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POND CONSERVATION

Freshwater diatom and macroinvertebrate diversity of coastal permanent ponds along a gradient of human impact in a Mediterranean eco-region

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Abstract Mediterranean coastal areas are characterised by heavily transformed landscapes and an ever-increasing number of ponds are subjected to strong alterations. Although benthic diatoms and macroinvertebrates are widely used as indicators in freshwater ecosystems, little is still known about the diatom communities of lowland freshwater ponds in the Mediterranean region, and, furthermore, there are few macroinvertebrate-based methods to assess their ecological quality, especially in Italy. This article undertakes an analysis of benthic diatom and macroinvertebrate communities of permanent freshwater ponds, selected along a gradient of anthropogenic pressures, to identify community indicators (taxa and/or metrics) useful to evaluate the effect of human impacts. A series of 21 ponds were sampled along Tyrrhenian coast in central Italy. Five of these ponds, in a good conservations status and surrounded by woodland were selected as 'reference sites' for macroinvertebrates and epipelic diatoms. The remaining sixteen ponds were located in an agricultural landscape subject to different levels of human impact. The total number of macroinvertebrate taxa found in each pond was significantly higher in reference sites than in both the intermediate and heavily degraded ones, whereas the diatom species richness did not result in a good community variable to evaluate the pond ecological quality. The analysis revealed a substantial difference among the compositions of diatom communities between reference ponds and degraded ponds. The former were characterised by the presence of several species belonging to genera, such as *Pinnularia* sp., *Eunotia* sp., *Stauroneis* sp., *Neidium* sp., all of which were mostly absent from degraded ponds. Furthermore, the taxonomic richnesses of some macroinvetebrate groups (Odonata, Ephemeroptera, Trichoptera, Coleoptera), and taxa composition attributes of macroinvertebrate communities (total abundance, percentages of top three dominant taxa, percentages of Pleidae, Ancylidae, Hirudinea, Hydracarina) significantly correlated with variables linked with anthropogenic pressures. The results of the investigation suggested that diatoms tended more to reflect water chemistry through changes in community structure, whereas invertebrates responded to physical habitat changes primarily through changes in taxonomic richness. The methodologies developed for the analysis of freshwater benthic diatom and macroinvertebrate communities may have a considerable

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potential as a tool for assessing the ecological status of this type of water body, complying with the European Union Water Framework Directive 2000/60/EC.

Keywords Algae · Bacillariophyceae · Macrofauna · Lowland ponds · Environmental modifications and land cover conversions · Pond conservation status

Introduction

Ponds are now widely recognised as an important biodiversity resource (Nicolet et al., 2004; Della Bella et al., 2005; Oertli et al., 2005), especially at landscape scale (Williams et al., 2004). Moreover, recent developments have contributed to a better understanding of their economic value, in particular, the role of ponds in delivering ecosystem services (EPCN, 2008). Although broadly distributed across the regions of Europe, ponds and others small water bodies are seriously threatened by anthropogenic pressures such as increasing urbanisation and agricultural development which has resulted in both a sharp decline in numbers and ecological quality (Wood et al., 2003; Biggs et al., 2005; EPCN, 2008). This situation can be witnessed along the Tyrrhenian coast of central Italy where many ponds and other small water bodies have been subjected to severe pressure as a result of environmental modifications and land cover conversion. This highly vulnerable coastal system is faced with increasing urban development and intensive agriculture practices which has a negative impact upon river basins and small water bodies (Mancini & Arcà, 2000).

In view of the above, it is vital that pristine ponds are maintained and that degraded ponds are restored wherever possible. The protection strategies of inland surface water bodies in Europe is outlined in the current European regulation on water, the Water Framework Directive 2000/60/EC (WFD) which establishes a Framework for Community Action in the Field of Water Policy (CEC, 2000). The EU WFD aims, among others, to achieve good ecological status for inland waters. Small still waters and wetlands, ecologically and functionally important elements of aquatic ecosystems, are acknowledged as playing a strategic role in the achievement of WFD objectives (CEC, 2000, 2005) and the restoration and creation of wetlands and ponds are included in the listed actions to achieve its objectives. In its assessment of water bodies, the Directive defines different biological quality elements, including macroinvertebrates and benthic algae, and these are the biological indicators taken into account in this research.

Diatoms are often the main element of phytobenthos biodiversity in inland waters, representing an important component in freshwater ecosystems and are one of the most important groups of algae for monitoring purposes (Kelly et al., 1998; Mancini, 2005; King et al., 2006). Although they are widely used as indicators in rivers (for a review see Prygiel, 1999), lakes (Blanco et al., 2004; DeNicola and Eyto, 2004) and large wetlands (Gell et al., 2002; Gaiser et al., 2005; Wang et al., 2006), little is known about the benthic diatom communities of Mediterranean coastal ponds (Della Bella et al., 2007).

Macroinvertebrates play key roles in the freshwater ecosystem functioning and are often the main components of animal biodiversity in small water bodies. Together with algae, they are the most often recommended group of organisms for water monitoring activities (Hellawell, 1986). While consolidated standard methods based on macroinvertebrates exist for flowing (Armitage et al., 1983; De Pauw & Vanhooren, 1983; Alba-Tercedor & Sánchez-Ortega, 1988; Ghetti, 1997) and standing waters (Wiederholm, 1980; Verneaux et al., 2004; Rossaro et al., 2007), and for broad wetland areas in Australia and North America (Hicks & Nedeau, 2000; Apfelbeck, 2001; Helgen & Gernes, 2001), there are still few macroinvertebrate-based methods proposed for assessing ecological quality of small lentic bodies in Europe (Biggs et al., 2000; Menetrey et al., 2005; Solimini et al., 2008), and, in particular, of lowland ponds in the Mediterranean eco-region (Trigal et al., 2009).

Although some studies on both macroinvertebrates and diatoms in other aquatic ecosystems have shown that they could provide consistent and complementary information on environmental quality (Apfelbeck, 2001; Triest et al., 2001; Chessman et al., 2006; Chipps et al., 2006; Feio et al., 2007), to date these biological components are not usually studied together in ponds, as recommended by the integrated approach of the WFD. Among the different typologies of wetlands, this study has focussed on lowland freshwater ponds. These ecosystems represent one of the biotopes most severely threatened by human impact and, therefore, worthy of urgent restoration actions.

The main purpose of this study is to carry out an analysis of both benthic diatom and macroinvertebrate communities of permanent ponds, selected along a gradient of anthropogenic pressures in the Mediterranean coastal area of central Italy to analyse the relationships between diatom and macroinvertebrate diversity and environmental variables (surrounding land use, pond conservation status, habitat condition, human disturbances and water chemistry), and to identify the community indicators (taxa and/or metrics) useful to evaluate the effect of human impact. The methodologies developed in this study may also provide valuable tools for assessing the ecological quality of these aquatic ecosystems.

