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PRIMARY RESEARCH PAPER

Characterization of diatom assemblages in mid-altitude streams of NW Italy

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Abstract Following the European Water Framework Directive, this study aims to be the first step to (i) identify diatom type assemblages in unpolluted streams in NW Italy, and (ii) find which ecological factors explain most of the variation. To achieve this, we collected physical, chemical and benthic community data from four streams in NW Italy from 2001 to 2004, for a total of 72 samples. All sampling sites were between 200 m a.s.l. and 800 m a.s.l., but differed in the dominant lithological substrate, i.e. alluvial or siliceous. Relationships between diatom communities and environmental factors were examined using canonical correspondence analysis (CCA), while Indicator Species Analysis was used to define characterizing species and accompanying species of three environmental groups identified by CCA: (1) high water quality and medium saline content, (2) high water quality and low saline content, (3) poor water quality. The diatom assemblages of the three groups of sites differed significantly, as shown by the Multi-Response Permutation Procedure. There were strong correlations between diatoms and environmental factors, especially chlorides (also highly correlated with sulphates and carbonate hardness), nitrate concentration and conductivity. The group 1 assemblage was typical of the alluvial Alpine streams with medium saline content and was characterized by mostly oligosaprobic/β-mesosaprobic taxa such as *Cymbella affinis*, *Diatoma ehrenbergii* and *Staurosira pinnata.* The species assemblage found in the siliceous Alpine rivers with good water quality make them suitable reference sites for a benthic diatom community.

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Introduction

Due to their sensitivity, wide distribution and relative ease of identification, periphytic diatoms are widely used for the biological assessment of running waters. Diatoms quickly respond to changes of environmental variables, thus reflecting overall ecological quality and the effects of different stressors. Many indices have been proposed to relate diatom assemblage composition to water quality, such as SPI (Coste in Cemagref, 1982), IBD (Prygiel & Coste, 1998), IDG (Coste & Ayphassorho, 1991) and EPI-D (Dell'Uomo, 2004). Moreover, the European Water Framework Directive 2000/60 (EU-WFD) recommended to EU member states that periphyton be included among the biological descriptors of water quality and that reference conditions for each stream typology be defined in terms of the climate, geology and relief within a specific ecoregion.

A few studies on river diatom communities have been carried out in Italy, covering some central Apennine streams (Grandoni & Dell'Uomo, 1996; Dell'Uomo & Grandoni, 1997; Dell'Uomo, 1999; Dell'Uomo et al., 1999), some springs of the Eastern Alps in the Trentino Region (Cantonati, 1998; Cantonati & Pipp, 2000) and of the Western Alps in the Piedmont Region (Battegazzore et al., 2004; Bona et al., 2005; Falasco et al., 2005). However, none of these studies defined reference diatom communities within their river typology, although this has recently been done in France (Campeau et al., 2003; Tison et al., 2005) and Luxembourg (Rimet et al., 2004). Assessment of the ecological status, as recommended by the EU-WFD, will be a long process in Italy because of the scarcity and scattered nature of existing data.

The first step in this task is to define different river typologies for each ecoregion on the basis of important environmental parameters (principally hydromorphological and geological), thus establishing the proper reference communities.

The comparison between an observed community and its potential reference status should give a preliminary quantification of the level of environmental alteration (Rimet et al., 2004).

The aim of this study is to detect major patterns in the species composition of diatom assemblages from four rivers of NW Italy and to find which ecological factors explain most of the variation. Sites were chosen to cover different river types representative of this region within the 200–800 m altitudinal range. This research is intended as a first step in identifying diatom type assemblages for unpolluted mid-altitude streams in the Western Alps, following the classification proposed by the AQEM project (Hering et al., 2004). Remarks on the use of diatoms as bioindicators are also provided.

