A benthic quality index for European alpine lakes

Bruno Rossaro[1], Angela Boggero[2], Brigitte Lods-Crozet[3], Gary Free[4], Valeria Lencioni[5], Laura Marziali[6] and Georg Wolfram[7]

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The development of benthic quality indices for European lakes is hindered by the lack of information concerning many national lake types and pressures. Most information is from north European lakes stressed by acidification and from deep lakes subjected to eutrophication; for other lake types (the ones included in the Mediterranean areas for example) and for other pressures (hydro-morphological alteration, toxic stress) there is practically no information about the response of benthic macro-invertebrates; this hinders the possibility of an intercalibration of the indices among the member states (MS) in the EU. In the present communication three benthic quality indices are proposed considering the littoral, sublittoral and profundal zone in 5 reference and 7 non reference lakes from the Alpine region in response to eutrophication. The sensitivity values of the 177 species found in these lakes were calculated taking a weighted average of the values of environmental variables from lakes in which the species were present. The indicator taxa which prevailed in these lakes were Chironomids and Oligochaetes. A coinertia analysis emphasized the importance of trophic variables (transparency, nitrates, total phosphorous) in explaining the species distribution, but geographic (altitude) and morphometric (depth, volume) variables were also important. The indices enabeled a separation of reference from non-reference lakes and to assign the non-reference lakes to different quality classes in agreement with the Water Framework Directive.

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1. DeFENS - Department of Food Environmental and Nutritional Sciences, Università degli Studi di Milano, Via Celoria 2 - I 20133 Milano, Italy
2. CNR-ISE - Istituto per lo Studio degli Ecosistemi, Largo V. Tonolli 50, I-28922 Verbania Pallanza, Italy
3. Service des Eaux, Sols et Assainissement, Chemin des Boveresses 155, CH-1066 Epalinges, Switzerland.
4. Aquatic Environment, Environmental Protection Agency, Mc Cumiskey House, Richview, Clonskeagh Road, Dublin 14, Ireland.
5. MTSN - Museo Tridentino Scienze Naturali, via Calepina 14, I-38122 Trento, Italia
6. CNR-IRSA - Water Research Institute, U.O.S. Brugherio, Via del Mulino 19, 20861 Brugherio (MB), Italy
7. DWS Hydro Oekologie GmbH, Zentagasse 47, A-1050 Vienna, Austria

Corresponding author: Bruno Rossaro
E-mail: bruno.rossaro@unimi.it

INTRODUCTION

Benthic macroinvertebrates were extensively used as indicators of ecological status in lakes. Naumann (1921), Lenz (1925), Lundbeck (1936), Thienemann (1953) and Brundin (1949) observed a distribution of different chironomid species according to depth, oxygen saturation and trophic conditions. Brundin (1974) revised the state of knowledge about the indicator value of chironomids in the zoobenthos. Oligochaetes

(Lang 1990) together with chironomids were another well represented taxonomic group in the soft bottom substrates of lakes used in bio-indication. Indicator values were assigned to different species of both groups (Brinkhurst 1974; Sæther 1979; Wiederholm 1980). Kansanen et al. (1984), Aagaard (1986), Johnson et al. (1993), Rossaro et al. (2006, 2007, 2011) and many others have made contributions to the use of benthic macroinvertebrates as indicators in lakes, using sophisticated multivariate statistical analysis.

According to the assessment criteria outlined in the European Union's Water Framework Directive (WFD) the taxonomic composition and abundance of benthic invertebrate fauna, the ratio of sensitive to tolerant taxa and the diversity of invertebrate fauna must be recorded. Five ecological status classes must be defined: high (H), good (G), moderate (M), poor (P) and bad (B). The ecological status should be expressed as an ecological quality ratio (EQR), that is a ratio between the observed value and a reference value.

With the aim of developing indices compliant with the WFD, many algorithms were proposed (Wiederholm 1980; Johnson et al. 1993; Rosenberg *et al.* 2004; Leonardsson *et al.* 2009) and were reconsidered for use in lake monitoring (Rossaro 2011).

The aims of this paper were: 1) to propose sensitivity values for benthic macroinvertebrate species calculated from a database including both reference and non-reference lakes, 2) to test if species with different sensitivity values characterized reference and non-reference lakes, 3) to calculate different indices including or excluding the calculated sensitivity values, 4) to compare different benthic quality indices with multivariate analysis (coinertia) results, 5) to assign non-reference lakes to different quality classes, 6) to calculate the uncertainty of classification.

MATERIALS AND METHODS

Study site, sampling and methods

Twelve lakes in the Alps were selected for the present study: 5 reference lakes all located in Austria and 7 non reference lakes all located in Northern Italy. At present no lake was selected in Italy satisfying the criteria to be considered a reference lake. The lakes considered are all in the Alpine (AL) Ecoregion. All these lakes belong to the intercalibration L_AL-3 group. For Italian lakes system B was used to classify lake types according to morphometrical, geographical and geological characteristics (Buraschi *et al.* 2005; Tartari *et al.*, 2006): according to this system the Italian lakes belong to AL-3, AL-6, AL-9 and AL-10 groups. Details of the geographical and chemical characteristics of the investigated lakes are on the web sites: http://www.ise.cnr.it/limno/limno.htm for Italian sites and from http://www.lebensministerium.at for Austrian sites; some features are summarized in Table 1 (morphometric characters) and in Table 2 (chemical data). The 5 Austrian lakes were considered reference sites from the trophic point of view according to criteria defined by Austrian Lebensministerium. In these lakes sites classified in an almost "natural" condition were found.

The data available belong to samples collected during full circulation in spring and during stratification in summer in the period between 2006 and 2010. The Austrian lakes were sampled in late spring 2008. The database includes soft substrate samples in areas free of macrophytes, collected with an Ekman (Ek) grab mostly in the littoral and sublittoral with few profundal stations in Como and Iseo.

Data analysis

In 148 samples 177 species were present in ≥ 5 samples and were selected for data analysis.

Table 1. The lakes investigated, morphometric characteristics. Abbreviations: bac, watershed area; vol, volume; altit, altitude; depthm, lake mean depth; depth, minimum and maximum depth sampled.

