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A comparison of different biotic indices based on benthic macro-invertebrates in italian lakes

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ABSTRACT

Benthic macroinvertebrates samples were taken from Italian lakes with different geological, morphological and chemical characteristics. Thirty-two lowland small and large lakes sampled using a grab in soft substrate were selected to develop biotic indices. Diversity indices based on species numbers - abundances and indices using species sensitivity values were compared. The lakes selected were all situated in the Alpine Ecoregion below 800 m a.s.l. and had similar chemical composition but different levels of anthropogenic pressure. Lakes with data available in different years were included as separate lakes in the analysis; littoral-sublittoral samples of large lakes were also separated from profundal samples yielding a total of 41 sites for analysis. Seven different biotic indices were compared: (1) Shannon diversity index (H), (2) weighted Shannon diversity index (Hw) including in the calculation a sensitivity value assigned to each species, (3) a benthic quality index based on means of three different environmental variables, measuring trophic status, weighted by species abundances (BQITS), (4) an index based on weighted means using a larger set of environmental variables (BQIENV), (5) a modified BQITS, which included both species numbers and total abundance of individuals (BQIES), (6) an index calculated according to a rarefaction method (ES), (7) an index considering indicator species based on experts judgment (BQIEJ). The indices were compared with a trophic status index (TSI) constructed by joining three environmental variables: O_2% saturation in the hypolimnion during summer stratification, total phosphorous and transparency during full circulation. Comparisons were also made with another environmental stress index (ENI) constructed on a larger number of variables. All the biotic indices had significant correlations with both TSI and ENI. BQIES, WFD compliant and well correlated with TSI and ENI, was selected to tentatively assign the investigated lakes into 5 quality classes: high (H), good (G), moderate (M), poor (P) and bad (B). The statistical power of the classification was estimated. Assuming tentatively equal intervals for each of the five quality classes, 2 lakes were classified at high status, 7 lakes were classified as good, 13 were classified as moderate, 13 were classified as poor and 4 were classified as bad. Fifteen lakes were classified with a power less than 80%. Some of the lakes resampled in different periods displayed a shift of class in the different years. Future work should focus on extending the database to test the indices in other lake types subjected to different pressures.

Key words: Chironomidae, Oligochaeta, diversity, eutrophication, WFD 60/2000

1. INTRODUCTION

Benthic macroinvertebrates are well known indicators of ecological status both in marine and inland waters (Johnson *et al*. 1993). Benthic quality indices developed to classify lakes have been based on: 1) indicator values of a few selected species (Wiederholm 1980; Lafont 1991); 2) indicator values of chironomid (Sæther 1979) or oligochaete species (Lang 1990); 3) calculation of species numbers and abundances (Verneaux *et al*. 2004); 4) means of environmental variables, weighted by species abundances (Rossaro *et al*. 2007). Recently the multimetric approach was gaining favor among scientists (Blocksom *et al*. 2002). An application of RIVPACS methods to lakes was also attempted (Johnson 2003).

The European Union Water Framework Directive (WFD) requires the analysis of the taxonomic composition and abundance of benthic invertebrate fauna, a measure of the ratio of sensitive taxa to tolerant taxa and of diversity of invertebrate taxa. According to the WFD the ecological status of European rivers, lakes, transitional and coastal waters should be classified as: high (H), good (G), moderate (M), poor (P) or bad (B). So WFD compliant indices should be based on all three requirements: 1) species indicator values, 2) species abundances and 3) diversity and should be able to classify water bodies into five quality classes: H, G, M, P, B. The WFD also requires that the classification of ecological status be expressed as an ecological quality ratio (EQR). An EQR represents the relationship between the values of the biological parameters observed for a given surface water body type and the values for these parameters in reference conditions applicable to that body. The ratio shall be expressed as a numerical value between zero and one, with high ecological status represented by values close to one and bad ecological status by values close to zero.

With the aim of developing indices compliant with the WFD, many algorithms were proposed for marine communities (Rosenberg *et al*. 2004), and some of them (Leonardsson *et al*. 2009) are particularly attractive for freshwaters also.

The aims of this paper were: 1) to test the validity of classification of Italian lakes based on morphometrical, geographical and geological characteristics, for benthic macroinvertebrates and 2) to compare different benthic quality indices. In the present paper the focus will be on 32 Italian lakes within the Alpine Ecoregion, below 800 m a.s.l., for which a reasonably large database is available; in these lakes eutrophication is the most serious pressure.

2. STUDY SITE, SAMPLING AND METHODS

In compliance with the WFD, the Italian classification system B was defined according to morphometrical, geographical and geological characteristics and 18 types were defined (Buraschi *et al*. 2005; Tartari *et al*. 2006). An abbreviation was used to identify these lake types: AL was used for lakes belonging to the Alpine Ecoregion (above the 44° parallel of North latitude), ME for those belonging to the Mediterranean Ecoregion below the 44° parallel. Within each ecoregion different lake types were labeled with a different number added to the AL and ME abbreviations. The dataset used here included the abundances per m^2 of 62 species of benthic macroinvertebrates and environmental data from different lake types belonging to 4 of the 18 types. The lakes considered were all lowland lakes (lower than 800 m a.s.l.) situated in the AL Ecoregion. In the Alpine Ecoregion, AL3 represents large and deep lakes (Maggiore, Como, Iseo and Garda), these lakes were divided into littoral-sublittoral and profundal zones and analyzed as separate lakes, except Lake Como for which no data were available. AL5 are smaller lakes with mean depth lower than 15 m, and AL6 those with mean depth higher than 15 m; within the AL6 lake type Lake Mergozzo was also separated into littoral-sublittoral and profundal zones, because samples of both zones were available. For all the other small lakes, with a maximum depth lower than 50 m (with few exceptions) a true profundal zone was not present. AL4 are polymictic lakes less than 15 m mean depth (Tab. 1, Tab. 2). For a few lakes (Maggiore, Garda, Iseo, Varese, Annone Est and Endine) data were available for different periods and were treated as separate lakes.

The data available belong both to historical and recently collected samples. Historical samples refer to collections made mainly in the '60s, '70s and '80s, recent samples belong to the period 2000-to date (Rossaro *et al*. 2006, table 1; Lencioni *et al*. 2007; Rossaro *et al*. 2007, table 1; Marziali *et al*. 2008, table 1; Free *et al*. 2009). The complete database included 1063 soft substrate samples in areas free of macrophytes, with 203 samples collected with an Ekman (Ek), 467 with a Petersen (Pt), 393 with a Ponar (Pn) grab (Tabs 3a, b, c).

Tab. 1. The lakes investigated with abbreviations used in tables and figures; 50, 60, 70, 80, 00, 02, 05 represent decades or years sampled, 50 means 1950, 00 means 2000 etc. l = littoral (sublittoral), p = profundal, for example Merg70p means Lake Mergozzo sampled in 1970-1980 in the profundal zone.

Lake	abbreviation	Lake	abbreviation
Alserio	Als60	Idro	Idro
Annone Est	AnnE00	Iseo	Iseo60p
	AnnE60		Iseo08p
Annone Ovest	AnnO60	Lamar	Lamar
Avigliana Grande	AvigG	Lases	Lases
Avigliana Piccolo	AvigP	Levico	Levico
Caldaro	Caldar	Maggiore	Magg50p
Caldonazzo	Caldon		Magg60l
Canzolino	Canzol		Magg80l
Cavedine	Cavedi	Mergozzo	Merg70l
Comabbio	Comabb		Merg70p
Como	Como05	Monate	Monate
Endine	End70	Montorfano	Montor
	End00	Piano	Piano
Frassino	Frassi	Pusiano	Pusian
Garda	Garda70l	Sartirana	Sartir
	Garda70p	Segrino	Segrin
	Garda08l	Tenno	Tenno
Garlate	Garlat	Varese	Var02
Ghirla	Ghirla		Var05
		Viverone	Viver

3. DATA ANALYSIS

In 1063 samples 62 species were present in ≥5 samples and were selected for data analysis.