Study area

The study area encompassed four rivers in the Piedmont region (Fig. 1) originating from the Western Alps (Bormida, Chiusella, Orco, Po). All of them are characterized by a typical nivopluvial regime, with maximum flow in spring and autumn

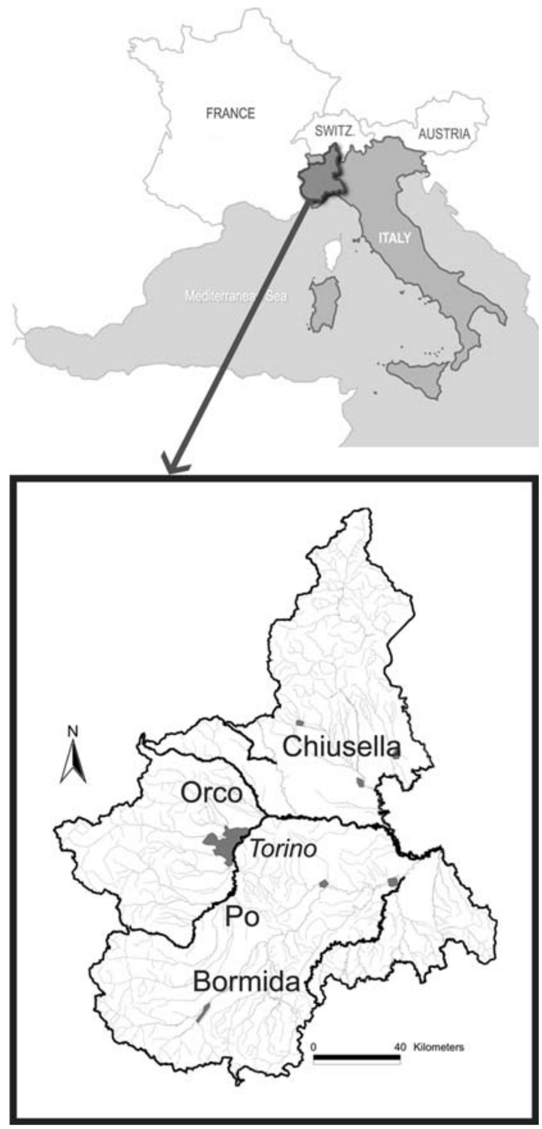

Fig. 1 Study area

Table 1 Catchment area, altitude and geology of the studied rivers

River	Stream type	Size class	Altitude class	Geology class
Po River (8 sites)	Large-sized hill streams	1000–10,000 km^2	200–800 m	Alluvial deposits
Orco river (4 sites)	Mid-sized hill streams	100–1,000 km^2	200–800 m	Siliceous
Chiusella river (4 sites)	Mid-sized hill streams	100–1,000 km^2	200–800 m	Siliceous
Bormida river (5 sites)	Mid-sized hill streams	100–1,000 km^2	200–800 m	Alluvial deposits

The definition of Stream types partially follows the AQEM project (Hering et al., 2004)

and low flow in late summer and winter. Table 1 reports the main features of the rivers in terms of catchment area, altitude and geology.

Sampling sites were further characterized by the ''Index of Anthropisation'' (Mancini et al., 2005), based on land use categories in a circular area (1 km radius) around each sampling site. Land use data were obtained from the CORINE Land Cover database (Krynitz, 2000) using a Geographic Information System. The Index of Anthropisation is calculated as follows:

$Ia = \Sigma k_i p_i$, where k_i is the specific coefficient for each CORINE land use category and p_i is the relative frequency for each category. The index ranges between 1 (100% natural woods) and 4 (100% urban and industrial areas).

Materials and methods

Samples for chemical analyses and diatom identifications were taken from September 2001 to September 2004, for a total of 72 samples. Each site was sampled at least twice at low flow time, as recommended by Kelly et al. (1998).