Methods

Study area and site selection

The study area is located in central Italy, a region containing several lakes (volcanic, coastal, man-made and Apennine lakes), small still waters and wetlands, and a number of designated protected areas have been created specifically to protect these lentic freshwater ecosystems.

Among the permanent freshwater lowland ponds of the Tyrrhenian coast of central Italy having a surface area less than one hectare, a homogeneous group of ponds were selected, based on water permanence and conductivity, low altitude and a surface area between 500 and 8,000 m^2. Temporary ponds have been excluded from the sample because previous studies indicated differences in the structure and composition of the biological communities, due to the effects of wet phase duration (Della Bella et al., 2005, 2008). A preliminary survey of the study sites was undertaken in spring 2007 following pond identification through cartographic search, previous studies made on potential suitable ponds, information gathered from staff attached to the Protected Areas together with information from local landowners. For each pond, surrounding land use up to 500 m radius was evaluated including the pond habitat condition, the presence/absence of human disturbances, and the application of ECELS index. This is a rapid methodology, developed and applied in Spain to assess the conservation status of Mediterranean wetlands (Sala et al., 2004), and is based upon five main components: (1) basin littoral morphology, (2) human activity, (3) water characteristics, (4) emergent vegetation and (5) hydrophytic vegetation. Although this index was developed as a tool to measure only the conservation status of wetlands, through the evaluation of hydromorphological, biological and physico-chemical characteristics, it can be used to establish the ecological status of wetlands, as considered under the WFD, together with assessments of other quality elements. From this evaluation, the percentage of land use under different categories (woodland, other natural vegetation such as shrub and grass, pasture, agricultural and artificial surface, water basins) surrounding the basin within a radius of 500 m was measured. In addition, the riparian vegetation width (as minimum distance between pond and land use conversion from wood or natural vegetation) using field observations, aerial photo interpretation and Corine Land Cover 2000 (CLC2000) was also measured. The percentage of pond surface covered by aquatic macrophyte (emergent and submerged) was estimated, and the percentage of perimeter covered by emergent vegetation, shore slope, and recorded the number of macrophyte species for each pond were noted. The maximum depth and area of ponds was assessed with a graduated pole and a measuring tape and, finally, the number of visible human disturbances was assessed and included, for example, the presence of exotic species, irrigation use of pond, pond shore cleaning, livestock grazing, presence of rubbish, fire, transport infrastructures within 100 m, pond digging, salinisation, farm activities, pesticides and herbicides use (see Table 1).

Out of this, 21 ponds were selected along the Tyrrhenian coast of the Tuscany and Latium Regions, a heavily transformed landscape which has been subject to strong alterations, due to environmental changes and land use conversions (Fig. 1). All study ponds are located inside Protected Areas: Maremma Regional Park (Tuscany); Litorale Romano Natural State Reserve (Latium), and in particular Macchiagrande di Ponte Galeria (SIC IT 6030025) and LIPU Oasis Castel di Guido; Decima-Malafede Natural Reserve; Presidential Estate of Castelporziano (SIC IT6030027-8, IT6030084); Wood of Foglino (SIC IT6030047). Out of the 21 sites, five were undegraded/unimpaired ponds and were identified as ‘reference sites’, surrounded by woodlands, in a high/good conservation status and without human disturbance. The other 16 ponds were located in the

Table 1 Pond variables taken into account in the study and their codes

Environmental variables	Code
Land use surrounding (Buffer radius = 500 m)	
Agriculture, artificial surface, pasture	AG_ART_P
Woodland and natural vegetation	BOS_NAT
Water bodies	ACQ
Conservation status	
ECELS index	ECELS
Habitat condition	
Macrophyte cover	CMAC
N° of macrophyte species	NVEG
Shore slope	RIVE
Riparian zone width	FRIP
Pond morphometry	
Depth	PROF
Surface	SUP
Water characteristics	
pH	PH
Dissolved oxygen	DO
Nitrate	NO_3^-
Phosphate	PO_4^{3-}
Conductivity	COND
BOD_5	
Number of human disturbances	DIST
Exotic species	
Irrigation use	
Shore cleaning	
Livestock grazing	
Rubbish	
Fire	
Roads	
Farm activities	
Salinisation	
Use of pesticides, herbicides	
Pond digging	

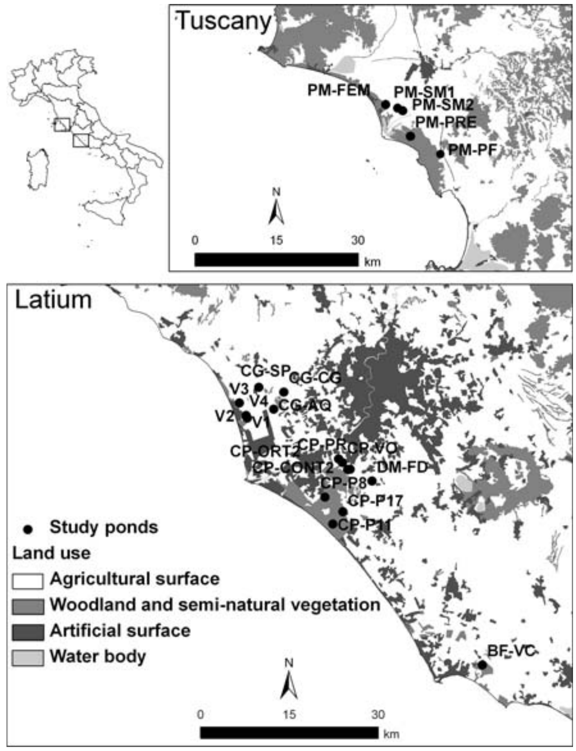

Fig. 1 Study area, studied ponds location and land use surrounding. Reference ponds are PM-PRE, CP-P11; CP-P8, CP-17, BF-VC

agricultural landscapes and were subject to varying levels of human alteration.

Sampling and laboratory methods

Floristic and faunistic materials and water samples were taken from all the ponds between late spring and early summer 2007, covering the period of the year generally characterised by the highest richness of diatom and macroinvertebrate species (Bazzanti et al., 1996; Dell'Uomo, 2004; Della Bella et al., 2005, 2007; Trigal et al., 2006). Conductivity, pH and dissolved oxygen were recorded by field meters (Table 1). Water samples were collected and analysed in laboratory for nitrate (NO_3), phosphate (PO_4^{3-}) contents and BOD_5, as per standard methods reported in IRSA (1994) and APHA (1998).

Diatoms

Diatom sampling, sample treatment, and laboratory analysis were carried out according to the European recommendations (Kelly et al., 1998; EN 1394, 2003; EN 14407, 2004, King et al., 2006) and national guidelines (APAT, 2008a). Epipelic forms present on the upper surface of the littoral sediment were sampled with a pipette, and five samples were taken for each pond. This sampling substratum was chosen because cobbles and emergent macrophytes were not present in all the water bodies visited, whereas it is very important that comparisons between water bodies are based on samples from the same substratum. The epipelon was the only substratum available in all the study ponds. In standing waters where the substrata types recommended for sampling diatoms are not an option, the use of the collection of soft sediment is justified (King et al., 2006).