For the same sampling points 29 environmental variables were available (Tab. 4). The data were measured in the field during the sampling of benthic macroinvertebrates or were derived from the web site: http:// www. ise.cnr.it/limno/limno.htm.

The day of the year, photoperiod and air temperature were calculated (Marsili-Libelli 1989) using the sampling day, latitude and altitude of the sampling site.

Correlation coefficients were first calculated between all variables, then a correspondence analysis (CA) was performed using the $\log_{10}(x+1)$ transformed abundances per m^2 of benthic macroinvertebrate species found in the samples, with environmental variables included as passive variables. CANOCO 4.55® was used to perform calculations. CA was preferred to constrained ordinations such as canonical correspondence analysis (CCA) because the focus of the work was on the response of the benthic macroinvertebrate community and it was not desirable that the ordination should be influenced by the limited number of environmental variables available.

Tab. 2. The morphometric characteristics of the investigated lakes.

Lake	Latitude	Longitude	Altitude (m)	Catchment area (km^2)	Volume (m^3 10^6)	Lake surface (km^2)	Depth mean (m)	Depth max (m)
Alseri	45°48'15"	9°13'17"	260	18	6.81	1.26	5.0	8
AnnE	45°47'35"	9°19'26"	224	28	24.04	3.81	6.0	11
AnnO	45°48'50"	9°18'30"	224	15	6.80	1.70	4.0	10
AvigG	45°03'54"	7°23'12"	352	12	16.20	0.83	20.0	26
AvigP	45°03'54"	7°23'12"	352	8	4.50	0.58	8.0	12
Caldar	46°22'59"	11°16'12"	214	49	5.50	1.51	4.0	7
Caldon	46°01'12"	10°15'39"	450	48	140.40	5.38	26.0	49
Canzol	46°04'53"	11°13'37"	540	1	0.49	0.07	7.0	15
Cavedi	46°00'00"	10°57'02"	241	85	24.50	1.02	24.0	50
Comabb	45°46'18"	8°40'36"	243	15	16.50	3.58	5.0	8
Como	45°51'10"	9°15'30"	198	4508	22500.00	145.00	155.0	410
Endine	45°46'33"	9°56'25"	334	37	11.90	2.13	6.0	10
Frassi	45°39'51"	10°39'51"	74	6	2.42	0.30	8.0	15
Garda	45°41'00"	10°42'10"	65	2360	49030.00	368.00	133.0	346
Garlat	45°48'14"	9°21'30"	198	4552	70.00	4.53	15.0	34
Ghirla	45°52'60"	8°44'11"	442	15	3.00	0.25	12.0	14
IdroE	45°44'60"	10°26'11"	368	555	33.50	11.40	3.0	122
Iseo	45°39'27"	9°57'24"	186	1785	7600.00	61.00	125.0	251
Lamar	46°06'27"	10°34'55"	714	2	0.26	0.04	6.0	16
Lases	46°08'27"	11°13'11"	629	2	1.60	0.12	13.0	26
Sartir	45°43'00"	9°25'32"	318	0.83	0.15	0.11	1.4	3
Levico	46°00'40"	11°16'37"	440	27	20.00	1.16	17.0	38
Magg	45°53'16"	8°33'50"	198	6526	37125.00	213.00	176.0	370
Merg	45°57'17"	8°27'36"	194	10	83.00	1.83	45.0	73
Monate	45°46'41"	8°39'33"	266	6	45.00	2.51	18.0	34
Montor	45°46'17"	9°07'21"	397	2	1.90	0.46	4.0	7
Piano	46°02'13"	9°00'00"	276	26	4.60	0.63	7.0	13
Pusian	45°48'43"	9°16'15"	259	94	69.20	4.95	14.0	24
Segrin	45°49'38"	9°16'16"	374	3	1.20	0.38	3.0	9
Tenno	45°56'19"	10°47'30"	570	18	3.90	0.24	16.0	48
Varese	45°50'90"	8°40'14"	238	112	162.00	14.52	11.0	26
Vivero	45°24'00"	8°03'05"	230	21	125.00	5.78	22.0	50

Tab. 3a. Number of samples (n.s.) collected for each level of the 5 factors.

Lake type		Tool		Years		Months		Depth	
Lake	n. s.	Tool	n. s.	Years	n. s.	Months	n. s.	Depth (m)	n. s.
AL3	406	Ekman	203	50-'60	96	January	18	0-4.5	317
AL4	49	Petersen	467	60-'70	73	February	99	4.5-20	321
AL5	309	Ponar	393	70-'80	334	March	144	20-50	180
AL6	299			80-'90	58	April	216	50-150	106
				2002	16	May	62	>150	139
				2003	16	June	79		
				2005	367	July	59		
				2006	86	August	70		
				2007	8	September	159		
				2008	9	October	33		
						November	95		
						December	29		

Tab. 3b. Number of samples collected with each sampling tool at different depths.

Depth (m)	0-4.5	4.5-20	20-50	50-150	>150
Ekman	94	76	29	4	0
Petersen	39	68	119	102	139
Ponar	184	177	32	0	0

Tab. 3c. Number of samples collected with each sampling tool in different months and in different years.

Months	Ekman	Petersen	Ponar	Years	Ekman	Petersen	Ponar
January	7	11	0	50-'60	0	96	0
February	4	66	29	60-'70	41	32	0
March	24	27	93	70-'80	53	281	0
April	71	41	104	80-'90	0	58	0
May	28	27	7	2002	16	0	0
June	4	75	0	2003	16	0	0
July	9	46	4	2005	0	0	367
August	22	14	34	2006	67	0	19
September	1	51	107	2007	1	0	7
October	4	29	0	2008	9	0	0
November	17	66	12				
December	12	14	3				

Tab. 4. The environmental variable used, with unit of measures and abbreviations.

environmental variable	unit of measure	abbreviation
altitude	m	altit
watershed area	m^2 10^3	bac
volume	m^3 10^6	vol
surface area	m^2 10^3	sup
mean depth	m	depthmean
maximum depth	m	depthmax
depth (sampling point)	m	depth
pH (water column)		pHcolu
pH		pH
conductivity (water column)	µs cm^{-1}	condcolu
conductivity (hypolimnion)	µs cm^{-1}	condhypo
conductivity (sampling point)	µs cm^{-1}	cond
alkalinity	mg L^{-1} $CaCO_3$	alcal
permanent Inhabitants		hinp
temporary inhabitants		hinf
transparency (Secchi disk)	m	trasp
dissolved oxygen (water column)	mg L^{-1}	O_2colu
dissolved oxygen (hypolimnion)	mg L^{-1}	O_2hypo
dissolved oxygen (water column)	% saturation	O_2percsatcolu
dissolved oxygen (hypolimnion)	% saturation	O_2percsathypo
nitrogen (ammonia)	mg L^{-1}	NH4
nitrogen (nitrate)	mg L^{-1}	NO3
water temperature (sampling point)	°C	temp
total phosphorous (water column)	µg L^{-1}	TPcolu
total phosphorous (sampling point)	µg L^{-1}	TP
chlorophyll	µg L^{-1}	chla
day of the year	1-365	gganno
photoperiod	hours	fotop
air temperature (calculated from photoperiod)	°C	tempcalc

A factorial multivariate analysis of variance (MANOVA) (Morrison 1967) was carried out using benthic macroinvertebrates as dependent variables and lake types, sampling tools, sampling years, sampling month and depths as factors. The analysis was planned as an unbalanced block design (Searle 1987) because a different number of cells was available for each combination of factor levels (Tab. 3). MANOVA was carried out calculating the model and error sum of squares matrices; five models were tested, each model including one factor.

