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DIATOMS AND QUALITY OF WATERCOURSES IN NORTH-CENTRAL SARDINIA

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PERIPHYTIC ALGAE
DIATOMS
BIOLOGICAL QUALITY
DIATOM INDICES
EPI-D
IBD
WATERCOURSES
SARDINIA

ABSTRACT . – Eight watercourses in North-Central Sardinia were studied between 2008 and 2009 to ascertain their environmental quality and to gain insights into the requirements for their proper management. In addition to physicochemical and microbiological parameters, epilithic diatoms were analyzed at 15 stations. Data on the diatom community of each sample were used to calculate the diatom-based eutrophication/pollution index (EPI-D) and the biological diatom index (IBD). In the 21 collected samples, 153 taxa belonging to 36 genera were identified, but the diatom flora also included species that could not be determined with certainty. Most of the observed diatoms are considered cosmopolitan and were common to various sites, with *Cocconeis placentula* var. *pseudolineata* and *Diademsis confervacea* among the most frequent and sometimes the most abundant taxa. *Platessa hustedtii*, which like *Diademsis confervacea* is considered a tropical species, was recorded for the first time in Sardinia. While these species are not considered by the EPI-D proposed for use in Italian watercourses, their integration will improve the usefulness of this index in the assessment of islands of Mediterranean typology. The applicability of the EPI-D and IBD was confirmed with water quality at nearly all the considered stations ranging from mediocre to bad according to both indices. Moreover, these results were supported by physicochemical and microbiological variables and were consistent with the typology of the investigated environments.

INTRODUCTION

Many European countries regularly use benthic diatoms as an indicator of the biological quality of running water. In addition, some countries, in order to comply with the objectives of Water Framework Directive 2000/60/CE, have introduced diatom-based methods into the annual samplings of their hydrographic networks (Rimet *et al.* 2005b). The success of these methods, however, fundamentally depends on extensive knowledge of this algal component. In Italy, the diatom flora of watercourses has been studied in detail in the central Apennine region, where the diatom-based eutrophication/pollution index (EPI-D) was first developed (Dell'Uomo & Tantucci 1996, Grandoni & Dell'Uomo 1996, Dell'Uomo & Grandoni 1997, Dell'Uomo *et al.* 1999, 2007, Torrisi & Dell'Uomo 2001a, b, 2006, Scuri *et al.* 2006, Torrisi *et al.* 2008, 2010). Recently, studies have been carried out in watercourses of different typologies in other regions of northern Italy. Their results have improved current knowledge of diatom communities, verified the applicability of the EPI-D index, and allowed standardization of the method as well as its comparison with other European indices (Battezzore *et al.* 2004a, b, 2005, 2007, Ciutti *et al.* 2004, Bona *et al.* 2005, 2007, Cappelletti *et al.* 2005, 2007, Falasco *et al.* 2005a, b, Griselli *et al.* 2005, Lösch & Alber 2009). Studies in the southern regions of Italy have focused on the watercourses of Calabria and

Lucania and those of the islands of Sicily and Sardinia (Nughes *et al.* 2005, Battezzore & Gastaldi 2007, De Filippis *et al.* 2007, Finocchiaro & Ferlito 2007, Lai *et al.* 2007). Some of these studies have reported the presence of taxa not yet considered by the EPI-D, highlighting the need for deeper knowledge of the distribution and ecology of diatom species before this index can be applied routinely to all Italian monitoring regions. The aims of the present study were to verify the applicability of the EPI-D and of the biological diatom index (IBD), to evaluate the environmental quality of the examined watercourses, and to obtain preliminary insights into their management. The results contribute to a deeper knowledge of diatom flora in Sardinia, the second largest Mediterranean island and an important biodiversity hot-spot at the biogeographic basin level (Médail & Quézel 2000).