Diatom samples were immediately examined in the laboratory or fixed and stored in 4% formalin. 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©) and up to 400 valves were counted and identified to species or variety level in each sample using a light microscope with 1,000× magnification. Measurements were made with the aid of image analysis software (Leica IM1000). Images of diatoms were digitised using a video camera (Leica DC 300) connected to a microscope (Optiphot-2) and to a computer. The main references for diatom taxonomy were Krammer & Lange-Bertalot (1986, 1988, 1991a, b, 2000), Krammer (2000), Lange-Bertalot (2001) and Prygiel & Coste (2000). Their nomenclature is used here, but is updated to include changes. Qualitative and structural attributes of algae communities evaluated as candidate metrics for assessing biotic responses to pond alteration were (1) species richness, (2) species composition: relative abundance of some genera (Hill et al., 2000; Chipps et al., 2006; Watchorn et al., 2008).

Macroinvertebrates

Macroinvertebrates were qualitatively and quantitatively sampled with a hand net (dimension: 20 × 27 cm; mesh size: 0.5 mm) which was worked over replicates of a known surface of 0.135 m^2. The area of each replicate sample was obtained by multiplying the width of the hand net by the sweep length (0.5 m). The number of replicates was calculated in proportion to the surface area of the each pond according to some surface area classes: a minimum of five replicates for surface lower than 1,000 m^2 (eight ponds); seven replicates for surface between 1,000 and 3,000 m^2 (five ponds), 10 replicates for surface between 3,000 and 4,000 m^2 (five ponds), 12 replicates for surface between 4,000 and 5,000 m^2 (one pond) and up to a maximum of 17 replicates for surface of 8,000 m^2 (two ponds).

The pond was netted in all the mesohabitats according to their relative extent within each pond following a 'multihabitat' sampling methodology previously applied in streams (Hering et al., 2004; APAT, 2008b). The concept of mesohabitat has been successfully adopted in recent studies on ponds (Biggs et al., 2005; Della Bella et al., 2005; Oertli et al., 2005). Mesohabitats identified in this study, defined as visually distinct and easily identifiable habitats within the freshwater body, were: filamentous algae; aquatic emergent and submersed vegetation; fine and coarse organic matter; roots of living riparian vegetation; dead wood; sediment dominated by silt and clay (diameter lower than 6 μm) or sand (between 6 μm and 2 mm) or gravel (between 2 mm and 2 cm). Material was preserved in alcohol (75%) until sorting. Invertebrate macrofauna was identified at the same level in each pond. Macroinvertebrates were usually identified to genus and species level, sometimes to family, and rarely to a higher taxonomic level (i.e. Oligochaeta, Turbellaria, Hirudinea and Hydracarina). Samples from different substrates were then pooled obtaining one combined sample for each site for next data analysis. Invertebrate 'density' was calculated by the number of sampled individuals divided by the total surface area of all replicate samples taken in each pond, and was expressed as individuals per square metre (ind/m^2). Some macroinvertebrate responses to pond alteration were evaluated including a variety of qualitative and structural attributes of communities known to respond to environmental degradation (Hicks & Nedeau, 2000; Biggs et al., 2000; Apfelbeck, 2001; Helgen & Gernes, 2001; Tangen et al., 2003; Menetrey et al., 2005; Gerecke and Lehmann, 2005; Solimini et al., 2008). In detail, we evaluated total taxa and family richness, some groups richness at genus and family level (Odonata, Ephemeroptera, Trichoptera, Coleoptera), total abundance (ind/m^2), percentage of top three dominant taxa, and percentages of some family and higher groups (Corixidae, Pleidae, Ancylidae, Hirudinea, Hydracrina). Community richness was calculated at family and a lower taxonomical level in order to evaluate the possibility in reduction of taxon identification effort.

Data analysis

Both multivariate and univariate approaches in statistical analyses were used to analyse the data (Reynoldson et al., 1997). Principal Component Analysis (PCA) was employed, based on environmental characteristics of the 21 study ponds, to summarise variations among sites and to highlight environmental gradients. Before the analysis, all variables were standardised following $X_{st} = (X - \overline{X})/SD$. The counts of each diatom taxon were expressed into relative abundance as a percentage of the total valves counted and identified in each sample. The macroivertabrate density data were expressed as individuals/m^2. In order to relate the diatoms and macroinvertebrate data to environmental data, a Canonical Correspondence Analysis (CCA), with Monte Carlo permutation tests, was applied. All the 18 environmental variables were included in the analysis. The ordination analyses were performed at the species level for diatoms and at the taxa and family level for macrofauna. The removal of all the taxa with less than two occurrences reduced the size of the original data set from 196 to 128 species and varieties of diatoms, and from 74 to 55 taxa of macroinvertabrate. Before the analyses, the relative abundance of diatom species were arcsin $\sqrt{p}$ transformed and macroinvertabrate densities were log $(x + 1)$ transformed to stabilise the variance and environmental variables were standardised as for PCA above. Besides observed total macroinvertebrate taxa of study ponds, the real richness estimator Chao1 (Chao, 1984) was used to take into account a possible underestimation of the real value due to sampling. The nonparametric Kruskal–Wallis analysis was used to test significant differences in observed and estimated richness, and in surface area, among pond groups, and Wilcoxon Matched Pairs Test to check significant differences between diatom and invertebrate richness. Spearman rank correlation (r_s) with Bonferroni's correction was used to explore relationships between two taxonomic richness (diatom and invertebrate), between both richness and sampling date and number of replicate samples, and between environmental and community variables or metrics. Data analyses were conducted with STATISTICA (version 5), PRIMER 5 (version 5.2.0), EstimateS (version 8.0.0) and PC-ORD (version 3.09) for Windows softwares.

Results

Environmental characteristics of ponds

The first two components extracted in the PCA performed on land use, pond habitat condition, number of human disturbances, ECELS index, and morphology and water variables, accounted for 45.8% of variance in the original data (Fig. 2). The ordination analysis PCA pointed out a clear separation among reference ponds, the most degraded ponds and the ponds with intermediate level of human alteration along the first axis (PC1). The PC1 axis represented the environmental gradient positively correlated with the number of human disturbances, percentages of agriculture and artificial surface in the land use surrounding, shore slope, conductivity, and negatively with ECELS index, percentage of land use occupied by woodland and natural vegetation, number of macrophyte species and percentage of macrophyte cover. Three pond groups (reference ponds, intermediately impaired ponds and grossly impaired ponds) did not significantly differ in area. In detail the results of 18 environmental characteristics of ponds taken into account in the study are reported in Table 2 and in Appendices 1 and 2—Supplementary Material.

