

Mediterranean river biomonitoring in Central Italy: Diatom biodiversity and characterization of communities

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ABSTRACT

In compliance with the European and Italian regulations, the Environmental Protection Agency of Umbria Region (ARPA Umbria) defined specific river monitoring programs and networks based on river type definition, human pressures and risk analysis. The Umbria Region lies in Central Italy and it can be split into three hydro-ecoregions belonging to the Mediterranean area. Data on diatom community composition were collected in five different Mediterranean macrotypes (M1-M5) throughout the diatom-based river monitoring network that is composed by 52 sampling stations in 36 watercourses. The main aim of this study was to characterise and to analyse diatom diversity across the different regional river macrotypes. Specifically, we investigated if: i) there were differences in species diversity (species richness and Shannon Index) among macrotypes; ii) there was difference in three water quality indexes (ICMi, IPS, and TI) among sites; and iii) there was a relationship between the observed ICMi, IPS and TI value and the diatom diversity. Two-hundred diatom species and varieties were identified, and the number of species *per* sampling station ranged from a minimum of 10 to a maximum of 38 species. The most frequent and abundant species were *Amphora pediculus*, *Achnanthydium minutissimum*, *Navicula cryptotenella*, *Nitzschia dissipata*, and each macrotype showed some peculiar species. The ecological status evaluation based on Intercalibration Common Metric Index (ICMi) classified 69% of the water bodies in high or good class. Significant differences in diversity and ICMi value among stream macrotypes were found, with M4 (small and medium mountain) and M5 (small, lowland, temporary) typologies showing the lowest species richness, and with M5 showing the lowest Shannon Index. Conversely, M2 (small and medium lowland) and M5 showed the highest ICMi value. Lastly, significant correlations between Shannon Index and the ICMi, IPS and TI indexes were found.

Key word: Benthic algae; indicator species; Bacillariophyceae; diversity metrics; Water Framework Directive 2000/60/EC.

INTRODUCTION

Ecological status assessment based on benthic algal communities is one of the requirements of the European Water Framework Directive 2000/60/EC (WFD; European Commission, 2000), implemented in Italy with Legislative Decree 152/2006 (Italian Regulation, 2006). Diatoms are the algal group most widely used as indicators of river quality. They are the main component of phytobenthos of river water bodies and have biological and ecological characteristics that make them a good indicator for the characterization of water quality (Kelly *et al.*, 1998). They have a cosmopolitan distribution and high sensitivity to water physico-chemical characteristics and environmental conditions (for a review see Prygiel *et al.*, 1999).

In Italy, studies on diatom communities have been mainly focused on Alpine streams (Cantonati and Pipp, 2000; Battagazzore *et al.*, 2004; Bona *et al.*, 2007; Rott *et al.*, 2006; Zorza and Honsell, 2008; Beltrami *et al.*, 2009; Falasco *et al.*, 2012) and some main watercourses in Southern Italy (Battagazzore *et al.*, 2003; Finocchiaro *et al.*, 2011). Existing data on benthic diatom assemblages

of Mediterranean rivers of Central Italy are scattered, covering some central Apennine streams (Dell'Uomo, 1999; Scuri *et al.*, 2006; Torrisi *et al.*, 2008, 2010), some volcanic-siliceous streams (Della Bella *et al.*, 2012), and the main river water body, the Tiber River (Cappelletti *et al.*, 2005; Ciutti *et al.*, 2007). A few studies on river diatom communities have been carried out on main streams of Umbria Region, Central Italy (Mancini *et al.*, 2008).

In compliance with the European and national regulations, the Environmental Protection Agency of Umbria Region (ARPA Umbria) defined specific monitoring programs and networks based on river type definition, human pressures, and risk analysis (ARPA Umbria, 2008). ARPA Umbria accomplished the first biomonitoring of river diatoms between 2009 and 2012 and calculated the Intercalibration Common Metric Index (ICMi), developed in Italy for the assessment of benthic algal ecological status (Mancini and Sollazzo, 2009). The main purposes of this study were to analyse diatom diversity of regional river types and to identify the characterising diatoms of different river types in Umbria (Mediterranean river typologies defined by altitude, river basin area, and river hydrology).

This study represented a contribution to diatom-based river quality assessment following the WFD in Italy and to evaluation of differences in diversity of diatom communities in Mediterranean river types. In detail, we investigated if: i) there were differences in species diversity (species richness and Shannon Index) among river types, ii) there was difference in ICMi, IPS, and TI value among sites, and iii) there was a relationship between the observed ICMi, IPS and TI value and the diatom diversity.

METHODS

Study area

The Umbria Region is located in the Mediterranean area of Central Italy and it is included in three hydroecoregions: Tuscan Hills, Apennines Centre and Italian Volcanics (Wasson *et al.*, 2006; Italian Regulation, 2008; Fig. 1). The morphology varies from lowlands in the central area to highlands in the eastern part and it is mainly characterized by a temperate climate with hot, dry summers and cool, wet winters. Almost the entire area belongs to the Tiber River basin, in the hydrographic district of Central Italy. The Tiber River begins in the northern part of the Apennine Mountains and crosses the Umbria Region from North to South, collecting waters from several tributaries and with a length of 400 km before draining into the Tyrrhenian Sea in the Lazio Region. Tributaries from the eastern part of the region show steady flows due to carbonate sources from the

Apennines and a good ecological quality. On the contrary, all over the central and western area the rivers show a high flow variability and significant impacts on water quality, due to human activities.

