

J. Limnol., 2013; 72(1): 35-51
DOI: 10.4081/jlimnol.2013.e4

Recent findings regarding non-native or poorly known diatom taxa in north-western Italian rivers

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ABSTRACT

Diatoms of the major rivers of North-Western Italy were investigated to highlight the presence of species of particular ecological interest but not as yet recorded. The survey area included streams belonging to seven different hydroecoregions (HERs) with a wide range of physical characteristics. Between 2008 and 2010, 200 samples were taken for the study of the diatom community composition, while a larger set of samples was examined to determine the presence or absence of the nuisance diatom species *Didymosphenia geminata* (Lyngbye) Schmidt. A specific field study was performed in two rivers characterized by persistent blooms of this species to evaluate the effects of its proliferation on the benthic communities. *D. geminata* was present in almost 20% of the samples. From a comparison with published data, we can confirm that *D. geminata* has recently been expanding its ecological range, as it has been found also in mesotrophic lowlands water. In some instances the formation of massive proliferation has been recorded. The calculation of autecological values confirmed its preference for oligotrophic waters with low mineral content and organic loading, although with a wider ecological amplitude than recorded in the first studies on this species. Another four taxa of particular interest were detected: *Achnanthydium subhudsonis* (Hustedt) Kobayasi (in 15 sites), *Cymbella tropica* Krammer (11 sites), *Mayamaea cahabaensis* Morales and Manoylov (2 sites) and *Reimeria uniseriata* Sala, Guerrero and Ferrario (18 sites). The first three species must be considered new records for Northern Italy. *A. subhudsonis* and *C. tropica* reached up to 20% relative abundance. From the analysis of their distribution and autecological values, we can assert that *A. subhudsonis* and *M. cahabaensis* show a preference for high values of nitrogen, this latter preferring also quite high values of total phosphorus. *C. tropica* prefers intermediate values of nitrogen nutrients and *R. uniseriata* is the least demanding species in terms of water quality. All the taxa studied have a wide ecological range, confirming their potential invasive behaviour. Finally, considerations are provided with respect to Italian and European historical data in order to understand whether these species can be considered non-indigenous and/or bloom forming, in the study area. The results may help improve the process of ecological classification of water bodies in the seven HERs, and the water protection actions introduced by the Water Framework Directive (2000/60).

Key words: diatoms, NW Italy, *Didymosphenia geminata*, non-indigenous taxa, river ecosystem.

Received: April 2012. Accepted: June 2012.

INTRODUCTION

Over the last 40 years, a considerable amount of ecological research has been devoted to biological invasions and their effects on ecosystems. Non-indigenous species (NIS) are considered one of the major menaces to ecosystem integrity worldwide (Mack *et al.*, 2000).

The biogeographical distribution of freshwater diatoms is still a controversial issue. According to Finlay *et al.* (2002), most diatom species are cosmopolitan, and species that are locally rare or abundant are like that worldwide. According to this hypothesis, it is inappropriate to assert the presence of endemic or exotic diatom species, since many of them have likely been underestimated, and would show a wider geographical distribution with further sampling efforts. On the other hand, several studies support the idea that diatoms have a biogeographical distribution (Vanormelingen *et al.*, 2007; Vyverman *et al.*, 2007). Telford *et al.* (2006) demonstrated that the dispersal of diatoms was limited, and timescales of isola-

tion allowed for regional genetic differences to develop and endemic taxa to evolve. Kilroy *et al.* (2007) supported this idea by defining endemic and exotic taxa and relating them to different disturbance levels. In the Palaearctic region, the only comprehensive study focussing on invasive diatom taxa considered historical data recorded in France (Coste and Ector, 2000). The authors defined exotic diatoms as *taxa not yet found in France until 1990 but showing an important proliferation and rapid dispersal since that time*.

Because of its bloom forming nature, *Didymosphenia geminata* (Lyngbye) Schmidt is by far the most studied non-indigenous diatom worldwide. *D. geminata* was first collected by Lyngbye in the Faroe Islands and described in 1819 (Lyngbye, 1819). The original distribution range of *D. geminata* is restricted to northern Europe and North America (in particular the northern hemisphere above the 30°N parallel; Blanco and Ector, 2009). In recent years, the presence of this diatom has also been confirmed at

lower latitudes, as far as New Zealand where it has been considered invasive since 2004. This expansion of the distribution range is probably due to a broadening of the ecological preferences of *D. geminata*. The older literature describes this species as typical of mountain streams with cold waters, fast flows and low nutrient content and conductivity (Patrick and Reimer, 1975; Krammer and Lange-Bertalot, 1986; Kawecka and Sanecki, 2003; Kilroy *et al.*, 2005). However, recent publications have reported *D. geminata* growing at lower altitude and higher temperatures, albeit never exceeding 20°C (Kara and Sahin, 2001; Kawecka and Sanecki, 2003; Rieberger, 1991), and not inhibited by elevated levels of nutrients, such as dissolved reactive phosphorus and nitrates (Kawecka and Sanecki, 2003; Noga, 2003; Subakov-Simić and Cvijan, 2004). The high hydrological stability produced by the increasing number of flow regulators, like dams, can be linked to massive proliferation of *D. geminata* and, as a consequence, with its spread (Kirkwood *et al.*, 2009).

Fossil and recent records of *D. geminata* in Italy are fully presented in Blanco and Ector (2009). Fossil and subfossil records came from Toscana and Lombardia regions (Mandl, 1839; Pritchard, 1842; Griffith and Henfrey, 1883; Corti, 1893), while recent records involve Valle d'Aosta (Brun, 1880), Lombardia (Bonardi, 1888; De Toni and Levi, 1886), Lazio (De Toni and Levi, 1886; Giaj-Levra and Abate, 1994), Trentino (Beltrami *et al.*, 2008a,b,c), and Friuli-Venezia Giulia regions (Zorza and Honsell, 2008). Old records of *D. geminata* in Piemonte region date to the early 1900s and concern Canavese and Verbano districts (Forni, 1925; Forti, 1900; Giaj-Levra, 1927; Monti, 1904). In recent years, biomonitoring programs in the Cuneo and Alessandria districts have highlighted the presence of *D. geminata* in many rivers (Battezzore, 2008; Battezzore *et al.*, 2007, 2009, 2010; Maffiotti, *personal communication*), especially in the Cuneo Province. In the Po river, a massive development of *D. geminata* was found at the Crissolo sampling station (Cuneo, 1380 m asl), characterized by oligotrophic conditions. Lower abundances were found in downstream stations where nutrient levels were slightly higher. In the Varaita river, *D. geminata* never exceeded 3% of the total community abundance (Battezzore *et al.*, 2007). Huge proliferations and macroscopic blooms have been found in the Erro river since 2007 (Maffiotti *personal communication*). Also in this case, the water quality was so good that the upper part of the watercourse was proposed as a potential reference site for this type of river. In their most recent paper, Battezzore *et al.* (2009) reported a distribution range expansion of *D. geminata*, with high peak abundances in the Corsaglia, Tanaro, and Maira rivers. According to Blanco and Ector (2009), the only record of *D. geminata* in Valle d'Aosta concerns the Courmayeur Valley (Brun, 1880), while there are no literature data on its presence in Liguria region.

The effects of *D. geminata* blooms on river biotic communities are somewhat controversial. There is general agreement on the effects of mats on macroinvertebrates (especially chironomids), *i.e.* species richness decreases and the community shifts toward tolerant taxa (Blanco and Ector, 2009; Brown, 2008; Larned *et al.*, 2007; Mundie and Crabtree, 1997; Whitton *et al.*, 2009). This is probably due to the microhabitat modifications induced by the thick biofilm and changes in food material availability, mainly consisting of polysaccharide stalks during blooms. On the other hand, the effects of massive proliferations on fish communities are contradictory. Several authors have highlighted the lack of habitat for spawning, feeding or rearing, and diurnal dissolved oxygen fluctuations caused by *D. geminata* blooms as possible threats to fish communities and the fishery industry (Beeson and Mitchum, 2006; Boubée *et al.*, 2008; Cook *et al.*, 2008; Jónsson *et al.*, 2000; Kilroy *et al.*, 2005; Shearer and Erickson, 2006; Shelby, 2006; Stohlgren *et al.*, 2007). In contrast, no effects on salmon or trout populations have been observed in NW England, Norway or Iceland (Jónsson *et al.*, 2008; Lindstrøm and Skulberg, 2007, 2008).