MATERIALS AND METHODS

Eight watercourses in four hydrographic basins of North-Central Sardinia were studied to describe their diatom assemblages and to verify the applicability of the EPI-D and IBD (Fig. 1, Table I). In the Mannu of Porto Torres, Padrongianu, and Tirso systems, environmental alterations are caused by urban wastes from Sassari and Olbia and industrial wastes from Ottana. In the Mare Foghe system the effects of intensive agricultural activities along canals that flow into Cabras Lagoon, the

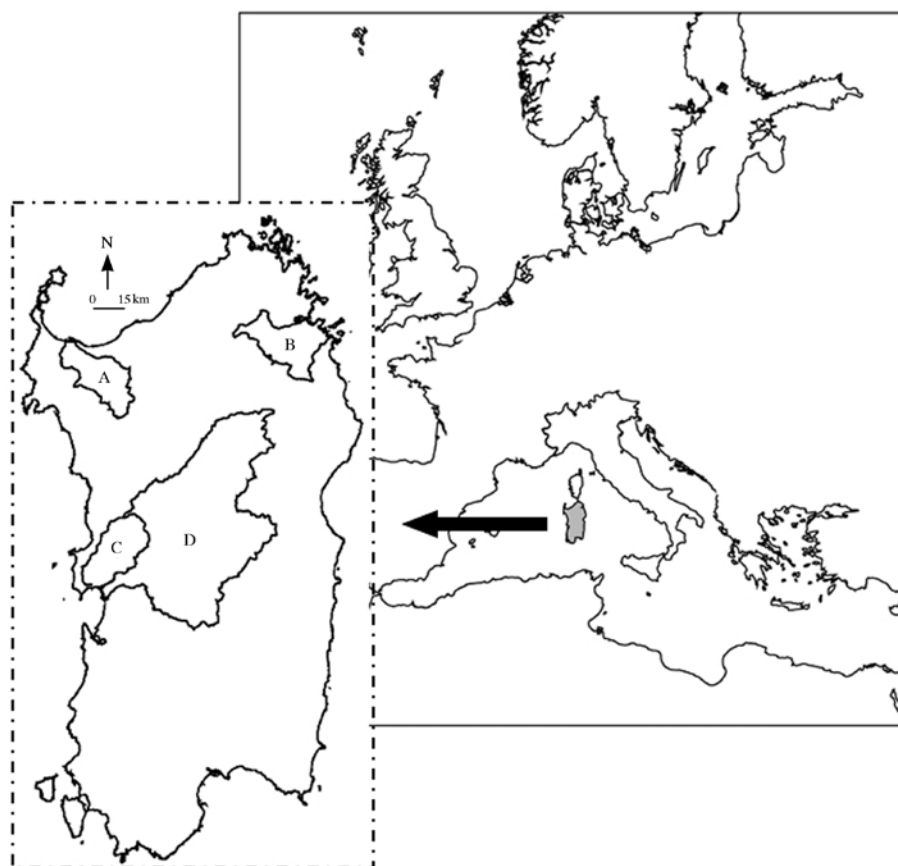


Fig. 1. – Study areas and respective catchments (A = Mannu of Porto Torres; B = Padrongianu; C = Mare Foghe; D = Tirso).

Table I. – Catchments, surface, geology, watercourses, and number of stations. ° = without name; * = Canals.

	Catchments							
	Mannu of Porto Torres		Padrongianu		Mare Foghe		Tirso	
Code	A		B		C		D	
Surface km²	671		450		286		3400	
Prevalent geological substratum	Calcareous		Granitic		Basaltic		Granitic	
Name of watercourses	Tributary of Mascari°	Mascari	Padrongianu	Pauli Gippa*	Tanui*	Iscas*	Mannu*	Tirso
Number of stations	4		3		1		1	
Stations	1A-2A-3A-4A	5A-6A	1B-2B-3B	1C	2C	3C	4C	1D-2D

largest lagoon in Sardinia and one of the most important in the Mediterranean, were assessed. Samplings were carried out at 15 stations, located in the middle or in the final stretches of four natural watercourses and four canals (Table I). Water and epilithic diatoms samples were collected at different times between April 2008 and April 2009. At all stations, the most significant physicochemical and microbiological factors were measured. Temperature, pH, conductivity and dissolved oxygen were analyzed *in situ* using a multiparameter probe (YSI556). Alkalinity, Cl⁻, BOD₅, soluble reactive phosphorus (SRP) and total phosphorus (TP), N-NH₃, N-NO₃, N-NO₂, total nitrogen (TN), reactive silica (RSi), and total suspended solids were determined in the laboratory according to Italian Standards (IRSA 1994). Microbiological analysis included the quantitative evaluation of *Escherichia coli* as well as fecal and total coliform and was

carried out using a membrane filtration method (IRSA 1994). Diatom samples were collected by scraping the substrate, principally rocks and stones, with a small hard brush and always from fast-flowing reaches (Kelly *et al.* 1998, ISPRA 2008). The 21 samples collected were immediately fixed *in situ* with 4 % neutralized formaldehyde. In the Rio Mannu of Porto Torres system, which is influenced by intense anthropogenic pressures, diatoms were collected in two samplings, one in winter and the other in spring, in order to follow the temporal evolution of biological water quality. The winter sampling started in December 2008 and was not completed until March 2009 because of the notable water flow, resulting from the very intense rainfall at three of the six stations. Diatom samples were treated in the laboratory with hydrogen peroxide (H₂O₂) and acetic acid (CH₃COOH) under heat, until complete oxydation of organic substances was