The multivariate test results were expressed: 1) as the highest eigenvalue from the product of the sum-of-squares matrix of the model (H) and the inverse of the sum-of-squares matrix of the errors (E^{-1}), 2) as trace of the same matrix (HE^{-1}) (Tab. 5) (Morrison 1967).

The same dataset was used to develop biotic indices.

Nine indices were calculated, two using environmental data, two using biological data alone and five using both.

A Trophic Status Index (TSI) was calculated as a mean of three parameters: the O_2 value expressed as % saturation in the hypolimnion during summer stratification (O_2percsat), transparency measured by Secchi disk (trasp), total phosphorous measured as mean value in the water column in the center of the lake during full circulation (TPcolu); the three parameters were rescaled

Tab. 5. Factorial multivariate analysis of variance results: largest eigenvalue (λ) and % trace of HE^{-1} grab samples.

Factor	λ	% trace	levels	Description
Lake type	2.473	10.867	4	AL3, AL4, AL5, AL6
Tool	2.540	8.867	3	Ekman, Petersen, Ponar grab
Year	6.816	17.731	10	'50 '60 '70 '80 '02 '03 '05 '06 '07 '08
Month	0.571	7.100	12	Months: January … December
Depth	1.907	8.040	5	m: 0-4.5, 4.5-20, 20-50, 50-150, >150

between 0 and 1 (Rossaro *et al*. 2006, 2007); because TPcolu increases with eutrophication the rescaled value was transformed calculating its complement to 1.

Another index (environmental stress index = ENI) was calculated using 11 environmental variables grouped into 2 sets according to their positive or negative influence on water quality.

A first group of environmental variables with values increasing with decreasing water quality was created including: 1) number of permanent inhabitants (hinp), 2) number of temporary inhabitants of the municipality (hinf) where the sampling site is located, 3) point measure of total phosphorous taken jointly with biological sampling (TP), 4) TPcolu, 5) ammonium (NH_4) and 6) chlorophyll-*a* (chl-*a*) concentration; these last 3 measures (4- 5- 6-) were mean values taken from the water column during full circulation.

A second group of variables included parameters increasing with water quality: 1) transparency (trasp), 2) dissolved oxygen measured as a mean of the water column at full circulation (O_2colu) and 3) dissolved oxygen measured in the hypolimnion at summer stratification (O_2hypo), both expressed as mean values in mg L^{-1}, the oxygen variables were also expressed as % saturation (variables 4), O_2percsatcolu, 5) O_2percsathypo); the weighted means calculated using the first set of variables (hinp, hinf, TPcolu, TP, NH_4, chl-*a*) were rescaled between 0 and 1 and transformed taking the complement to 1, so the highest values corresponded to the lowest values of the original variables and indicated good water quality, the means of the second set (trasp, four O_2 measures) were rescaled without taking the complement to 1, the highest values indicating good quality.

The pressure environmental index (ENI) was calculated as a mean value of the above environmental variables rescaled.

Seven biotic indices were calculated.

1) Shannon diversity index (*H*) (Magurran 1988), measuring species numbers and frequencies;

2) Weighted Diversity Index (*Hw*) including in the Shannon formula an indicator value (BQIWDIV) for each species (Ozzola *et al*. 1992):

$$H_w = \sum_{j=1}^{p} \left(\frac{y_{ij}}{\sum_{j=1}^{p} y_{i_j}} * \log_2 \frac{y_{ij}}{\sum_{j=1}^{p} y_{i_j}} * BQIWDIV_j \right) \quad (1$$

where y_{ij} is the number of individuals belonging to the species *j* in the site *i*, *p* is the number of the species present in a site and BQIWDIVj is the weight assigned to a species; it was based on expert judgment; it took into account the information that could be derived from both lotic and lentic waters data present in the database (Rossaro 1993; Marziali *et al*. 2010) and from historical data.

3) Benthic Quality Index calculated taking into account the response of species to O_2percsat, trasp, TP; the weighted means of each of these 3 parameters was calculated, using as weight the abundance of each species and then pooled to give a single sensitivity value to each species (BQIWTS), the sensitivity value was rescaled between 0 and 1 (Tab. 6, BQIWTS); a benthic quality index reflecting trophic status (BQITS) for each site was then calculated multiplying the log(x+1) of species abundances (x) in each site by the BQIWTS (Rossaro *et al*. 2006, Rossaro *et al*. 2007, 2009), divided by the total number of specimens (always log transformed) found in a site.

4) Another set of benthic quality index weights (Tab. 6, BQIWENV) was prepared starting from the weighted means calculated from the larger set of environmental variables used to calculate ENI; the biotic index in each site (BQIENV) was then calculated by multiplying the BQIWENV by log(x+1) of species abundances divided by the log transformed total number of specimens in a site, with the same algorithm used to calculate BQITS.

5) The BQITS and the BQIENV calculation (Rossaro *et al*. 2007) did not take into account the total fauna abundance, as required by the WFD, for this reason the index was modified including the number of species and the total faunal abundance. For benthic marine fauna an index was proposed (Rosenberg *et al*. 2004; Leonardsson *et al*. 2009), which calculated the sensitivity values using the Rarefaction method (Sanders 1968; Hurlbert 1971; Magurran 1988). It estimated the number of species expected in a sample if all samples were of a standard size; it was called ES_{50} because it was calculated assuming the samples composed of 50 individuals (Rosenberg *et al*. 2004).

Species tolerance values ($ES_{50,0.05}$) (Rosenberg *et al*. 2004), here renamed ESW, were calculated as the value of ES_{50} which separates 5% of the frequency distribution curve of each species. The sensitivity values of each species were then calculated assuming that the most tolerant species are associated to sites with the

Tab. 6. Sensitivity values of the 62 species; BQIWTS: sensitivity values of trophic status; BQIWENI: sensitivity values responding to more environmental variables, ESW: sensitivity values calculated from rarefaction method, BQIWEJ: sensitivity values based on expert judgment (see text for more explanation).