Lake	Lake code	latitude N	longitude E	bac m^2 10^6	vol m^3 10^6	altit m	depthm m	depth m	Reference
Altausseer See	Alta	47°38'26"	13°47'07"	55	73	712	35	7-50	Y
Anterselva	Ante	46°53'08"	12°09'58"	20	9	1642	22	1-29	N
Braies	Brai	46°41'35"	12°05'38"	20	9	1642	22	4.5-17	N
Como	Como	45°48'17"	9°04'51"	4508	22500	198	155	4-100	N
Faaker See	Faak	46°34'45"	13°56'37"	36	35	555	16	4-26	Y
Grundlsee	Grun	47°38'05"	13°52'01"	125	169	708	41	6-59	Y
Iseo	Iseo	45°39'27"	9°57'24"	1785	7600	186	125	15-130	N
Levico	Levi	46°02'48"	11°26'41"	27	20	440	19	.5-28	N
Monate	Mona	45°47'58"	8°40'06"	6	45	266	15	5-30	N
Viverone	VLan	45°52'20"	8°25'02"	21	128	529	23	.8-40	N
VordLangbath	Viver	47°50'04"	13°41'01"	12	5	664	15	5-30	Y
Weißensee	Weis	46°43'27"	13°21'15"	50	226	929	37	3-27	Y

Table 2. The lakes investigated: chemical characteristics: mean values of all the sampled period. Abbreviations: alk, alkalinity; cond, conductivity; TP, total phosphorus; O_2, dissolved oxygen; trasp, transparency; Chla, chlorophyll-a; NO_3, nitrates.

Lake	alk mgl^{-1} $CaCO_3$	cond µScm^{-1}	TP µgl^{-1}	O_2 mgl^{-1}	O_2 % sat	trasp m	Chla µgl^{-1}	NO_3 µgl^{-1}
Altausseer See	80	147	4	12.00	100.00	8.70	1.20	430
Anterselva	25	97	9	3.75	25.50	8.39	3.68	103
Braies	23	97	4	2.49	14.41	9.21	2.53	99
Como	69	172	20	9.69	80.25	4.00	2.65	859
Faaker See	150	351	6	10.00	80.00	3.50	1.50	240
GrundlSee	95	221	3	12.00	100.00	9.60	0.93	400
Iseo	46	221	60	0.80	6.30	3.50	2.40	609
Levico	126	266	29	5.23	46.01	5.00	7.22	224
Monate	74	122	8	5.15	62.49	8.60	8.10	158
Viverone	101	227	68	6.62	47.64	2.50	7.25	64
VordLangbath	120	260	5	8.00	70.00	11.40	1.80	400
Weißensee	140	292	5	12.00	100.00	9.50	1.10	50

For the same sampling points 13 environmental variables were considered (Table 1-2). Latitude and longitude were not included in data analysis while depth of sampling station was. Environmental data were collected in the field during the sampling of benthic macroinvertebrates, while morphometric data were taken from the web site: http://www.ise.cnr.it/limno/limno.htm for Italian sites and from http://www.lebensministerium.at for Austrian sites.

A coinertia analysis (COI) (Dolédec & Chessel, 1994) was performed relating log10 (x+1) transformed abundances per m^2 of the 177 benthic macro-invertebrate species and the 13 environmental variables. The program R (version 2.14.0, R Development Core team, Vienna, Austria) with the package ADE-4 was used to perform calculations. COI was preferred to other multivariate analysis, because it is able to relate species abundances with environmental variables using datasets in which the species number is larger than the number of observations (Dray et al 2003).

The same dataset was used to develop biotic indices. Four indices were calculated, one using only environmental data, two using environmental and biological data, one using biological data only. These indices were described in a recent paper (Rossaro et al., 2011). In the present paper only a short explanation of the indices used is given.

Indices based on environmental data alone:

1. TSI: Trophic Status Index (*TSI*) calculated as an average of three variables: dissolved oxygen in mgl^{-1}, transparency measured by Secchi disk in m, total phosphorus (TP) in µgl^{-1}; the variables were rescaled between 0 and 1; because TP increases with eutrophication it was inverted and rescaled before being used in the average calculation.

Three biotic indices were calculated.

1- *H*: Shannon diversity index (*H*) (Magurran 1988);

2- BQIES was calculated with the following formula:

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

where y_{ij} is the number of individuals belonging to the species *j* in the site *i*, In this formula *p* is the number of species for which a sensitivity value is known, *m* the number of all the species present, including the ones for which no sensitivity value is known. The last term is about one when the number of specimens in a sample is large, while it is significantly less than one when there are few specimens in a sample, in this manner the index is sensitive to total abundance also, as required by WFD. $BQIW_j$ was calculated as weighted means of the three environmental variables used to calculate TSI; 3- as an alternative a *BQIEJ* index can be calculated with the same formula used for *BQIES* using new weight, which could be assigned to each species according to the expert judgment ($BQIWEJ_j$) (Sæther 1979; Wiederholm 1980; Lang 1990; Rossaro *et al.* 2000; Lods-Crozet & Reymond 2005); it took into account the information derived from both lotic and lentic waters, when available in http://www.freshwaterecology.info/; the values are in agreement with the saprobic index (si); when si was not available, weights (= sensitivity values) were based on the information present in an ACCESS database filed by the first author.

To classify lakes according to the WFD it was necessary to define boundaries (L) between 5 quality classes. The definition of L was based on the correlation of *BQIES* with *TSI* trophic status.

The *TSI* values calculated from the 12 lakes were divided into 5 equal intervals (with boundaries L equal to 0.2, 0.4, 0.6 and 0.8) and the corresponding *BQIES* values were defined as boundaries of benthic macroinvertebrate classes. The

Table 3a. The species found (except chironomids) and their sensitivity values (*BQIWTS*). Tolint: T, tolerant; I, intolerant species (see text).