In Italy, the definition of native or exotic species is complicated by the scarcity of historical data. Most of the scientific publications on diatoms of Italian rivers regard data collected over the last ten years, making it difficult to determine whether a taxon is non-indigenous or simply has not yet been recorded or has been misclassified. In this respect, the hydrographic network of North-Western Italy is of particular interest because it includes seven hydroecoregions (HER); therefore, marked differences in climate, geology and relief are found in a relatively small area. During our research, particular attention was given to other taxa of special interest that can be considered potentially non-indigenous according to the following criteria: i) not reported in the European diatom floras up to the beginning of the 1990s; ii) commonly widespread in other continents but not in Europe; iii) known as tropical, subtropical or invasive. We also focused on taxa subject to frequent misidentification (especially in the past). Indeed, apart from isolated reports, no studies have been carried out to establish the distribution of *D. geminata* and of non-indigenous taxa in North-Western Italy based on large-scale surveys.

The aims of this study were i) to shed light on the distribution of *D. geminata* in North-Western Italy based on data from regional biomonitoring programs; ii) to analyse the most important effects of macroscopic proliferation of *D. geminata* on benthic communities (both macroinvertebrates and diatoms) at two sampling sites affected by the presence of blooms for most of the year; iii) to highlight the presence of other taxa of ecological interest and newly recorded in the study area.

This study could provide an important contribution to

the knowledge of the diatom flora of this heterogeneous region, with particular regard to the presence and ecology of nuisance taxa. The findings may be useful for the management of river basins in terms of classification of water bodies and protection of their biotic integrity.

METHODS

All the surveys were conducted in North-Western Italy (Valle D'Aosta, Piemonte and Liguria regions). Sampling sites corresponded to environmental agency monitoring stations on account of their relevance and data availability.

The study area is highly heterogeneous, including different hydroecoregions (HERs) and rivers with different hydrological regimes, altitudes and impacts. It included the first part (approximately 40% of the total surface) of the Po river catchment (the largest in Italy) and all the main watercourses belonging to the Ligurian sea basin. Most of the Po river tributaries have a typical nivopluvial regime, with maximum flow in spring and autumn. Some significant watercourses (*e.g.*, Dora Baltea) with glacial feeding were also included. Ligurian rivers have a typical Apennine regime, with maximum flow in spring and autumn and marked summer droughts. The study area includes 7 HERs out of the 21 identified in Italy for implementation of the WFD 2000/60. The Valle d'Aosta region includes one HER, the Inner Alps (HER1), represented by mountain streams mainly flowing on siliceous substrate. The Piemonte region includes 6 HERs: Inner Alps, Po plain (HER 56), Monferrato (HER 62), Piemonte Apennines (HER 63), Apennines N (HER 64) and Inner Alps S (HER 107). The Ligurian rivers belong to 2 HERs, Apennines N and Ligurian Alps (HER 122). The sites and samples analysed in the study area are summarized in Tab. 1.

For the analysis of the *D. geminata* distribution, 411 samples were examined to assess the presence/absence of this species. The Valle d'Aosta samples numbered 234, collected between 2007 and 2010 in a very wide altitudinal range (300-2000 m asl). The dataset for Piemonte consisted of 97 samples, all collected in 2010 at sites between 100 and 800 m asl, almost half of the stations belonging to HER 56 (Po plain). The remaining samples were collected in 2008-2010 in Liguria at altitudes between 2 and 965 m asl. Given the considerable size of this species, the criterion for discriminating between presence and absence was based on the examination of the whole slide. A distribution map was elaborated: for sites with seasonal replicates, *D. geminata* was considered present even when detected only once.

Water quality parameters at the sampling sites were provided by the environmental agencies (ARPA Valle d'Aosta and ARPA Liguria) and by Piemonte Region according to IRSA (1994) Italian Standard methods. For Valle d'Aosta, 134 of the 234 original samples were chosen based on the availability of water quality parameters. To highlight possible differences at sites with and without *D. geminata*, Mann-Whitney tests were performed for each of the following physical-chemical parameters: ammonium (N-NH_4^+), nitrate (N-NO_3^-), total nitrogen (TKN), biochemical oxygen demand (BOD), chemical oxygen demand (COD), calcium (Ca^{++}), chloride (Cl^-), conductivity, total phosphorus (TP), total suspended solids (TSS), dissolved oxygen (DO), pH. For Piemonte, additional parameters (sulphates, alkalinity, magnesium, potassium, sodium) were available. Samples were divided into 5 groups, corresponding to the 5 HERs where *D. geminata* was detected, before the Mann-Whitney test was carried out on each group.

Some macroscopic blooms of *D. geminata* were ob-

Tab. 1. Number of sampling sites and samples used for the *D. geminata* distribution map and Mann-Whitney tests (presence/absence), and the calculation of the species optima inventories. In Valle d'Aosta region, several sites were sampled during all four years.

			2007	2008	2009	2010
Valle d'Aosta	Presence/absence of DGEM	no. sites	17	24	26	68
		no. samples	34	39	27	134
	Inventories	no. sites			22	
		no. samples			23	
Piemonte	Presence/absence of DGEM	no. sites				97
		no. samples				97
	Inventories	no. sites				97
		no. samples				97
Liguria	Presence/absence of DGEM	no. sites		8	19	17
		no. samples		12	34	34
	Inventories	no. sites		8	19	17
		no. samples		12	34	34

DGEM, *D. geminata*.

served during samplings. Two sites with the most persistent blooms were monitored for a 5-week period in order to analyse the effects of thick mat production on benthic communities (both macroinvertebrates and diatoms), and to highlight the most important physical and chemical parameters associated with blooms. We chose the Germanasca and Pellice rivers (Piemonte), performing sampling every 7 days for 5 weeks. Characterization of the two sites was based on water quality data provided by Regione Piemonte, supplemented with field data collected specifically for this study, namely bed velocity, water depth (three replicates in the same transect), chlorophyll *a* and pH. Chlorophyll *a* was analysed according to the procedure proposed in Bona *et al.* (2008); periphyton for the analyses was obtained by scraping a known substrate surface (at least 20 cm²) from cobbles. The samples were immediately filtered (Whatman GFC) and stored frozen in the dark until analysis. Bed velocity (0.05 m from the bottom) was measured with a current meter (Mod RHCM Idromar). We took three velocity measurements for each site following the diatom sampling transect.

Ten diatom and macroinvertebrate samples were collected. The macroinvertebrates were sampled with a hand-net sampler with a 250 µm mesh according to the procedure suggested by Ghetti (1997). Macroinvertebrates were always collected along the same transect during the whole survey in order to avoid spatial variability of the community composition. When possible, the collected organisms were identified in the field to the family or genus level; otherwise they were preserved in 70% alcohol and analysed under a stereo-microscope. Each identified taxon was assigned to the respective functional feeding groups (Merritt and Cummins, 1996) and respiratory system. Diatom samplings and treatments followed the procedure proposed in the guidelines for routine monitoring with diatoms (EN Norm 13946; UNI, 2005). Diatoms were identified mainly according to Blanco *et al.* (2010), Hofmann *et al.* (2011); Krammer (1997a, 1997b, 2000, 2002, 2003), Krammer and Lange-Bertalot (1986, 1991a, 1991b), Lange-Bertalot (2001), Lange-Bertalot and Metzeltin (1996), Lavoie *et al.* (2008), Reichardt (1999) and Werum and Lange-Bertalot (2004). Each identified taxon was assigned to an ecological guild following Berthon *et al.* (2011). Mann-Whitney tests were performed to assess differences between bloom/no bloom samples in terms of macroinvertebrate community (metrics: number of families, feeding functional groups and respiratory systems) and diatom community (taxa composition, guilds).

Periphyton samples for the diatom community analysis were collected at all the above-mentioned sites in Piemonte and Liguria and at 22 sites in Valle d'Aosta following the procedure and the bibliographic references described above. We focused on the presence and autecology of some particular taxa due to their floristic and

biogeographical interest. For these taxa, we calculated the values of the autecological parameters in the study area with regard to main water quality variables: N-NH₄⁺, N-NO₃⁻, TKN, TP, BOD, Ca⁺⁺, Cl⁻, conductivity, pH, TSS, DO and temperature. For each variable, we calculated the environmental range (*i.e.* the minimum and maximum values for that variable in all the samples in which the species was recorded), optimum and tolerance. Optima were calculated using the weighted average method proposed by Birks *et al.* (1990), whereby the ecological optimum for a given species is the average of the values observed at the sites where the species is present weighted by the relative abundances of the species in each sample.