SPECIES	BQIWTS	BQIWENI	ESW	BQIWEJ	SPECIES	BQIWTS	BQIWENI	ESW	BQIWEJ
A. aquaticus	0.497	0.669	2.693	2	*P. acuta*	0.221	0.457	2.216	2.5
A. monilis	0.778	0.877	6.010	2.5	*P. albimanus*	0.615	0.727	4.019	3
A. pluriseta	0.513	0.628	4.126	2	*P. austriacus*	0.922	0.866	8.893	3
B. sanguinea	0.476	0.688	4.868	4	*P. barbatus*	0.387	0.536	3.000	4
B. sowerbyi	0.273	0.372	2.522	2	*P. bathophila*	0.702	0.749	6.222	4
B. tentaculata	0.480	0.631	2.770	2.5	*P. camptolabis*	0.691	0.830	4.615	2.5
B. vejdovskyanum	0.546	0.714	5.032	3	*P. casertanum*	0.431	0.572	1.986	2.5
C. annulator	0.779	1.000	8.578	2	*P. choreus*	0.380	0.486	2.652	1
C. anthracinus	0.494	0.572	2.282	2	*P. flavipes*	0.452	0.721	7.453	2.5
C. atridorsum	0.672	0.692	3.820	2.5	*P. hammoniensis*	0.186	0.316	2.529	1
C. defectus	0.459	0.512	2.998	2.5	*P. heuscheri*	0.245	0.498	3.031	1
C. flavicans	0.009	0.000	1.712	1	*P. nigritulum*	1.000	0.754	3.522	4
C. pallidula	0.612	0.707	4.761	2.5	*P. nigrohalteralis*	0.449	0.572	2.226	4
C. plumosus	0.055	0.124	1.990	1	*P. nubeculosum*	0.543	0.650	2.995	2
C. vermiformes	0.102	0.251	2.071	2	*P. olivacea*	0.457	0.504	3.303	4
C. viridulum	0.256	0.396	2.727	2.5	*P. orophila*	0.627	0.673	7.322	4
D. digitata	0.395	0.528	5.047	2.5	*P. oxyura*	0.598	0.703	2.943	2.5
D. nervosus	0.546	0.648	2.282	2.5	*P. prasinatus*	0.589	0.639	2.966	3
D. tigrina	0.383	0.523	2.967	2.5	*R. coccineus*	0.593	0.679	6.550	4
D. vulneratus	0.398	0.458	3.994	3	*S. bausei*	0.685	0.736	6.008	4
E. tetraedra	0.549	0.656	3.729	2.5	*S. ferox*	0.521	0.676	2.508	4
Echinogammarus	0.456	0.704	2.813	2	*S. heringianus*	0.452	0.630	3.061	3
G. pallens	0.014	0.000	2.000	2.5	*S. lacustris*	0.492	0.732	5.182	2.5
H. marcidus	0.467	0.616	13.377	4	*S. lemani*	0.497	0.668	2.538	3
H. stagnalis	0.564	0.744	5.143	2.5	*S. pictulus*	0.470	0.642	13.819	2
Hydracarina	0.503	0.531	2.893	2.5	*Sialis*	0.365	0.440	2.000	2.5
L. hoffmeisteri	0.294	0.426	2.309	1	*T. fluviatilis*	0.227	0.593	4.304	2.5
L. peregra	0.625	0.744	5.516	2.5	*T. gregarius*	0.538	0.675	2.927	3
M. atrofasciata	0.000	0.238	3.082	2.5	*T. tubifex*	0.262	0.350	2.000	1
M. nebulosa	0.465	0.618	10.068	4	*U. uncinata*	0.522	0.567	6.110	2.5
M. pedellus	0.372	0.613	2.796	2	*V. piscinalis*	0.511	0.633	2.000	2.5

lowest ES_{50} values and the most intolerant associated to sites with the highest ES_{50} (Tab. 6, ESW).

In this index only p of m species present in a site have a sensitivity value available. To account also for species not having a known sensitivity value, the total number of species and the total number of individuals divided by the total number of individuals + 5 was included; this ratio is about one when the number of individuals is large and becomes low when there are few individuals (Leonardsson *et al*. 2009). In this manner an estimation of the faunal total abundance and not only its composition is included in the index. The advantage of this index is that it does not need environmental measures to calculate the sensitivity values, because the sensitivity values are estimated from ES diversity of samples. The formula used (Leonardsson *et al*. 2009) was rewritten using different symbols:

$$ES_i = \left[\sum_{j=1}^{p} \left(\frac{y_{ij}}{\sum_{j=1}^{p} y_{i_j}} * ESW_j \right) \right] * \log_{10}(m+1) * \left(\frac{\sum_{j=1}^{m} y_{ij}}{\sum_{j=1}^{m} y_{ij} + 5} \right) \quad (2$$

where p is the number of species for which an indicator value was known and m the number of all the species; ESWj is the indicator weight assigned to species j calculated with the rarefaction method ($ES50_{0.05}$ in Rosenberg *et al*. 2004; "sensitivity value" in Leonardsson *et al*. 2009); the other symbols have the same explanation as in formula (1).

The European Union Water Framework Directive (WFD) states that tolerant and sensitive taxa must be considered along with diversity and abundance of benthic macroinvertebrates for measuring the status of the benthic habitat. All these requirements are satisfied in this index.

6) Instead of the ESW sensitivity values, BQIWTS and BQIWENV calculated as weighted means of environmental variables could be used in equation (2). So the calculation could be performed using a formula similar to equation (2), were a BQIW[TS] or [ENV] substitutes the ESW:

$$BQIES_i = \left[\sum_{j=1}^{p} \left(\frac{y_{ij}}{\sum_{j=1}^{p} y_{i_j}} * BQIW[TS]or[ENV]_j \right) \right] \quad (3$$

$$* \log_{10}(m+1) * \left(\frac{\sum_{j=1}^{m} y_{ij}}{\sum_{j=1}^{m} y_{ij} + 5} \right)$$

7) Lastly, an index (BQIEJ) was calculated using as species weights (BQIWEJ, Tab. 6), based on expert judgments. In this case only information derived from lentic waters (Sæther 1979; Wiederholm 1980; Lang 1990; Rossaro *et al*. 2000; Lods-Crozet & Reymond 2005) was used:

$$BQIEJ_i = \sum_{j=1}^{p}\left(\frac{y_{ij}}{\sum_{j=1}^{p} y_{i_j}} * BQIWEJ_j\right) \quad (4$$

The last step was to select the most appropriate index. Two criteria were considered to select the best index:

1) a criterion was to select the biotic index with the highest correlation with the anthropogenic impact; at present the most reliable measure of the anthropogenic impact is the TSI or ENI;

2) another criterion was to select an index fully compliant with the WFD.

The boundaries (L) between the 5 quality classes required by the WFD were defined assigning equal intervals to the 5 quality classes; so the following boundaries between classes were considered: 0.8 High - Good, 0.6 Good - Moderate, 0.4 Moderate - Poor, 0.2 Poor - Bad. These boundaries were proposed as tentative, prior to an intercalibration with other EU member states and further data collection.

The statistical power of classification was estimated assuming a non central t distribution of values, using as non-centrality parameter δ the difference between the observed mean m and the lower boundary L nearest to the observed values, the difference being rescaled by dividing it by the standard error of mean ($s/\sqrt{n}$), where n is the number of samples and s the standard deviation of the measures (Carstensen 2007):

$$\delta = \frac{(m-L)}{s/\sqrt{n}} \quad (5$$

The δ value was calculated from sample mean (m), standard deviation (s) and number of samples (n) available within each lake. The non-central t distribution was built around the δ value. The cutoff separated 20% area of the central t student distribution and established the extension of the power area 1-β, were the power is the probability that the decision rule rejects the null hypothesis when the alternative hypothesis is true (Winer 1962). In the present case it is the probability to correctly assign a lake to the class above the boundary, when the index value calculated is above this boundary.

Microsoft ACCESS 2010 (MSA)® was used to store information (Rossaro *et al*. 2001). Data were processed with Matlab R2010b®. Matlab scripts and functions performing the calculations of indices are available on request to the first author.