Order	Family	Genus	Species	Author	Code	*BQIWTS*	Tolint
Hydrozoa	Hydrozoa	*Hydra*	sp.		Hydra	0.996	I
Turbellaria		*Triclada*	sp.		Triclad	0.662	
		Dugesia	*tigrina*	(Girard, 1850)	D.tigri	0.619	
		Polycelis	*nigra*	(Müller, 1774)	P.nigra	0.519	
Nematoidea	Mermitidae		sp.		Mermith	0.451	T
Nematoda			sp.		Nematod	0.796	I
Oligochaeta	Naididae	*Amphichaeta*	sp.		Amphich	0.872	I
		Chaetogaster	*diaphanus*	(Gruithuisen, 1828)	C.diaph	0.660	
		Chaetogaster	*langi*	(Bretscher, 1896)	C.langi	0.765	I
		Nais	*barbata*	(Müller, 1774)	N.barba	0.186	
			bretscheri	(Michaelsen, 1899)	N.brets	0.694	I
			communis	Piguet, 1906	N.commu	0.618	
			elinguis	Müller, 1774	N.eling	0.777	I
			pseudobtusa	(Müller, 1774)	N.parda	0.555	I
			simplex	Piguet, 1906	N.simpl	0.735	I
		Stylaria	*lacustris*	(Linnæus, 1767)	S.lacus	0.627	
		Ophidonais	*serpentina*	(Müller, 1774)	O.serpe	0.440	
		Dero	*digitata*	(Müller, 1774)	D.digit	0.201	T
		Uncinais	*uncinata*	(Orsted, 184)	U.uncin	0.807	
		Vejdovskyella	*intermedia*	(Bretscher, 1896)	V.inter	0.919	I
	Tubificidae	*Psammoryctides*	*albicola*	(Michaelsen, 1901)	P.albic	0.996	I
			barbatus	(Grube, 1861)	P.barba	0.255	
		Spirosperma	*ferox*	(Eisen, 1879)	S.ferox	0.386	
		Embolocephalus	*velutinus*	(Grube, 1879)	E.velut	0.000	T
		Aulodrilus	*pluriseta*	(Piguet, 1906)	A.pluri	0.306	
		Branchiura	*sowerbyi*	Beddard, 1892	B.sower	0.458	
		Tubifex	*ignotus*	(Stolc, 1886)	T.ignot	0.517	
			tubifex	(Müller, 1774)	T.tubif	0.495	
		Limnodrilus	*claparedianus*	Ratzel, 1869	L.clapa	0.555	I
			hoffmeisteri	Claparède, 1862	L.hoffm	0.265	
			profundicola	(Verrill, 1871)	L.profu	0.262	T
			udekemianus	Claparède, 1826	L.udeke	0.180	
		Ilyodrilus	*templetoni*	(Southern, 1909)	I.templ	0.510	
		Potamothrix	*bedoti*	(Piguet, 1916)	P.bedot	0.075	T
			hammoniensis	(Michaelsen, 1901)	P.hammo	0.255	
			heuscheri	(Bretscher, 1900)	P.heusc	0.396	T
			vejdovskyi	(Hrabe, 1941)	P.vejdo	0.000	T
	Haplotaxidae	*Haplotaxis*	*gordioides*	(Hartmann, 1821)	*H.*gordi	0.099	
	Lumbriculidae	*Stylodrilus*	*heringianus*	Claparède, 1862	S.herin	0.278	
	Enchytraeidae		sp.		Enchytr	0.602	
		Cernosvitoviella	*atrata*	(Bretscher, 1903)	C.atrat	0.991	I
	Lumbricidae	*Eiseniella*	*tetraedra*	(Savigny, 1826)	E.tetra	0.068	T
Rhyncob-dellida	Glossiphoniidae	*Helobdella*	*stagnalis*	(Linnæus, 1758)	*H.*stagn	0.111	T
		Glossiphonia	*complanata*	(Linnæus, 1758)	G.compl	0.410	T
	Hirudinidae	*Limnatis*	*nilotica*	(Savigny, 1822)	Limnati	0.411	T
	Erpobdellidae	*Erpobdella*	*octoculata*	(Linnæus, 1758)	E.octoc	0.572	
		Dina	*lineata*	(O.F.Muller, 1774)	D.linea	0.106	T
Peracarida	Asellidae	*Asellus*	*aquaticus*	(Linnæus, 1758)	A.aquat	0.560	
	Gammaridae	*Gammarus*	*fossa rum*	(Koch, in Panzer, 1835)	G.fossa	0.956	I
		Gammarus	*lacustris*	Sars, 1836	G.lacus	0.997	I
		Gammarus	*roeseli*	Gervais, 1835	G.roese	0.555	I
		Echinogammarus	*stammeri*	(Karaman S., 1931)	E.stamm	0.019	T

Continued on next page.

Table 3a. Continued.