According to national legislation (DM 131/08; Italian Regulation, 2008), 135 river waterbodies were included in the monitoring network and assigned to 19 types, which were grouped in five main Mediterranean macrotypes (M1-M5) defined in the European Intercalibration exercise (European Commission, 2008; Tab.1). Most of the regional water bodies belongs to macrotypes M5 (39%) or M1 (34%) and are characterized by small basins and low or temporary discharges, while there are few big rivers (8%) with significant flows in floodplain areas (macrotype M3). Several rivers, altered by human activities such as land drainage, dredging, flood protection, water abstraction, building of dams to create reservoirs, have been designated as “artificial” (1; AWB) or “heavily modified” (20; HMWB) water bodies (Fig. 2).

In order to define monitoring networks and programs, a risk analysis and an anthropic pressure assessment have been carried out (ARPA Umbria, 2008). Data were collected using the diatom-based river monitoring network that is composed by 52 sampling stations distributed in 36 watercourses. Data were collected between 2009 and 2012. Almost 100 diatom samples were collected and analysed. Data gathered allowed to calculate for 48 out of 52 sites the Intercalibration Common Metric Index (ICMi), developed for the assessment of benthic algal ecological status follow-

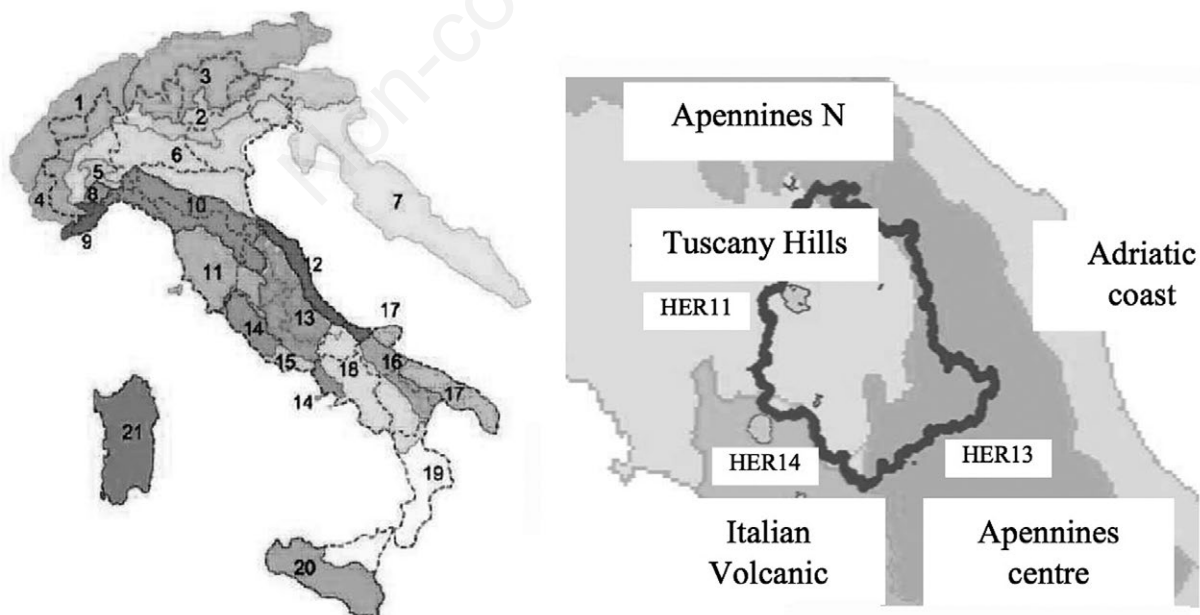


Fig. 1. Hydro-ecoregions (HER; WFD 2000/60/CEE) defined by CEMAGREF for Italy (Wasson *et al.*, 2006), then verified at the local/regional scale and identified according to Basin Authority, Regions, Regional Environmental Agency and Italian Ministry of Environment (DM 131/08; Italian Regulation, 2008). HER11, Tuscany Hills; HER13, Apennine Centre; HER14, Italian volcanic regions.

ing the formula: $ICMi \text{ index} = (RQE_IPS + RQE_TI) / 2$ (Mancini and Sollazzo, 2009). The ICMi is based on two sub-indices: the IPS index (CEMAGREF, 1982), which mainly assesses the sensitivity of species to organic pollution and the TI Index (Rott *et al.*, 1999), which is based on the species sensitivity to trophic pollution. For the diatom-based ecological status assessment, the value of the two sub-indices have to be expressed as Ecological Quality Ratio (RQE_IPS and RQE_TI) with the respective refer-

ence values for each river macrotype following the DM 260/2010 (Italian Regulation, 2010). Boundaries between quality classes adopted for river macrotypes M1-M2-M3-M4 are: High/Good = 0.80, Good/Moderate = 0.61, Moderate/Poor = 0.51, Poor/Bad = 0.25; and for M5 are: High/Good = 0.88, Good/Moderate = 0.65, Moderate/Poor = 0.55, Poor/Bad = 0.26 (reported values are the lowest value of higher class). We evaluated the ecological status on the basis ICMi for 38 out of 48 sites because ten sites

Tab. 1. River Mediterranean macrotypes identified in the Region Umbria.

