (Krammer & Lange-Bertalot 1986, Hürlimann & Niederhauser 2007). The species Diatoma tenuis Agardh is uniquely present in group 7. It consists of a planktonic species originating in Lake Geneva and leading to the Rhône River (Rimet et al. 2009).

Group 8 is well characterized in terms of season and location: it groups the summer samples from the enclave of Céligny. Its waters are at a lower limit compared to the average quality class and the "Jura" geotype. The most

GROUPS	1	2	3	4	5	6	7	8	9
Achnanthidium minutissimum (Kützing) Czarnecki	3	2	1	1	1	1	1	1	1
Achnanthes minutissima var. inconspicua Oestrup		1							
Achnanthes lanceolata ssp. lanceolata (Brebisson) Grunow				1					
Amphora pediculus (Kützing) Grunow		1	3	2	2	1	1	2	1
Achnanthidium pyrenaicum (Husted) Kobayasi		3			1			1	
Caloneis bacillum (Grunow) Cleve						1			
Encyonema minutum (Hilse in Rabenhorst) D.G.Mann		1							
Cocconeis placentula Ehrenberg var. placentula		1	1	1	2				1
Cocconeis placentula var. euglypta Ehrenberg								3	
Cocconeis placentula var. lineata (Ehrenberg) van Heurck				1					
Diatoma tenuis Agardh							1		
Denticula tenuis Kützing	1	1							
Gomphonema olivaceum Reichardt					1	1	1		1
Gomphonema parvulum (Kützing) Kützing									1
Gomphonema tergestinum (Grunow) M. Smith									1
Mayamaea atomus var. permitis (Hustedt) Lange-Bertalot									1
Navicula cryptotenella Lange-Bertalot	1			1		1	1	1	1
Navicula cryptotenelloïdes Lange-Bertalot									
Nitzschia dissipata (Kützing) Grunow	1	1	1	2	1	3	2		1
Nitzschia fonticola Grunow in Cleve & Möller		1			1	1	1		1
Navicula gregaria Donkin					2	1	1		
Navicula lanceolata (Agardh) Ehrenberg				1			2		
Eolimna minima (Grunow) Lange-Bertalot									1
Adlafia minuscula var. muralis (Grunow) Lange-Bertalot				1					
Nitzschia palea (Kützing) W. Smith									1
Navicula reichardtiana Lange-Bertalot						1	1		1
Eolimna subminuscula (Manguin) Moser, Lange-Bertalot & Metzeltin					1				2
Nitzschia sociabilis (Hustedt)				1		1			
Navicula tripunctata (O.F. Müller) Bory de Saint Vincent		1	1	1			1		
Rhoicosphenia abbreviata (Agardh) Lange-Bertalot			1	1			1		1
Surirella brebissonii Krammer Lange-Bertalot				1		1			
Reimeria sinuata (Greg.) Kociolek & Stroemer								1	
Ntaxon		10	6	13	9	11	12	6	15

Table II. – The dominant species of diatoms ($\geq 2\%$) in each of the 9 groups. Abundance classes: 1 = 2-9%; 2 = 10-29%; $3 = \geq 30\%$.

abundant species is *Cocconeis placentula* var. *euglypta* Ehrenberg (40 %). This species supports levels of pollution that are quite diverse. Rather rare in environments of low saprobic levels, it could indicate a rise in trophic level and is frequently found in calcareous waters of the Swiss Plateau (Schmedtje *et al.* 1998, Monnier *et al.* 2007). It is an epiphytic species, often associated with filamentous algae, such as *Cladophora* sp., which are numerous in Group 8. This species, as well as *Reimeria sinuata* (Gregory) Kociolek & Stroemer, can only be found in this group.

Average to poor water quality

Groups 5 and 9 are composed of samples from globally poor water quality, with some concentrations that can be elevated in DOC, BOD and Nmin total (Fig. 3). These are predominantly summer samples, the summer being the period of low water levels, when the water quality deteriorates. The dominant diatoms are principally species resistant to strong pollution, whether it is of agricultural, domestic or industrial origin: *Navicula gregaria* Donkin et *Eolimna subminuscula* (Manguin) Moser, Lange-Bertalot & Metzeltin.

Group 5 is linked to various geotypes ("Plaine", "Marais", "Alpes") while Group 9 only contains "Plaine" stations, belonging to the catchment area of the Drize and the Aire Rivers.