Diatom species richness and assemblages

A total of 196 species and varieties of diatoms belonging to 53 genera were identified (Table 3). The ponds with intermediate level of human alteration had significantly higher number of species than reference and the most degraded ponds (Kruskal–Wallis test: $H_{2,N=21} = 8.12$; $P < 0.05$) (Fig. 3). The total number of species found in samples of each study pond was significantly correlated with pond surface area ($r_s = 0.64$; $P < 0.001$) and macrophyte cover ($r_s = 0.63$; $P < 0.01$). No significant relationship was found between diatom number of species and sampling date.

The CCA revealed a substantial difference among diatom communities of reference ponds, degraded ponds, and ponds with intermediate level of human alteration (Fig. 4). The first two axes were significant (Monte Carlo test $P < 0.05$) and explained 25.5% of cumulative variance in diatom data. Indeed, communities of undegraded reference ponds were characterised

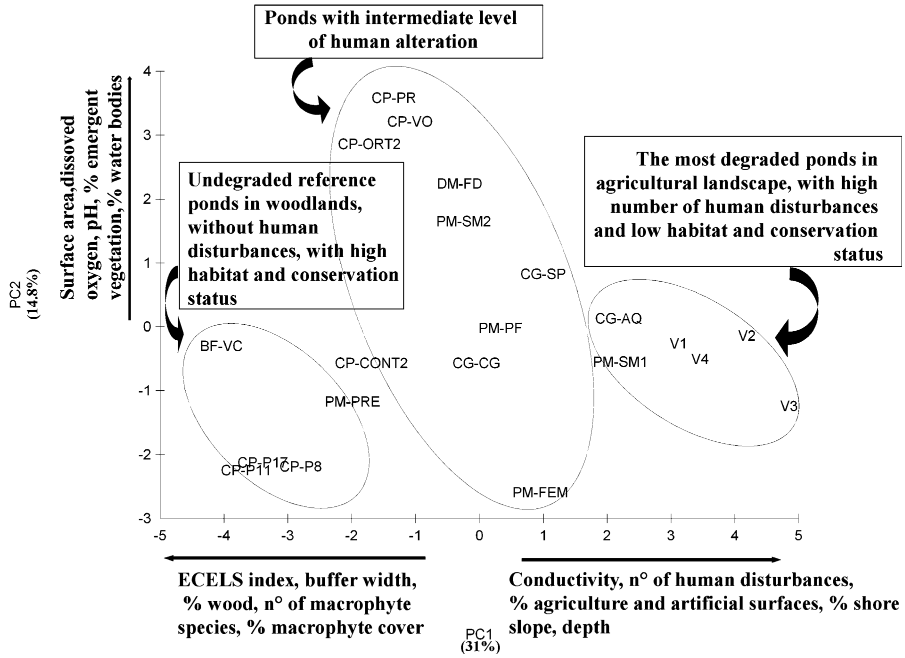

Fig. 2 PCA performed on environmental variables of studied ponds. *Arrows* indicate the correlation (r_s) with a significance at least $P < 0.05$ between axis pond scores and environmental variables

by a presence of several species belonging to genera, such as *Pinnularia* sp., *Eunotia* sp., *Neidium* sp., *Stauroneis* sp., which were almost totally absent from disturbed ponds. In contrast, the most impaired ponds were characterised by species of *Fragilaria* and *Pseudostaurosira* genera, such as *Pseudostaurosira brevistriata*, *Fragilaria ulna* var. *arcus* and *Fragilaria* cf. *nanana*.

The relative abundances of species belonging to genera *Fragilaria* were significantly correlated with surrounding land use, in particular, positively correlated with agriculture, pasture and artificial surfaces, and negatively correlated with surfaces occupied by woodland and natural vegetation ($r_s = 0.81$, $P < 0.0001$). Species abundances of this genera were negatively correlated also with the ECELS index, used to evaluate pond conservation status ($r_s = -0.78$, $P < 0.0001$). A positive relationship was found between this genera abundances and number of human disturbances present ($r_s = 0.72$, $P < 0.001$), pond water conductivity ($r_s = 0.79$, $P < 0.0001$) and shore slope ($r_s = 0.71$, $P < 0.001$). On the contrary, the total of abundances of species belonging to genera *Eunotia*, *Stauroneis*, *Neidium* and *Pinnularia*, were positively correlated with the ECELS index ($r_s = 0.62$, $P < 0.005$), riparian zone width ($r_s = 0.58$, $P < 0.01$), woodland and natural vegetation surface ($r_s = 0.50$, $P < 0.05$), and negatively correlated with pond water conductivity ($r_s = 0.57$, $P < 0.05$), pastureland, agricultural and artificial surfaces ($r_s = 0.52$, $P < 0.05$).

Macroinvertebrates

A total of 74 taxa, belonging to 15 main zoological groups were collected from the 21 study ponds (Table 4). Macrofauna was qualitatively dominated by insects with 60 taxa, most of which belonged to Coleoptera (a total of 27 taxa) and Diptera (11 taxa), and, secondly, to the Hemiptera (10 taxa) and Odonata (nine taxa). The observed and estimated total number of macroinvertebrate taxa are strongly correlated to each other ($r_s = 0.96$, $P < 0.0001$), and richness was not significantly correlated with diatom richness. There were significant differences between diatom and two invertebrate richness (Wilcoxon test $P < 0.001$). Unlike diatoms, the reference ponds had significantly higher number of observed (Kruskal–Wallis test: $H_{2,N=21} = 13.71$; $P < 0.001$) and estimated ($H_{2,N=21} = 12.83$; $P < 0.005$) number of taxa of macroinvertebrates than both the most degraded ponds and ponds with intermediate level of human alteration (Fig. 5). The total number of taxa of macroinvertebrates found in each pond as positively correlated with the ECELS index ($r_s = 0.83$, $P < 0.0001$), number and cover of macrophytes ($r_s = 0.84$, $P < 0.0001$ and $r_s = 0.64$, $P < 0.001$,

Table 2 Median, mean, minimum and maximum of the distribution of pond environmental variables in reference, intermediate and impaired groups of ponds

	Morpholgy			Water chemistry					
Variable:	PROF	SUP	RIVE	NO_3^-	PO_4^{3-}	PH	COND	DO	BOD_5
Units:	cm	m^2	%	mg/l	mg/l		µS	mg/l	
Reference ponds									
Median	55	800	5	1.22	0.45	7.83	173.8	8.60	7
Mean	99	2226	23	1.39	0.52	8.10	156.3	8.68	8
Min.	45	690	5	0.86	0.14	7.14	73.0	6.97	3
Max.	200	8000	80	2.00	0.84	9.95	222.0	10.70	14
Ponds with intermediate level of human alteration									
Median	150	3195	30	0.77	0.47	8.49	611.0	8.95	8
Mean	144	3349	25.5	1.32	0.52	8.96	867.3	9.93	8.1
Min.	60	700	5	0.50	0.11	8.19	337.0	5.25	3
Max.	300	8000	50	5.44	1.08	10.25	2960.0	15.87	16
Very degraded pond									
Median	200	1100	99	12.88	0.51	8.71	1361.0	9.82	7.5
Mean	210	1042	97.5	13.64	0.61	8.67	1329.0	9.89	8.7
Min.	110	500	90	0.63	0.12	8.27	1114.0	7.85	5
Max.	300	1600	99	28.62	1.29	8.90	1460.0	12.36	14