Water quality data

Water temperature, conductivity and pH were measured in the field with a WTW Multiline probe. Water samples from each site were analyzed at the Regional Environmental Protection Agency (ARPA Piemonte) laboratories for total phosphorus (TP), $N–NO_3$, BOD_5, COD, Cl^-, carbonate hardness, total suspended solids (TSS) and SO_4^{2-}, according to IRSA (1994) Italian Standard methods. The Water Resource Department of the Piedmont Region provided us with data concerning the Extended Biotic Index (IBE, Ghetti, 1997), based on macroinvertebrates, and the SECA index (Ecological Status of rivers) required by Italian Water legislation (D. Leg. 152/99 and successive ones), which combines an organic pollution index based on the concentration of total phosphorus, $N–NO_3$, $N–NH_4^+$, BOD_5 and COD with the IBE. Five quality classes are defined by this index, corresponding to the following judgments: high, good, moderate, poor and bad.

Diatom sampling and laboratory identification

Diatom samples were collected according to French and European standard sampling methods (Kelly et al., 1998; AFNOR, 2000). Samples were stored in 4% formaldehyde solution and processed within one week from sampling. In the laboratory, we centrifuged the samples 3 times at 1,500 rpm for 10 min, replacing the overlying layer with deionized water each time and treating the sample with 0.1 N HCl before rinsing. Subsamples were placed on cover slips and incinerated at 450°C for 1 h. This method allowed the frustules to remain intact. The cover slips were then mounted on microscope slides by means of a synthetic resin (Naphrax®).

Diatoms were identified under a Carl Zeiss optical microscope (1,000× magnification) according to Krammer & Lange-Bertalot (1991–2000). Species composition and relative abundances were based on the observation of at least 400 frustules per sample.

Data analysis

Relationships between diatom communities and environmental factors were examined using canonical correspondence analysis (CCA, Ter Braak, 1986). Ordinations were performed with PC-ORD software (McCune & Mefford, 1999). A diatom species x sample data matrix, excluding rare species (≤5% in each sample), was produced using log $(x + 1)$ transformation to stabilize variances.

At the same time, an environmental variable x sample data matrix was produced. This matrix included 11 variables: altitude, water temperature, pH, conductivity, TSS, Cl^-, TP, $N–NO_3$, BOD_5, COD, SECA. Carbonate hardness and SO_4^{2-} concentration were excluded from the environmental matrix since they were highly correlated ($P < 0.001$) with other variables: carbonate hardness with chlorides (Pearson's correlation coefficient $r = 0.631$), conductivity ($r = 0.657$) and SO_4^{2-} ($r = 0.681$); SO_4^{2-} with chlorides ($r = 0.907$), conductivity ($r = 0.633$) and pH ($r = 0.628$).

The resulting diatom matrix consisted of 72 observations and 50 species; the environmental matrix consisted of 72 observations and 11 variables.

A Monte Carlo test of significance was performed to test the null hypothesis of no linear relationship between matrices, using 1,000 randomizations.

Indicator Species Analysis (ISA, Dufrene & Legendre, 1997) was used to establish ecological values of different species in relation to environmental conditions. This analysis combines information on the concentration of species abundances in a particular group of samples and the faithfulness of occurrence of a species in this group. We used ISA to define the characterizing species of three environmental groups identified by CCA: (1) high water quality and medium saline content; (2) high water quality and low saline content; (3) poor water quality.

To test the hypothesis of no difference between the diatom assemblages of the three groups, we applied the MRPP (Multi-Response Permutation Procedure, Mielke, 1984; Mielke & Berry, 2001), a nonparametric procedure to test the hypothesis of no difference between two or more groups of entities. MRPP was performed with PC-ORD, using Sørensen distance as the distance measure between matrices.

Table 2 Physical and chemical characteristics (mean and range values) of each river typology