4. RESULTS

Some of the 62 species analysed had a positive correlation with depth: *Stylodrilus lemani*, *Potamothrix heuscheri*, *Psammoryctides barbatus*, *Spirosperma ferox*, *Stylodrilus heringianus*, *Bichaeta sanguinea*, the same species had an inverse correlation with water temperature and number of inhabitants. Other species were positively correlated with the number of inhabitants and water temperature: *Chaoborus flavicans*, *Chironomus plumosus*, *Glyptotendipes pallens*, Ceratopogonidae *"vermiformes"*, *Tubifex tubifex* and *Branchiura sowerbyi*. The same species except *B. sowerbyi* were correlated with conductivity, the first 3 species were also correlated with TP.

A correspondence analysis with 1063 records as samples and 62 species as variables was carried with environmental variables as passive variables.

The species characteristic of profundal stations had high factor loadings in the first axis and were plotted on the top right (Fig. 1). The species characteristic of oligotrophic condition had negative loadings in the second axis and were plotted on the bottom, in agreement with correlation results.

The first axis separated profundal stations from littoral-sublittoral ones, whereas the second axis separated lakes with different trophic status. Profundal stations were plotted on the top right, eutrophic lakes were plotted on the top left (Fig. 2). For reason of clarity the mean values of the stations belonging to the same lake are plotted, but a high scatter between stations of the same lake was sometimes observed (lakes Mergozzo and Maggiore).

The morphometrical parameters were correlated with the first axis and were related to lake size and were plotted on the top right. Environmental variables measuring trophic status were correlated with the second axis and were plotted on the top left. Dissolved oxygen was inversely correlated with the second axis (Fig. 3).

A factorial MANOVA was carried out with the same 62 species as dependent variables and 5 factors as criterion variables. The factors were: lake type, sampling tool, sampling year or decade, season, depth. The number of samples available in each cell are in table 3.

Years were responsible of the largest source of variation, months accounted for the smallest source, whereas all the other sources of variation were intermediate as indicated by the eigenvalues and by the trace (Tab. 5).

Using the same dataset a trophic status index (TSI), an environmental stress index (ENI) and 7 biotic indices were calculated. Correlations between all indices were then examined.

All the indices had a highly significant correlation to each other and with the TSI and ENI index (Tab. 7), but ES, Shannon, Shannon weighted and BQIEJ indices had lower correlation values, even if always highly significant.

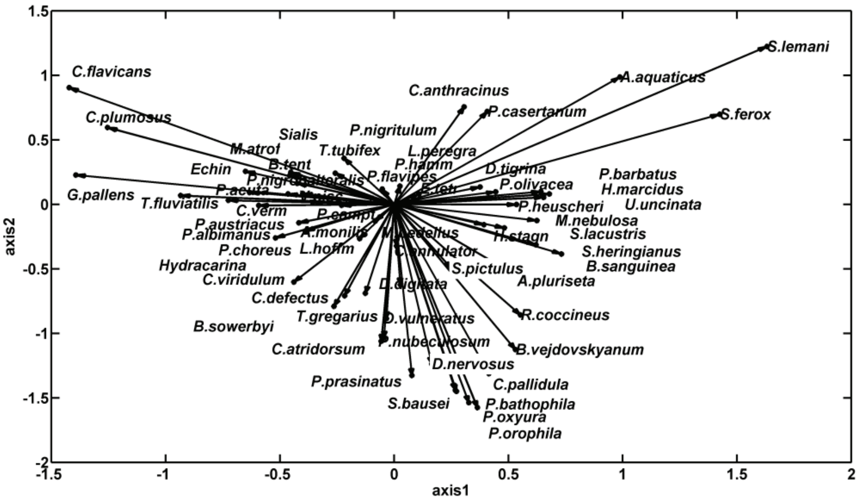

Fig. 1. Correspondence analysis results in the plan of the first two axes, species scores.

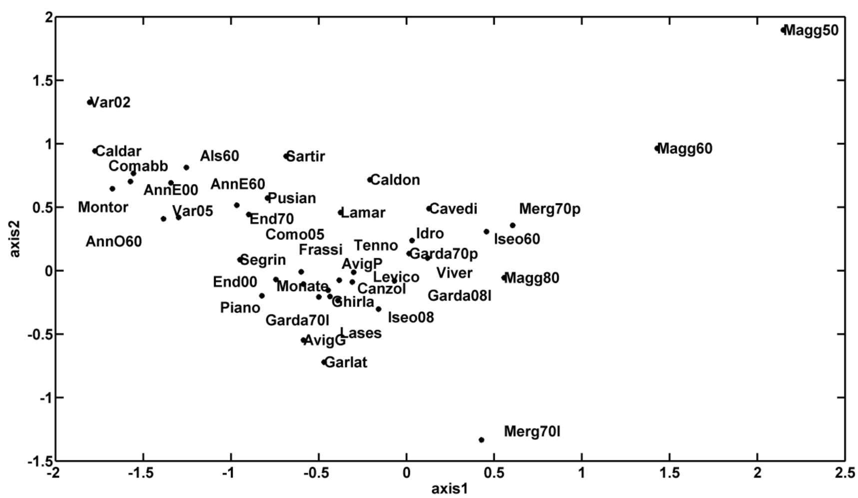

Fig. 2. Correspondence analysis results in the plan of the first two axes, sites scores (mean values of all sites from each lake).

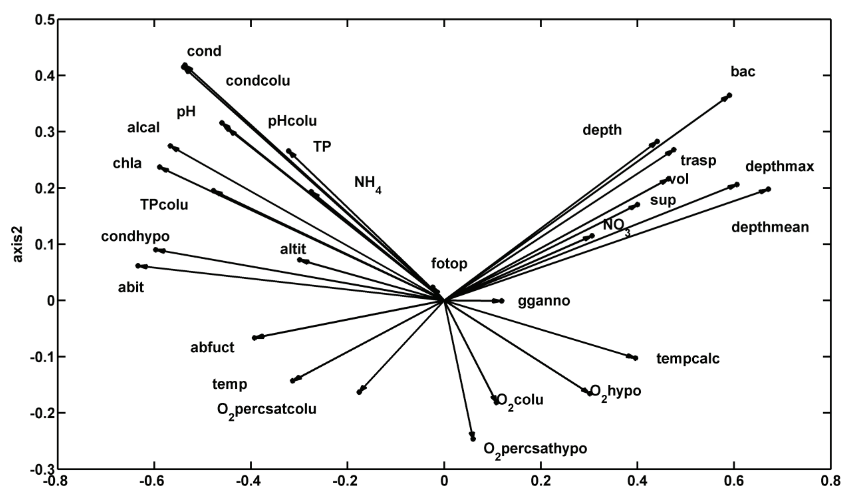

Fig. 3. Correspondence analysis results in the plan of the first two axes, environmental variables (included as passive variable).

Tab. 7. Correlation between indices, TSI: trophic status index, ENI: environmental index based on a larger number of variables; BQITS: benthic quality index based on trophic status; BQIENV: benthic quality index based on more environmental variables, BQIES: benthic quality index based on trophic status, including an abundance factor; ES: index based on rarefaction method, H: Shannon diversity; Hw: weighted Shannon diversity; BQIEJ: index based on expert judgment (see text for more explanation).