	Habitat condition				Conservation status	Human Disturbances	Land use		
Variable:	NVEG	CMAC	VEM	FRIP	ECELS	DIST	ACQ	ARG_ART_P	BOS_NAT
Units:		%	%	m	Value	N°	%	%	%
Reference ponds									
Median	7	80	5	700	80	0	0	0	100
Mean	7.6	55	25	900	83	0	0	0	100
Min.	5	5	0	500	75	0	0	0	100
Max.	14	90	90	2000	95	1	0	0	100
Ponds with intermediate level of human alteration									
Median	4	80	80	20	61.5	4	1	78	22
Mean	7.8	64	56	18.4	60.5	4	3	71	27
Min.	3	20	0	1	40	2	0	10	0
Max.	19	95	99	50	74	6	10	95	90
Very degraded pond									
Median	1	0	10	0	17	7	0	97	2
Mean	1.5	8	27	0.83	13.3	6	1	96	4
Min.	0	0	0	0	2	2	0	87	0
Max.	4	30	99	3	21	9	5	99	13

For code variables see Table 1

respectively), percentages of woodland and natural vegetation surfaces ($r_s = 0.79$, $P < 0.001$), riparian zone width ($r_s = 0.71$, $P < 0.001$), and negatively with pastureland, agricultural and artificial surfaces ($r_s = 0.77$, $P < 0.001$), conductivity ($r_s = 0.67$, $P < 0.001$) and shore slope ($r_s = 0.60$, $P < 0.005$). No significant relationship was found between the total number of macroinvertebrate taxa and sampling date, nor between total number of macroinvertebrate taxa and number of replicate samples taken per pond.

As for diatoms, the CCA analyses performed on densities (ind/m^2) of macroinvertebrates at family

Table 3 Number of diatom species and varieties identified for each genera

Genera	Number of species
Nitzschia	30
Navicula	28
Gomphonema	13
Amphora	8
Pinnularia, *Stauroneis*	7
Eunotia, *Cyclotella*, *Cymbella*, *Gyrosigma*, *Fragilaria*, *Neidium*, *Planothidium*	5
Achnanthidium, *Craticula*, *Caloneis*, *Surirella*	4
Sellaphora, *Tryblionella*	3
Diadesmis, *Encyonema*, *Encyonopsis*, *Epithemia*, *Mayamaea*, *Cymatopleura*, *Rhopalodia*, *Anomoeoneis*, *Luticola*, *Diploneis*, *Cocconeis*	2
Brachysira, *Achnanthes*, *Cymbopleura*, *Denticula*, *Adlafia*, *Bacillaria*, *Cyclostephanos*, *Hippodonta*, *Synedra*, *Staurosira*, *Rhoicosphenia*, *Rhizosolenia*, *Pseudostaurosira*, *Fallacia*, *Mastogloia*, *Discostella*, *Hanztschia*, *Parlibellus*, *Ulnaria*, *Fistulifera*, *Eucocconeis*, *Eolimna*, *Frustulia*	1
Total No. of species	196

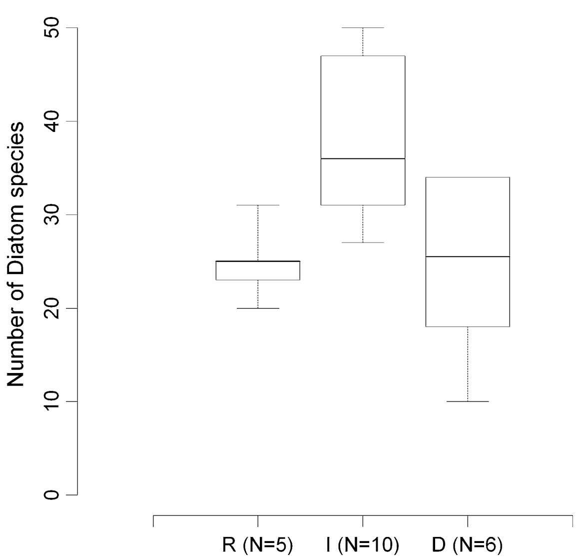

Fig. 3 *Box plot* of number of diatom species found in reference ponds (R), the most degraded ponds (D) and ponds with intermediate level of human alteration (I). *Box* is interquartile range (25–75%), *black line* is median value, and *whiskers* are minimum and maximum values

and at lower taxonomic level revealed a substantial difference between communities of reference ponds and those of ponds with intermediate level of human alteration and the most degraded ones. Alternatively, the analysis did not highlight a clear separation between the latter two groups of ponds. Figure 6 shows the plot of CCA performed on family densities. The first two axes explained 32.3% of cumulative variance but were not significant after Monte Carlo permutation test. The evaluated invertebrate metrics were significantly correlated with pond conservation status (ECELS index), land use (percent woodland and natural vegetation, and percent agriculture, pasture and artificial surfaces), shore slope, conductivity, nitrate and number of human disturbances (Table 5). Among the tested richness metrics, the total number of taxa and family had the strongest correlation with pond conservation status (ECELS Index). Total richness metrics were also strongly correlated with percentage of woodland and natural vegetation in land use surrounding. Then, the number of taxa and family of Odonata and EOT (Ephemeroptera, Odonata and Trichoptera) highly responded to pond conservation status level and the surrounding land use variables. The number of Coleoptera was weakly correlated, and only at genera level. In general, taxa composition metrics showed to be slightly less significant than most of the richness metrics. Among the tested composition metrics, the top three dominant taxa and percentage of Pleidae were strongly correlated with the ECELS index. The percentage of Pleidae and Hydracarina had the highest positive correlation with the percentage of woodland and natural vegetation in the surrounding land use. Moreover, a very high correlation was found also between the total density of all the sampled macroinverterbrate individuals (ind/m^2) and the ECELS Index and the percentage of surrounding land use variables (Table 5).

