J. Limnol., 65(1): 41-51, 2006

Tools for the development of a benthic quality index for Italian lakes

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ABSTRACT

In this paper, we propose a methodology to develop a benthic quality index useful for Italian lakes. The existing data about benthic macroinvertebrates of the Italian lakes were collected over a period of 50 years, but only a few lakes such as the Maggiore and the Mergozzo have been intensely studied. Some large lakes such as Lake Como are still almost uninvestigated. In total, 570 benthic macroinvertebrate taxa were identified; of which 373 belong to Chironomidae and 85 to Oligochaeta. With the aim of relating environmental variables with macrobenthos assemblages, we carried out a canonical correlation analysis (CANON) using a database that included 1060 sampling points. Both environmental (13 variables describing morphometry and hydrochemistry) and biological data (57 taxa) were available, but only taxa present in at least 10 samples were selected for data analysis. Three canonical variates were ecologically significant. The first one was correlated with conductivity, pH and alkalinity and accounted for 20% of the total variation. The second one was positively correlated with total phosphorus and $N\text{-}NH_4$*, and inversely with dissolved oxygen, and accounted for 18% of the total variation. The third one showed a direct correlation with maximum lake depth and volume and an inverse correlation with water temperature, and accounted for 17% of the total variation. A Trophic Status Index (TSI), based on the table 11 of the Italian Law 152/99 (without including chlorophyll), was calculated by ranking percent oxygen saturation, transparency and total phosphorus. TSI was used to test a Benthic Quality Index for Italian Lakes (BQIL) which is proposed in the present paper. The algorithm considered three steps. First, the means of three variables were calculated: percent oxygen saturation, transparency and total phosphorus weighted by the taxa abundances. These values are interpreted as optimum for each taxon and used to assign an indicator weight (BQIW). Second, the mean of these three variables was calculated for each taxon (mean BQIW). Third, the mean BQIW was multiplied by taxa abundance and divided by the total number of specimens present at each site for which the BQIL was obtained. Using a regression between BQIL and TSI values, lake sites were assigned to 5 quality classes as required by the Italian Law 152/99 and the WFD 2000/60/CE. This assignment must be considered as tentative, because different lake types should be considered separately to develop an index. At present the lack of information from different lake typologies hinders the development of a more sophisticated index such as the French Lake Biotic Index (LBI).*

Key words: bioindicators, lakes, chironomids, oligochaetes, multivariate analysis, trophic status

1. INTRODUCTION

1.1. State of knowledge on the study sites

Northern sub-alpine and Central volcanic lakes constitute two of the largest Italian lake districts and include more than 90% of the entire Italian freshwater volume. They have high ecological and environmental value and are valuable resources of water within densely populated areas. These characteristics explain the high interest that researchers have had in lowland lakes. The management and conservation of the quality characteristics and the maintenance of biodiversity currently represents a topic of major importance because of the need for technical support and scientific data for planning necessary interventions.

The papers included in the present database (Tab. 1) only represent a part of the studies carried out on the macrofauna of Italian lakes; thus, this is not a complete review of the knowledge about this theme. For example, the volcanic lakes sampled in Central Italy (Seminara *et al*. 1990; Bazzanti *et al*. 1998) were not considered, because the detailed data were not available. The samples selected for the present analysis included quantitative benthic macroinvertebrate counts, water chemical analyses and environmental variables.

The macrobenthos of some Italian lakes was investigated in the past, but there are many gaps in knowledge. Many contributions are in Memorie dell'Istituto Italiano di Idrobiologia (now Journal of Limnology) concerning the macrozoobenthos of Italian lakes since the '50s. Macrozoobenthos was analyzed in littoral, sublittoral and profundal zones with different sampling strategies and time schedules (Tab. 1).

It must be emphasized that, in general, the investigations were limited to large taxonomic groups without detailed taxonomic information, or they were restricted to a single group (e.g. chironomids, Mietto *et al*. 2000). Moreover, the data available are from a very small proportion of lakes such as the ones reported in "Catasto dei laghi Italiani" (Gaggino & Cappelletti 1984) and most investigations refer to '60s and '70s.

Tab. 1. List of lakes and papers considered to develop the database and concerning: L = littoral, SL = sublittoral, P = profundal.

Lakes	L / SL	P	Sampling method	N. taxa
Alserio	Ceretti & Nocentini 1996	Bonomi *et al.* 1967	Ekman grab, net sludge	44
Annone Est	Ceretti & Nocentini 1996	Bonomi *et al.* 1967	Ekman grab, net sludge	27
Annone Ovest		Bonomi *et al.* 1967	Ekman grab, net sludge	10
Bolsena	Nocentini 1973, 1974	Bonomi & Ruggiu 1968	Petersen grab	74
Bracciano	Nocentini 1973, 1974		Petersen grab	60
Comabbio	Ceretti & Nocentini 1996		net sludge	42
Endine	Nocentini *et al.* 1974		Petersen grab	29
Garda		Bonomi 1974	Petersen grab	279
Garlate	Ceretti & Nocentini 1996		net sludge	48
Ghirla	Ceretti & Nocentini 1996		net sludge	59
Iseo		Bonomi & Gerletti 1967	Petersen grab	25
Maggiore	Lenz 1954; Nocentini 1963, 1988,1991	Corbella *et al.* 1956; Bonomi *et al.* 1979	Ekman&Petersen grab, net sludge	131
Mergozzo	Nocentini 1966, 1979	Ruggiu & Saraceni 1972	Petersen grab	104
Monate	Ceretti & Nocentini 1996		net sludge	36
Montorfano	Ceretti & Nocentini 1996	Bonomi *et al.* 1967	Ekman grab, net sludge	30
Pertusillo		Bonomi & Andreani 1978	Petersen grab	15
Pusiano	Ceretti & Nocentini 1996	Bonomi *et al.* 1967	Ekman grab, net sludge	55
Sartirana	Ceretti & Nocentini 1996		net sludge	24
Segrino	Ceretti & Nocentini 1996	Bonomi *et al.* 1967	Ekman grab, net sludge	24
Varese	Ceretti & Nocentini 1996	Bonomi 1962, 1964	grab, net sludge	72
Vico	Nocentini 1973, 1974		Petersen grab	49

The database available is not suitable to build a model to forecast lake evolution over long scales. New samplings are necessary to test a benthic quality index. The development of a biotic index must be considered tentative with the present state of knowledge.