RESULTS

Distribution of *D. geminata* in North-Western Italy

A distribution map of *D. geminata* in NW Italy was produced on the basis of the 411 samples (Fig. 1). In Valle d'Aosta, the first records of *D. geminata* overlap with the beginning of the diatom survey carried out by the environmental agency (ARPA VdA) in 2007. In that year, *D. geminata* was observed all along the Dora Baltea river from Dolonne to Point Saint Martin (on the border with Piemonte region), and in 7 lateral valleys including the highest sampling site, Marmore river in Valtournenche (1830 m asl). Subsequent studies highlighted the rapid spread of *D. geminata* all over the region (Falasco *et al.*, 2008 a,b). In 2008, *D. geminata* colonized two more rivers, Artavanaz and Buthier. In total, *D. geminata* was present at least once at 46 sites out of 85. Despite this, no macroscopic bloom was ever observed in this region. In general, sites with the presence of *D. geminata* show lower BOD, Ca⁺⁺ and TKN and slightly higher N-NO₃⁻, TP, DO, and Cl⁻. The geological substrate is not a key feature for *D. geminata*, which is able to colonize both siliceous and calcareous streams. In general, the ecological status of Valle d'Aosta rivers is high, and all watercourses can be considered oligotrophic, with circumneutral or slightly basic pH. The only disturbance affecting these rivers is probably physical, with strong morphological modifications of riverbanks and bank tops. In Piemonte, we found *D. geminata* at 20 sampling sites out of 97; 17 of the 20 positive sites were characterized by artificial flow regulators, in particular concrete or rock weirs. Land use surrounding the sites generally comprised pastures and agricultural areas, and low urbanization. *D. geminata* preferred sites with lower alkalinity, Ca⁺⁺, Cl⁻, conductivity, magnesium, N-NO₃⁻, pH, potassium, sodium, sulphates, and TKN. In Liguria, the diatom survey started in 2008 and *D. geminata* was detected in 15 samples out of 80 (11 sampling sites out of 36) in three years of monitoring.

Environmental features related to the presence/absence of *D. geminata*

The Mann-Whitney test applied to the HERs where *D. geminata* was present highlighted the importance of different environmental parameters (Tab. 2). In particular, the nutrient content in the Alpine HER did not seem a key factor in determining the presence of *D. geminata*, probably due to the general oligotrophic condition. On the other hand, BOD and Cl^- differed between the two groups of sites, although the ranges of variation were quite narrow (maximum value for BOD was 5 mg L^{-1} ; Cl^- concentration was generally lower than 25 mg L^{-1}). In the Po plain, Ca^{++} , Cl^- and conductivity were important variables for the presence of *D. geminata*, which was mainly restricted to non-calcareous sites with low conductivity and Cl^- . In the North Apennines, the nitrogen level was an important factor, since the presence of

D. geminata was restricted to waters with low N-NO_3^- and TKN contents.

Macroscopic blooms of *D. geminata*

Macroscopic blooms were detected in some rivers in Piemonte. In particular, thick mats were observed for several months in the Germanasca and Pellice rivers. From a general evaluation of the historical data, both stations present high water quality in terms of nutrient and salinity content, with the exception of N-NO_3^- whose concentrations were slightly higher in the Pellice river. The low calcium content reflected the presence of a siliceous substrate. The pH was circumneutral, always between 6.45 and 8; TSS were always very low. Temperature was generally low during the survey, with a peak of 14.4°C in June. During our study, chlorophyll *a* values were between 0.57 and $19.6 \mu\text{g cm}^{-2}$, and were significantly

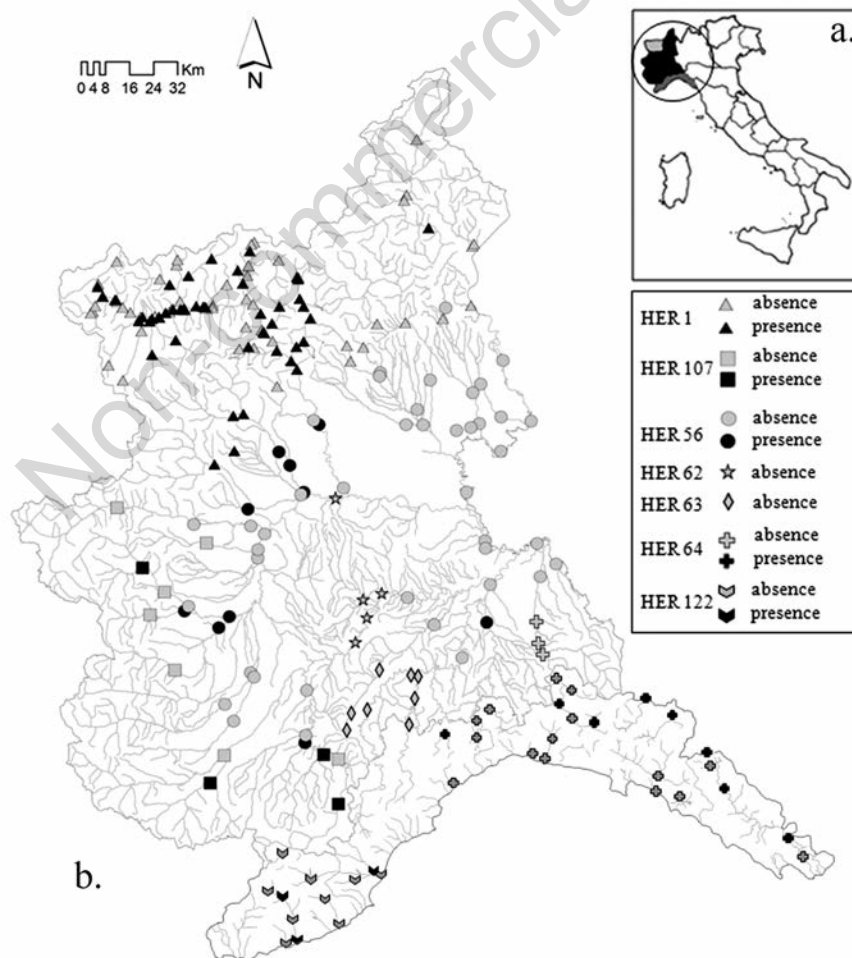


Fig. 1. Distribution map of *Didymosphenia geminata* in the study area, with classification of sampling sites based on the HER to which they belong. a) geographical position of the study area; b) Valle d'Aosta (2007-2010); Piemonte (2010); Liguria (2008-2010).

higher during *D. geminata* blooms ($P < 0.05$). Bed velocity never exceeded 1.5 m s^{-1} , and blooms were generally observed during high-speed flow conditions (mean value 0.91 m s^{-1}). On the other hand, there was a negative relation between cell density and water depth, as blooms were never observed in water deeper than 0.35 m. There was no difference in the number of families or genera making up the macroinvertebrate communities during the blooms or in normal conditions. Despite this, we observed an increase of the number of taxa belonging to Diptera and a significant decrease of Tricoptera, in particular Hydropsychidae and Heptageniidae, during *D. geminata* blooms. In general, we observed an increase of collectors and a decrease of scrapers. In terms of respiratory groups, there was no increase of taxa characterized by cutaneous respiration with respect to those with branchial respiration (*i.e.* external tracheal gills).

In general, diatom communities consisted of oligo and β -mesosaprobous taxa typical of mountain streams, and denoting good water quality, *i.e.* *Achnantheidium pyrenaicum* (Hustedt) Kobayasi, *Achnantheidium minutissimum* species group, *Encyonema silesiacum* (Bleisch) Mann, *Gomphonema elegantissimum* Reichardt and

Lange-Bertalot, *Gomphonema tergestinum* Fricke, and *Reimeria sinuata* (Gregory) Kociolek and Stoermer. The abundance of some species decreased when *D. geminata* produced blooms. In particular, *A. minutissimum*, *G. elegantissimum* and *G. tergestinum* were inhibited by massive mats. On the other hand, *Diatoma mesodon* (Ehrenberg) Kutzing increased in abundance when a *D. geminata* bloom was present. Overall, there was an increased abundance of motile taxa and erect forms when a bloom was present, while stalked and prostrate forms decreased. However, no statistically significant differences were observed among guilds in the case of a bloom.