River typology	Altitude (m)	IA[a]	t °C	pH	Conductivity (µS/cm)	Hardness (mg/l $CaCO_3$)	TSS (mg/l)	SO_4^{2-} (mg/l)	Cl^- (mg/l)	COD (mg/l)	BOD_5 (mg/l)	$N–NO_3$ (mg/l)	TP (mg/l)	SECA[b]
Mid-sized hill streams alluvial deposits	335 (256–400)	2.4 (2.4–2.5)	13.8 (11.9–14.8)	8.4 (8.3–8.5)	418 (370–452)	179 (146–210)	11.4 (<10–20.1)	67.3 (62–71)	21.9 (21–23.6)	5.4 (<5–6.3)	2.16 (<2–2.7)	0.74 (0.66–0.82)	0.06 (<0.06–0.13)	2.1 (2.0–2.2)
Mid-sized hill streams, siliceous	402 (202–800)	2.2 (1.1–3.0)	11.2 (8.6–13.1)	7.8 (7.6–8.1)	73 (36–112)	41 (28–65)	<10	5.3 (3.5–8.6)	1.3 (0.5–2.4)	<5.0	<2.0	0.75 (0.41–1.3)	<0.06	2.1 (1.5–3.0)
Large-sized hill streams, alluvial deposits	216 (201–251)	2.8 (1.9–3.4)	14.4 (12.1–19.0)	7.9 (7.6–8.2)	380 (258–492)	160 (125–222)	21.3 (<10–83.8)	47.6 (20.3–73.3)	10.4 (3.3–16.7)	6.6 (<5–10.8)	1.2 (<2–2.2)	2.7 (2.2–3.3)	0.16 (<0.06–0.28)	3.3 (2.6–4.0)

[a] Index of Anthropisation (Mancini et al., 2005)

[b] Index of river ecological status

Results

Water quality

The mean values of the physical and chemical characteristics of the sampling sites are summarized in Table 2. The table also reports the Index of Anthropisation as a measure of human impact at each sampling site: it ranged from 1.1 (pristine conditions with almost natural land cover) to 3.4 (agriculture and urban development are dominant). The Bormida River sites showed the narrowest index range (2.4–2.5), corresponding to a mix of woods and agriculture with small urban areas. The Po River had the highest values with a peak of 3.4, while the lowest values were for the siliceous streams. The Orco River had the lowest values, with a minimum of 1.1.

The highest pH values were found in the alluvial mid-sized stream (around 8.4). In all the other sites, the water was slightly alkaline, with a maximum mean value of 8.2.

All the sites with alluvial deposit had similar mean values of conductivity, carbonate hardness, SO_4^{2-} and Cl^- concentration, while the lowest values for all these parameters were found in streams with siliceous substrate.

With regard to organic pollution, the sites could be classified into two main categories. The first was characterized by no evidence of organic pollution, as shown by the low BOD_5, COD, total phosphorus and nitrate concentrations. The mean SECA index for this set of sites ranged from 1.5 (very high quality) to 2.5 (good quality). This category included all sites belonging to mid-sized hill streams, regardless of the lithology. The second category consisted of the sites in the large hill streams, characterized by a higher concentration of nitrates and in some cases of total phosphorus, but with BOD_5 and COD values comparable to those of the first category. Mean SECA values ranged from 2.6 to 4, the latter corresponding to the worst class of water quality. In this stream, we also found peaks of TSS up to 83 mg/l.

Diatom communities

In total, 59,073 individuals belonging to 174 species were identified. *Cocconeis placentula*, *Reimeria sinuata* and *Encyonema minutum* were present in more than 90% of the samples. Indeed, these diatoms are widely distributed in mid-altitude streams. Forty-one species were present in at least 25% of the samples; among them, *Achnanthidium biasolettianum*, *Achnanthidium minutissimum*, *Gomphonema olivaceum*, *Cymbella affinis* and *Diatoma mesodon* are typical of unpolluted rivers.

The main summary statistics of the CCA are shown in Table 3. The total explained variance in species frequency was 26.4%.

Axes 1, 2 and 3, evaluated with a Monte Carlo test with 1,000 permutations, were highly significant ($P < 0.01$). There was a strong inverse relationship between the first axis and $N–NO_3$, SECA and TP, while the second axis was mostly related to Cl^- and conductivity. The third axis did not show a strong relationship with any of the variables; hence, we chose the first two for the ordinations reported in Fig. 2a, b. These figures show the directions and lengths of the vectors of the environmental variables.