1.2. The benthic quality indices

Several indices and classification systems were developed using benthic macroinvertebrates. Oligochaetes and chironomids were considered the most useful indicators of oxygen conditions (Brundin 1949) and trophic status (Sæther 1979).

Lake classification using benthic macroinvertebrates has been developed for Central European and Scandinavian lakes (Wiederholm 1981; Aagaard 1986; Kansanen *et al*. 1990; Johnson *et al*. 1993). Chirononomids and oligochaetes showed a different distribution according to depth, oxygen saturation and trophic conditions (Lenz 1925; Naumann 1932; Lundbeck 1936; Thienemann 1954; Brundin 1956, 1974).

The trophic indices developed for lakes of Northern Europe relied on the relative abundances of chironomid taxa, the ratio of tolerant to intolerant tubificid oligochaetes, and the ratio of oligochaetes to chironomids (Wiederholm 1980). Wiederholm (*op. cit.*) also developed a Benthic Quality Index (BQI) based on chironomids, giving 6 different score levels as indicator values:

$$BQI_i = \sum_{j=0}^{5} \frac{h_j y_{ij}}{\sum_{j=0}^{5} y_{ij}} \qquad (1$$

where y_{ij} = number of individuals of each indicator group j in site i, $\sum_{j=0}^{5} y_{ij}$ = total number of individuals of all indicator groups j in site i, h_j is the score level which ranges from 0 to 5 according to the indicator value given to different taxa.

Sæther (1979, 1980) developed a classification system identifying 15 lake groups using profundal, sublittoral and littoral chironomid assemblages from Nearctic and Palaearctic lakes. Community structure varied in relation to the increasing ratio between phosphorus concentration and depth. The chironomid assemblages proposed include many taxa never recorded in Italy, so the system cannot be applied to Italian lakes without a substantial revision. Wiederholm (1980) and Lang (1985) developed benthic quality indices for oligochaetes as well. Verneaux *et al*. (2004) and Borderelle *et al*. (2005) discussed a Lake Biotic Index (LBI) based on the comparison of littoral and profundal macroinvertebrate communities sampled in soft sediments. A recent review of benthic macroinvertebrate indices is in Le Foche *et al*. (2005).

The Water Framework Directive 2000/60/CE (WFD) requires an assessment of either high, good, moderate, poor or bad ecological status using different components of biotic community. The aim of the present paper is to propose a Benthic Quality Index which uses the same conceptual framework proposed by Wiederholm (1980), summarized in equation (1, and that uses all the most common taxa among macroinvertebrates living in the Italian lakes (acronym: BQIL).

2. MATERIALS AND METHODS

2.1. Sampling methods

For the papers considered in this data analysis benthic macroinvertebrates were collected from soft bottom samples with an Ekman or a Petersen grab (Corbella *et al*. 1956; Nocentini 1979, 1989) or with a net sludge (Tab. 1). Samples were collected in late winter – early spring during the period of full circulation and in summer during stratification. Samples were sieved on a 250 µm mesh and fixed in 10% neutralized formaldehyde. The number of specimens for each taxon was counted using a stereomicroscope. For details see the original papers.

2.2. The taxa and stations analyzed

In the lakes examined 570 macroinvertebrate taxa were captured, 373 of which belonged to chironomids. Among the chironomid taxa, 43 belonged to the subfamily of Tanypodinae, 18 to Diamesinae, 3 to Prodiamesinae, 151 to Orthocladiinae (including 31 terrestrial taxa), 158 to Chironominae (63 to Tanytarsini, 94 to Chironomini and 1 to Pseudochironomini). The most represented group after Chironomidae was Oligochaeta with 85 taxa, and the other aquatic insects with 67 taxa. Mollusca were present with 37 taxa, Crustacea with 8 taxa.

At present a database is available with almost 20,000 records of macroinvertebrates collected in small and large Italian lakes. Samples were selected considering the availability of both environmental variables and quantitative benthic samples: 1060 samples were used including 13 environmental variables (Tab. 2) and 57 taxa, mostly oligochaetes and chironomids; the taxa present in at least 10 samples were selected for data analysis.

Tab. 2. Environmental variables used in data analysis.

Description	Abbreviation	Unit of measure
Latitude (large set)	lat	Gauss Boaga
Longitude (large set)	long	Gauss Boaga
Lake volume	vol	m3
Maximum depth of lake	max depth	m
Depth of sampling site	depth	m
Water temperature	temp	°C
Transparency	transp	m
Conductivity	cond	$\mu S\ cm^{-1}$
Alkalinity	alkal	$mg\ l^{-1}$
pH	pH	
Oxygen content	O2	$mg\ l^{-1}$
Percent O_2 saturation	O_2 % sat	%
Total phosphorus	TP	$\mu g\ l^{-1}$
Nitrate	$N\text{-}NO_3$	$\mu g\ l^{-1}$
Ammonia	$N\text{-}NH_4$	$\mu g\ l^{-1}$

The database included 21 lakes that included small and large lakes in Northern Italy, volcanic lakes in Central Italy and one artificial lake in Southern Italy (Basilicata Region). The lakes considered were divided into 6 groups:

1. small lakes with volumes lower than 70×10^6 m^3: Monate, Comabbio, Montorfano, Alserio, Pusiano, Annone Est, Annone Ovest, Segrino and Endine;
2. L. Mergozzo with a volume of 73×10^6 m^3 constitutes the second group; it was well analysed in the '70s and is characterised by a very low conductivity;
3. L. Pertusillo, an artificial lake from Basilicata Region (Southern Italy);
4. L. Varese, for which an historical data series is available since the '50s;
5. L. Vico, L. Bracciano and L. Bolsena (volcanic lakes in Central Italy);
6. large lakes (L. Maggiore, L. Iseo and L. Garda) with a volume larger than 5000×10^6 m^3.