River macrotype	River macrotype description	Number of water bodies	Sampling sites
M1	Small mid-altitude streams (200-800 m asl)	45	20
M2	Small and medium lowland streams (<400 m asl)	23	12
M3	Large lowland rivers	11	10
M4	Small and medium mountain streams (400-1500 m asl)	2	2
M5	Small, lowland, temporary (<300 m asl)	54	8

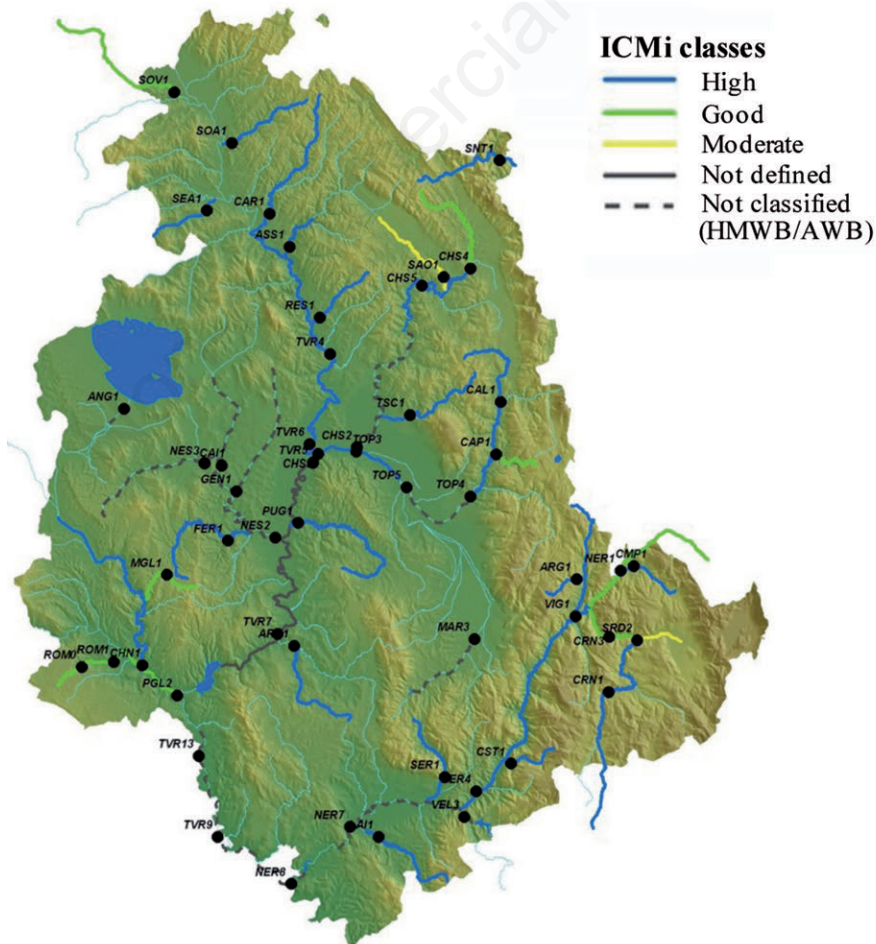


Fig. 2. Diatom-based river classification using ICMi Index.

were defined as heavily modified/artificial water bodies (HMWB/AWB).

Sampling activities, data analysis and statistics

Diatom sampling, sample treatment and laboratory work were carried out according to the European recommendations (European Committee for Standardization, 2003, 2004) and national guidelines (APAT, 2007). In order to sample epilithic forms, the upper surface of five stones was brushed with a toothbrush in each sampling site. Diatom samples were immediately placed in an ice bag and carried to the laboratory. In order to identify the diatom frustules, the diatom valves were cleaned using hydrogen peroxide to eliminate organic matter and with hydrochloric acid to dissolve calcium carbonate. Clean diatom frustules were mounted in a synthetic resin with high-refraction index (Naphrax[®]). Successively in each sample up to 400 valves were counted and classified at the species or the variety level using a light microscope with 1000x magnification. Morphometric measurements were made with the aid of image analysis software (CellB, Imaging Software for Life Sciences Microscopy[®] OLYMPUS Soft Imaging Solutions GmbH, Münster, Germany). The main references for diatom taxonomy were Krammer and Lange-Bertalot (1986, 1988, 1991a, 1991b, 2000), Krammer (2000), Lange-Bertalot (2001), and Hofmann *et al.* (2011).

In order to analyse diatom diversity and identify the characteristic species of different river macrotypes, we performed two types of statistical analyses. To define the characterizing species of river macrotypes, we applied the Indicator Species Analysis (ISA, Dufrene and Legendre, 1997). This analysis establishes indicator values for different species combining the information on relative abundances of species in a particular group of samples with the relative frequency of the species occurring in the group.