Table II. – Diatom genera identified.

Genus	Taxa	Genus	Taxa
<i>Achnanthes</i>	10	<i>Frustulia</i>	1
<i>Amphipleura</i>	1	<i>Gomphonema</i>	10
<i>Amphora</i>	6	<i>Gyrosigma</i>	1
<i>Anomoeoneis</i>	1	<i>Hantzschia</i>	1
<i>Aulacoseira</i>	1	<i>Melosira</i>	1
<i>Bacillaria</i>	1	<i>Meridion</i>	1
<i>Caloneis</i>	3	<i>Navicula</i>	40
<i>Cyclostephanos</i>	2	<i>Neidium</i>	1
<i>Cyclotella</i>	3	<i>Nitzschia</i>	21
<i>Cymatopleura</i>	1	<i>Opephora</i>	1
<i>Cymbella</i>	6	<i>Pinnularia</i>	4
<i>Cocconeis</i>	5	<i>Platessa</i>	1
<i>Diadesmis</i>	1	<i>Pleurosira</i>	1
<i>Diatoma</i>	1	<i>Rhoicosphenia</i>	1
<i>Diploneis</i>	3	<i>Stephanodiscus</i>	1
<i>Epithemia</i>	1	<i>Surirella</i>	3
<i>Eunotia</i>	3	<i>Synedra</i>	2
<i>Fragilaria</i>	12	<i>Thalassiosira</i>	1
Total genera 36		Total taxa 153	

achieved (Schrader 1973). The cleaned frustules were mounted on permanent slides using Styrax resin (index of refraction 1.59). Taxonomic determination was carried out using an inverted

optical microscope (Zeiss, Axiovert 10) at 1000× magnification and the criteria in published monographs by Bourrelly (1981), Germain (1981), Hustedt (1985), Krammer and Lange-Bertalot (1986, 1988, 1991a, b), Lange-Bertalot *et al.* (2003). Species abundances were evaluated by counting about 400 valves and/or frustules, as indicated by Ciutti *et al.* (2004), in accordance with the procedure used in other European countries. To calculate the EPI-D and IBD indices, the manuals published by Dell'Uomo (2004) and Prygiel & Coste (2000) were used. The EPI-D data were converted to a scale of 1-20 and the results compared with those obtained by application of the IBD.

RESULTS

The diatom flora of watercourses

Among the detected genera, seven belonged to the order Centrales and 29 to Pennales, with the majority of species belonging to the genera *Navicula* (40), *Nitzschia* (21), *Fragilaria* (12), *Achnanthes* (10), and *Gomphonema* (10) (Table II). The total number of taxa for each sample varied, from a minimum of 12 at station 4A (Rio Mannu of Porto Torres) to a maximum of 48 at station 2D (Tirso). Most of the observed diatoms were common to several stations but some taxa were exclusive to only one of them. Generally, the detected species are considered