Intraset correlations (sensu Ter Braak, 1986) of the environmental variables indicated that all the variables except water temperature and BOD_5 significantly influenced the ordination, the most important being Cl^- (0.883 with the second axis), $N–NO_3$ (–0.826 with the first axis) and conductivity (0.788 with the second axis).

The ordination of sites based on species composition (Fig. 2a) showed three clearly separated groups of sites. The first was located in the first quadrant: all the sites showed a positive correlation with pH, conductivity and Cl^-, and a negative correlation with TSS, TP, $N–NO_3$, SECA and altitude; for this reason, we can define these sites as having high water quality and medium saline content. There is a perfect correspondence between this group and the type ''Mid-sized stream with alluvial deposits''.

The second group was in the second quadrant and was characterized by higher altitudes, even though the range of values was 200–800 m a.s.l. These sites showed a negative correlation with conductivity, Cl^-, TSS, TP, $N–NO_3$ and SECA. We can conclude that this group was characterized by high water quality and low saline content.

Table 3 Canonical correspondence analysis (CCA) axes summary statistics

	Axis 1	Axis 2	Axis 3
Eigenvalue	0.219	0.151	0.078
Variance in species data			
Cumulative % explained	12.9	21.8	26.4
Pearson correlation, Spp-Envt	0.891	0.918	0.789
Intraset correlation for environmental variables			
SECA	–0.478	0.138	0.298
Altitude	0.393	–0.436	0.166
$N–NO_3$	–0.826	0.129	–0.211
BOD_5	0.197	0.420	–0.099
COD	–0.168	0.427	–0.012
Cl	0.340	0.883	0.002
Conductivity	–0.023	0.788	–0.030
TSS	–0.444	0.153	0.053
pH	0.435	0.445	–0.104
TP	–0.526	0.183	0.458

All the remaining sites were spread throughout the third and fourth quadrants, characterized by the lowest altitude values and highest values of nutrients, TSS, COD and SECA.

The species ordination in Fig. 2b indicated that *Craticula accomoda* and *Gyrosigma scalproides* were particularly associated with high values of nutrients, TSS and SECA, and were more abundant in low-altitude sites. The same pattern, although not as strong, was shown by *Gomphonema angustatum*, *Nitzschia amphibia*, *Amphora veneta*, *Cyclotella meneghiniana* and *Navicula veneta*. Six species, *Cymbella affinis*, *Encyonopsis microcephala*, *Diatoma ehrenbergii*, *Staurosira pinnata*, *Navicula recens* and *Nitzschia capitellata*, exhibited a clearly different response to the environmental factors, being associated with the highest Cl^-, conductivity and pH values and low organic content. *Fragilaria arcus* and *Diatoma mesodon* showed the strongest association with high altitudes, low saline content, nutrients and pH.

Indicator Species Analysis was carried out to define the characterizing species for each of the three groups of sites identified by CCA (Table 4). Sites characterized by high water quality and medium saline content (group 1) had a highly significant presence of *Cocconeis pediculus*, *Cymbella affinis*, *Diatoma ehrenbergii*, *Staurosira pinnata*, *Navicula gregaria*, *N. reichardtiana*, *Nitzschia capitellata* and *Surirella brebissonii*. Sites with high water quality and low saline content (group 2) had a highly significant presence