Successively, in order to test if there were differences in species diversity among macrotypes and hydro-geographic regions (HER), we used a GLM procedures including the diatom species richness or the Shannon Index as the response variables (assuming a Poisson distribution for species richness and a Gaussian for Shannon) and using in turn the macrotype (5 categories) and HER (2 categories) as independent variable. The diatom species richness and Shannon Index were calculated at site level. Successively, six separate GLMs were performed in order to test the presence of a significant difference among macrotypes and HERs for the three indexes ICMi, TI and IPS. Lastly, GLM was used in order to test if there are any significant relationship between ICMi, IPS and TI and diatom diversity (the observed species richness and Shannon at the site level). These analyses were also replicated excluding the 10 sites not classified because defined as heavily modified/artificial water bodies (HMWB/AWB). Significance of all GLMs was tested against a null model (*i.e.*, no significant relationships with factors) by chi-squared test. All the statistical analyses were performed using the R statistical environment ver. 3.30 (R Core Team, 2015).

RESULTS AND DISCUSSION

A total of 200 diatom species and varieties were identified (Supplementary Tab. 1) in 96 collected samples (almost 41,000 counted frustules). The number of species *per* sample varied from 10 to 38 with a mean of 22 species. The most frequent and abundant species were *Amphora pediculus* (Kützing) Grunow, *Achnantheidium minutissimum* (Kützing) Czarnecki, *Navicula cryptotenella* Lange-Bertalot, *Nitzschia dissipata* (Kützing) Grunow ssp. *dissipata* (Tab. 2).

A. pediculus and *A. minutissimum* are cosmopolitan

Tab. 2. List of most frequent and abundant species. Only species found in more than 2/3 of sampling sites are listed.

Species	Sites	Samples	Mean relative abundance (%)	Median relative abundance (%)	Max relative abundance (%)
<i>Amphora pediculus</i> (Kützing) Grunow	47	91	10.52	6.00	55.67
<i>Achnantheidium minutissimum</i> Kützing	47	87	20.09	10.27	79.12
<i>Navicula cryptotenella</i> Lange-Bertalot	45	79	4.53	2.10	23.90
<i>Nitzschia dissipata</i> (Kützing) Grunow	45	72	2.58	0.67	25.24
<i>Cocconeis placentula</i> Ehrenberg	42	64	8.84	0.85	86.82
<i>Navicula tripunctata</i> (Müller) Bory	39	60	2.27	0.25	22.76
<i>Gomphonema parvulum</i> (Kützing) Kützing	37	57	0.98	0.25	10.82
<i>Gomphonema olivaceum</i> (Hornemann) Brébisson	40	54	1.65	0.25	19.01
<i>Rhoicosphenia abbreviata</i> (Ag.) Lange-Bertalot	34	52	2.40	0.23	69.51
<i>Nitzschia palea</i> (Kützing) W. Smith	35	47	2.14	0.00	21.13

species, very common and abundant in the Italian rivers and streams, often dominant in diatom communities and considered pioneer species (Falasco and Bona, 2013). *A. pediculus* can tolerate high concentration of nutrients in water with low organic load, while *A. minutissimum* has a wide ecological range as it can tolerate large ranges of organic and inorganic pollution (Falasco *et al.*, 2013). The high abundance and frequency of these both pioneer species were probably due to the high flow variability of rivers for the most part of the region in analysis. *N. cryptotenella* is also a cosmopolitan and mobile species. Like *A. pediculus*, this species is quite sensitive to organic pollution, and it can be found in oligotrophic to eutrophic waters (Falasco *et al.*, 2013). *N. dissipata*, also found in several sampling sites though with less abundance, may become a dominant species in diatom community in site with medium - high content of nutrients such as nitrate and total phosphorus ($P_{\text{tot}} > 46.5 \mu\text{g L}^{-1}$; Falasco *et al.*, 2013; Hofmann, 1994). Among the most common and abundant species there were also *Gomphonema olivaceum* (Hornemann) Brébisson, and *Gomphonema parvulum* (Kützing) Kützing. *G. olivaceum* is sensitive to organic pollution but can tolerate a moderate trophic load. Hence, it is generally abundant in the limestone streams with high conductivity (Falasco *et al.*, 2013). *G. parvulum*, belonging to a complex of species with a large ecological value and a high tolerance to trophic and organic pollution, it is also widespread in sites affected by anthropogenic pressure due to urbanization and agriculture practices (Della Bella *et al.*, 2007; Falasco *et al.*, 2013).

Among the species found in more than half of the monitoring sites was *Rhoicosphenia abbreviata* (Agardh) Lange Bertalot, an epiphytic species which can be often found on aquatic macrophytes in brackish water and/or characterized by limestone substrates. It is characterized by a moderate tolerance to eutrophication (Della Bella *et al.*, 2007). Both *N. dissipata* and *R. abbreviata* are characteristic species of sites with a surrounding land use of the catchment area devoted to agriculture that reflected in high values of nutrients in waters, as shown by studies carried out in other Italian regions (Bona *et al.*, 2007; Della Bella *et al.*, 2012). During the study, we also identified *Reimeria uniseriata* Sala Guerrero & Ferrario, a diatom species that is considered an alien species in Italy (Falasco *et al.*, 2013), and *Diadesmis confervacea* Kützing var. *confervacea*, a taxon considered non-native in Europe coming from tropical or subtropical areas (Coste and Ector, 2000). Both these species were found with low abundances (<5.5%) and in only four samples belonging to M1, M2 and M3 river typologies.