Discussion

This study contributed to a better knowledge of benthic diatom of ponds. Indeed, this pond biological component in the Mediterranean eco-region is still little studied at both national (Della Bella et al., 2007) and international levels, especially associated with the macroinvertebrate component analysis. We

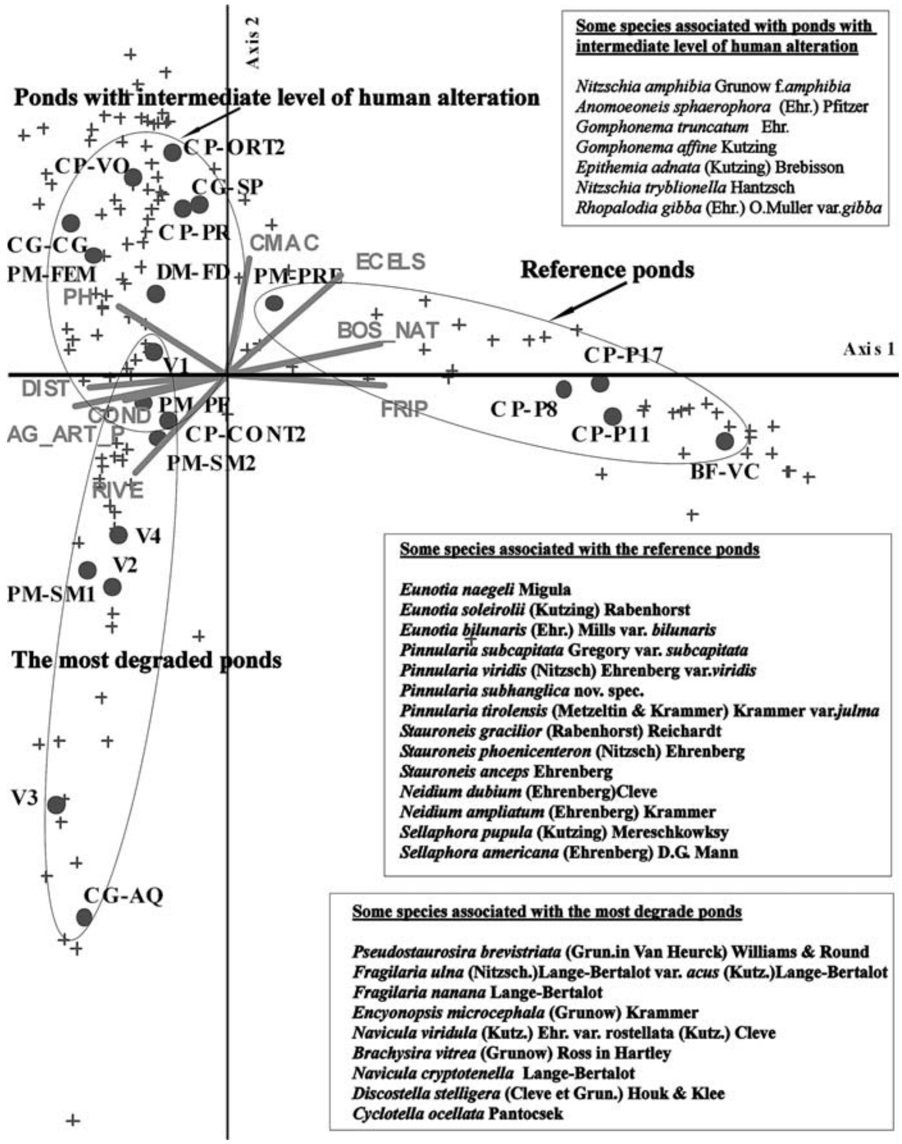

Fig. 4 CCA performed on relative abundances of diatom species and varieties found in each study ponds. For variable and pond codes see Table 1 and Fig. 1, respectively

analysed for the first time the epipelic diatom assemblages and associated aquatic macrofauna of some of the few last remnant pristine ponds along the Thyrrenian coast of central Italy. They are still not or minimally impaired by human activities, and we used them as reference condition to compare with those of impacted ponds.

Principal Component Analysis highlighted that the five reference ponds with a good/high conservation status and surrounded by woodland and shrubs showed the highest number of macrophytes and greatest macrophyte cover. In addition, they had a lower conductivity than degraded ponds with high number of human disturbances, high shore slope, and high percentage of agriculture in its surroundings. Although the highest nitrate contents were found in some of most impaired ponds, the present analysis did not indicate the water column nutrients as an important environmental gradient from low impact site to disturbed sites. Some studies on wetlands also found that conductivity, or specific conductance, were positively correlated with the percentage of agriculture land or negatively with the percentage of natural areas (Stewart et al., 2000; Carrino-Kyker & Swanson, 2007). Wetlands, when influenced by agricultural activity, often have significantly higher conductivity

Table 4 List of main zoological groups collected in studied ponds, with their total number of identified systematic units and their identification level

Zoological groups	Identification taxonomic level	Number of taxa
TURBELLARIA	Class	1
OLIGOCHAETA	Class	1
HIRUDINEA	Class	1
ISOPODA	Genera	1
DECAPODA	Species	2
HYDRACARINA	Order	1
EPHEMEROPTERA	Genera (2)/Species (1)	2
ODONATA	Family (1)/Genera (7)/Species (1)	9
HEMIPTERA	Genera (5)/Species (5)	10
TRICHOPTERA	Genera	1
COLEOPTERA	Family (8)/Genera (17)/Species (2)	27
DIPTERA	Family (7)/subFamily (2)/Species (2)	11
GASTROPODA	Family (1)/Genera (3)/Species (1)	5
BIVALVIA	Species	1
BRIOZOA	Class	1
Total taxa		75

The number of taxa for each identification level is indicated in brackets

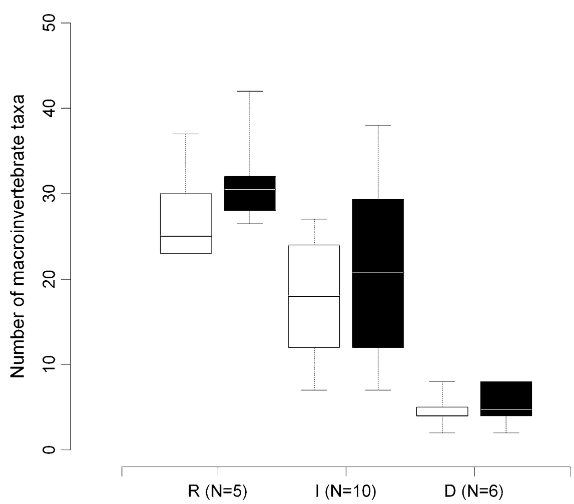

Fig. 5 *Box plot* of number of macroinvertebrate taxa in reference ponds (R), the most degraded ponds (D) and ponds with intermediate level of human alteration (I). *White boxes* indicate observed number of taxa, *black boxes* indicate estimated number of taxa with Chao1. *Box* is interquartile range (25–75%), *black* and *white line* their median values, and *whiskers* are minimum and maximum values

than reference wetlands (Helgen & Gernes, 2001; Chipps et al., 2006). Our reference ponds were characterised by conductivity lower than 225 µS/cm^{-1}, confirming these findings.