Order	Family	Genus	Species	Author	Code	*BQIWTS*	Tolint
Plecoptera	Leuctridae	*Leuctra*	sp.		Leuctra	0.997	I
	Nemouridae	*Nemoura*	sp.		N.morto	0.848	
Epheme-roptera	Baetidae	*Baetis*	*rhodani*	(Pictet, 1843)	B.rhoda	0.406	
		Centroptilum	*luteolum*	(Muller, 1776)	C.luteo	0.799	I
	Caenidae	*Caenis*	*horaria*	(Linnæus, 1758)	C.horar	0.755	
			luctuosa	(Burmeister, 1839)	C.luctu	0.855	I
	Ephemeridae	*Ephemera*	*danica*	Müller, 1764	E.danic	0.936	I
	Leptophlebiidae	*Habrophlebia*	*lauta*	Eaton, 1884	*H.*lauta	0.899	I
		Leptophlebiidae	sp.		Leptoph	0.567	I
Odonata	Anisoptera		sp.		Anisopt	0.944	I
	Corduliidae	*Somatochlora*	*metallica*	(Vander Linden, 1825)	S.metal	0.895	I
	Gomphidae	*Gomphus*	*vulgatissimus*	(Linnæus, 1758)	G.vulga	0.765	I
	Libellulidae	*Libellulidae*	sp.		Libellu	0.834	I
	Platycnemididae	*Platycnemis*	*pennipes*	(Pallas, 1771)	P.penni	0.623	I
Hemiptera	Corixidae	*Micronecta*	*poweri*	(Douglas & Scott, 1869)	M.power	0.925	I
		Micronecta	sp.		Microne	0.835	I
Neuroptera	Megaloptera	*Sialis*	*lutaria*	(Linnæus, 1758)	S.lutar	0.711	
Trichoptera	Ecnomidae	*Ecnomidae*	sp.		Ecnomus	0.481	T
	Hydroptilidae	*Hydroptila*	sp.		Hydropt	0.995	I
		Oxyethira	sp.		Oxyethi	0.961	
	Leptoceridae	*Athripsodes*	sp.		Athrips	0.872	I
			aterrimus	(Stephens, 1836)	A.aterr	0.982	I
			cinereus	(Curtis, 1834)	A.ciner	0.881	I
		Leptoceridae	sp.		Leptoce	0.768	I
		Mystacides	*azureus*	(Linnæus, 1761)	M.azure	0.645	I
	Psychomyiidae	*Tinodes*	*waeneri*	(Linnæus, 1758)	T.waene	0.765	I
	Limnephilidae	*Limnephilidae*	sp.		Limneph	0.715	T
	Polycentro-podidae	*Cyrnus*	*trimaculatus*	(Curtis, 1834)	C.trima	0.794	I
		Polycentropus	sp.		Polycen	0.530	
	Serico-stomatidae	*Sericostoma fla-vicorne personatum*	sp.		Sericos	0.870	
	Chaoboridae	*Chaoborus*	*flavicans*	(Meigen, 1830)	C.flavi	0.403	
	Ceratopogonidae	*Ceratopogonidae*	sp.		C.vermi	0.706	
		Bezzia	sp.		Bezzias	0.763	I
		Dasyhelea	sp.		Dasyhel	0.715	I
Coleoptera	Haliplidae	*Haliplus*	sp.		Haliplu	0.826	I
	Dytiscidae	*Dytiscus*	*marginalis*	Linnæus, 1758	D.margi	0.809	I
		Graptodytes	sp.		Graptod	0.995	I
		Graptodytes	*pictus*	(Fabricius, 1787)	G.pictu	0.997	I
		Platambus	*maculatus*	(Linnæus, 1758)	P.macul	0.995	I
	Hydrophilidae	*Hydrophilidae*	sp.		Hydropo	0.997	I
Hydrachnidia	Hydrachnidia	*Hydracarina*	sp.		Hydraca	0.750	
Bivalvia	Dreissenidae	*Dreissena*	*polymorpha*	(Pallas, 1771)	D.polym	0.267	
	Pisidiidae	*Pisidium*	*casertanum*	(Poli, 1791)	P.caser	0.604	
			milium	Held, 1836	P.miliu	0.572	I
			subtruncatum	Malm, 1855	P.subtr	0.672	I
Prosobranchia	Valvatidae	*Valvata*	*piscinalis*	(Müller OF, 1774)	V.pisci	0.478	
Pulmonata	Bithyniidae	*Bithynia*	*tentaculata*	(Linnæus, 1758)	B.tenta	0.290	
	Lymnaeidae	*Lymnaea*	sp.		Lymnaea	0.408	T
	Planorbidae	*Gyraulus*	*albus*	(Müller, 1774)	G.albus	0.553	

Table 3b. The chironomid species found and their sensitivity values (*BQIWTS*). Tolint: T, tolerant; I, intolerant species (see text).

Tribe	Genus	Species	Author	Code	BQIWTS	Tolint
TANYPODINI	*Tanypus*	*punctipennis*	(Meigen, 1818)	T.punct	0.245	T
PROCLADIINI	*Procladius*	*choreus*	(Meigen, 1804)	P.chore	0.428	
MACROPELOPIINI	*Macropelopia*	*nebulosa*	(Meigen, 1818)	M.nebul	0.673	
COELOTANYPODINI	*Apsectrotanypus*	*trifascipennis*	(Zetterstedt, 1838)	A.trifa	0.917	
PENTANEURINI	*Ablabesmyia*	*longistyla*	Fittkau, 1962	A.longi	0.742	
		monilis	(Linnæus, 1758)	A.monil	0.590	
	Thienemannimyia	*carnea*	(Fabricius, 1805)	T.carne	0.997	I
	Pentaneurella	sp.		Pentane	0.320	T
	Zavrelimyia	sp.		Zavreli	0.995	I
	Larsia	*atrocincta*	(Fittkau, 1962)	L.atroc	0.998	I
	Conchapelopia	*pallidula*	(Meigen, 1818)	C.palli	0.458	T
PROTANYPINI	*Protanypus*	*morio*	(Zetterstedt, 1838)	Protany	0.566	I
PRODIAMESINI	*Prodiamesa*	*olivacea*	(Meigen, 1818)	P.oliva	0.446	
ORTHOCLADIINI	*Orthocladius*	sp.		Orthocl	0.748	I
	Psectrocladius	*limbatellus*	(Holmgren, 1869)	P.P.lim	0.555	I
		psilopterus	(Kieffer, 1906)	P.P.psi	0.699	I
	Synorthocladius	*semivirens*	(Kieffer, 1909)	S.semiv	0.886	I
	Paratrichocladius	*rufiventris*	(Meigen, 1830)	P.rufiv	0.704	T
	Paracladius	*conversus*	(Walker, 1856)	P.conve	0.775	I
	Cricotopus	*albiforceps*	(Kieffer in Thienemann & Kieffer, 1916)	C.albif	0.642	I
		annulator	Goetghebuer, 1927	C.annul	0.768	I
		fuscus	(Kieffer, 1924)	C.fuscu	0.633	
	Cricotopus(Isocladius)	*reversus*	Hirvenoja, 1973	I.rever	0.875	I
METRIOCNEMINI	*Heterotrissocladius*	*marcidus*	(Walker, 1856)	H.marci	0.684	
	Epoicocladius	*flavens*	(Malloch, 1915)	E.flave	0.961	I
	Parakiefferiella	*bathophila*	(Kieffer, 1912)	P.batho	0.810	I
		coronata	(Edwards, 1929)	Parakie	0.815	I
		gracillima	(Kieffer, 1924)	P.graci	0.765	I
CORYNONEURINI	*Corynoneura*	*lacustris*	Edwards, 1924	C.lacus	0.762	I
TANYTARSINI	*Stempellinella*		Brundin, 1947	Stempel	0.555	I
	Stempellinella	*minor*	(Edwards, 1929)	S.minor	0.555	I
	Stempellina	*bausei*	(Kieffer, 1911)	S.bause	0.712	I
	Constempellina	*brevicosta*	(Edwards, 1937)	C.brevi	0.562	I
	Tanytarsus	*bathophilus*	Kieffer, 1911	T.batho	0.794	I
		brundini	Lindeberg, 1963	T.brund	0.676	I
		ejuncidus	(Walker, 1856)	T.ejunc	0.794	I
		gregarius	Kieffer, 1909	T.grega	0.673	
		recurvatus	Brundin, 1947	T.recur	0.671	I
	Cladotanytarsus	*atridorsum*	Kieffer, 1924	C.atrid	0.506	
		mancus	(Walker, 1856)	C.mancu	0.753	I
	Paratanytarsus	*austriacus*	(Kieffer in Albrecht, 1924)	P.austr	0.766	
		bituberculatus	(Edwards, 1929)	P.bitub	0.680	I
		laccophilus	(Edwards, 1929)	P.lacco	0.995	I
		lauterborni	(Kieffer, 1909)	P.laute	0.731	T
	Micropsectra	*atrofasciata*	(Kieffer, 1911)	M.atrof	0.552	
		contracta	Reiss, F., 1965	M.contr	0.028	T
	Pagastiella	*orophila*	(Edwards, 1929)	P.oroph	0.843	
PSEUDO-CHIRONOMINI	*Pseudochironomus*	*prasinatus*	(Stäger, 1839)	P.prasi	0.637	
CHIRO-NOMINI		sp.		CHIRONO	0.802	I
	Chironomus	*anthracinus*	Zetterstedt, 1860	C.anthr	0.406	
		lacunarius	Wülker, 1973	C.lacun	1.000	I
		plumosus	(Linnæus, 1758)	C.plumo	0.224	T