The Indicator Species Analysis (ISA) suggested that in each river type some typical species occur (Tab. 3). For example, temporary rivers (M5) are characterized by *A. minutissimum*, which is a pioneer and mobile species, ca-

pable of a fast river substratum recolonization after repeated annual dry phases. Plain large rivers (M3), instead, are characterized by slow water flow, which allows the development of planktonic species that can be also found in benthic communities, like *Cyclotella meneghiniana* Kützing.

According to the GLM analyses we found significant difference among macrotypes in terms of species richness (df=4, deviance=16.271, P=0.003; Fig. 3) and Shannon Index (df=4, deviance=2.523, P=0.019; Fig. 3). When we evaluated how species richness and Shannon Index were distributed across macrotypes (Fig. 3), we found that M4 and M5 showed a significant lower species richness. M5 showed also a significant lower value of the Shannon indexes (Tab. 4). By contrast, when we focussed on HERs (Fig. 3), we found that only the species richness was significant different between HER 11 and HER 13 (df=1, deviance=5.1931, P=0.023) with the last showing a significant lower estimate of species richness values (Tab. 4).

Tab. 3. Characteristic species, defined by Indicator Species Analysis, for the five Mediterranean river macrotypes in analysis.

River macrotype	Species
M1 - Small mid-altitude streams	<i>Achnanthydium pyrenaicum</i> (Hustedt) Kobayasi <i>Amphora inariensis</i> Krammer <i>Encyonema silesiacum</i> (Bleisch in Rabh.) D.G. Mann
M2 - Small and medium lowland streams	<i>Cymatopleura solea</i> (Brébisson) W. Smith <i>Gomphonema parvulum</i> (Kützing) Kützing <i>Navicula tripunctata</i> (Müller) Bory
M3 - Large lowland rivers	<i>Cyclotella meneghiniana</i> Kützing <i>Navicula capitatoradiata</i> Germain <i>Navicula cincta</i> (Ehrenberg) Ralfs <i>Navicula cryptocephala</i> Kützing <i>Navicula cryptotenella</i> Lange-Bertalot <i>Nitzschia frustulum</i> (Kützing) Grunow
M4 - Small and medium mountain streams	<i>Denticula tenuis</i> Kützing <i>Encyonema minutum</i> (Hilse in Rabh.) D.G. Mann <i>Nitzschia fonticola</i> Grunow <i>Nitzschia palea</i> (Kützing) W. Smith
M5 - Small, lowland, temporary	<i>Achnanthydium minutissimum</i> (Kützing) Czarnecki <i>Encyonopsis microcephala</i> (Grunow) Krammer <i>Fragilaria recapitellata</i> Lange-Bertalot & Metzeltin <i>Gomphonema tergestinum</i> Fricke

Tab. 4. Estimated richness and Shannon index coefficients. GLM results were reported for the three significant models. Estimates and their standard errors, t or z test values and associate significance for each level of the fixed factors were reported.

Macrotypes richness estimates				
Coeff.	Estimate	Std. Error	z value	Pr(> z)
(Intercept)	3.129	0.033	94.596	<0.001
M2	0.017	0.054	0.323	0.747
M3	0.080	0.067	1.196	0.232
M4	-0.281	0.125	-2.252	0.024
M5	-0.168	0.066	-2.556	0.011
Macrotypes Shannon index estimates				
Coeff.	Estimate	Std. Error	t value	Pr(> z)
(Intercept)	2.008	0.073	27.460	<0.001
M2	0.032	0.119	0.268	0.79
M3	0.246	0.152	1.614	0.11
M4	-0.271	0.243	-1.117	0.28
M5	-0.310	0.137	-2.266	0.026
Hydro-ecological regions richness estimates				
Coeff.	Estimate	Std. Error	z value	Pr(> z)
(Intercept)	3.143	0.026	122.888	<0.001
HER 13	-0.110	0.049	-2.261	0.024