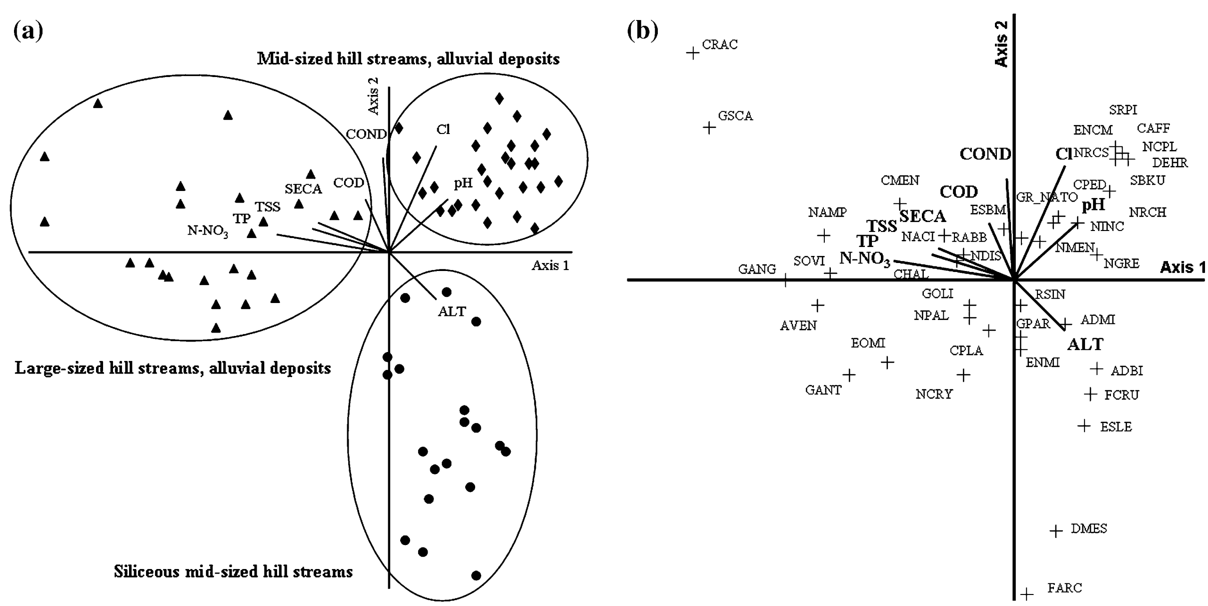

Fig. 2 Canonical correspondence analysis (CCA) diagram showing environmental variables and sites (**a**) or diatom taxa centroids (**b**) in the ordination space. CRAC = *Craticula accomoda*, EOMI = *Eolimna minima*, NRCS = *Navicula recens*. All the other diatom abbreviation codes are given in Electronic Supplementary Material. Environmental variables that had low correlations with ordination axes are not shown, ALT = altitude, COND = conductivity

of Achnanthidium biasolettianum, Encyonema silesiacum, Diatoma mesodon and Fragilaria arcus. Sites characterized by low water quality (group 3) had a highly significant presence of Navicula tripunctata, Nitzschia amphibia, N. dissipata, Rhoicosphenia abbreviata and Surirella ovalis.

We defined as ‘‘accompanying species’’ those present in at least 75% of the samples of each group and presenting >5% of relative abundance in at least 50% of them. Table 5 reports the diatom type assemblages, including characterizing and accompanying species, for the three river typologies.

The MRPP applied to species composition of the three groups revealed a highly significant difference between them ($T = -30.3$, $A = 0.19$, $P = 0.00$), thus confirming the response of diatoms to the selected physico-chemical parameters.

Discussion

In terms of water quality, all of the sites belonging to the first (mid-sized streams with alluvial deposits) and second groups (mid-sized siliceous hill streams) can be considered potential reference sites within their respective stream typology. The third group (large streams with alluvial deposits) had high nutrient concentrations and SECA indices, making it unfit to be classified as unpolluted. The CCA ordination demonstrated the importance of the different environmental factors in determining diatom assemblages. The summary statistics reported in Table 3 for the first three CCA axes showed a strong correlation between diatoms and environmental factors (Pearson correlations: 0.891, 0.918 and 0.789 respectively). Chlorides (also highly correlated with sulphates and water hardness), nitrate concentrations and conductivity were the most important variables influencing species distribution. In fact, there were two major gradients to which diatom assemblages responded: organic enrichment and saline content of the water.