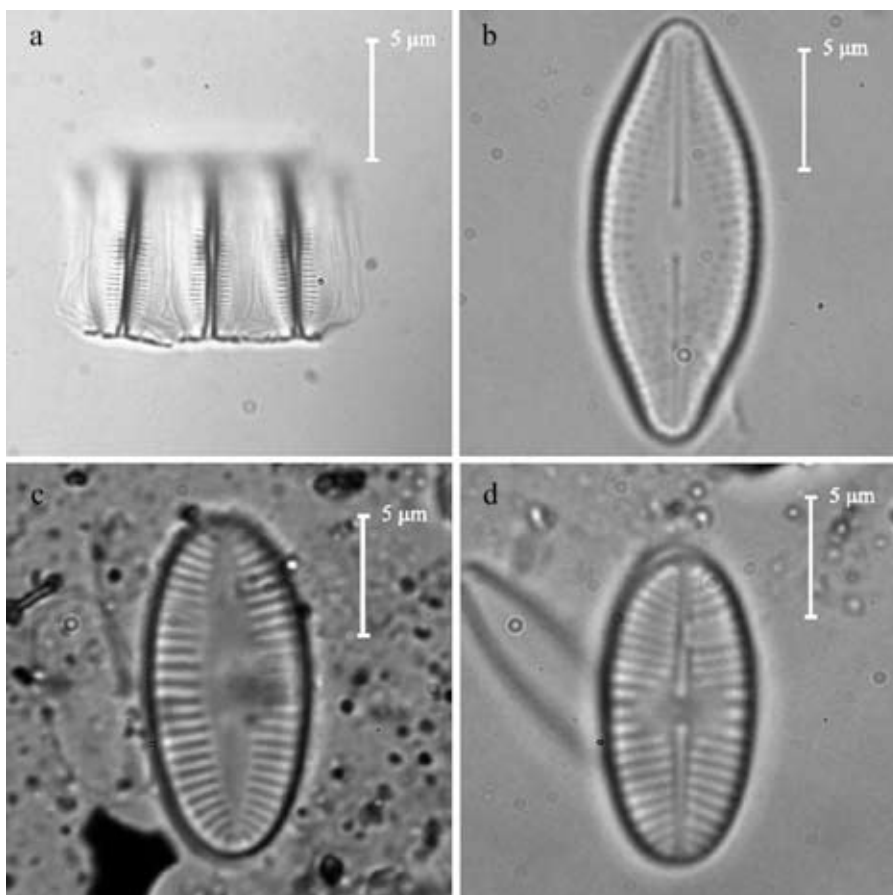


Fig. 2. – Light micrographs, scale bars = 5 µm. **2a**, Colony of *Diadesmis confervacea*, girdle view. **2b**, *Diadesmis confervacea*, valve view. **2c**, *Platessa hustedtii*, view of rapheless valve. **2d**, *Platessa hustedtii*, view of raphe valve.

cosmopolitan and typical of environments of mediocre to bad quality. The taxa most frequently observed and also the most abundant were two, *Cocconeis placentula* var.

pseudolineata Geitler and *Diademsis confervacea* Kützing, that are not part of the list compiled by the EPI-D.

Diademsis confervacea (Fig. 2a, b), an invasive species (Coste & Ector 2000) already observed in the north of Sardinia island (Lai *et al.* 2007), was present also in the Tirso River and in the canals of the Mare Foghe system (central Sardinia). This species appeared in the spring and summer samples, when the water temperature was in the range of 11.9-22.6 °C. The detection of *Platessa hustedtii* (Krasske) Lange-Bertalot *in* Krammer & Lange-Bertalot (Fig. 2c, d), also a tropical taxon, was the first in Sardinia. A few individuals of this species were observed in both winter and spring at two stations upstream of the Rio Mascari, one of the most important tributaries of the Rio Mannu in Porto Torres. Diatom assemblages in the canals of the Mare Foghe system consisted of species such as *Pleurosira laevis* (Ehrenberg) Compère, *Thalassiosira weissflogii* (Grunow) Fryxell & Hasle, *Achnanthes inflata* (Kützing) Grunow, *A. brevipes* C. Agardh, *Cymatopleura solea* var. *apiculata* (W. Smith) Ralfs, and *Neidium dubium* (Ehrenberg) Cleve. Although never abundant at these sites, these species were not detected at any of the stations of the other watercourses considered.

The quality of the watercourses

The physicochemical and microbiological variables are reported in Table III. The results obtained by EPI-D and IBD indices in the sampling sites are provided in Table IV and in Figs 3 and 4. The values of two diatom indices were interpreted according to Table V, where the five classes of water quality on a scale of 1-20 are reported. In the system of Rio Mannu of Porto Torres, all the investigated stations were affected by contamination, as reflected in the significant values of algal nutrients and of the microbio-

Table III. – Results of physical, chemical, and microbiological variables analyzed. Results of the Mannu of Porto Torres (1A-6A) system are the mean values of different samplings.