Non-indigenous taxa: distribution and site characterisation

In total, 200 samples were collected for the diatom community analysis from 2008 to 2010, and 305 taxa were identified. The most abundant taxa and present in at least 50% of the samples are (in order of abundance): *A. minutissimum*, *A. pyrenaicum*, *Nitzschia fonticola* Grunow, *R. sinuata*, *E. silesiacum*, *Cocconeis placentula* var. *euglypta* Ehrenberg, *Nitzschia dissipata* (Kützing) Grunow, *Amphora pediculus* (Kützing) Grunow, *Ency-*

Tab. 2. Mann Whitney test applied to the different hydroecoregions. Grouping variable: presence of *D. geminata*. In italics: significant difference ($P < 0.05$). The Monferrato and Piemonte Apennines hydroecoregions were excluded from the analysis since *D. geminata* was not detected in any of the sampling sites.

	HER	Mann-Whitney U	P	HER	Mann-Whitney U	P	HER	Mann-Whitney U	P
N-NH ₄ ⁺	Alpine	2330.5	0.122	Po plain	198	0.867	Apennines N	154.5	0.214
N-NO ₃ ⁻		2392	0.270		138	0.112		<i>48.5</i>	<i>0.000</i>
TKN		2349.5	0.144		128	0.068		<i>79.5</i>	<i>0.004</i>
BOD		<i>1950.5</i>	<i>0.004</i>		148	0.150		165	0.432
COD		2692.5	0.894		154	0.204		191	0.826
Ca ⁺⁺		2403.5	0.290		<i>122.5</i>	<i>0.049</i>		101.5	0.618
Cl ⁻		<i>1934.5</i>	<i>0.003</i>		<i>103.5</i>	<i>0.016</i>		154.5	0.313
Conductivity		2382.5	0.255		<i>109.5</i>	<i>0.023</i>		178	0.522
TP		2317	0.128		131.5	0.073		145	0.114
SS		2490.5	0.408		145.5	0.145		194	0.790
DO		2384	0.257		149	0.184		191.5	0.836
pH		2525	0.486		179	0.537		136	0.098
Temperature		2239.5	0.074		204.5	0.991		158	0.265
N-NH ₄ ⁺	Inner Alps S	198	0.262	Ligurian Alps	12	0.501			
N-NO ₃ ⁻		138	0.071		2	0.060			
TKN		128	0.218		42.5	0.300			
BOD		148	0.229		55.5	0.778			
COD		154	0.189		48	0.359			
Ca ⁺⁺		122.5	0.850		28	0.657			
Cl ⁻		103.5	0.450		54	0.950			
Conductivity		109.5	1.000		42	0.299			
TP		131.5	0.548		49	0.314			
SS		145.5	0.303		35	0.166			
DO		149	0.776		31	0.094			
pH		179	0.705		52	0.481			
Temperature		204.5	0.059		53.5	0.537			

HER, hydroecoregion; BOD, biochemical oxygen demand; COD, chemical oxygen demand; TP, total phosphorus; SS, suspended solids; DO, dissolved oxygen.

onema minutum (Hilse) Mann. In addition to *D. geminata*, the analysis focused on four taxa poorly known in Italy and with a potentially expanding distributional range. They are, in order of occurrence, *Reimeria uniseriata* Sala Guerrero and Ferrario, *Achnantheidium subhudsonis* (Hustedt) Kobayasi, *Cymbella tropica* Krammer and *Mayamaea cahabaensis* Morales and Manoylov. A map with the distribution of these taxa is reported in Fig. 2. Tab. 3 summarizes data on records of *D. geminata*, *A. subhudsonis*, *C. tropica*, *M. cahabaensis*, and *R. uniseriata* in the study area. Light and electron microscope photographs of the last four taxa are shown in Fig. 3.

A. subhudsonis was recorded only in Piemonte, at 15 sites distributed mainly in the northern part of the region (Inner Alps and Po plain; HERs 1 and 56). The relative abundance of *A. subhudsonis* reached 20% in the Chiusella and Ticino rivers, highlighting the invasive be-

haviour of the species. *C. tropica* was widespread throughout Piemonte and reached its highest abundance in the Chisone river (25.7%). Several rivers were contemporaneously affected by *A. subhudsonis* and *C. tropica* (i.e. Cervo, Chiusella, Roggia Busca, and Sessera). These two non-indigenous taxa were mainly recorded at sites with similar environmental and chemical features. All the sites were surrounded by agricultural zones (cereal production or rice paddies). In some cases, the presence of industrial areas (chemical, metallurgical or paper mills) affected the water chemistry of sampling sites, e.g., presence of heavy metals in the water column (Cd, Cr, Cu, Hg, Ni, Pb, and Zn, data provided by Regione Piemonte). *A. subhudsonis* seems to be able to survive in habitats with reduced light penetration; indeed the transparency of the water column was limited or nil in some cases. Although cobbles were the dominant substrate where *A. subhudo-*

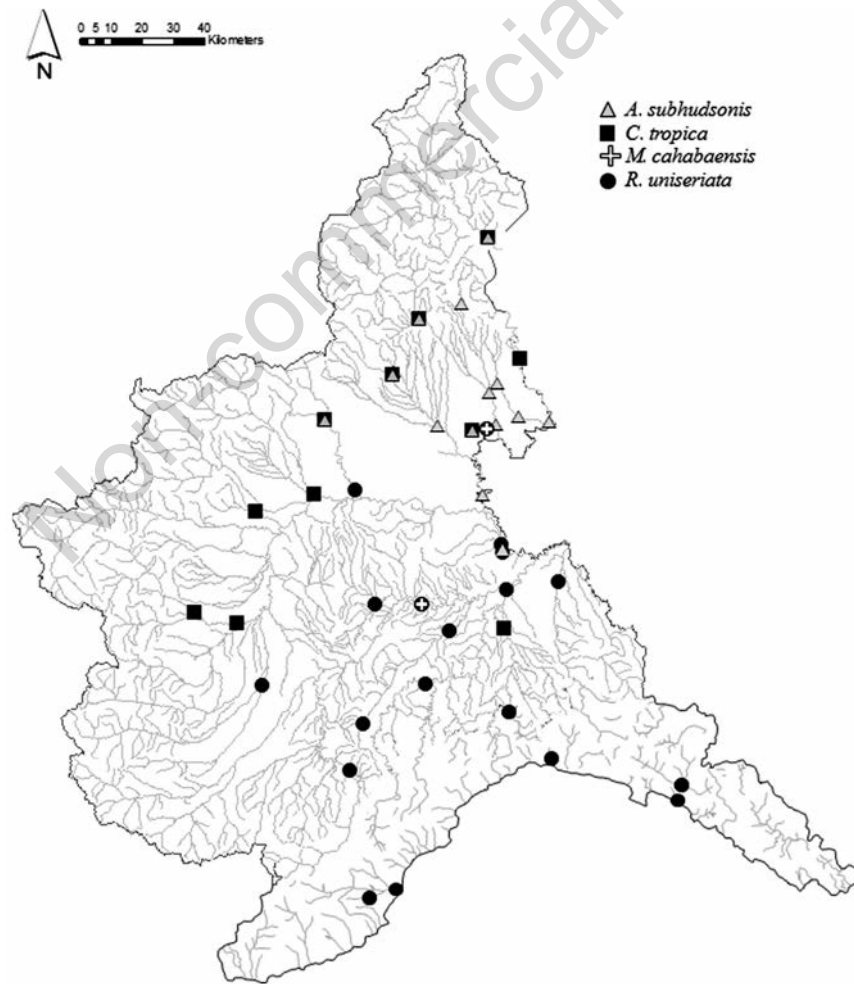


Fig. 2. Distribution map of *Achnantheidium subhudsonis* (Hustedt) Kobayasi, *Cymbella tropica* Krammer, *Mayamaea cahabaensis* Morales and Manoylov, *Reimeria uniseriata* Sala Guerrero and Ferrario in Piemonte and Liguria updated to 2010. These taxa were not detected in Valle d'Aosta.

Tab. 3. Location (Universal Transverse Mercator projection, European Datum ED50) and hydrocoregions classification of sampling sites where the studied species were detected.