3. DATA ANALYSIS

Physical (lake volume, depth, water temperature, etc.), chemical (pH, conductivity, oxygen, TP, $N\text{-}NO_3$, $N\text{-}NH_4$, etc.) (Tab. 2), and biological variables (macroinvertebrate taxa, including oligochaetes, crustaceans, aquatic insects, and molluscs) were analyzed. Chemical measures referred to either hypolimnic values during stratification or to the mean water column values during full circulation. Environmental data expressed using different units of measurement were standardized by subtracting the mean and dividing by the standard deviation. Taxa abundances per square meter were log transformed before data analysis. Microsoft ACCESS (MSA)® was used to store information (Rossaro *et al*. 2001). Data were exported from MSA into Matlab 7.2® for all the other data analysis. Calculations of Trophic Status Index (TSI), Benthic Quality Index Weight (BQIW) and Benthic Quality Index Lakes (BQIL) were performed using a Matlab program written by the senior author.

3.1. Trophic Status Index and Benthic Quality Index

Total phosphorus (TP), Secchi transparency, chlorophyll-*a* content and different forms of organic and inorganic nitrogen were used to describe and summarize the trophic status of lakes (Carlson 1977). The application of these concepts to the eutrophication of the Italian lakes was considered by Chiaudani *et al*. (1983). At present the Italian Law 152/99 (table 11) defines five quality classes (where class 1 is the best and class 5 is the worst) using TP, transparency, percent of hypolimnetic oxygen saturation and chlorophyll-*a* to describe the trophic status of lakes. In comparison with Carlson's TSI, percent of hypolimnetic oxygen saturation was also included. These variables were rescaled in an interval from 1 to 100, the lowest values of TP, chlorophyll-*a* and the highest values of transparency, and percent oxygen saturation were set to 100. The highest values of TP, chlorophyll-*a* and the lowest values of transparency, and percent oxygen saturation were set to 1. The lakes can be ranked and the mean of the variables for each lake can be calculated to summarize the ecological status using a TSI value. In Gaggino *et al.* (1985), chlorophyll-*a* was not included in the analysis because of the reduced number of observations. As in Gaggino *et al.* (*op. cit.*), we also considered three variables to calculate the TSI: TP, transparency and percent of oxygen saturation.

The weighted means and standard deviations of the three variables for each taxon were calculated, using the species abundances as the weighting factor, according to the following formula:

$$\bar{z}_{jk} = \frac{\sum_{i=1}^{n} y_{ij} z_{ik}}{\sum_{i=1}^{n} y_{ij}} \qquad \bar{s}_{jk} = \sqrt{\frac{\sum_{i=1}^{n} y_{ij}(z_{ik} - \bar{z}_{jk})^2}{\frac{(n-1)}{n}\sum_{i=1}^{n} y_{ij}}} \qquad (2$$

where z_{ik} is the value of the environmental variable k measured in a locality i, y_{ij} is the abundance of the taxon j in the same locality i, $\bar{z}_{jk}$ is the weighted mean and $\bar{s}_{jk}$ is the standard deviation calculated for the taxon j and the environmental variable k. Weighted means and standard deviations can be interpreted as optimum and tolerance values for each taxon (Ter Braak & Prentice 1988). To develop the benthic quality index (BQIL), the means were used as a weight (BQIW: Benthic Quality Index Weight) and assigned to each taxon. The weighted means were rescaled to between 5 and 1 according to the following formula (Lek & Guégan 2000): (this facilitates the assignment of lakes to 5 quality classes as requested by the Italian Law 152/99 and the WFD 2000/60/CE):

$$\breve{z}_{jk} = \frac{(\bar{z}_{jk} - z_{\min})}{(z_{\max} - z_{\min})} * (5-1) + 1 \qquad (3$$

where $\bar{z}_{jk}$ is the weighted mean of each taxon j and environmental variable k as above and $\check{z}_{jk}$ is the rescaled weighted mean. In the present case k refers to one of the q = 3 environmental variables selected to build TSI. TP is assumed to decrease with water quality, whereas transparency and percent of oxygen saturation are assumed to increase, so $\check{z}_{jTP}$ was rescaled:

$$\breve{z}_{jTP} = 5 - \breve{z}_{jTP} + 1 \qquad (4$$

The indicator weight $BQIW_j$ was obtained by taking the means of the rescaled $\check{z}_{jk}$ according to the formula:

$$BQIW_j = \sum_{k=1}^{q} \frac{\breve{z}_{jk}}{q} \qquad (5$$

where: q = number of environmental variables used to calculate *BQIW* (3 in the present case); $\check{z}_{jk}$ = rescaled mean value of the environmental variable k weighted by the abundance of the taxon j. At this point, $BQIW_j$ assumes values comprised between 1 and 5.

As a last step, $BQIL_i$ for each site i was calculated using the modified Wiederholm's (1980) formula (see Equation 1), using the *BQIWj* weights instead of the h values in the equation (1:

$$BQIL_i = \frac{\sum_{j}^{p} BQIW_j \, y_{ij}}{\sum_{j}^{p} y_{ij}} \qquad (6$$

where: p = the number of taxa in the site i; $BQIW_j$ = the indicator weight of the taxon j; y_{ij} = the abundance of the taxon j in the site i; $BQIL_i$ = the biotic index of site i.