Continued on next page.

Table 3b. Continued.

Tribe	Genus	Species	Author	Code	BQIWTS	Tolint
TANYPODINI	*Tanypus*	*punctipennis*	(Meigen, 1818)	T.punct	0.245	T
		lateralis	(Spies & Sæther, 2004)	C.later	0.657	I
		viridulum	(Linnæus, 1767)	C.virid	0.372	T
	Cryptochironomus	*defectus*	(Kieffer, 1913)	C.defec	0.340	
	Cryptotendipes	*pseudotener*	(Goetghebuer, 1922)	C.pseud	0.795	
	Demicrypto-chironomus	*vulneratus*	(Zetterstedt, 1838)	D.vulne	0.627	
	Dicrotendipes	*modestus*	(Say, 1823)	D.modes	0.337	
	Einfeldia	*pagana*	(Meigen, 1838)	E.pagan	0.654	
	Endochironomus	*tendens*	(Fabricius, 1775)	E.tende	0.555	I
	Glyptotendipes	*paripes*	(Edwards, 1929)	G.parip	0.765	I
	Microchironomus	*tener*	(Kieffer, 1818)	M.tener	0.236	T
	Microtendipes	*chloris*	(Meigen, 1818)	M.chlor	0.589	I
		pedellus	(De Geer, 1776)	M.pedel	0.247	
	Paracladopelma	*camptolabis*	(Kieffer, 1913)	P.campt	0.273	
		nigritulum	(Goetghebuer, 1942)	P.nigri	0.946	I
	Paralauterborniella	*nigrohalteralis*	(Malloch, 1915)	P.nigro	0.604	
	Paratendipes	*albimanus*	(Meigen, 1818)	P.albim	0.267	
	Phaenopsectra	*flavipes*	(Meigen, 1818)	P.flavi	0.613	
	Polypedilum	*nubeculosum*	(Meigen, 1804)	P.nubec	0.564	
	Polypedilum (Tripodura)	*bicrenatum*	Kieffer, 1921	P.T.bic	0.940	
		pullum	(Zetterstedt, 1838)	P.T.pul	0.999	I
		scalaenum	(Schrank, 1803)	P.T.sca	0.576	I
	Sergentia	sp.	Kieffer, 1922	Sergent	0.633	
	Stictochironomus	*pictulus*	(Meigen, 1830)	S.pictu	0.691	

boundaries, according to the WFD should really be placed at points of ecological change relating to the 'normative definitions' in annex 5 of the WFD. The boundaries and their widths should then be standardised afterwards.

The statistical power of classification was estimated assuming a non-central *t* distribution of values of *BQIES*, 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):

$$\delta = \frac{(m - L)}{s/\sqrt{n}} \qquad (2)$$

The non-central t distribution was built around the δ value. A cutoff separated 20 % area of the central t student distribution and established the extension of the power area $1\text{-}\beta$, 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 the power is the probability to correctly assign a lake to the class above the boundary, when the index value calculated is indeed above this boundary.

It is here remembered that in statistical inference the non central t distribution is used to calculate the power of a statistical test of hypothesis, to represent the probability distribution of the alternative hypothesis a non centrality parameter δ is needed, hence the term "non central t", while the central t distribution is used to represent the probability distribution of the null hypothesis (Winer, 1962).

Microsoft ACCESS 2010 (MSA)® was used to store information (Rossaro *et al.* 2001). Data were processed with Matlab R2011a®. Matlab scripts and functions performing the calculations of indices are available on request to the first author.

RESULTS

84 species were found only in reference lakes, 25 species were exclusive to non-reference lakes. The species list is given in Table 3 with the sensitivity values for each species (*BQIWTS*) and an indication if the species is "tolerant", that is the species has an exclusive presence in non reference sites or is "intolerant", that is the species is exclusively present in reference sites.

With the exception of *Echinogammarus stammeri* and *Lymnaea* sp. all the tolerant species belonged to Oligochaetes, Hirudinea and Chironomids. Odonata, Plecoptera, Ephemeroptera, Hemiptera and Trichoptera included intolerant species above all, with the exception of *Ecnomus* and Limnephilidae sp. which were exclusive to non reference sites

Table 4. COI analysis: eigenvalues.

axis	eigen-value	% variance	axis	eigen-value	% variance
1	3.0291	66	7	0.0228	0
2	0.7090	15	8	0.0070	0
3	0.4998	11	9	0.0020	0
4	0.2011	4	10	0.0011	0
5	0.0838	2	11	0.0004	0
6	0.0659	1	12	0.0001	0

and were classified as "tolerant".

COI analysis was carried out with 13 environmental variables and 177 species present in 148 samples from 12 lakes. The first axis explained 66 % of total inertia, the second axis 15 % and the third axis 11 % (Table 4).

The first axis separated the Austrian reference lakes from the Italian non reference ones, Braies and Anterselva in Sud Tyrol (Italy) were only moderately impacted and were plotted in an intermediate position (Figure 1). High nutrient concentrations separated impacted lakes from reference ones, with high oxygen and transparency, but morphometric parameters were also included in the first axis, separating large deep lakes from smaller ones (Figure 2); 85 species had a row weight larger than 1 and were plotted on the right; tolerant species were plotted on the left (Figure 3).