Region	Year	HER	River	Location	UTM_X	UTM_Y	DGEM	RUNI	MCAH	ADSH	CTRO
Valle d'Aosta	2010	1	Buthier	Ferreres	379980	5082431	+				
	2010	1	Colombaz	Foce	348164	5068787	+				
	2010	1	Petit Monde	Loditor	388730	5078545	+				
	2009; 2010	1	Savara	Introd	359447	5061619	+				
	2008	1	Artanavaz	Allein	366065	5073747	+				
	2007; 2008	1	Ayasse	Hone	401716	5051820	+				
	2010	1	Ayasse	Vignat	392283	5053152	+				
	2008	1	Buthier	Thoules	372265	5076705	+				
	2010	1	Buthier	Foce	370934	5065780	+				
	2010	1	Chateau de Quart	Foce	374852	5066641	+				
	2010	1	Clusellaz	Foce	364744	5064396	+				
	2010	1	Comboè	Foce	370924	5065430	+				
	2007; 2008; 2010	1	Dora Baltea	Pont Saint Martin	406504	5048368	+				
	2007; 2008; 2009; 2010	1	Dora Baltea	Pré Saint Didier	343788	5069928	+				
	2010	1	Dora Baltea	Champex	343400	5070183	+				
	2007; 2008; 2009	1	Dora Baltea	Aosta	367160	5065446	+				
	2010	1	Dora Baltea	Monte CAS	369677	5065550	+				
	2010	1	Dora Baltea	Plan Felinaz	370556	5065479	+				
	2010	1	Dora Baltea	Les Iles	377937	5066359	+				
	2010	1	Dora Baltea	Quart	377284	5066432	+				
	2010	1	Dora Baltea	Favà	397169	5058181	+				
	2010	1	Dora Baltea	Dolonne	342082	5073104	+				
	2010	1	Dora Baltea	Sarriod de la tour	362389	5062981	+				
	2010	1	Dora Baltea	Morgex	347808	5068637	+				
	2010	1	Dora Baltea	Marais	348565	5068028	+				
	2007; 2009; 2010	1	Dora Baltea	Borgo di Montjovet	396297	5064175	+				
	2007; 2008	1	Dora di Rhemes	Villeneuve	360098	5062498	+				
	2007	1	Dora di Valgrisenche	Leverogne	355768	5061657	+				
	2010	1	Dora di Valgrisenche	Foce	356993	5062968	+				
	2007; 2008	1	Evançon	Arcesaz	402595	5066504	+				
	2010	1	Evançon	Isollaz	400070	5060859	+				
	2010	1	Evançon	Foce	397572	5057732	+				
	2007; 2008; 2009	1	Grand Eyvia	Cogne	368074	5055371	+				
	2010	1	Grand Eyvia	Foce	362692	5062829	+				
	2007	1	Lys	Gaby	412950	5062582	+				
	2008; 2010	1	Lys	Fontainemore	410655	5054880	+				
	2010	1	Lys	Bessesse	408205	5053124	+				
	2010	1	Lys	Tschossil	410657	5066522	+				
	2010	1	Lys	Tache	408821	5075594	+				
	2010	1	Lys	Ejo	408451	5076384	+				
2010	1	Lys	Tschoarde	409265	5069137	+					
2009	1	Lys	Fontainemore	410655	5054880	+					
2008	1	Lys	Pont Saint Martin	341970	5049975	+					
2008	1	Marmore	Filey	390403	5074245	+					
2007	1	Marmore	Perrères	392683	5084973	+					
2010	1	Savara	Degioz	360182	5050509	+					
Piemonte	2010	1	Malone	Rocca Canavese	387584	5018405	+				
	2010	1	Stura di Lanzo	Lanzo Torinese	380983	5013876	+				
	2010	1	Curone	Pontecurone	494618	4980962	+				
	2010	1	Dora Baltea	Settimo	408403	5045493	+				
	2010	1	Orco	Pont-Canavese	387187	5030109	+				
	2010	1	S. Bernardino	Verbania	466732	5086599					+
	2010	1	S. Giovanni di Intra	Verbania	467274	5087417				+	
	2010	1	Sessera	Borgosesia	444512	5061036				+	+
	2010	1	Toce	Premosello-Chiovenda	452169	5092720	+				
	2010	56	Malesina	San Giusto Canavese	406014	5013548	+				

To be continued on next page.

Tab. 3. Continued from previous page.

Region	Year	HER	River	Location	UTM_X	UTM_Y	DGEM	RUNI	MCAH	ADSH	CTRO
Piemonte	2010	56	Orba	Casal Cermelli	471572	4961515	+				+
	2010	56	Orco	Chivasso	410724	5004642	+				+
	2010	56	Pellice	Villafranca Piemonte	385855	4963372	+				+
	2010	56	Pellice	Garzigliana	370974	4965289	+				
	2010	56	Agogna	Briga Novarese	457896	5066137					+
	2010	56	Agogna	Novara	469024	5027557					+
	2010	56	Belbo	Castelnuovo Belbo	454035	4960704			+		
	2010	56	Bormida	Alessandria	472683	4974110			+		
	2010	56	Ceronda	Venaria	392087	4999203	+				+
	2010	56	Cervo	Cossato	435884	5043337					+
	2010	56	Chisone	Garzigliana	372268	4966857					+
	2010	56	Chiusella	Strambino	413982	5028585					+
	2010	56	Dora Baltea	Saluggia	423844	5006090			+		
	2010	56	Dora Baltea	Strambino	415703	5027182	+				
	2010	56	Ellero	Bastia Mondovì	411066	4921394	+				
	2010	56	Grana	Valenza	471094	4986167			+		+
	2010	56	Grana-Mellea	Savigliano	394086	4943259			+		
	2010	56	Marchiazza	Collobiano	450367	5027114					+
	2010	56	Orco	Feletto	402425	5018044	+				
	2010	56	Po	Villafranca Piemonte	382362	4959682	+				
	2010	56	Po	Valenza	471025	4988687			+		
	2010	56	Roggia Biraga	Novara	466243	5026191			+	+	
	2010	56	Roggia Busca	Casalino	461450	5025218					+
	2010	56	Roggia Mora	San Pietro Mosezzo	466861	5037793					+
	2010	56	Scrvia	Castelnuovo	489345	4976740			+		
	2010	56	Sesia	Motta De' Conti	464813	5004710					+
	2010	56	Strona	Cosstato	436176	5044496					+
	2010	56	Tanaro	Castello Di Annone	445180	4969789			+	+	
	2010	56	Terdoppio	Caltignaga	469539	5040650					+
	2010	56	Terdoppio	Tredate	476323	5029754					+
	2010	56	Ticino	Oleggio	476900	5048490					+
	2010	56	Ticino	Cerano	486288	5028271					+
	2010	62	Triversa	Asti	430365	4969192			+		
2010	63	Belbo	Feisoglio	426535	4931207			+			
2010	63	Bormida di Millesimo	Monastero Bormida	446382	4943933			+			
2010	107	Corsaglia	Lesegno	417324	4917401	+					
2010	107	Germanasca	Pomaretto	357062	4979691	+					
2010	107	Tanaro	Priola	422210	4900947	+					
2010	107	Tanaro	Ceva	422163	4915889			+			
2010	107	Vermenagna	Roccavione	379528	4907933	+					
Liguria	2008;2009	64	Aveto	Rezzoaglio	532636	4933287	+				
	2009	64	Erro	Sassello	457511	4926456	+		+		
	2008	64	Magra	Sarzana	575141	4884454			+		
	2008	64	Pentemina	Montoggio	506581	4930596	+				
	2010	64	Scrvia	Ronco Scrvia	495341	4936937	+				
	2009	64	Taro	Tornolo	544050	4920477	+				
	2008	64	Trebbia	Gorreto	523551	4939317	+				
	2010	64	Vara	Vezzano Ligure	570955	4889831	+				
	2008	64	Vara	Varese Ligure	549897	4906958	+				
	2010	64	Varenna	Genova	485911	4919727			+		
	2010	122	Argentina	Molini di Triora	404311	4869936	+				
	2010	122	Argentina	Taggia	408361	4854486	+				
	2009	122	Centa	Albenga	436329	4878102			+		
	2009	122	Lerrone	Garlenda	427872	4876043			+		
2010	122	Neva	Albenga	434716	4878630	+					

HER, hydroecoregions; UTM, universal transverse mercator; DGEM, D. geminata; RUNI, R. uniseriata; MCAH, M. cahabaensis; ADSH, A. subhudsonis; CTRO, C. tropica.

nis and *C. tropica* were collected, we noticed a significant percentage of finer materials (gravel, sand, and silt), especially at sites with *A. subhudsonis*. Water velocity was generally low, typical of lowland rivers, and the mean depth was approximately 25 cm. The agricultural activities slightly affected the water quality in terms of nitrates. *M. cahabaensis* was detected in two rivers far from each other but belonging to the same HER (Po plain). Although different in size and substrate, the two rivers have common characteristics in terms of anthropogenic pressures, mainly related to intensive agriculture. *R. uniseriata* currently appears to have a rather wide distribution, as the 18 stations in which it was found belong to all the HER in-

cluded in the study area. The affected rivers have very disparate characteristics, often with a depositional character.