Equation (6 can be used to calculate the $BQIL_i$ of a new site starting from the $BQIW_j$ and the abundances y_{ij} of the taxon in the new site.

3.2. Canonical correlation analysis (CANON)

A CANON was carried out to analyse the relationships between benthic macroinvertebrates and environmental variables and to summarize the results (Gittins 1979).

The canonical analysis investigates the relationships between variables of two distinct but associated sets; it searches for linear combinations for a set (taxa) of dependent variables which have the maximum correlation with a linear combination of a set of independent variables (environmental variables):

$$_{n}\mathbf{Y}_{p}\mathbf{A}_{k} = {}_{n}\mathbf{Z}_{q}\mathbf{B}_{k} \qquad (7$$

where Y are the dependent variables (taxa), Z are the independent variables (environmental variables), A and B are the factor loadings estimated to maximize the correlation. The products YA and ZB are the factor scores for the biological and environmental sets respectively. The model is developed for n sites, p taxa and q environmental variables; k linear combinations are calculated (= canonical variates) that are independent of one another.

4. RESULTS

4.1. Canonical correlation analysis

The three canonical axes had an eigenvalue greater than 0.5 (Tab. 3). The first axis was associated with a gradient related to ionic concentration, the second with a trophic – hypolimnetic oxygen gradient (Fig. 1), and the third with a morphometric (lake volume and depth) gradient that separated large and deep lakes from small and shallow ones that had higher water temperature and ammonia content. A fourth axis separated large, profundal, transparent lakes from small eutrophic lakes. The factor loadings of the environmental variables are in table 4.

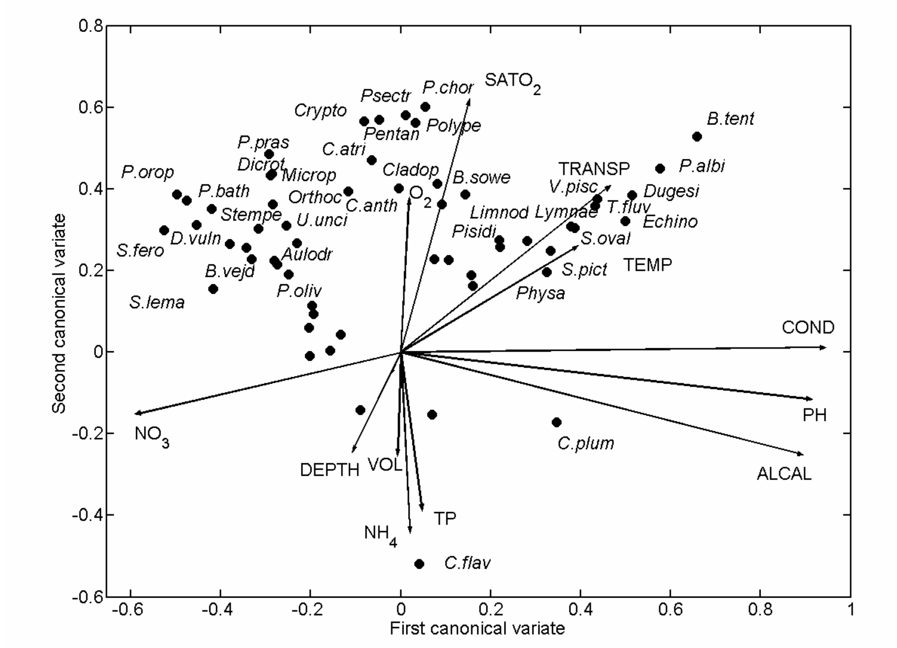

Fig. 1. Biplot of the factor loadings of environmental variables (arrows) and taxa (circles) of the canonical correlation analysis, in the plane defined by the two first axes. Taxa names with absolute values <0.3 in both axes were not evidenced.

Tab. 3. Canonical analysis: eigenvalues.

canonical axis	eigenvalue	% total
1	0.853	20.068
2	0.750	17.655
3	0.708	16.673
4	0.466	10.960
5	0.354	8.335
6	0.248	5.847
7	0.228	5.369
8	0.173	4.080
9	0.154	3.616
10	0.136	3.212
11	0.090	2.113
12	0.057	1.341
13	0.031	0.731

The first canonical axis separated (Fig. 2 and Tab. 4) volcanic lakes in Central Italy with a high conductivity, pH and alkalinity from subalpine lakes such as L. Mergozzo with a very low conductivity. The trophic condition (Fig. 2 and Tab. 4) was associated with the second axis: the oligotrophic L. Mergozzo and volcanic lakes had low TP content, while the other lakes (as L. Varese) had high TP content.

The factor loadings of taxa are in tables 5 and 6, and in figure 1. Taxa with high negative values on the first canonical axis (*Spirosperma ferox*, *Pagastiella orophila*, *Parakiefferiella bathophila*, *Stylodrilus lemani*, *Demicryptochironomus vulneratus*, *Stempellina bausei*, *Bothrioneurum vejdovskianum*, *Aulodrilus* sp., *Prodiamesa olivacea*, *Uncinais uncinata*, *Dicrotendipes* spp.) were characteristic of waters with low alkalinity, pH and conductivity, whereas taxa with high positive values on the first canonical axis (*Bithynia tentaculata*, *Paratendipes albimanus*, *Dugesia* sp., *Echinogammarus* sp., *Theodoxus fluviatilis*, *Valvata piscinalis*, *Sphaerium ovale*, *Chironomus plumosus*, *Lymnaea* spp., *Physa* spp.) were positively correlated with pH, conductivity and alkalinity. High alkalinity was related to high hardness which favoured the Mollusca. Taxa characteristic of eutrophic lakes are plotted in the low part of the plane (Fig. 1).