DISCUSSION

The dominant species

Considering only the dominant species in the nine groups (Table II), two observations may be made:

1) The flora of the nine groups is mainly composed of



Fig. 4 – Occurrence of geotypes in each of 9 groups.

Fig. 5 – Number of samples winter-spring or summer in each of 9 groups.

cosmopolitan species, frequent in Swiss Plateau surface waters of good to average quality $(3.5 \le \text{DI-CH} \le 5.49)$: *Amphora pediculus*, *Achnanthidium minutissimum*, *Navicula cryptotenella* Lange-Bertalot and *Nitzschia dissipata*.

2) However, some species are more closely related to particular environmental conditions. They have a smaller ecological spectrum. This is the case of Achnanthidium pyrenaicum, Achnanthes minutissima var. inconspicua Oestrup, Encyonema minutum ((Hilse in Rabenhorst)) D G Mann, Navicula cryptotenelloïdes Lange-Bertalot or Denticula tenuis Kützing, present in Groups 1 and/or 2. These diatoms are often confined to low-nutrient rivers in calcareous regions. Group 1 is composed of samples from the Allondon River while Group 2 consists of reference samples from the Versoix and the Allondon Rivers (stations at the head of the basin). These two rivers contain the "Jura" (calcareous) geotype. This combination of species has already been noted in several studies on the oligo-mesotrophic rivers of the calcareous Alps. More particularly the composition of Group 2 joins the aforementioned species in stations of close proximity to the source (Cordonier et al. 2003, Rimet et al. 2007).

The season

Many studies have shown the influence of the season on the specific composition of diatoms. They have mainly been carried out on planktonic diatoms, for which the season, through nutrients, temperature and stratification influences the growth and favors certain species, but also benthic diatoms in rivers (Anderson 2000, Soininen & Eloranta 2004, Leira & Sabater 2005, Rimet *et al.* 2009).

Given our low numbers and the unequal distribution of the seasons (50 winter-spring / 22 summer samples), we were not able to evaluate the influence of the season on the diversity and the specific composition of the diatoms.

The geotypes

Groups 1, 2, 6, 7, 8 and 9 have a unique or dominant geotype, at most of 50 % (Fig. 4). The dominant "Jura" geotype in Groups 1,2 and 8 is the only one that contains species exclusively belonging to this particular geotype and being rarely sampled in Geneva's hydrogeographic network: Achnanthes minutissima var. inconspicua, Encyonema minutum, Navicula cryptotenelloïdes, Denticula tenuis, Reimeria sinuata et Cocconeis placentula var. euglypta.

The "Jura" (calcareous) geotype therefore has a floristic composition that can be considered to be unique compared to other geotypes.

Note that the best indices of water quality are obtained in the "Jura" (Groups 1 and 2) geotype, while the worst are found in the "Alpes", "Plaine" and "Marais" (Groups 5 and 9) geotypes.

The "Marais" geotype is underrepresented in our study. It would be interesting to see if the dominant diatom species in this geotype is characteristic, rare, or even considered in the calculation of the DI-CH index.

Finally our results show that in addition to the quality of water, which selects the species according to their pollution tolerance, the geochemical signature also appears to influence the floral composition in a comparable manner. In fact, Groups 1, 2 (very good to good quality) and 8 (good to average quality), where the "Jura" geotype dominates, are composed of species that are not present in other groups and that are known for being indentured to calcareous waters. Similar to other studies taken at the regional level, these results have shown the influence of the geology and the ecoregions on the assemblage of diatoms (Vyverman 1996, Rimet *et al.* 2004, Tison *et al.* 2004).

This work and its initial conclusions are based on the data collected in the main watercourses of Geneva's surface waters. At the Swiss level, so as to adapt our objectives of biological quality, it would be interesting to define some ecoregions based on the geochemistry patterns and to measure their influence on the assemblage of diatoms. At the European level, the Water Framework Directive stipulates that its member states are required to qualify the ecological status of their surface waters according to the objectives adapted to the ecoregions (European Commission 2000). Switzerland, as well, could follow this process to account for factors other than the simple gradient of chemical pollution. Only the use of bioindicators allows to estimate the anthropic impacts on the aquatic ecosystems and its capacity for resilience.

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