Autecology of taxa of special interest

Tab. 4 reports the comparison between the ranges of the main water parameters calculated for the whole database (200 records covering the entire study area), and the range of the same parameters where the single taxon was recorded. Optimum and tolerance values for each species are also reported.

The samples covered a wide range of trophic levels for phosphorus, nitrogen, and organic loading. The min-

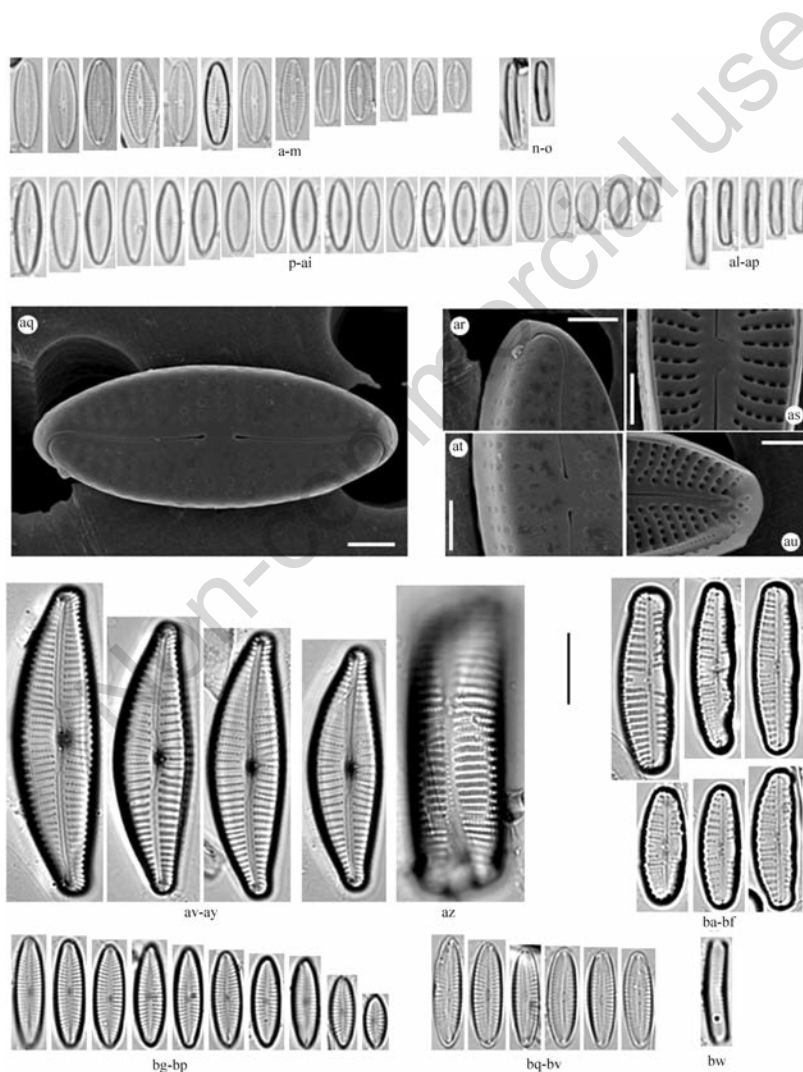


Fig. 3. Taxa of particular interest detected during the surveys. Light micrographs of different populations of *M. cahabaensis*. Tanaro river: valvar (a-m) and girdle (n-o) views. Mincio river: valvar (p-ai) and girdle (al-ap) views. Scanning electron micrographs of *M. cahabaensis* from Mincio river. External view (aq). Detail of the valve apex showing the terminal raphe ending: external (ar) and internal (au) views. Details of the central part of valve: external (at) and internal (as) views. Light micrographs of *C. tropica*: valvar (av-ay) and girdle (az) views; *R. uniseriata* (ba-bf); *A. subhudsonis*: rapheless (bg-bp) and raphe (bq-bv) valves; girdle view (bw). LM scale bar: 10 μ m; SEM scale bar: 1 μ m.

eral content was generally low, as highlighted by conductivity, Ca^{++} and Cl^- , with the exception of sites in the Ligurian catchments. pH was generally between neutral and alkaline values. TSS content was generally moderate (below 100 mg L^{-1}), with values above 100 confined to the Po plain (HER 56), characterized by fine substrate.

A. subhudsonis showed a marked preference for waters low in ammonia, TP and BOD, while its optimum for N-NO_3^- corresponded to a mesotrophic-eutrophic condition. Its optimum for conductivity and Cl^- was low but the range for conductivity was wide (up to $407 \mu\text{S cm}^{-1}$). pH was circumneutral. This species showed a high tolerance to TSS (up to 202 mg L^{-1}), with a preference for low values. *C. tropica* seemed to tolerate quite high nitrate concentrations, although the optimum value was low; regarding the other nutrients and BOD, this taxon was found in waters with oligotrophic values. It showed a rather narrow range for conductivity, Cl^- , and

TSS. *D. geminata* generally colonized oligotrophic stretches with low organic loading, a non-calcareous substrate and low TSS values. Data on conductivity showed an optimum at $229 \mu\text{S cm}^{-1}$. Despite this, a rather high ecological amplitude emerged from our data. The autecological data on *M. cahabaensis* must be considered preliminary, due to the very limited distribution in the study area at the time of the sampling (2008-2010). This taxon showed a higher preference for nutrient and organic enrichment than the other taxa. The mineral content at the stations with this species was moderate. Its presence did not seem to be influenced by the TSS concentration. *R. uniseriata* seems to be a euryoecious species typical of lowland stretches. In many cases, the range of environmental parameters in which it was present overlapped with that of the entire database. The nutrient and BOD optima corresponded to a II - III class. The optimum for calcium was 53 mg L^{-1} with a wide range ($18\text{-}109 \text{ mg L}^{-1}$).

Tab. 4. Ranges, optima (in italics) and tolerance (in parentheses) values for nutrients, biochemical oxygen demand, calcium, chloride, conductivity, pH, total suspended solids, dissolved oxygen and temperature calculated from 200 samples.