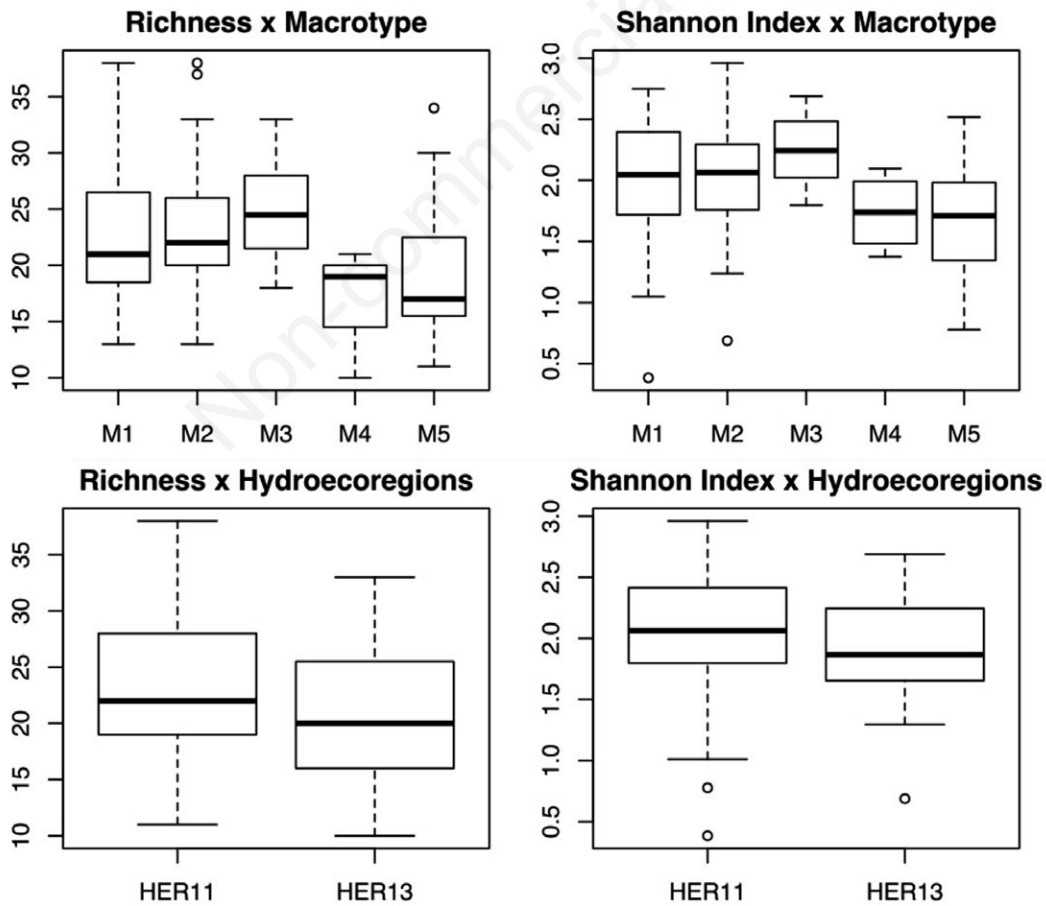


Fig. 3. Box plots of species richness and Shannon Index (on Y axis) among river macrotypes and among hydroecoregions (respectively, M and HER on X axis). The inner line is the median, the box margins are the 25th and 75th percentile, bars extend to 5th and 95th percentile.

Intercalibration Common Metric Index (ICMi) classified 69% of water bodies in high or good class. Forty-two species (about 20% of the total) were not included in the list of taxa for the calculation of ICMi Index, because these diatom species were not included in the TI Index (Rott *et al.*, 1999), as their sensitivity (TW) and reliability (G) values are still unknown.

We found a significant ICMi ($df=4$, deviance=0.67824, $P<0.001$), IPS ($df=4$, deviance=55.867, $P<0.001$) and TI ($df=4$, deviance=3.8236, $P<0.001$) variation among macrotypes (Fig. 4; Tab. 5). Particularly, the highest ICMi was found in M2 and M5, IPS was found to be significant lower in M2, M3 and M4 while M3 and M5 showed the lowest TI (Tab. 5). When we in-

vestigated variation between HERs we found that only the TI was significant different ($df=1$, deviance=1.9075, $P=0.008$) with HER13 showing a significant lower value.

Finally, we did not find any significant correlation between species richness, Shannon Index and the three indexes when we took into account the total of 48 sites. However, when we limited the analysis to the 38 sites for which was possible to evaluate the ecological status on the basis of ICMi, we found that only the Shannon Index showed a significant negative correlation with the ICMi Index and IPS, while TI showed a significant positive correlation with both species richness and Shannon Index (Fig. 5).