The second axis separated lakes of higher altitude (Braies, Anterselva) (Figure 1), with low alkalinity and conductivity (Figure 2), from other lakes. The third axis separated well the most impacted lakes (Figure 1) with high total phosphorus and chlorophyll concentration and low oxygen and transparency (Figure 2);

Trophic status index (*TSI*) and three biotic indices were calculated. Correlations between all indices are in Table 5. All the indices had a highly significant correlation to each other and with the *TSI*, but Shannon diversity index *H* and *BQIEJ* indices had lower correlation with *TSI*. *BQIES* was also significantly correlated with the COI axes. The correlation coefficient between *BQIES* and the first biological coinertia axis was 0.665 ($p<0.01$), with the environmental coinertia axis was 0.739 ($p<0.01$).

BQIES was selected for use in lake classification because it satisfied all WFD criteria and was well correlated with the indicators of trophic status (*TSI*).

Figure 4 shows boxplots for each lake with its median value of *BQIES*, its 25 and 75 % percentile, maximum and minimum values and outliers. The lakes were plotted in order of their *TSI* value. It is possible to see an increasing trend in *BQIES* with the *TSI* index, also reflected by its significant correlation coefficient(see Table 5). Lake Monate had a lower than expected biotic index value given its *TSI* index.

In Table 6 the mean values, the number of samples and the standard deviation of the *BQIES* indices values estimated in all stations for each of the 12 lakes are reported. On the basis of the mean value each lake was assigned to a quality class, each defined by a central value and an upper and a lower boundary. The power of each classification is given in the last column.

The boundary values between classes were defined based on the relationship with *BQIES* with *TSI* (see Data Analysis). The central values of each class were: poor = 0.189, moderate = 0.412, good = 0.635, high = 0.858.

In this manner it was possible to assign each lake to a quality class according to benthic macrofauna indices. As

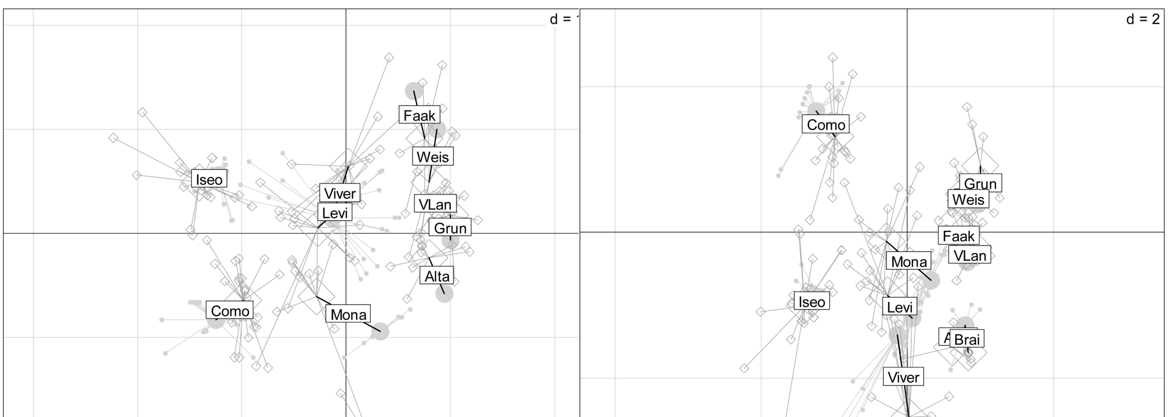

Figure 1. COI plot of first and second axis (left) and of first and third axis (right); large filled grey circles: lake means, small filled circles : environmental scores; diamonds: species scores.

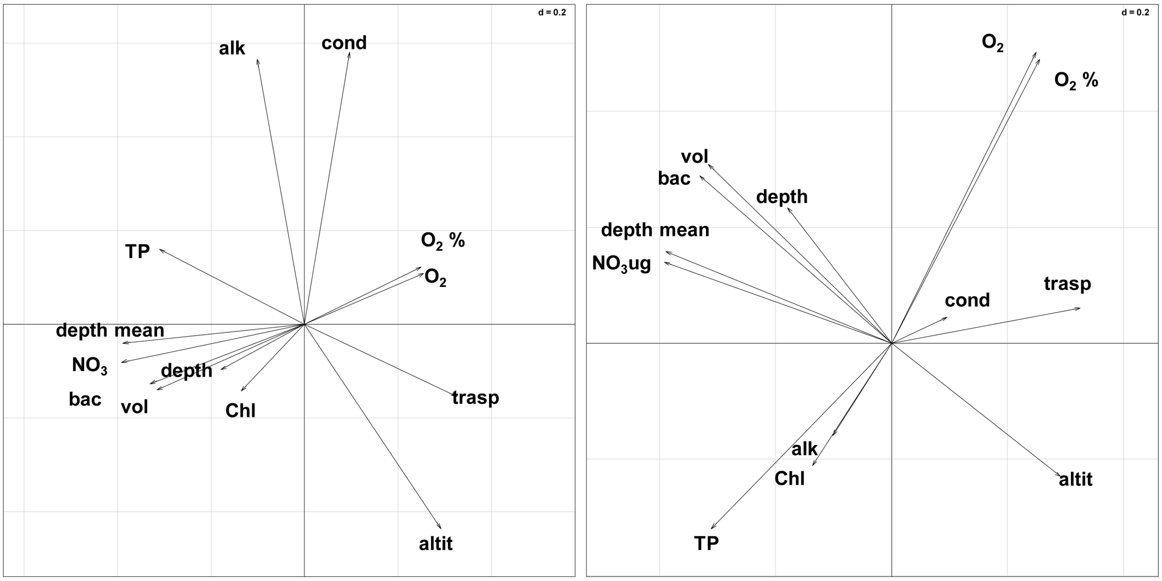

Figure 2. COI plot of environmental variables; first and second axis (left) and of first and third axis (right).

Table 5. Correlation between indices (upper right triangle) and significance of correlations (lower left triangle). *TSI*, trophic status index; *BQIES*, benthic quality index based on trophic status including an abundance factor; *H*, Shannon diversity; *BQIEJ*, index based on expert judgment (see text for more explanation).