Parameters	Stations of watercourses														
	1A	2A	3A	4A	5A	6A	1B	2B	3B	1C	2C	3C	4C	1D	2D
Temperature	15.6	14.0	15.4	15.4	11.2	13.2	18.0	20.3	19.2	21.9	22.4	22.6	22.4	14.1	15.5
Conductivity	1016	1121	1098	954	1045	1157	1008	1345	595	756	832	1227	818	480	638
pH	7.69	8.07	7.99	7.83	8.11	8.14	7.75	7.18	7.45	7.44	7.68	8.31	8.03	7.69	7.74
Alcalinity	4.27	2.80	4.14	3.01	4.54	4.71	1.37	1.80	1.53	4.22	4.20	4.78	2.06	1.28	3.00
Dissolved oxygen	8.3	9.4	8.9	9.0	9.5	8.3	9.04	8.23	7.82	4.6	6.4	11.8	10.0	9.0	8.2
Oxygen saturation	84	91	88	90	87	78	95	100	85	52	73	137	115	88	82
BOD ₅	2.1	2.2	2.1	7.9	2.2	3.9	2.0	5.7	1.1	1.2	3.1	5.4	3.4	3.1	3.3
Cl ⁻	117	144	128	125	125	129	60	142	81	99	96	177	113	-	-
SRP	0.106	0.228	0.141	0.581	0.234	0.508	0.020	0.928	0.270	0.114	0.146	1.199	0.392	0.117	0.164
TP	0.143	0.277	0.247	0.771	0.392	0.680	0.050	1.282	0.331	0.187	0.301	1.462	0.484	0.282	0.348
N-NH ₃	0.060	0.034	0.074	0.878	0.078	0.328	0.046	0.067	0.051	0.042	0.364	0.056	0.020	0.086	0.105
N-NO ₂	0.036	0.040	0.064	0.148	0.044	0.172	0.008	0.021	0.027	0.010	0.160	0.207	0.005	0.055	0.044
N-NO ₃	6.324	6.405	6.501	3.101	2.113	5.721	0.460	3.320	1.356	0.342	0.366	3.810	2.086	0.492	0.629
TN	9.641	7.967	8.838	6.037	4.652	5.012	1.197	8.151	2.068	1.287	2.068	5.520	3.046	1.641	1.907
RSi	3.62	3.47	2.30	3.30	6.91	4.55	9.24	7.76	8.91	6.04	8.03	7.66	11.02	7.41	7.83
Suspended solids	3.5	7.9	6.7	22.7	28.6	11.0	1.8	32.2	4.2	8.5	27.0	13.0	15.5	-	-
<i>Escherichia coli</i>	17667	7333	28800	20033	1453	31500	650	10600	610	92	1700	1000	700	8000	1000
Fecal coliforms	17867	10489	23934	44014	9100	48350	580	14800	770	88	6700	8100	1100	12000	1000
Total coliforms	55467	26934	54800	73000	28534	118500	1460	40000	1760	600	26000	30700	6800	67000	24000

Table IV. – Results of EPI-D and IBD at the sampling stations. Results of the Mannu of Porto Torres system are the mean values of different samplings.

Catchments	Watercourses	Stations	Date of sampling	EPI-D	IBD
Mannu of Porto Torres	Tributary of Rio Mascari	1A	02/12/2008 - 07/04/2009	6.91	6.9
		2A	02/12/2008 - 07/04/2009	4.94	7.2
		3A	02/12/2008 - 07/04/2009	8.32	9.8
		4A	10/03/2009 - 07/04/2009	9.46	11.1
	Rio Mascari	5A	10/03/2009 - 07/04/2009	10.76	13.6
	6A	10/03/2009 - 07/04/2009	6.56	9.4	
Padrongianu	Rio Padrongianu	1B	06/06/2008	13.54	15.5
		2B	06/06/2008	10.74	13.8
		3B	06/06/2008	9.27	8.4
Mare Foghe	Rio Pauli Gippa	1C	28/08/2008	10.00	11.8
	Rio Tanui	2C	28/08/2008	9.36	8.6
	Rio Iscas	3C	28/08/2008	7.65	5.6
	Rio Mannu	4C	28/08/2008	8.27	6.5
Tirso	Tirso	1D	23/04/2009	10.83	12.2
		2D	23/04/2009	10.64	13.6

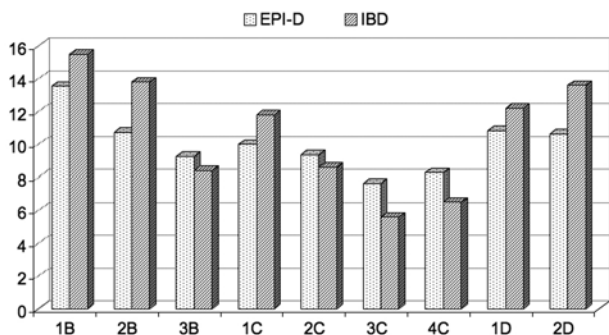


Fig. 3. – EPI-D and IBD results at the different stations of the three catchments: Padrongianu (B), Mare Foghe (C), Tirso (D).