	N-NH ₃ (mg L ⁻¹)	N-NO ₃ (mg L ⁻¹)	TKN (mg L ⁻¹)	TP (mg L ⁻¹)	BOD (mg L ⁻¹)	Ca ⁺⁺ (mg L ⁻¹)
Range for the whole study area	0.000-2.470	0.05-7.1	0.025-8.15	0.002-0.657	0.5-20	0.25-120
ADSH	0.015-0.140 <i>0.04</i> (0.035)	0.4-3.4 <i>1.62</i> (0.77)	1.7-5.3 <i>2.58</i> (0.612)	0.003-0.203 <i>0.05</i> (0.043)	1-7.3 <i>1.66</i> (1.22)	4.4-62.1 <i>19.9</i> (8.96)
CTRO	0.015-0.097 <i>0.02</i> (0.012)	0.5-3.0 <i>0.81</i> (0.523)	0.95-3.70 <i>1.48</i> (0.762)	0.003-0.053 <i>0.04</i> (0.016)	1.0-3.7 <i>1.23</i> (0.678)	8.1-63.2 <i>29.1</i> (12.95)
DGEM	0.005-0.380 <i>0.03</i> (0.03)	0.14-3.03 <i>0.54</i> (0.57)	0.24-3.70 <i>1.06</i> (0.838)	0.015-0.171 <i>0.03</i> (0.025)	0.50-3.51 <i>1.16</i> (0.703)	0.25-65.10 <i>28.6</i> (21.97)
MCAH	0.075-0.177 <i>0.17</i> (0.044)	1.4-3.1 <i>1.58</i> (0.745)	3.07-4.83 <i>3.25</i> (0.759)	0.1-0.3 <i>0.27</i> (0.082)	3.0-4.0 <i>3.1</i> (0.430)	33.9-68.7 <i>37.5</i> (14.97)
RUNI	0.003-2.470 <i>0.13</i> (0.460)	0.005-4.450 <i>1.26</i> (1.01)	0.46-5.95 <i>2.38</i> (1.732)	0.015-0.657 <i>0.09</i> (0.135)	1.0-20.0 <i>3.17</i> (3.08)	18-109 <i>53.8</i> (25.46)
	Cl ⁻ (mg L ⁻¹)	Conductivity ($\mu\text{S cm}^{-1}$)	pH	TSS (mg L ⁻¹)	DO (%)	Temperature (°C)
Range for the whole study area	0.5-96	33-924	6.3-8.93	0.3-242	70-129	1-23
ADSH	1.9-15.9 <i>9.22</i> (4.43)	56-407 <i>163</i> (62.9)	6.8-7.8 <i>7.2</i> (0.26)	5-202 <i>11.3</i> (20.8)	90.5-121.5 <i>100</i> (8.22)	5.6-20.1 <i>15.5</i> (5.49)
CTRO	1.15-17.17 <i>3.18</i> (1.98)	66-337 <i>177</i> (63.4)	6.5-7.9 <i>7.6</i> (0.42)	5.0-12.0 <i>5.3</i> (1.38)	86-121.5 <i>110</i> (8.41)	5.9-20.1 <i>14.9</i> (3.11)
DGEM	0.67-28 <i>7.33</i> (5.22)	56-552 <i>229</i> (129)	6.5-8.9 <i>7.8</i> (0.74)	0.5-30 <i>4.5</i> (5.79)	91-129 <i>102</i> (6.70)	2-20.1 <i>14.1</i> (4.43)
MCAH	12.7-21.5 <i>13.6</i> (3.75)	253-448 <i>274</i> (83.3)	7.3-7.9 <i>7.3</i> (0.28)	18-66 <i>23.6</i> (20.5)	94-96 <i>95</i> (1.00)	12.3-19.3 <i>13.0</i> (3.03)
RUNI	6.1-90.8 <i>23.9</i> (21.9)	227-925 <i>416</i> (169)	7.3-8.5 <i>7.9</i> (0.38)	0.3-202 <i>29.2</i> (55.8)	70-113 <i>94</i> (12.8)	10.1-21.6 <i>14.4</i> (2.53)

TKN, total nitrogen; TP, total phosphorus; BOD, biochemical oxygen demand; ADSH, *A. subhudsonis*; CTRO, *C. tropica*; DGEM, *D. geminata*; MCAH, *M. cahabaensis*; RUNI, *R. uniseriata*; TSS, total suspended solids; DO, dissolved oxygen.

DISCUSSION

Historical data concerning the first record of *D. geminata* in Italy date to 1880 (Brun, 1880). Indeed, the definition of *D. geminata* as a non-indigenous species for Italy should probably be reconsidered. Nevertheless, if we look at the distribution pattern of this species in Italy, the frequency of its records, its relative abundance and the formation of massive proliferations, we can confirm that the distribution range of *D. geminata* has recently been expanding. The records of *D. geminata* are undoubtedly limited to those basins included in the regional monitoring programs and to the implementation of the WFD. Despite this, the first records of macroscopic blooms of *D. geminata* in Piemonte date only to 2003 (personal observation, unpublished data) and they were never observed earlier.

Our results confirm that *D. geminata* is widespread throughout North-Western Italy. In the surveys conducted in Piemonte, Valle d'Aosta, and Liguria, *D. geminata* was detected in 123 samples out of 411 (ca. 30% of the samples collected from 2007 to 2010). Valle d'Aosta rivers seem to represent the most suitable habitat for *D. geminata* colonization for several reasons: the oligotrophic status of the waters, their good oxygenation, and the flow regulators (weirs and dams) in most hydrological networks of the region. During the three-year survey, *D. geminata* was detected in almost the entire Dora Baltea watercourse (160 Km), from Dolonne (Aosta) to Strambino (Turin), with the exception of 3 sites in Valle d'Aosta and 1 in Piemonte immediately before its confluence with the Po river. Nevertheless, no macroscopic blooms were ever observed in Valle d'Aosta. It is possible that the high-speed flow typical of watercourses in this region does not favour the production of stalks and thus of blooms, which require more stable and constant conditions (Whitton *et al.*, 2009).

From the comparison with literature data, the presence of *D. geminata* in Piemonte was confirmed at 4 sampling sites out of 12 (Ellero, Corsaglia, Vermegnana, and the upper part of the Tanaro river). *D. geminata* was detected all along the Tanaro and Bormida di Millesimo rivers during the 2007 surveys (Battezzatore *et al.*, 2009), but its presence was not confirmed in 2010 (apart from the upper sampling station on the Tanaro, *i.e.* Priola, Cuneo).

Historical and literature data from Liguria are completely absent. The first results concerning diatom communities come from 2008. Up to 2010, *D. geminata* was found in 15 samples out of 80. Mann-Whitney test provided some general indications on the role of some chemical parameters (mainly nitrogen and mineral content) limiting the presence of *D. geminata*. Despite this, the role of other characteristics favouring its spread, such as river morphological alterations, diffusion pathways or closeness to already contaminated sites, cannot be neglected. The autecological data on *D. geminata*, based on the optimum calculations, confirmed the literature data. *D. gem-*

inata shows a preference for cold and oligotrophic waters with low mineral content (Krammer and Lange-Bertalot, 1986; Patrick and Reimer, 1975; De Wolf, 1982; Potapova and Charles, 2007; Rabenhorst, 1853; Rawson, 1956; Sládeček, 1973). However, the ecological amplitude of the species seems to be wider than hypothesized in the old literature, confirming recent data (Battezzatore *et al.*, 2009; Gunde-Cimerman *et al.*, 2005; Kawecka and Sanecki, 2003; Kilroy *et al.*, 2005; Sterrenburg *et al.*, 2007).

Macroscopic blooms of *D. geminata* were limited to a few sites: they were never observed in Valle d'Aosta or Liguria, while they were present in several stretches in Piemonte. Literature data confirmed the presence of mats in the Po and Varaita rivers (Battezzatore *et al.*, 2007) and high relative abundances were reported in the Corsaglia, Tanaro, Maira and Bormida rivers (Battezzatore *et al.*, 2009). During our 2010 survey, macroscopic blooms covering the whole river bottom were observed only in the Germanasca and Pellice rivers. A weekly survey of these two sites confirmed the statements by several authors that *D. geminata* prefers a low-medium and stable discharge (mean value $5.73 \text{ m}^3 \text{ s}^{-1}$) and a shallow streambed ($<0.35 \text{ m}$) (Kirkwood *et al.*, 2007; Miller *et al.*, 2009). The artificial dam upstream of the sampling site on the Germanasca river probably favoured the establishment of these conditions, and the consequent massive proliferation (Kawecka and Sanecki, 2003; Kirkwood *et al.*, 2007; Kirkwood *et al.*, 2009; Skulberg, 1982; Spaulding, 2007; Whitton *et al.*, 2009). The pH of these two rivers was generally neutral to slightly basic, in agreement with most references. River waters were well oxygenated, with DO values always above 90% and 10 mg L^{-1} , and SO_4^{2-} concentrations never below 5.2 mg L^{-1} (in agreement with information contained in Whitton *et al.*, 2009). Nutrient concentrations were generally low, in agreement with average values found in the literature (Whitton *et al.*, 2009). Riparian vegetation at these two stream sites was completely absent due to the bank and bank top modifications: the increase of ultraviolet radiation would probably favour the growth of *D. geminata*, as suggested by Sherbot and Bothwell (1993).

In accordance with several authors (Larned *et al.*, 2007; Mundie and Crabtree, 1997; Whitton *et al.*, 2009), we recorded a significant decrease of the EPT index followed by a huge increase of chironomids during blooms (Brown, 2008). In these conditions, the abundance of scrapers decreased, and thus *D. geminata* proliferation was not contrasted. Therefore, we tested whether mats could somehow limit the presence of taxa characterized by external respiratory mechanisms via tracheal gills, but no statistical evidence for this hypothesis was obtained. In disagreement with some publications (Whitton *et al.*, 2009; Wyatt *et al.*, 2008), we observed a decreased abundance of *A. minutissimum* during *D. geminata* blooms.