	TSI	*BQIES*	*H*	*BQIEJ*
TSI	1.000	0.780	0.238	0.266
BQIES	0.000	1.000	0.439	0.448
H	0.004	0.000	1.000	0.635
BQIEJ	0.001	0.000	0.000	1.000

an example the uncertainty of classification of a reference lake (GrundlSee) and of a non reference lake (Viverone) was estimated and reported in Figures 5-6. The central and the non central t distribution of the *BQIES* values for each lake are given. The central t distribution assumes that the null hypothesis is true, that is the lake belong to the class with central value immediately lower than the BQIES observed, while the non central t distribution assumes that the alternative hypothesis is true, that is the lake belong to the quality class immediately higher. In the figures the value of *BQIES* of each lake is represented on the abscissa with its value δ (that is the non centrality parameter, which is a rescaled *BQIES*, obtained subtracting the *L* boundary of the lower class and dividing the

Table 6. *BQIES* benthic quality index; m, mean; n, number of samples; std, standard deviation; L, central value of the nearest lower class; pw020, statistical power; italics, lakes assigned with a power less than 80 %. Class: quality class: H: high, 0.858; G: good, 0.635; M: moderate, 0.412; P: poor, 0.189.

	m	*n*	*std*	*L*	*pw020*	*Class*
Altausseer See	1.072	9	0.070	0.858	100	H
Anterselva	0.903	6	0.153	0.858	44	H
Braies	0.988	6	0.002	0.858	100	H
Como	*0.633*	*24*	*0.079*	*0.412*	100	*M*
Faaker See	1.015	9	0.123	0.858	100	H
Grundlsee	1.127	9	0.116	0.858	100	H
Iseo	0.243	27	0.124	0.189	92	P
Levico	0.319	12	0.175	0.189	96	P
Monate	*0.476*	*12*	*0.131*	*0.412*	80	*M*
VordLangbath	0.955	9	0.059	0.858	100	*H*
Viverone	*0.398*	*18*	*0.252*	*0.189*	100	P
Weißensee	*0.945*	*7*	*0.051*	*0.858*	100	*H*

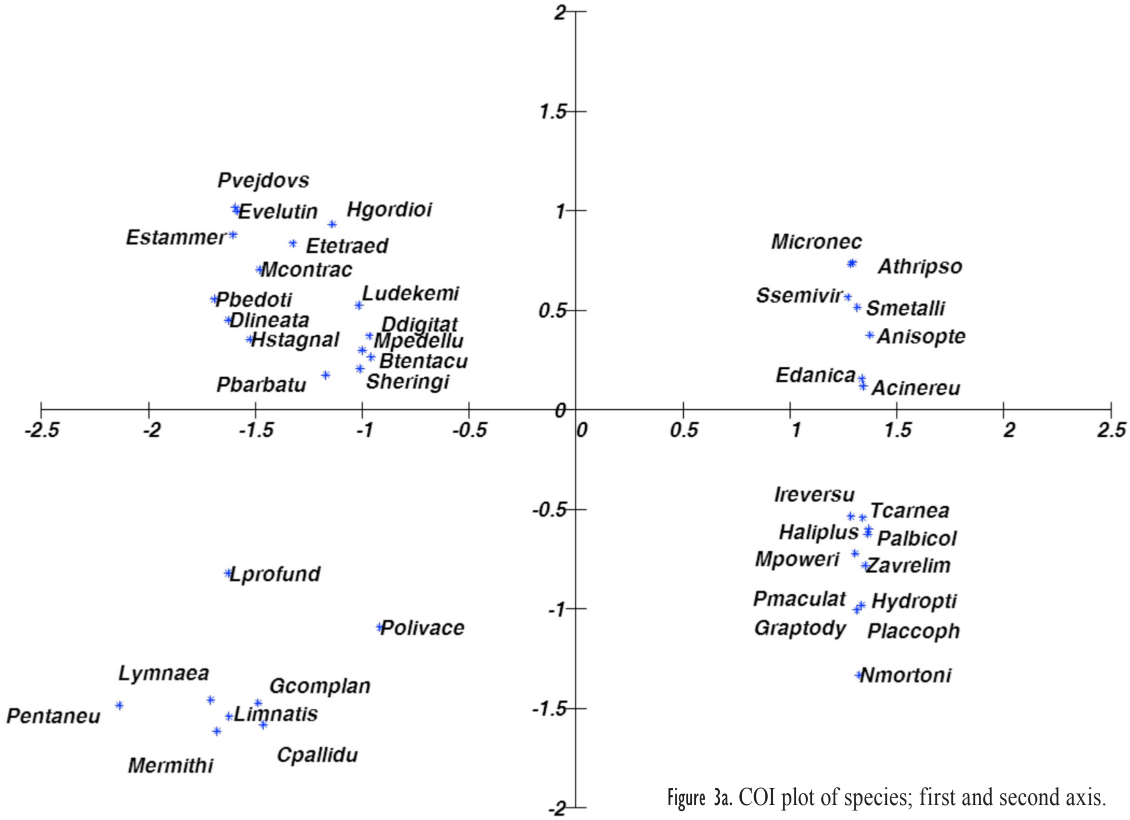

Figure 3a. COI plot of species; first and second axis.