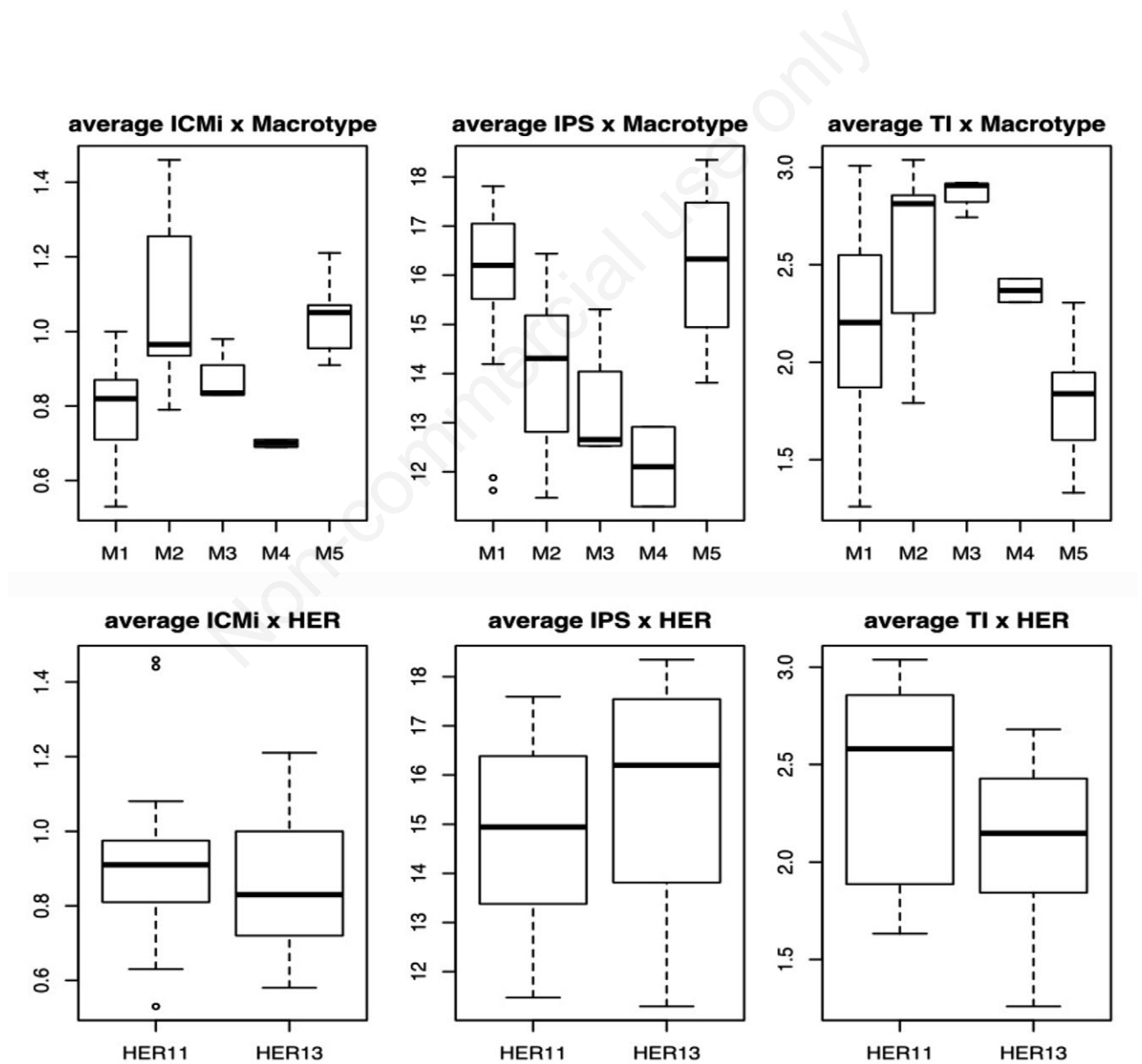


Fig. 4. Box plots of ICMi, IPS, TI values (Y axis) among macrotypes and among hydroecoregions (respectively, M and HER on X axis). The inner line is the median, the box margins are the 25th and 75th percentile, bars extend to 5th and 95th percentile.

Tab. 5. Estimated values for the three indexes among macro-types and hydro-ecological regions.

	Macrotypes ICMi estimates			
	Estimate	Std. Error	t value	Pr(> t)
(Intercept)	0.777	0.037	20.885	<0.001
M2	0.288	0.066	4.382	<0.001
M3	0.089	0.085	1.049	0.302
M4	-0.082	0.115	-0.717	0.478
M5	0.253	0.069	3.671	0.001
	Macrotypes IPS estimates			
	Estimate	Std. Error	t value	Pr(> t)
(Intercept)	15.785	0.423	37.313	<0.001
M2	-1.720	0.748	-2.300	0.028
M3	-2.499	0.969	-2.578	0.015
M4	-3.682	1.304	-2.824	0.008
M5	0.405	0.783	0.518	0.608
	Macrotypes TI estimates			
	Estimate	Std. Error	t value	Pr(> t)
(Intercept)	2.225	0.101	22.137	<0.001
M2	0.359	0.178	2.018	0.052
M3	0.644	0.230	2.798	0.009
M4	0.142	0.310	0.460	0.649
M5	-0.429	0.186	-2.306	0.028
	Hydro-ecological regions TI estimates			
	Estimate	Std. Error	t value	Pr(> t)
(Intercept)	2.420	0.099	24.332	<0.001
HER 13	-0.367	0.168	-2.188	0.035

CONCLUSIONS

According to this result the diatom species richness did not result as a community variable related to the ecological quality of the studied river. A similar conclusion was already reported in previous works on other Italian (Della Bella *et al.*, 2012) and European catchments (Blanco *et al.*, 2012), and on other aquatic ecosystems (Cohen *et al.*, 1993; Chipps *et al.*, 2006; Della Bella and Mancini, 2009). Diatom diversity metrics exhibited poor linear correlations with environmental factors indicating ecological status because of complex environmental influences. The relationship between diatom diversity indices and productivity has remained unclear and then some authors suggested that these indices are not suitable for evaluating ecological conditions (Blanco *et al.*, 2012). Archibald (1972) found linear negative and Lavoie *et al.* (2008) found positive relationships between diversity and nutrients. Soininen (2009) indicated that other variables than nutrients determine diatom diversity. For example, Stenger-Kovács *et al.* (2014) found that stream order is a relevant typological parameter which can basically influence the diatom species number and diversity. Species

richness and diversity indices, like the Shannon Index, are two important aspects of diversity, but it is not obvious that both respond in a similar way to varying intensities of disturbance (Svensson *et al.*, 2012). Indices of diversity generally include both the number of species and their relative abundances, which make assessment of their responses more complex. Diversity indices based on species relative abundances (*e.g.*, Shannon Index) could show misleading responses and could be unsuitable for comparison of biological communities. A recent study on other biological community showed that the majority of the biodiversity metrics increased whereas the most abundant species declined and highlighted that increasing metrics of diversity may occur in parallel with substantial losses of individuals (Schipper *et al.*, 2016).