	TSI	ENI	BQITS	BQIENV	BQIES	ES	H	Hw	BQIEJ
TSI		0.847	0.641	0.561	0.593	0.429	0.310	0.454	0.435
ENI	p<0.01		0.671	0.610	0.607	0.419	0.292	0.429	0.456
BQITS	p<0.01	p<0.01		0.930	0.940	0.617	0.401	0.607	0.727
BQIENV	p<0.01	p<0.01	p<0.01		0.985	0.683	0.416	0.612	0.782
BQIES	p<0.01	p<0.01	p<0.01	p<0.01		0.685	0.415	0.613	0.768
ES	p<0.01	p<0.01	p<0.01	p<0.01	p<0.01		0.624	0.741	0.585
H	p<0.01	p<0.01	p<0.01	p<0.01	p<0.01	p<0.01		0.922	0.301
Hw	p<0.01	p<0.01	p<0.01	p<0.01	p<0.01	p<0.01	p<0.01		0.538
BQIEJ	p<0.01	p<0.01	p<0.01	p<0.01	p<0.01	p<0.01	p<0.01	p<0.01	

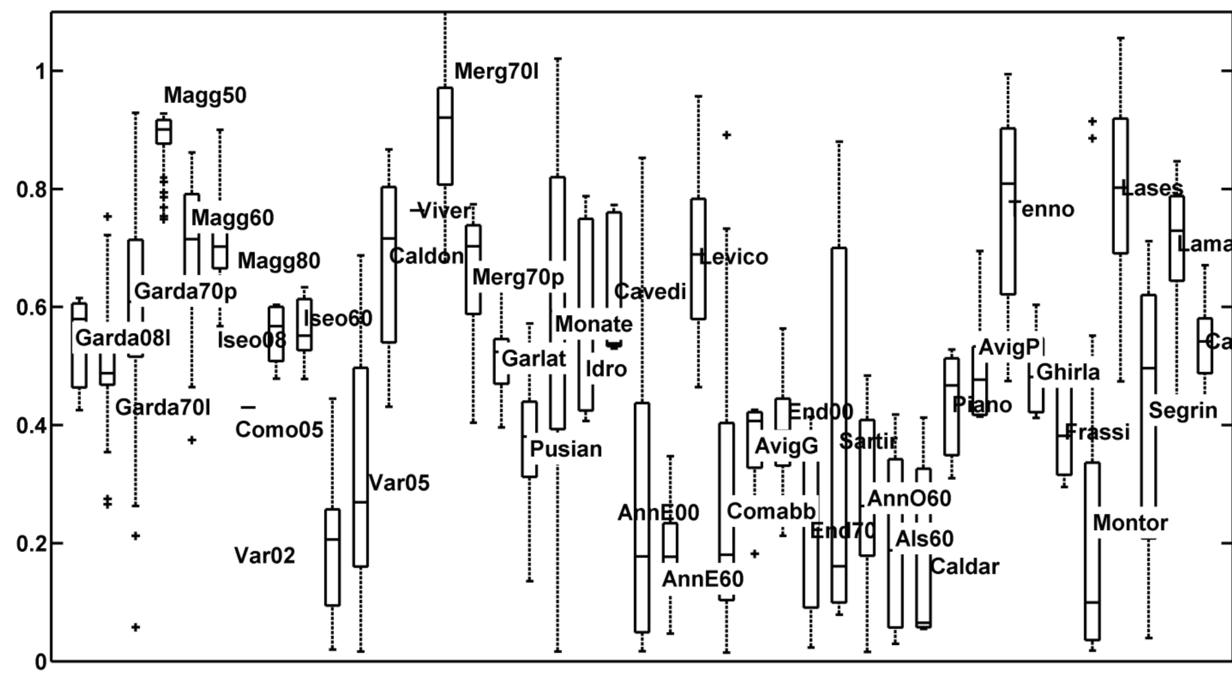

Fig. 4. Boxplot of benthic quality index BQIES in different lakes, median (bar in rectangles), 25 and 75 percentile (rectangles), maximum and minimum values (whiskers), outliers are expressed as + sign.

BQITS was the index with the highest correlation with TSI and ENI (Tab. 7), but it was not selected because it was not compliant with the WFD (see Data analysis). Observing table 7 it is possible to see that biotic indices calculated using weighted means of environmental variables (BQITS, BQIENV, BQIES) were highly correlated, so the selection of BQIES was justified, because it satisfied all WFD criteria and was in any case well correlated with the indicators of trophic status (TSI) and environmental stress (ENI).

Except the Shannon diversity index, all the other indices needed the calculation of a sensitivity value for each species; these values are reported in table 6.

The indices were correlated directly with dissolved oxygen and inversely correlated with total phosphorous, chlorophyll-*a* and conductivity. The diversity indices were less correlated with environmental variables indicative of trophic status (O_2, trasp, TP, chl-*a*) and environmental pollution. In any case the indices gave comparable values in different lakes.

The highest values (>0.8) were observed for Lake Mergozzo, followed by Maggiore, Tenno and Lases, the lowest values were observed for lakes Caldaro, Montorfano, Sartirana, Annone Est, Alserio, Varese, and Comabbio (Fig. 4). In figure 4 the BQIES values of all the 41 sites are given.

An exponential fitting between TSI and BQIES (R^2 = 0.38) was observed; the exponential function fitted better than the linear one (R^2 = 0.36). Despite the relation some lakes, Lake Mergozzo in particular, showed

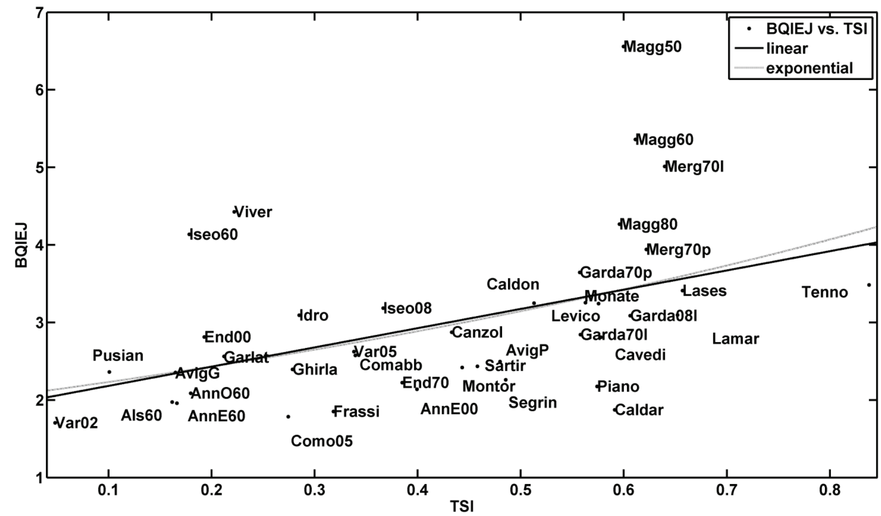

Fig. 5. Plot of benthic quality index BQIEJ against TSI.

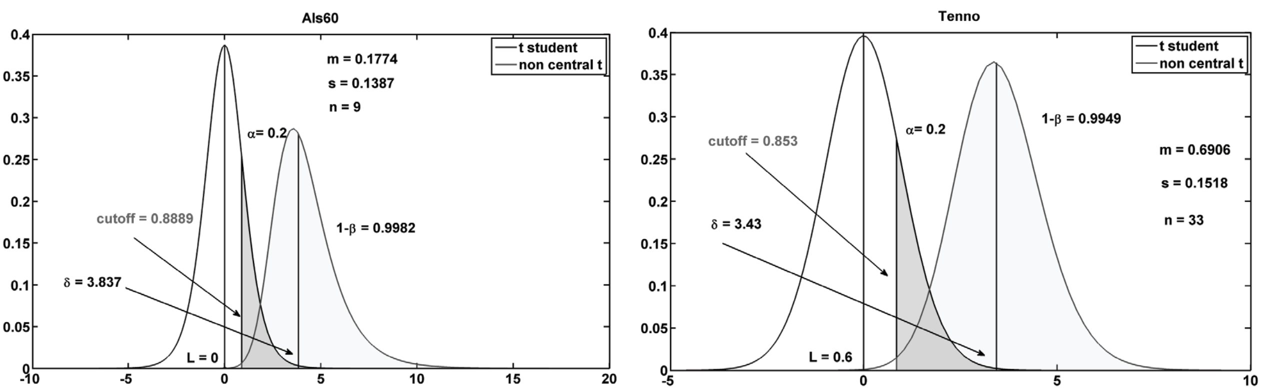

Fig. 6. Classification of the Lake Alserio (left) and Lake Tenno (right); L: boundary between two quality classes, cutoff: value of the abscissa separating the area α, which is the probability to reject the null hypothesis H_0 when it is true (α = 20% in the present case); δ: non centrality parameter, that is the mean value of the alternative hypothesis expressed in standard units; 1-β: probability to reject the null hypothesis when the alternative hypothesis is true, that is the power of the test (see text for other explanations).