Few taxa had low factor loadings in the second axis and are positively correlated with N-NH_4 and TP: *Chaoborus flavicans*, *Chironomus plumosus*, *Tubifex tubifex*, Ceratopogonidae and Lumbriculidae. In contrast, many taxa had high factor loadings in the second axis and were positively correlated with oxygen and transparency: *Procladius choreus*, *Polypedilum* spp., Pentaneurini spp., *Bithynia tentaculata*, *Psectrocladius* spp., *Cryptochironomus* spp., *Paratendipes albimanus*, *Pseudochironomus prasinatus*, *Micropsectra* spp., *Cladotanytarus atridorsum*, *Chironomus anthracinus* (Tab. 6, Fig. 1). Profundal taxa had high loading in the third axis: *Asellus aquaticus*, *Niphargus foreli*, *Stylodrilus lemani*, *Spirosperma ferox*.

4.2. TSI, and BQIL results

The values of the taxa weights (BQIW) are in table 7. The taxa known to be very tolerant (*C. flavicans*, *C. plumosus*) received very low weight, whereas the less tolerant taxa received high weights: *S. pictulus*, *P. albimanus* among chironomids, *S. ovale*, *T. fluviatilis*, *B. tentaculata* among molluscs and *Echinogammarus* sp. among Crustacea.

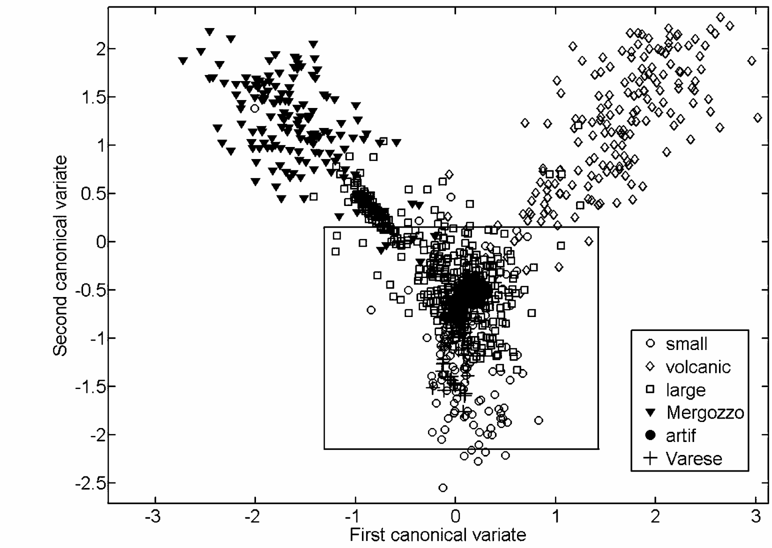

Fig. 2. Factor scores of sites in the plane of the first two axes in the canonical correlation analysis.

Tab. 4. Factor loadings of the first four canonical variates (environmental set).

	I		II		III		IV
NO_3	-0.592	NH_4	-0.445	NH_4	-0.421	NO_3	-0.373
depth	-0.109	TP	-0.390	temp	-0.273	NH_4	-0.369
maxdepth	-0.021	vol	-0.256	alkal	-0.240	TP	-0.179
vol	-0.007	alkal	-0.251	TP	-0.220	temp	-0.165
O_2	0.019	depth	-0.246	cond	-0.144	pH	-0.154
NH_4	0.020	NO_3	-0.152	sat O_2	-0.135	cond	-0.114
TP	0.048	pH	-0.115	transp	-0.074	sat O_2	-0.019
sat O_2	0.154	maxdepth	-0.053	pH	0.060	alkal	-0.006
temp	0.395	cond	0.012	O_2	0.136	O_2	0.217
transp	0.467	temp	0.261	NO_3	0.218	maxdepth	0.307
alkal	0.896	O_2	0.380	depth	0.311	depth	0.577
pH	0.915	transp	0.409	vol	0.354	vol	0.750
cond	0.946	sat O_2	0.622	maxdepth	0.693	transp	0.871

Tab. 5. Factor loadings of the first canonical variate and list of abbreviations (taxa set).

Spirosperma ferox Eisen, 1879	S.fero	-0.526	Pentaneurini spp.	Pentan	-0.048
Pagastiella orophila (Edward, 1929)	P.orop	-0.498	*Cladopelma* spp.	Cladop	-0.004
Parakiefferiella bathophila (Kieffer, 1912)	P.bath	-0.475	*Psectrocladius* spp.	Psectr	0.011
Demicryptochironomus vulneratus (Zetterstedt, 1838)	D.vuln	-0.455	*Polypedilum* spp.	Polype	0.032
Stempellina spp.	Stempe	-0.420	*Chaoborus flavicans* (Meigen 1830)	C.flav	0.041
Stylodrilus lemani (Grube, 1879)	S.lema	-0.417	*Procladius choreus* (Meigen, 1804)	P.chor	0.054
Bothrioneurum vejdoskianum Stolc, 1886	B.vejd	-0.380	*Tubifex tubifex* (Müller, 1774)	T.tubi	0.070
Aulodrilus spp.	Aulod	-0.343	*Dero digitata* (Müller, 1774)	D.digi	0.074
Prodiamesa olivacea (Meigen, 1818)	P.oliv	-0.331	*Branchiura sowerbyi* Beddard, 1892	B.sowe	0.081
Uncinais uncinata (Orsted, 1842)	U.unci	-0.316	*Pisidium* spp.	Pisidi	0.091
Pseudochironomus prasinatus (Staeger, 1839)	P.pra	-0.292	*Nais elinguis* Müller, 1774	N.elin	0.106
Micropsectra spp.	Tanyta	-0.289	*Limnodrilus* spp.	Limnod	0.144
Dicrotendipes spp.	Dicrot	-0.287	*Endochironomus* spp.	Endoch	0.157
Orthocladius spp.	Orthoc	-0.285	*Stylaria lacustris* (Linnaeus, 1767)	S.lacu	0.160
Micronecta sp.	Micron	-0.282	*Microtendipes* spp.	Microt	0.219
Caenis sp.	Caenis	-0.274	*Potamothrix* spp.	Potamo	0.221
Rhyacodrilus sp.	Rhyaco	-0.254	*Paratanytarsus* spp.	Parata	0.281
Paralauterborniella nigrohalteralis (Malloch, 1915)	P.nigro	-0.250	*Physa* spp.	Physa	0.325
Psammoryctides barbatus (Grube, 1861)	P.barb	-0.231	*Stictochironomus pictulus* (Meigen, 1830)	S.pict	0.332
Asellus aquaticus (Linnaeus, 1758)	A.aqua	-0.204	*Chironomus plumosus* (Linnaeus, 1758)	C.plum	0.346
Ceratopogonidae sp.	Cerato	-0.202	*Lymnaea* sp.	Lymnea	0.378
Paracladopelma spp.	Paracl	-0.197	*Sphaerium ovale* (Férussac, 1807)	S.ova	0.387
Slavina appendiculata (Udekem, 1855)	S.appe	-0.193	*Theodoxus fluviatilis* (Linnaeus, 1758)	T.fluv	0.432
Bichaeta sanguinea Bretscher, 1900	B.sang	-0.157	*Valvata piscinalis* (Müller, 1774)	V.pisc	0.438
Niphargus foreli Humbert, 1877	N.fore	-0.133	*Echinogammarus* spp.	Echino	0.499
Chironomus anthracinus Zetterstedt, 1860	C.anth	-0.117	*Dugesia* sp.	Dugesi	0.514
Lumbriculidae spp.	Lumbri	-0.090	*Paratendipes albimanus* (Meigen, 1818)	P.albi	0.576
Cryptochironomus spp.	Crypto	-0.082	*Bithynia tentaculata* (Linnaeus, 1758)	B.tent	0.659
Cladotanytarsus atridorsum Kieffer, 1924	C.atri	-0.065			