The present study highlighted differences in diversity metrics (species richness and Shannon Index) of diatom communities in Mediterranean river types, and this finding suggested that this aspect should be taken into account when comparison studies were made among different river typologies. Although our analyses could be affected by an imbalance in the number of observations among macrotypes and further testing with additional data of different hydroecoregion are necessary, our results indicated

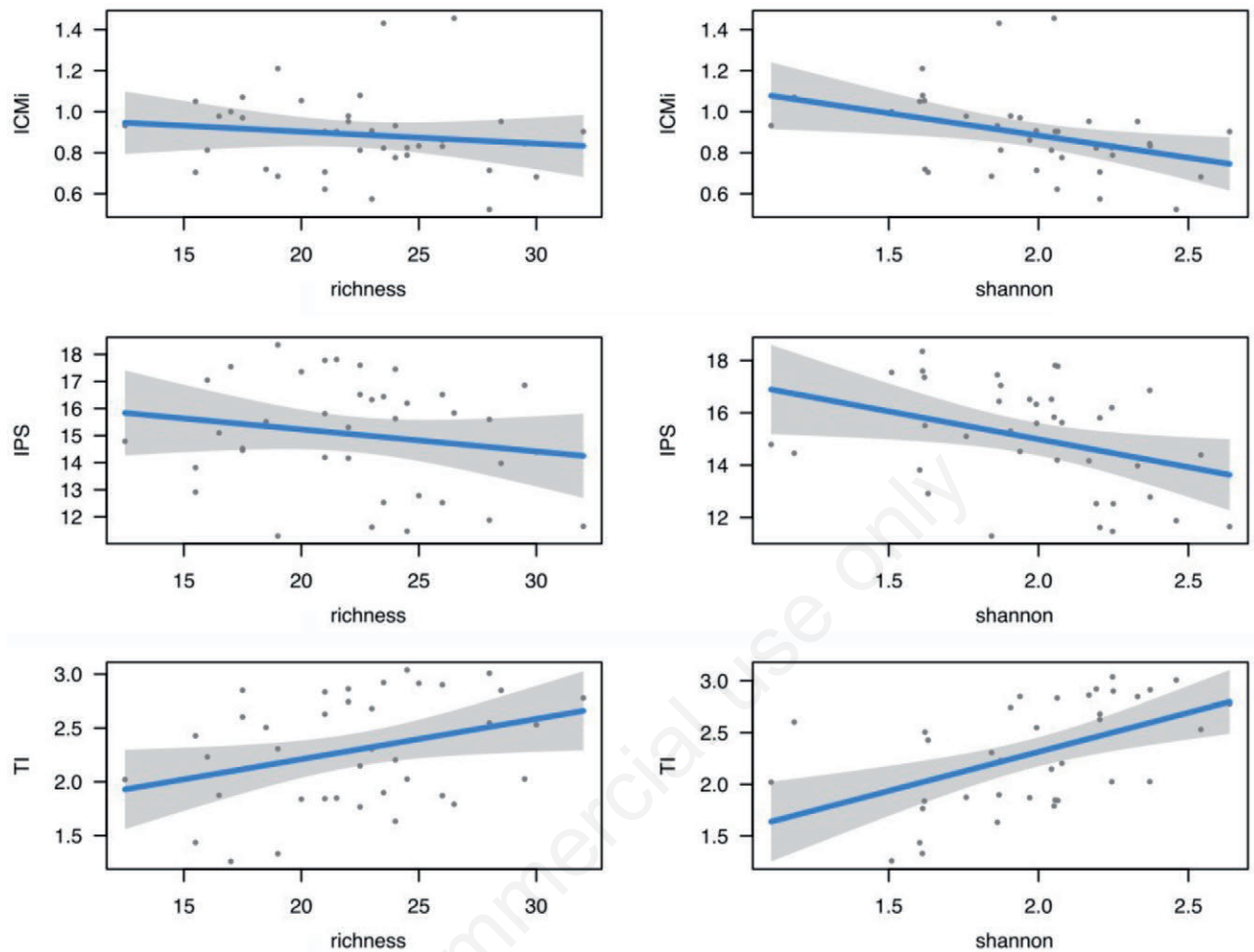


Fig. 5. Predicted values (blue continuous line) and confidence intervals (95%, light grey area) for species richness and Shannon Index of diatoms in relation to the ICMi, IPS and TI index values.

that the diatom diversity metrics could be considered complementary parameters in river biomonitoring for the ecological status assessment based on diatoms.

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