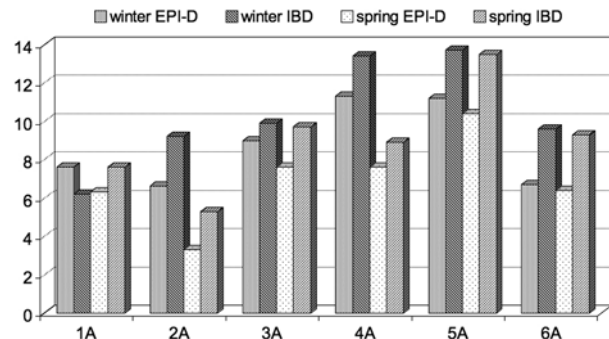


Fig. 4. – Seasonal variations (winter and spring) of EPI-D and IBD in the Rio Mannu of Porto Torres catchment.

logical parameters. The seasonal variations in the biological quality of the Rio Mannu of Porto Torres system are reported in Fig. 4. For the Padrongianu River, the diatom indices confirmed a decline in water quality from upstream to downstream. This trend was also highlighted by the physicochemical and microbiological factors. The water quality of the most upstream station was good, according to both the EPI-D and the IBD. At station 2, influenced by wastewater discharge of the town of Olbia, water quality as determined by the EPI-D and IBD was, respectively, mediocre and good. Station 3, the most downstream station, was the most polluted, with a mediocre to bad quality according to the EPI-D and a bad

quality according to the IBD. The dominant species was *Nitzschia inconspicua* Grunow, a good indicator of mesotrophic environments (Dell'Uomo 2004).

The catchments of Rio Mare Foghe, Rio Iscas and Rio Mannu were the worst in terms of biological quality. All were characterized by the highest recorded values of BOD₅, N-NO₂, SRP, and TP, while the microbiological variables likewise indicated contamination with organic compounds. For all three watercourses, the EPI-D and IBD unanimously showed the bad quality of these waters. The dominant species were *Nitzschia inconspicua* Grunow, *Cyclotella meneghiniana* Kützing and *Bacillaria paxillifera* (O.F. Müller) Hendey. In particular, the lat-

Table V. – Ranges of EPI-D and IBD values, classes and water quality assessment.

EPI-D ₍₁₋₂₀₎	IBD	Quality	Class	Color
20 > EPI-D > 15	IBD ≥ 17.0	Excellent	I	Blue
15 > EPI-D > 12	17.0 > IBD ≥ 13.0	Good	II	Green
12 > EPI-D > 9	13.0 > IBD ≥ 9.0	Mediocre	III	Yellow
9 > EPI-D > 6	9.0 > IBD ≥ 5.0	Bad	IV	Orange
6 > EPI-D > 1	IBD < 5.0	Very bad	V	Red

ter two taxa are considered excellent indicators of eutrophic environments (Dell'Uomo 2004). The EPI-D and IBD also pointed out the mediocre-bad and bad quality, respectively, of the Rio Tanui, in line with its higher values of nutrients and microbiological variables compared to Rio Pauli Gippa, its tributary. In the latter, both indices indicated a mediocre quality.

Finally, for the two stations at Tirso River, the quality was mediocre although both indices showed a slight improvement of the biological quality at station 2, downstream from the industrial area of Ottana. At this station, algal nutrient levels were higher and microbiological variables lower than at station 1.