Tab. 6. Factor loadings of the second canonical variate (taxa set).

Chaoborus flavicans (Meigen 1830)	-0.519	*Uncinais uncinata* (Orsted, 1842)	0.302
Chironomus plumosus (Linnaeus, 1758)	-0.171	*Sphaerium ovale* (Férussac, 1807)	0.304
Tubifex tubifex (Müller, 1774)	-0.153	*Lymnaea* sp.	0.308
Lumbriculidae spp.	-0.141	*Rhyacodrilus* sp.	0.309
Ceratopogonidae sp.	-0.009	*Demicryptochironomus vulneratus* (Zetterstedt, 1838)	0.311
Bichaeta sanguinea Bretscher, 1900	0.004	*Echinogammarus* spp.	0.321
Niphargus foreli Humbert, 1877	0.044	*Stempellina* spp.	0.350
Asellus aquaticus (Linnaeus, 1758)	0.059	*Theodoxus fluviatilis* (Linnaeus, 1758)	0.359
Slavina appendiculata (Udekem, 1855)	0.094	*Orthocladius* spp.	0.361
Paracladopelma spp.	0.114	*Pisidium* spp.	0.363
Stylodrilus lemani (Grube, 1879)	0.154	*Parakiefferiella bathophila* (Kieffer, 1912)	0.371
Stylaria lacustris (Linnaeus, 1767)	0.162	*Valvata piscinalis* Müller, 1774	0.376
Endochironomus spp.	0.188	*Dugesia* sp.	0.386
Paralauterborniella nigrohalteralis (Malloch, 1915)	0.190	*Limnodrilus* spp.	0.386
Physa spp.	0.196	*Pagastiella orophila* (Edward, 1929)	0.387
Caenis sp.	0.216	*Chironomus anthracinus* Zetterstedt, 1860	0.394
Micronecta sp.	0.224	*Cladopelma* spp.	0.401
Nais elinguis Müller, 1774	0.225	*Branchiura sowerbyi* Beddard, 1892	0.412
Prodiamesa olivacea (Meigen, 1818)	0.228	*Micropsectra* spp.	0.432
Dero digitata (Müller, 1774)	0.228	*Dicrotendipes* spp.	0.437
Stictochironomus pictulus (Meigen, 1830)	0.248	*Paratendipes albimanus* (Meigen, 1818)	0.450
Aulodrilus spp.	0.256	*Cladotanytarsus atridorsum* Kieffer, 1924	0.470
Potamothrix spp.	0.258	*Pseudochironomus prasinatus* (Staeger, 1839)	0.486
Bothrioneurum vejdoskianum Stolc, 1886	0.264	*Bithynia tentaculata* (Linnaeus, 1758)	0.528
Psammoryctides barbatus (Grube, 1861)	0.268	*Polypedilum* spp.	0.562
Paratanytarsus spp.	0.273	*Cryptochironomus* spp.	0.565
Microtendipes spp.	0.274	Pentaneurini spp.	0.569
Spirosperma ferox Eisen, 1879	0.298	*Psectrocladius* spp.	0.580
		Procladius choreus (Meigen, 1804)	0.601

Tab. 7. BQIW of 57 taxa calculated on the basis of 1060 samples.