The total number of diatom species found in each pond did not provide a good community variable to evaluate pond ecological quality. Other studies have shown only minor and statistically insignificant reductions in diatom species richness between low and high disturbance sites of wetlands (Chipps et al., 2006) and lakes (Cohen et al., 1993). Indeed, reference ponds did not show a higher number of species than heavily degraded ponds. On the contrary, the diatom species number was significantly higher in ponds with an intermediate human impact. This result supports the intermediate disturbance hypothesis that predicted that environments under intermediate disturbances create more variable living conditions (e.g. available niche space, food resources, competition), which allow high levels of species diversity (Connell, 1978; Watchorn et al., 2008). Diatom species probably had a competitive advantage as a result of the habitat disturbance filling the available niche. Although Mackey & Currie (2001) suggest that the intermediate disturbance hypothesis has generally not been demonstrated, especially when few disturbances levels were examined, some researchers have reported increases in diatom richness under moderate stress (van Dam, 1982; Stevenson, 1984; Hill et al., 2000).

The CCA analysis indicated that diatom communities of minimally impaired ponds are characterised by the presence and abundance of some species belonging to genera *Pinnularia* sp. *Eunotia* sp.,

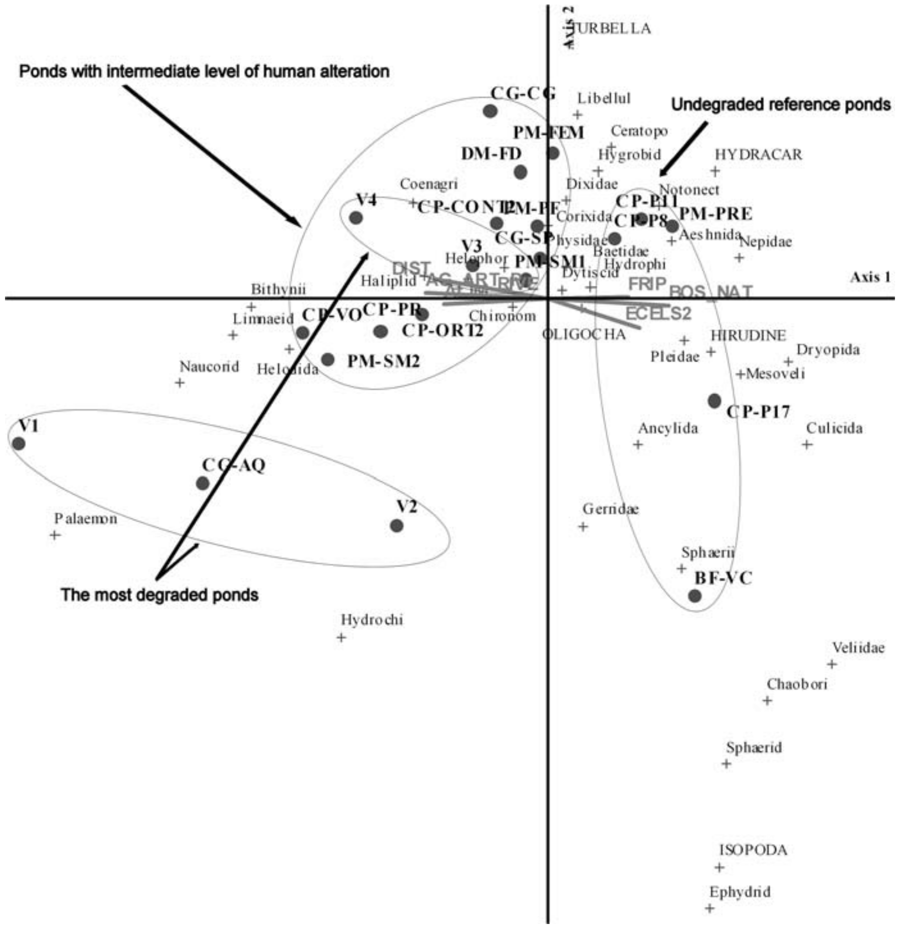

Fig. 6 CCA performed on relative abundances of macroinvertebrate family found in each study ponds. For variable and pond codes see Table 1 and Fig. 1, respectively. TURBELLA = Turbellaria; Libellul = Libellulidae; Ceratopo = Ceratopogonidae; Hygrodib = Hygrobidae; HYDRACAR = HYDRACAR INA; Coenagri = Coenagrionidae; Notonect = Notonectidae; Corixida = Corixidae; Aeshniida = Aeshnidae; Helophor = ; Helophoridae; Hydrophi = Hydrophilidae; Haliplid = Haliplidae; Dytiscid = Dytiscid; Bithiynii = Bithyniidae; Chironom = Chironomidae; Limnaeid = Limnaeidae; OLIGOCHA = OLIGOCHAETA; HIRUDINE; HIRUDINEA; Naucorid = Naucoridae; Helodida = Helodidae; Dryopida = Dryopidae; Ancylida = Ancylidae; Mesoveli = Mesovelia;Cuicida = Culicidae; Palaemon = Palaemonidae; Hydrochi = Hydrochidae; Sphaerii = Sphaeridiidae; Chaobori = Chaoboridae; Sphaerid = Sphaeridae; Ephydrid = Ephydridae

Neidium sp., *Stauroneis* sp., almost totally absent from degraded ponds. *Pinnularia* and *Eunotia* genera were inhabiting typically pristine water ecosystems such as Italian Alpine springs with siliceous substrate, and are important taxonomic groups in these habitats (Cantonati et al., 2005, 2006). On the other hand, diatom communities of the most disturbed ponds are characterised by the presence and high relative abundance of species belonging to genera *Fragilaria* and *Pseudostaurosira*. In their paleolimnological reconstruction using diatoms from sediments of the Swan Lake (Southern Ontario, Canada), Watchorn et al. (2006) found very similar changes in diatom community composition through time rather than in space (among sites). The diatom flora changed from an assemblage dominated by larger, benthic, acid-tolerant species including *Neidium* spp., *Eunotia* sp., *Pinnularia* spp., *Stauroneis* spp. and *Sellaphora pupula* to an assemblage characterised by smaller, and facultative planktonic taxa-like Fragilariaceae-type. The authors directly linked these changes in diatom assemblages to regional deforestation and agricultural activities associated with European settlements. In this study, these groups of species were

Table 5 Spearman rank correlation coefficients (r_s) for the relationships between invertebrate metrics and environmental variables in the studied ponds