DISCUSSION

Our study contributes to current knowledge of the diatom flora in Sardinia watercourses and, at a more general level, in the Mediterranean area. With the addition of our results, the previous list of taxa (Lai *et al.* 2007) has now been extended to 207, by the inclusion of 50 different taxa observed in this study. The analyses of epilithic diatoms carried out in this work revealed the presence of 153 taxa belonging to 36 genera. The detection of *Platessa hustedtii* and *Diademsis confervacea*, two exotic species, is of particular interest. It is also worth noting that *Didymosphenia geminata* (Lyngbye) M. Schmidt, another exotic taxon not yet observed in Sardinia, has been reported in watercourses of several northern Italian regions, and often with massive growth (Beltrami *et al.* 2008, Blanco & Ector 2009). While these three species were recently included in the floristic list of Italian diatoms based on studies carried out to date by Mancini and Sollazzo (2009), *Platessa hustedtii* had never been found in Sardinia before this study. Krammer and Lange-Bertalot (1991) described this species in tropical areas but found it to be widely distributed albeit uncommon in watercourses. Recently, it was observed in Alpine springs of northwestern Italy (Battezzore *et al.* 2004) and in Spanish Mediterranean watercourses (Gomà 2004). The indicator values in Van Dam *et al.* (1994) define *Platessa hustedtii* as oligosaprobous and oligotraphentic. This may explain the low number of individuals of this species found at stations 1A and 2A, where the levels of N-NO₃ and TN were significant. *Diademsis confervacea* is characteristic of

tropical and subtropical areas (Coste & Ector 2000) but nowadays is also found in many temperate regions and can thus be considered cosmopolitan. It shows a predilection for waters rich in organic matter and as a thermophile it may be a good indicator of the warming of continental waters in temperate regions (Coste & Ricard 1990, Coste & Ector 2000). Also according to the indicator values of Van Dam *et al.* (1994), *Diademsis confervacea* is

an α -mesosaprobic and eutraphentic taxon. This species was detected at high frequency in our samples. In Europe, it has been recorded in Great Britain, Germany, Hungary, Slovakia, and France (Coste & Ector 2000). In Italy, its first occurrence was in Trasimeno Lake (Granetti 1984). More recently, it has been observed in Lazio (Congestri *et al.* 2006, Della Bella *et al.* 2006), in Sicily (Mannino 2007), and in some fast-flowing rivers in northern Sardinia (Lai *et al.* 2007).

New studies will better define the presence and geographical distribution of the species reported in this work. A further objective is the enlargement of the list of taxa currently considered by the EPI-D, adding also species that are relevant in the Sardinian context. This study will contribute to a better application of the EPI-D index in this Italian region and, in general, in all of the Mediterranean islands. Indeed, most of the diatoms observed in this study are common to the flora of Sicilian and Corsican watercourses (Rimet *et al.* 2005a, Finocchiaro & Ferlito 2007). Thus, creation of a preliminary checklist of the species observed in the major Mediterranean islands should be particularly useful for future studies. The diatom list reported herein should also have included a few other species (not more than ten), represented by very small numbers of individuals and not yet identified with certainty. The identification of these taxa is of relevance to biodiversity because they form part of the periphytic communities of the watercourses investigated, even if their frequency of detection was low and they were not taken into account in the evaluation of biological water quality. Nonetheless, this investigation confirms the utility of diatom indices also in Sardinian rivers for the evaluation of the quality of running waters and for water management in this region.

Compared to traditional parameters, the EPI-D and IBD provide a more complete and coherent evaluation of a particular environment as well the impacts upon it. The results obtained were in agreement with physicochemical and microbiological parameters measured. Moreover, both indices, despite slight differences in their values, showed good applicability in all lotic contexts investigated although the EPI-D values seemed to be more reliable. According to the diatom indices, stations 1, 2 and 6 of Rio Mascari were those with the worst environmental quality. The former two stations are located upstream whereas the latter receives urban and industrial wastes. Both indices

indicated a decline in water quality at these stations during spring, coinciding with an important reduction of flow. Moreover, in the same period, a reduction in the number of taxa was observed at stations 1, 3, and 6. This probable simplification of the diatom assemblages corresponded with a decline in the biological quality of the water. Overall, among the 15 stations examined, the systems of Rio Mannu of Porto Torres and of Rio Mare Foghe had the greatest level of water contamination. The pollution was mostly of organic type and was largely due to the agricultural and livestock activities carried out in the basins as well as to civil wastes not adequately depurated.

Previous studies of Sardinian watercourses have focused on the impact of the high anthropogenic pressure, whereas little is known about the composition of diatom assemblages in the island's waters. Future studies will need to consider a larger number of stations along the island's watercourses in order to understand and assess the dynamics of diatom assemblages throughout the respective basins, including diatom presence in pristine conditions or in areas with very light human impact.

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