Chaoborus flavicans (Meigen, 1830)	1.000	*Stempellina* spp.	3.824
Chironomus plumosus (Linnaeus, 1758)	2.458	*Parakiefferiella bathophila* (Kieffer, 1912)	3.845
Asellus aquaticus (Linnaeus, 1758)	2.687	*Pagastiella orophila* (Edward, 1929)	3.851
Niphargus foreli Humbert, 1877	2.837	*Caenis* sp.	3.854
Slavina appendiculata (Udekem, 1855)	2.871	*Micronecta* sp.	3.873
Stylodrilus lemani (Grube, 1879)	3.102	*Dicrotendipes* spp.	3.905
Tubifex tubifex (Müller, 1774)	3.144	*Cladopelma* spp.	3.911
Ceratopogonidae spp.	3.183	*Orthocladius* spp.	3.923
Spirosperma ferox Eisen, 1879	3.196	*Cryptochironomus* spp.	3.927
Chironomus anthracinus Zetterstedt, 1860	3.352	*Polypedilum* spp.	3.983
Lumbriculidae spp.	3.359	*Nais elinguis* Müller, 1774	3.986
Psammoryctides barbatus (Grube, 1861)	3.369	*Pseudochironomus prasinatus* (Staeger, 1839)	3.994
Bichaeta sanguinea Bretscher, 1900	3.411	*Endochironomus* spp.	3.999
Prodiamesa olivacea (Meigen, 1818)	3.420	*Branchiura sowerbyi* Beddard, 1892	4.028
Paracladopelma spp.	3.455	Pentaneurini spp.	4.109
Potamothrix spp.	3.498	*Psectrocladius* spp.	4.140
Aulodrilus spp.	3.500	*Paratanytarsus* spp.	4.154
Demicryptochironomus vulneratus (Zetterstedt, 1838)	3.586	*Cladotanytarsus atridorsum* Kieffer, 1924	4.198
Uncinais uncinata (Orsted, 1842)	3.601	*Physa* spp.	4.272
Bothrioneurum vejdovskianum Stolc, 1886	3.608	*Lymnaea* sp.	4.393
Dero digitata (Müller, 1774)	3.622	*Dugesia* sp.	4.425
Pisidium spp.	3.661	*Valvata piscinalis* (Müller, 1774)	4.434
Rhyacodrilus sp.	3.723	*Paratendipes albimanus* (Meigen, 1818)	4.462
Paralauterborniella nigrohalteralis (Malloch, 1915)	3.724	*Echinogammarus* spp.	4.547
Limnodrilus spp.	3.724	*Stictochironomus pictulus* (Meigen, 1830)	4.549
Microtendipes spp.	3.747	*Bithynia tentaculata* (Linnaeus, 1758)	4.589
Procladius choreus (Meigen, 1804)	3.776	*Theodoxus fluviatilis* (Linnaeus, 1758)	4.591
Stylaria lacustris (Linnaeus, 1767)	3.795	*Sphaerium ovale* (Férussac, 1807)	4.809
Micropsectra spp.	3.802		

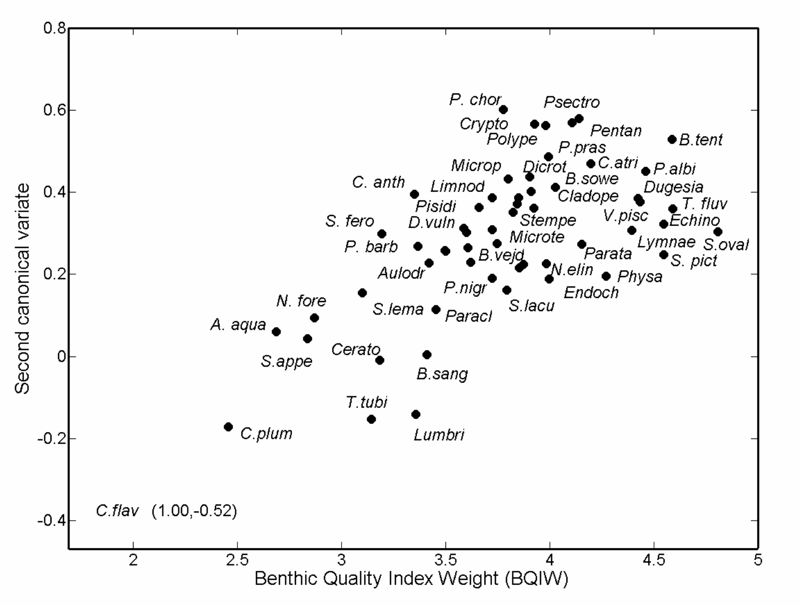

Fig. 3. Plot of BQIW against the factor loadings of the second canonical variate.

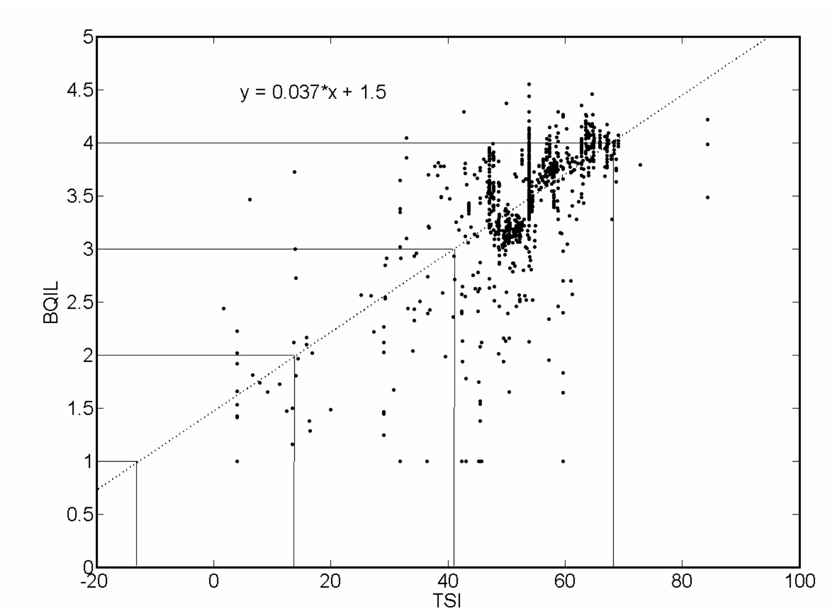

Fig. 4. Plot of the TSI value against the BQIL value calculated for each site. Minimum square line and linear correlation equation are reported.

BQIL was significantly correlated (p <0.001) with the second canonical axis (r = 0.598 and 1058 df): the relation between BQIW and the factor loadings of the second canonical variate are in figure 3.