BQIES values higher than expected on the basis of TSI values, whereas other lakes such as Caldaro, Montorfano, Endine, Annone Est and Comabbio had lower values than expected. It is emphasized that these lakes have a faunal composition not easily predictable from the environmental variables measured. It was probable that other factors not measured and not used to calculate the sensitivity values were responsible for the observed deviation from the regressed values. The plot of BQIEJ against TSI (Fig. 5) emphasized the higher values of BQIEJ with respect to the values predicted by TSI in lakes Mergozzo and Maggiore and the lower than expected values in many other lakes (Caldaro, Como, Piano, Annone Est, Segrino and Frassino). It may be considered that BQIES was correlated with TSI because BQIES was derived from weights calculated using the same environmental data used to calculate TSI, but similar results were obtained with BQIEJ, based only on expert judgment without considering TSI data in its calculation. BQIEJ was well related with TSI, according to an exponential function (R^2 = 0.22), again a bit better than a linear one (R^2 = 0.21).

The rescaled BQIES values and the boundaries (L), as defined in Data analysis, allowed the 41 sites to be assigned to different quality classes. The mean values of BQIES obtained for each site, the standard deviation and the number of samples available allowed the calculation of the statistical power of the classification; central and non central t distribution with non centrality values δ and the power of test calculated for lakes Alserio and Tenno (Fig. 6) are given as examples; results for all the other lakes are in table 8.

In table 8 the mean values, the number of samples and the standard deviations of BQIES indices values estimated in all stations of each of the 41 sites are reported. On the basis of the mean value each lake was assigned to a quality class, each defined with an upper and a lower boundary (L). In the last 2 columns there is the power of each classification and the quality class assigned. Only 2 lakes were assigned to high (H) class

Tab. 8. BQIES benthic quality index rescaled between 0 and 1; m: mean, n: number of samples; std: standard deviation; L: lower boundary between classes; pw: statistical power; C=class: H high, G good, M moderate, P poor, B bad; italics: lakes assigned with a power less than 80%; underlined: lakes not classified.

Lake	m	n	std	L	pw	C
Caldar	0.150	3	0.187	0	66	B
AnnE60	0.159	9	0.079	0	100	B
Var02	0.164	32	0.105	0	100	B
Als60	0.177	9	0.139	0	100	B
Montor	0.203	21	0.249	0.2	22	P
End70	0.204	18	0.118	0.2	25	P
AnnE00	0.213	76	0.193	0.2	40	P
Comabb	0.220	40	0.201	0.2	42	P
AnnO60	0.240	12	0.135	0.2	57	P
Var05	0.270	25	0.183	0.2	86	P
Pusian	0.317	10	0.122	0.2	98	P
AvigG	0.322	8	0.084	0.2	100	P
Sartir	0.329	3	0.405	0.2	37	P
End00	0.339	10	0.095	0.2	100	P
Frassi	0.344	5	0.075	0.2	100	P
Segrin	0.377	36	0.209	0.2	100	P
Como05	0.381	1	0.000	0.2		
Piano	0.386	3	0.103	0.2	97	P
Ghirla	0.437	5	0.072	0.4	60	M
AvigP	0.448	6	0.099	0.4	62	M
Garda70l	0.457	24	0.112	0.4	95	M
Garlat	0.458	14	0.067	0.4	99	M
Garda08L	0.482	3	0.092	0.4	71	M
Canzol	0.484	12	0.063	0.4	100	M
Iseo08	0.496	4	0.054	0.4	99	M
Iseo60	0.502	9	0.050	0.4	100	M
Idro	0.528	10	0.145	0.4	97	M
Monate	0.529	31	0.253	0.4	98	M
Garda70p	0.543	222	0.125	0.4	100	M
Cavedi	0.562	5	0.116	0.4	98	M
Merg70p	0.594	26	0.100	0.4	100	M
Levico	0.616	29	0.132	0.6	42	G
Caldon	0.617	24	0.126	0.6	43	G
Magg60l	0.624	23	0.120	0.6	55	G
Magg80l	0.636	24	0.074	0.6	94	G
Lamar	0.636	36	0.090	0.6	94	G
Viver	0.688	1	0.000	0.6		
Tenno	0.691	33	0.152	0.6	99	G
Lases	0.723	36	0.140	0.6	100	G
Magg50P	0.802	96	0.037	0.8	36	H
Merg70l	0.810	69	0.092	0.8	52	H

(Maggiore-profundal '50 and Mergozzo-littoral '70, but with a power less than 80%, 7 lakes were assigned to good (G) quality class (Levico, Caldonazzo, Maggiore–littoral '60, Maggiore-littoral '80, Lamar, Tenno and Lases), but 3 of them (Maggiore-littoral '60, Caldonazzo, Levico) were assigned with a power less than 80%. Thirteen lakes were assigned to the moderate quality class (M) (Ghirla, Avigliana Piccolo, Garda-littoral '70, Garlate, Garda-littoral '08, Canzolino, Iseo-profundal '08, Iseo-profundal '60 , Idro, Monate, Garda-profundal '70, Cavedine, Mergozzo-profundal '70), but 3 lakes (Ghirla, Avigliana Piccolo, Garda-littoral '80) were assigned with a power lower than 80%. Thirteen lakes were assigned to the poor (P) quality class (Montorfano, Endine '70, Annone Est '00, Comabbio, Annone Ovest '60, Varese '05, Pusiano, Avigliana Grande, Sartirana, Endine '00, Frassino, Segrino, Piano), 6 lakes with a power less than 80%. Caldaro, Annone Est '60, Varese '02 and Alserio '60 were assigned to the bad (B) quality class. For lakes Como and Viverone the number of samples was not enough to allow any classification.

5. DISCUSSION

The species composition of benthic macroinvertebrates is conditioned by natural factors at different spatial scale: physical and chemical variables (substrate, conductivity, hardness, water temperature), littoral and riparian habitat, lake morphometric parameters (Free *et al*. 2009) and may be responsible for quite different community composition. It was emphasized that factors such as water temperature, chemistry, including anthropogenic stress factors were responsible for the observed differences in benthic macroinvertebrate composition in Italian lakes (Rossaro *et al*. 2006, 2007).

Water quality modified by anthropogenic influences can remodel the community, with the disappearance of sensitive species and the appearance of tolerant ones. This concept is well expressed in the WFD stating that the ecological status must be determined only as a displacement from a reference condition (EQR).

For this reason it is expected that benthic macroinvertebrates communities differ in different lake types, but a classification of lake types does not necessarily agree using geomorphological, chemical and biological descriptors. Furthermore, it was underlined that classifications generated with different biological descriptors (phytoplankton, aquatic macrophytes, benthic macroinvertebrates and fishes) do not necessarily agree with each other (Johnson & Hering 2009).