The sensitivity of benthic diatoms to organic enrichment is well documented, leading to the classification of taxa in relation to water trophic levels, evaluated according to total phosphorus concentration (Van Dam et al., 1994; Soininen, 2002). In our study, total phosphorus concentration was typical of oligotrophic waters, with some exceptions for the Po River with a maximum of 0.28 mg/l. Nitrates and COD showed a wider variability, although mean concentrations never exceeded 3.1 mg/l and 10.8 mg/l respectively. Nitrate concentration affects diatom community composition because of the different nitrogen uptake metabolism of the various species. Thus,

Table 4 Results of the indicator species analysis

Species	River typology	Observed indicator value	Randomized Indicator Value		
			Mean	SD	*P*-value
Cocconeis pediculus Ehrenberg	1	55.6	21.9	5.07	0.0010
Cymbella affinis Kutzing	1	82.8	18.1	4.66	0.0010
Diatoma ehrenbergii Kutzing	1	55.3	15.0	4.43	0.0010
Staurosira pinnata Ehrenberg	1	55.2	14.1	4.85	0.0010
Navicula gregaria Donkin	1	56.3	27.5	4.31	0.0010
Navicula reichardtiana Lange-Bertalot	1	52.5	27.5	4.95	0.0010
Nitzschia capitellata Hustedt	1	82.8	18.4	4.79	0.0010
Surirella brebissonii Krammer & Lange-Bertalot	1	61.6	20.6	4.73	0.0010
Achnanthidium biasolettianum (Grunow) Lange-Bertalot	2	56.6	29.3	4.19	0.0020
Encyonema silesiacum (Bleish) Mann	2	66.1	24.5	4.80	0.0010
Diatoma mesodon (Ehrenberg) Kutzing	2	55.0	16.3	4.77	0.0010
Fragilaria arcus (Ehrenberg) Cleve	2	54.6	14.7	4.77	0.0010
Navicula tripunctata (O.F. Müller) Bory	3	62.5	27.4	4.52	0.0010
Nitzschia amphibia Grunow f. *amphibia*	3	57.6	14.8	4.49	0.0010
Rhoicosphenia abbreviata (C. Agardh) Lange-Bertalot	3	52.0	31.2	3.76	0.0010
Surirella ovalis Brèbisson	3	57.8	14.5	4.50	0.0010

Table 5 Summary of the environmental features of the three river typologies and corresponding diatom type assemblages

	Environmental features	Characterizing species	Accompanying species
River typology 1	Mid-sized hill streams, alluvial deposits, medium alkalinity, conductivity, carbonate hardness, SO_4^{2-} and Cl^- concentration. No evidence of organic pollution.	*Cocconeis pediculus* Ehrenberg, *Cymbella affinis* Kutzing, *Diatoma ehrenbergii* Kutzing, *Staurosira pinnata* Ehrenberg, *Navicula gregaria* Donkin, *Navicula reichardtiana* Kutzing, *Nitzschia capitellata* Hustedt, *Surirella brebissonii* Krammer & Lange-Bertalot	*Achnanthidium minutissimum* (Kutzing) Czarnecki, *Achnanthidium biasolettianum* (Grunow) Lange-Bertalot, *Reimeria sinuata* (Gregory) Kociolek & Stoermer
River typology 2	Mid-sized hill streams, siliceous, with low conductivity values, carbonate hardness, SO_4^{2-} and Cl^- concentrations. No evidence of organic pollution.	*Achnanthidium biasolettianum* (Grunow) Lange-Bertalot, *Encyonema silesiacum* (Bleisch) Mann, *Diatoma mesodon* (Ehrenberg) Kutzing, *Fragilaria arcus* (Ehrenberg) Cleve	*Cocconeis placentula* Ehrenberg, *Reimeria sinuata* (Gregory) Kociolek & Stoermer
River typology 3	Large-sized hill streams, alluvial deposits, with medium conductivity, carbonate hardness, SO_4^{2-},Cl^-, N–NO_3 and TP concentration. Moderate water quality.	*Navicula tripunctata* (O.F. Müller) Bory, *Nitzschia amphibia* Grunow f. *amphibia*, *Rhoicosphenia abbreviata* (Agardh) Lange-Bertalot, *Surirella ovalis* Brèbisson	*Cocconeis placentula* Ehrenberg, *Navicula cryptotenella* Lange-Bertalot, *Mayamaea atomus* group, *N. veneta* Kützing