BQIL was also significantly correlated (p <0.001) with the modified TSI (Fig. 4). The correlation coefficient was 0.656 with 1058 df. BQIL values could be tentatively assigned to 5 quality classes (see this paper, paragraph 3.1.). If we plot the number of sites in each class *vs* lakes (Fig. 5), most sites should be assigned to classes 2, 3, some in class 4, and very few are in classes 1 and 5. Most stations of the large lakes Maggiore and Garda were assigned to classes 2-3, as were the Mergozzo lake stations. Most stations of the volcanic lakes were in class 2; Varese, Annone and other small lakes had many stations in class 4.

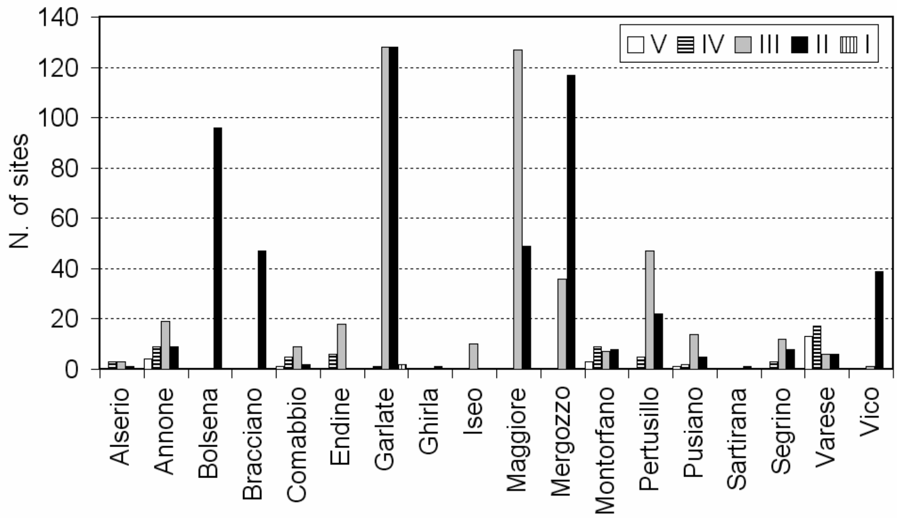

Fig. 5. Frequency of quality classes into which stations of different lakes were assigned according to BQIL values.

5. DISCUSSION

The benthic macroinvertebrates from Italian lakes were sampled since the '50s (Corbella *et al.* 1956; Nocentini 1979), but in Northern Italy few sampling campaigns were undertaken after the ’80s (Nocentini 1988, 1989, 1991; Bonacina *et al.* 1992).

The sampling effort varied considerably both in space and in time and often macroinvertebrate collection was not synchronous with water sampling. Rarely, samples were available for the same lake in different years (lakes Maggiore, Mergozzo and Varese); in these cases changes in community composition were detected (Nocentini 1979; Ruggiu & Saraceni 1972).

CANON was carried out as a preliminary multivariate analysis to emphasize the relationships between environmental variables and macrobenthos composition. The most significant results were that different lake types, with different morphology and water chemical composition, were responsible for the different distribution of macroinvertebrate taxa. Conductivity, alkalinity and pH were the environmental variables accounting for the largest source of variation in the first canonical variate. Transparency, nutrients and dissolved oxygen had the highest factor loadings in the second, and lake volume and depth contributed most to the third canonical variate.

A highly significant correlation coefficient between the BQIW and the second canonical variate was observed in the lakes investigated. The correlation between oxygen, phosphorus and transparency with the second variate justified the formulation of a single indicator weight (BQIW) that summarized the response of each taxon to lake trophic status (measured as TP and transparency) and oxygenation level without separating different lake types. This was only a very rough approximation because the interactions between lake's morphometry (volume, depth), natural chemical characteristics and anthropogenic factors are associated with a high number of potential macroinvertebrate colonizers, resulting in a quite variable and complex response. Seminara *et al.* (1990) and Borderelle *et al.* (2005) emphasized that the comparison between the littoral and the profundal communities were critical for developing a Biotic Index. Verneaux *et al.* (2004) stressed that a high content of allogenic matter in sediments can be present without high chlorophyll content in waters. The consequence is that very different lake types should require more sophisticated indexes that take into account different trophic or biogenic potential of the lake and the lake's ability to transfer available matter to consumers.

In the present paper BQIW characterized taxa, whereas BQIL characterized sites. The BQIL was used to assign different sites to different quality classes, as requested by the WFD. It must be emphasized that the assignment of BQIL into 5 quality classes must be considered very tentative, because of the heterogeneous database used. New and well planned sampling campaigns are required for the collection of new data from a larger spectrum of lake types to have a more extended range of variation to validate the indexes. In particular, the analysis of the response of deep communities in large profundal lakes (Maggiore, Como, Garda and Iseo) is needed, as is the response of Alpine lakes and brackish water ones.

The protocol developed for Italian lakes can obviously be extended to other countries. Attention should be paid to the choice of environmental variables that are aggregated to calculate BQIW. These variables should reflect the ability of species to transfer energy to different trophic levels in lakes of different natural and anthropogenic conditions.

ACKNOWLEDGEMENTS

This work was supported by: (I) European Commission Directorate General JRC Joint Research Centre Institute for Environment and Sustainability Inland and Marine Waters Unit contract No Imw-Eewai-20041119-1 "Support for a Research Project on the Assessment of the Ecological State of Lakes by Macroinvertebrates In Lombardy"; (II) Italian Murst First 2003-2005: "Taxonomy, Ecology, Biogeography of Diptera Chironomidae". A sincere acknowledgement is for A.M. Nocentini and C. Bonacina (CNR Institute for Ecosystem Study - Verbania Pallanza), and for G. Bonomi (University of Bologna), who contributed to the knowledge of benthos in Italian lakes.

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Received: January 2006
Accepted: March 2006

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