However this disagreement could arise from different identification within the *A. minutissimum* group. In contrast, no significant variation of *A. pyrenaicum* was recorded. Stalked taxa, such as *G. elegantissimum* and *G. tergestinum*, seemed to be inhibited by massive *D. geminata* proliferations, probably due to spatial competition, whereas erect, motile and tube-dwelling forms were favoured and increased in abundance.

During our surveys, other taxa of ecological interest and with nuisance behaviour were recorded for the first time in North-Western Italy. *A. subhudsonis* was described in Africa in the early 19th century (Hustedt, 1921). After this, it was recorded in Africa, Indonesia and South America, and since 1950 in the Antilles, Korea and Japan (Coste and Ector, 2000). According to Lange-Bertalot and Krammer (1989) and Coste and Ector (2000), the species was absent from Europe up to the 1990s. Indeed, starting from 1991 (Ector, 1991), *A. subhudsonis* was recorded in several European countries, such as Spain, Portugal, and France (Coste and Ector, 2000; Blanco *et al.*, 2010; Novais, 2011). Our research confirmed the presence of this species in several NW Italian rivers, with high relative abundance in two of them. As confirmed by Blanco *et al.* (2010), *A. subhudsonis* can reach a relative abundance of over 20% of the whole community and in some cases can dominate the sample. From our data, the optimum for *A. subhudsonis* is reached at low N-NH₄⁺, total phosphorus and BOD values but at relatively high N-NO₃⁻ concentrations. These results are slightly different from the data obtained for the Duero Basin in Spain, where the species was recorded with higher nutrient optima and organic load concentrations.

C. tropica was recorded for the first time in Brazil (Metzeltin and Lange-Bertalot, 1998) and wrongly identified as *Cymbella turgidula*. In 2002, Krammer defined the length/width ratio and the presence of one single stigma in *C. tropica* as the diagnostic character to distinguish it from *C. turgidula*. Starting from this publication, *C. tropica* seemed to be distributed mainly in tropical areas, *i.e.* Brazil, Venezuela, Costa Rica, and Ecuador. Recent publications reported *C. tropica* in Guadeloupe (Lefrançois *et al.*, 2010), Martinique (Desrosiers and Bargier, 2010), Central-Western Brazil (Da Silva, 2009), and New Jersey (Charles *et al.*, 2010). In recent years, *C. tropica* was recorded in France (Coste *et al.*, 2008) and Portugal (as *C. cf tropica*; Novais, 2011), but had never been detected in Italy before our study. Data on its ecology are poor. From our results, its range of tolerance for nutrients seems to be quite wide, but its optimum values are generally low, denoting a sensitive species with only adequate ecological reliability.

R. uniseriata was described by Sala *et al.* (1993) in South America but its diffusion has likely been underestimated, as it has probably been misclassified as *Reimeria sinuata* (Gregory) Kociolek and Stoermer. *R. uniseriata*

differs from *R. sinuata* by the uniseriate striae, C-shaped foramina and the apical pore field at each pole (Sala *et al.*, 1993). In Europe, this species has been recorded in Southern France (Coste and Ector, 2000), in the Danube river catchment (Ács *et al.* 2004, 2006) and in Portugal (Novais, 2011). A recent revision of the herbarium materials from Poland confirmed the presence and misidentification of the taxon in Polish waters starting from the early 1960s (Wilk-Woźniak and Wojtal, 2005), and thus it is probably no longer invasive according to our criteria. In Italy, it was recorded in the Dolomiti Bellunesi National Park (Cantonati and Spitale, 2009), and in two rivers of Central Italy, and one of Southern Italy (Torrisi and Dell'Uomo, 2009). The only published data regarding its presence in Piemonte concern the upper part of the Po basin (Battezzore *et al.*, 2007, 2009, 2010; Battezzore, 2008, 2012). Our results suggest that *R. uniseriata* is the most tolerant species in terms of nutrient concentration, mineral content and organic loading. It is widespread in both Piemonte and Liguria, with no geological and biogeographical preferences.

M. cahabaensis was recorded in 46 samples collected from 1997 to 2004 in southern US rivers (Morales and Manoylov, 2009). Up to 2010, there were no published records of this species for Europe (and Italy). Our results and literature data (Morales and Manoylov, 2009) indicate that *M. cahabaensis* tolerates intermediate values of nutrients and organic loading and can be considered a good indicator of meso-eutrophic conditions. The distribution range of this species has been expanding lately. Recent surveys in both Piemonte and Lombardia highlighted the presence of this species in several rivers (Falasco, *personal observations from samples collected in 2011*). These records of the species were particularly important due to the high relative abundance of *M. cahabaensis* in the samples. In some cases, particularly in the Mincio and Oglio rivers but also in the Tanaro, the species reached up to 50% relative abundance, with substantial effects on the biodiversity of the community, which strongly decreased. The small size of the species and the scarce literature data on this taxon do not favour its correct identification. Thus, we provide LM and SEM pictures of recently collected populations in order to supply further iconographical material on this species (Fig. 3). *M. cahabaensis* is morphologically very close to *Eolimna comperei* Ector *et al.*, and only scanning electron analyses can distinguish between them. The differences are related to the position of the hymens covering each areola, and the proximal and terminal end deflections of the raphe (Morales and Manoylov, 2009). These fine differences could have led to the misidentification of the two taxa during monitoring surveys. Indeed, *E. comperei* has frequently been recorded in Europe, in particular in France (Coste and Ector, 2000; Guillard and Ector, 2005), in the Duero basin (Blanco *et*

al., 2010) and in Portugal (Novais, 2011). Thus far, there have been no records of this species for Italy. From a comparison with the literature data (especially Blanco *et al.*, 2010), we can say that *E. comperei* tolerates intermediate values of nutrients and organic loading and can be considered a good indicator of mesotrophic conditions. The similar ecological preferences and morphological features, and the same nuisance behaviour suggest that more systematic investigations on the *E. comperei* type material and European samples are required in order to clarify its taxonomic position with respect to *M. cahabaensis* and their potential synonymy.

CONCLUSIONS

The results of this study provide detailed knowledge of the diatom flora in NW Italy, with some interesting implications for river management, and questions for future investigations.

Although *D. geminata* is not considered non-indigenous, its geographical range has expanded in recent years, with a preference for oligotrophic waters and regulated rivers. A tendency to produce blooms at sites depleted of riparian vegetation was observed in this study. Indeed, *D. geminata* is extremely resistant to drought conditions and is able to tolerate high UV radiation. River habitat modifications lead to a loss of ecosystem integrity, with effects on biodiversity and the spread of nuisance taxa such as *D. geminata*. Further research should be aimed at clarifying (also in quantitative terms) the relationship between *D. geminata* and human-induced physical modifications.

This is the first study recording the presence of *A. subhudsonis*, *C. tropica*, and *M. cahabaensis* in northern Italian rivers. These taxa must be considered non-indigenous in Italy with an increasing spread. Considering their relative abundance in the diatom community, their correct identification is essential to control their diffusion and their potential detrimental effects on the diatom community, as well as for a correct river classification following the WFD. Indeed the presence of these taxa is often associated with a decrease in species richness and biodiversity.

A focus on *R. uniseriata*, even though it is not considered bloom forming or non-indigenous, can shed further light on its identification and distribution, as this taxon has been misclassified as *R. sinuata* in some diatom floras.

ACKNOWLEDGMENTS

This study arises from a research collaboration between the University of Torino, and ARPA Valle d'Aosta and ARPA Liguria.

The authors are particularly grateful to Livia Mobili, Anna Risso and Rosella Bertolotto for providing data and

samples, Luca Cardillo and Elena Piano for help with the field work. The authors thank the Water Resources Department of the Piemonte Region for providing us with the water quality data. The authors would like to thank CRP "G. Lippmann" and in particular Luc Ector and Maria Helena Novais for taking SEM pictures of *Mayamaea cahabaensis*, as well as Saul Blanco and Eduardo Morales for their systematic expertise on this species. The authors also thank Peter Christie for the linguistic revision.

This research was partly funded by Fondazione Gorla and Banca CRT within the framework of "Master dei Talenti della Società Civile" and by ARPA Liguria.

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