To investigate the factors most responsible for benthic macroinvertebrate variation a CA and a MANOVA were carried out with 62 species of macroinvertebrates.

MANOVA emphasized that different lake types, grab type used, sampling years, sampling months and depth were all significant, but differences among years were the most important source of variation and differences among months the lowest.

Different tools were used in different periods and at different depths, so it was not easy to separate the importance of sampling tool with respect to the periods investigated and depth.

For lakes Mergozzo, Maggiore, Iseo and Garda profundal stations were separated from the littoral- sublittoral stations, but no species positively correlated with depth was exclusive to the profundal zone. Even if CA and factorial MANOVA confirmed the importance of depth on benthic macroinvertebrate distribution, depth was inversely correlated with water temperature and variation of species distribution with depth was dependent on oxygen concentration also; so it can be inferred that depth *per se* is not a critical factor. For these reasons it was decided to include in the present analysis the profundal zone of some lakes.

CA emphasized that lakes Mergozzo and Maggiore, all sampled with a Petersen grab in the '70s, were responsible for a large proportion of the observed dispersion. That is samples collected in the same lake and in the same period were responsible for a large source of variation (not evident in figure 2 because only mean values were given).

A long term trend could not be detected, because data collected from the same lake over a long period were rarely available. Examining the few data available and the CA results an impairment can be observed in Lago Maggiore from '50 to '60-'80, whereas lakes Annone Est and Endine displayed an amelioration from '60 to 2000. Recent data from Lago Maggiore using samples collected by scuba divers suggest an amelioration in recent years, but they were not included in the present analysis because of the different sampling tools (unpubl. data).

Excluding alpine types: AL1, AL2, AL7, AL8 and Mediterranean lakes (ME) and including only AL3, AL4, AL5 and AL6 lakes for the calculation of biotic indices reduced the sources of variation bound to natural factors, so anthropogenic influences could be better detected.

The rationale of the indices development was based on the general consensus that diversity is often a measure of ecosystem status, another assumption was that species respond to environmental gradients according to a Gaussian law of tolerance and optimum response can be measured with weighted means (Ter Braak & Prentice 1988). With these principles seven different biotic indices were tested. Two indices (H and ES) were based on species diversity, they do not need environmental variables to be measured; only species composition and abundances were required in their calculation; for this reason these two indices are very useful when synchronous environmental and biological data are not available.

Three indices (BQITS, BQIENV, BQIES) were based on weighted means of species with environmental variables; they have the advantage that their response is focused on the environmental variables (pressures) used to calculate the weights, so these indices are more related to specific pressures. In the present case the indices were based on species response to eutrophication pressure (BQIWTS) or on a combined set of variables (BQIWENV). The drawback was that they require that both environmental pressure measures and benthic macroinvertebrate counts be available. BQIEJ and Hw were based on expert judgment so they had the advantage of not being influenced by possible weaknesses present in the environmental measures available.

Hw indices are weighted diversity indices, their interest is bound to the fact that they are a measure of diversity, but include also sensitivity values of each species to stressors. The use of expert judgment is suggested in the WFD and may be a valid alternative when the environmental data available are not synchronous in space and time with biological data. In the present case the expert judgment was based on the information present in the copious literature about benthic macroinvertebrate responses to eutrophication (Johnson *et al*. 1993). The information about the response of benthic macroinvertebrates to toxicants is still scant, while attention is increasingly focusing on the response of macroinvertebrates to hydromorphological alteration (Solimini *et al*. 2006).

Rescaling the values between 0 and 1 implies that the database included both reference sites and the species characterizing them, no sensitive species being overlooked. If sensitive species were not included in the database, the indices will be too much optimistic, giving values overestimating the quality of a water body. It was also assumed that lakes in the worst condition were included in the database, these lakes should have a 0 value of indices and should be inhabited by species with sensitivity value near to 0. We are confident that these conditions were fulfilled by the present database.

Data analysis emphasized that all the indices (including the indices which did not use environmental variables in their calculation, H, Hw, ES, BQIEJ) were all correlated with physico-chemical measures of eutrophication (oxygen, transparency and total phosphorous), with the trophic status index (TSI) which summarizes the three indicators and with ENI, which summarized a larger set of environmental variables; the biotic indices were also correlated to each other, the H index being the least correlated. Some discrepancy in the response of the indices was found for some lakes: Lake Mergozzo showed higher values of all indices than the ones expected on the basis of TSI or ENI index; the reverse was observed for lakes Caldaro and Annone Est, with values of all the indices lower than expected. The intensive sampling of Lake Mergozzo (108 samples) may be responsible at least in part for the high number of species observed (44) and for the high value of all indices observed in this lake. Lake Caldaro results were possibly biased by the low number of samples available (3), in this lake the presence of few tolerant species (*Chaoborus flavicans* and *Limnodrilus hoffmeisteri*) with a low indicator weight lowered the index value; the introduction of an alien fish (*Ctenopharyngodon idella*) in the 70's could be an explanation of the low BQIES value observed, despite a relative high TSI value. In Lake Annone Est the biotic indices were low despite the 87 samples available, probably other pressures not measured by TSI are responsible for this result and will require further investigation.

The biotic indices allowed the tentative assignment of the lakes to five different quality classes. A statistical power test calculated the uncertainty of the assignment of each lake to a class; the highest uncertainty source was bound to the low number of samples available (lakes Caldaro and Sartirana with 3 samples), but it was not the case of lakes Annone Est '00 and Endine '70, where the observed value near the boundary was probably the reason for uncertainty. It must be emphasized that the results given in table 8 are very tentative, because for many lakes the small number of samples or the high variability within each lake suggests caution in translating these results into management criteria. The weakest point is the arbitrariness in defining the class boundaries L; an intercalibration exercise will be needed to better define these boundaries. Furthermore, additional data should be collected to allow boundaries to be established at points of ecological change to match normative definitions as laid out in Annex 5 of the WFD.

The WFD at point 1.3 (iii) suggests that type-specific biological reference conditions may be based on modeling or on expert judgment; at point 1.3 (v) predictive models or hindcasting methods with the use of historical data are also suggested. In the present analysis the use of historical data from lakes with low anthropogenic impact at the time of sampling (Mergozzo and Maggiore in '50, '60) suggest that at least some reference sites were included in the database, allowing the expression of the indices as ecological quality ratios (EQR).

In any case it is important for a future development of benthic macroinvertebrate indices to improve the database by collecting samples with a standard protocol, in strictly defined lake zones, including reference sites, with standardized sampling methods and with enough replicates, sampling different seasons, to produce robust indices sensitive to anthropogenic stress.

Future needs are also to test indices with other pressures (hydromorphological alteration, toxic substances) and in all lake types. An accurate taxonomic revision for a better definition of species sensitivity values to be included in the database is also recommended. For what concerns taxonomy it is well known that species belonging to the same genus have different sensitivity values, as evidenced by both Chironomidae (Rossaro *et al*. 2000) and Oligochaeta (Lang 1990). Unfortunately often larvae cannot be identified to species. To overcome this drawback samples of adults and pupal exuviae associated to larval collections, examination of the karyotype (in *Chironomus*) aid in species identification. It is recommended to encourage expertise in taxonomy, to improve the identification keys actually available for benthic macroinvertebrate species. The use of larger taxonomic groups should be avoided because often in this manner we lose the indicator value of the species.

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