See the text for the definitions of ''Characterizing species'' and ''Accompanying species''

Van Dam et al. (1994) classified diatoms into four groups, ranging from completely nitrogen-autotrophic species to obligately nitrogen-heterotrophic ones. In our study, *Craticula accomoda* and *Gyrosigma scalproides* were highly correlated with high nutrient concentration; the former is cited by Van Dam et al. (1994) and Dell'Uomo (2004) as polysaprobic, while most authors attribute intermediate values of sensitivity and ecological reliability to *G. scalproides*.

The second major gradient of ordination, which follows Axis 2, was related to water salinity. Conductivity has been found to be a significant factor by other authors (Sabater & Roca, 1990; Rott et al., 1998; Soininen, 2002; Potapova & Charles, 2003). In our samples, the saline content was never particularly high; nevertheless, the Bormida River sites had the highest values, probably related to their geological features, i.e. a mainly alluvial substrate with marls. Three species (*Nitzschia capitellata*, *Diatoma ehrenbergii* and *Navicula recens*) considered halobic by Van Dam et al. (1994) were associated with the highest chloride values. As expected, the siliceous streams showed low saline contents.

The diatom type assemblages for the three river typologies are reported in Table 5. The group 1 assemblage was characterized by mostly oligosaprobic/β-mesosaprobic species such as *Cymbella affinis*, *Diatoma ehrenbergii* and *Staurosira pinnata*, although there was one polysaprobic species (*Nitzschia capitellata*). The ''accompanying species'' of this group are considered sensitive taxa by most authors, with the exception of *Nitzschia capitellata* and *Navicula gregaria* which were particularly abundant at sites B1 and B2, where we recorded the peak values of nutrients in group 1. Moreover, these species were often associated with medium-high levels of chlorides (Van Dam et al., 1994).

Group 2 had a lower number of indicator species; three of them are considered xenosaprobic taxa by Dell'Uomo (2004), while the fourth is classified as oligo β-mesosaprobic. This species assemblage and the good water quality corroborate the decision to consider this group of sites as a reference within their river typology. Using Indicator Species Analysis, we found four species that characterized group 3, ranging between oligosaprobic and α-mesosaprobic. The accompanying species have a similar sensitivity value, reflecting the water quality conditions.

We use the terms ''characterizing'' and ''accompanying'' species according to the definition given by Pérès & Picard (1964). Our characterizing species can be more specifically defined as ''characterizing preferential species'' because they have a high level of faithfulness but are not exclusive to a given river typology. Species we defined as ''accompanying'' are generally ubiquitous, as shown by their low or intermediate reliability values (Dell'Uomo, 2004).

A comparison of these assemblages with other sites in Italy is not feasible due to the scarcity of published data. The most exhaustive work on the typology of diatom communities in nearby countries regards French rivers (Tison et al., 2005). Eleven groups of sites were classified as ''reference'' or ''disturbed''. Our Group 1 is very different from all the French groups, with a maximum number of shared species of only 2. Group 2 showed a higher level of similarity, sharing two of four species with the ''cluster no. 5'', a reference assemblage composed of undisturbed crystalline highland sites in the Pyrenees and southern Central Massif. French Alpine and pre-Alpine reference sites only shared *Achnanthidium biasolettianum* as characterizing species with our Group 2 assemblages.

In conclusion, our study is the first step in identifying the epilithic diatoms and diatom assemblages in mid-altitude streams in NW Italy. Diatom assemblages respond to the physicochemical conditions of the water, not only to organic enrichment but also to chlorinity, water hardness, sulphates and conductivity. For this reason, we suggest that the establishment of reference site conditions for phytobenthos, as prescribed by the EU-WFD, should take into account a wider set of river typologies than the one defined by the AQEM project for Northern Italy, with special emphasis on water geochemistry.

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