Metrics	Variables									
	Conservation status	Land use		Morphology	Habitat condition			Water chemistry		Disturbances
	ECELS index	ARG_ART_P	BOS_NAT	RIVE	NVEG	CMAC	FRIP	NO3	COND	DIST
Taxa richness										
Total number of taxa	0.83 ****	−0.77***	0.79***	−0.60*	0.84****	0.64*	0.71**		−0.63**	
Total number of family	0.85****	−0.75***	0.76***	−0.62*	0.90****	0.71**	0.68**		−0.68**	
Number of Odonata taxa	0.70**	−0.72**	0.77***		0.55*	0.68**	0.57*		−0.55*	
Number of Odonata family	0.73**	−0.73**	0.78***		0.67**	0.70**	0.55*			
Number of EOT taxa	0.68**	−0.66**	0.71**			0.74***	0.56*	−0.65*	−0.58*	
Number of EOT family	0.74***	−0.66**	0.71**		0.71**	0.78***	0.56*	−0.58*	−0.58*	
Number of Coleoptera taxa	0.62*	−0.60*	0.56*	−0.55*	0.69**		0.56*			
Number of Coleoptera family					0.58*					
Taxa composition										
Percent top 3 Dominant taxa	−0.76***				−0.71**	−0.64*				0.58*
Percent Corixidae				−0.58*						
Percent Pleidae	0.73**	−0.83****	0.84****	−0.60*	0.63*		0.75***			−0.55*
Percent Ancylidae	0.67**	−0.58*	0.59*		0.70**					
Percent Hirudinea + Bivalvia	0.62*	−0.60*	0.61*	−0.67**	0.68**		0.66*			
Percent Hydracarina	0.61*	−0.80***	0.81***				0.57*			−0.69**
Taxa abundance										
Total density (ind/m^2)	0.81****	−0.93****	0.94****	−0.74**	0.67**		0.83****		−0.70**	−0.66*

Only $|r_s| \geq 0.55$ are reported. *Total density* number of all the sampled individuals divided to total surface area of all the replicate samples taken in each pond

**** $P < 0.00001$; *** $P < 0.0001$; ** $P < 0.001$; * $P < 0.01$

correlated with pond water conductivity, percentage land use, pond conservation status, human disturbances, confirming they could be candidate metrics to assess the ecological quality of these types of habitat.

Concerning the multivariate approach, diatom communities were more clearly associated with each of the three pond types of reference, intermediate and highly altered ponds than macroinvertebrate assemblages. In fact, invertebrate communities did not point out a clear separation between ponds with intermediate level of human alteration and the most degraded ones. As this algae group is represented by several species and might provide a better ecological resolution, this analytical method could be promising for detecting human impact using diatoms.

On the contrary, the univariate approach indicated that among the considered attributes of macroinvertebrate community, several metrics significantly correlated with variables associated with human impact. Contrary to diatoms, the number of macroinvertebrate taxa was significantly higher in reference ponds than both intermediate and heavily degraded ponds. This finding does not support the intermediate disturbance hypothesis (Connell, 1978). The observed difference between macroinvertebrate and diatom richness response could depend on the different spatial scales involved. Because of their larger size compared to microalgae, macroinvertebrate are more sensitive to physical habitat characteristics such as the morphological alterations of the habitat conditions (presence of steep shore, destruction of riparian belt, loss of mesohabitats). Differences between diatom and macroinvertebrate responses were also found in studies on river ecosystems (Triest et al., 2001; Chessman et al., 2006; Feio et al., 2007). Our findings confirmed several studies that show decreasing macroinvertebrate diversity with an increasing anthropogenic alteration of wetlands and ponds, and that identified the taxonomic richness as a good community variable to evaluate their ecological integrity (Biggs et al., 2000; Apfelbeck, 2001; Hicks & Nedeau, 2000).

In this study, almost all the evaluated taxonomic richness attributes of macroinvertebrate communities significantly correlated with variables reflecting human disturbance. According to previous studies (Biggs et al., 2000; Menetrey et al., 2005; Solimini et al., 2008) , the total number of taxa and family, number of Odonata, number of Ephemeroptera, Odonata, Trichoptera, and number of Coleoptera significantly showed correlation with pond impairment. Pond conservation status (ECELS Index) had the strongest correlation with total macroinvertebrate richness metric which, in turn, highly responded also to variables associated with the surrounding land use. Among the other tested metrics, the number of Odonata and number of Ephemeroptera, Odonata and Trichoptera were also strongly correlated with pond conservation status level and the percentage of surrounding woodland and natural vegetation. The response of evaluated qualitative metrics obtained at family and a lower taxonomical level were not very different, except for the case of Coleoptera. In our study their richness correlated with variables linked with anthropogenic pressures only when they were identified to lower level than family.

Taxa composition metrics tended to show only slightly less significance than most of taxa richness-based metrics. The proportion of the three most abundant taxa (percent top three dominant taxa) in the studied communities was negatively correlated with pond conservation status and habitat condition variables, in agreement with previous studies which found an increase of dominance by few groups along the anthropogenic disturbance gradient (Hicks & Nedeau, 2000; Apfelbeck, 2001). Surprisingly, in our study we did not find, as expected, a correlation between relative abundance of water boatmen (Corixidae) and site degradation variables. This hemipteran group of herbivores and detritivores are tolerant to low dissolved oxygen in water and tend to increase with eutrophication impact (Hicks & Nedeau, 2000; Solimini et al., 2008), whereas the degraded ponds in our study were not clearly characterised by deoxygenated and eutrophic waters. On the other hand, percentage of Pleidae correlated with land use categories, riparian zone width and conservation status of ponds, where this group was represented exclusively by the pigmy back swimmer *Plea minutissima*. This predator and carnivorous species feed on small crustaceans such as *Cyclops*, Ostracoda, small *Gammarus*, and also larvae of aquatic insects such as Ephemeroptera, Chironomidae, and mosquitoes (Vafaei, 2004). Usually, higher proportion of predator bugs occurred in higher-quality wetlands (Helgen & Gernes, 2001). Also the percentage of another group of predators, the adults of Hydracarina, showed a positive correlation with the percentage of surface occupied by woodland and natural vegetation around ponds. This group is particularly sensitive to pollution and contaminant and was not usually found in ecosystems altered for water abstraction (Gerecke & Lehmann, 2005; Cantonati et al., 2006). Finally, we found an expected decrease of total macroinvertebrate density along with study site degradation. This community attribute showed a highly positive correlation with conservation status, percentage woodland and natural vegetation and riparian buffer width. Most often, organism density provided variable response to impact and is rarely used as considerable a metric for data analysis, but could be decreased with loss of habitat, siltation, and toxic substance presence (Hicks & Nedeau, 2000; Apfelbeck, 2001).

Our results suggested that the methodologies we developed for the analysis of freshwater benthic Diatom and macroinvertebrate communities may have a considerable potential as a tool for assessing the ecological status of this type of water body, complying with the WFD 2000/60/EC. Diatoms tended more to reflect water chemistry through changes in community structure, whereas invertebrates responded to physical habitat changes primarily through changes in taxonomic richness. Moreover, further applied studies, in particular on diatom component in the Mediterranean eco-region are necessary, especially for lowland freshwater ponds.

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