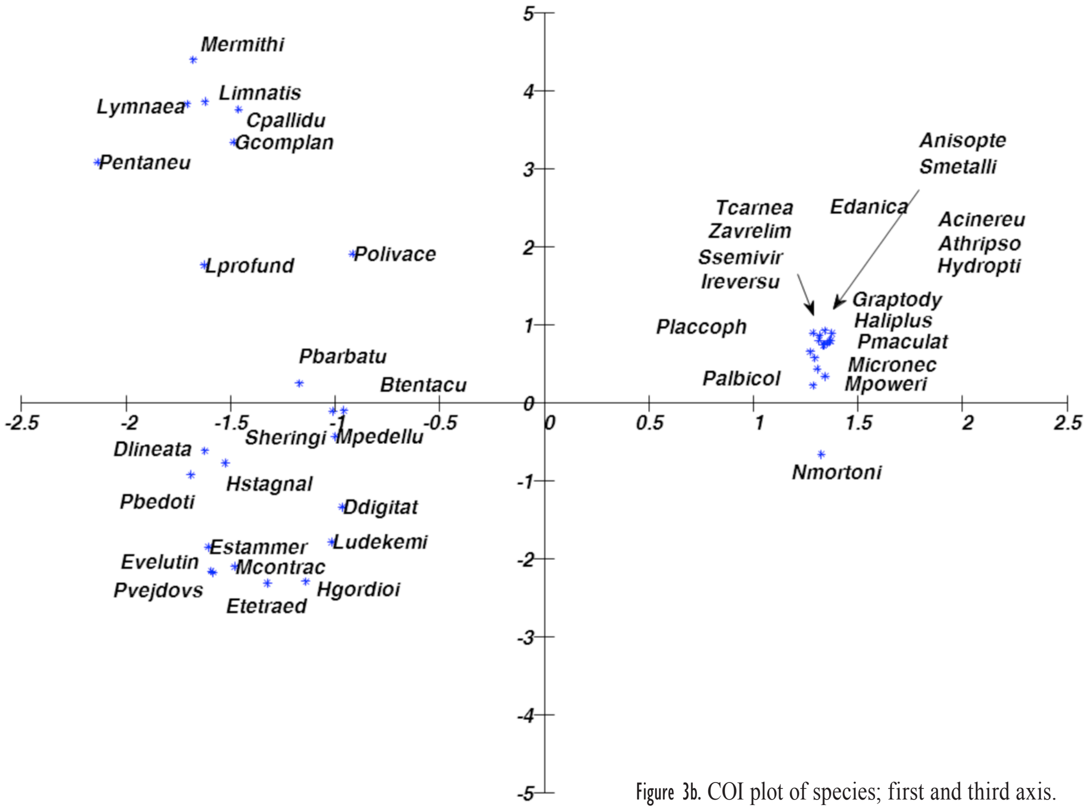

Figure 3b. COI plot of species; first and third axis.

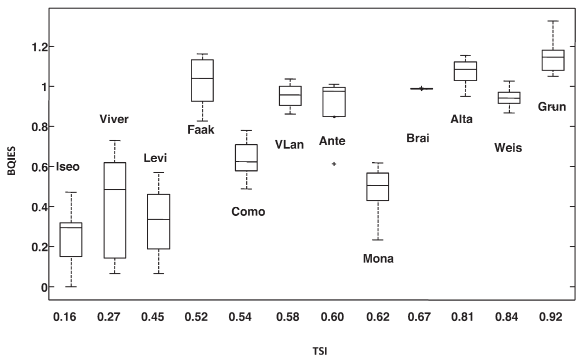

Figure 4. *BQIES* values plotted against *TSI* values; horizontal bar in rectangles: median, rectangles: 25° 75° percentile, whiskers: about 3 standard deviations, cross: outliers.

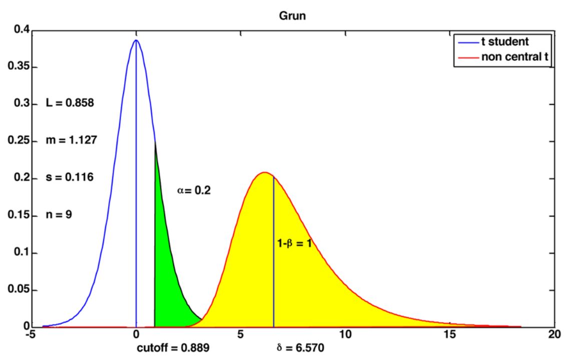

Figure 5. Power test of Grundlsee; abscissa: difference between the observed mean (**m**) BQIES index and the boundary **L** immediately lower than the observed value, the difference is rescaled by dividing it by the standard error of mean (**S**/√**n**), where **n** is the number of samples and **S** the standard deviation of the measures; ordinate: probability density under the null and alternative hypothesis; **L**: boundary between high and good classes, **cutoff**: value on the abscissa separating 20 % of the area of the null hypothesis H_0; **δ**: non centrality parameter, **α**: risk of type I error, **β**: risk of type II error.

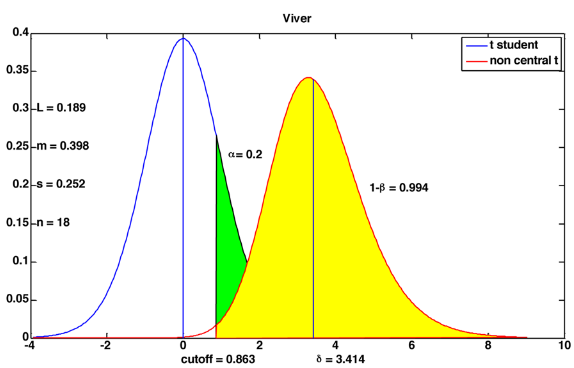

Figure 6. Power test of Viverone lake; see Figure 5 for explanation.

result by standard error of mean). The central t distribution is cut by the cutoff line to separate its 20 % area (in green), the power of classification is the area under the non central t distribution at the right of the cutoff line (yellow area). In figure 5 the 100% of the non central t distribution is on the right of the cutoff, so the power of test is 100%, in Figure 6 a small area is on the left, reducing the power of a small quantity.

DISCUSSION

The use of benthos in lake monitoring has a long history (Johnson et al., 1993) as outlined in Introduction, unfortunately the use of benthos to assess water quality of lakes, even if expressly required in WFD, has been somewhat overlooked by member states, and no official method is actually proposed to assess eutrophication using benthos, and emphasis is on the littoral zone (http://www.alpine-space.org/uploads/media/Alplakes_Ecological_indicator_of_Lake_status.pdf) or on other communities as phytoplankton (Wolfram et al. 2009).

Among the three biotic indexes here suggested, the BQIES is the one more related with TSI index, so it is the best candidate to be proposed as a WFD compliant index. The BQIES index is not yet WFD compliant because it is not expressed as ecological quality ratio (EQR), that is as a ratio between the observed value and the value of a reference site. At present the Austrian reference sites are only tentatively proposed as reference, so they were not used as denominators in the calculation of EQR.

It is unlikely that bio-geographical factors played an important role in the determining the composition and abundance of species. Previous work has found no evidence of a different species distribution in lakes belonging to the Northern and Southern side of the Alps bound to geographical factors (Reiss 1968, Lencioni et al 2011). Differences in the species composition of benthic macroinvertebrates in the soft substrate of the investigated lakes were therefore likely to be determined largely by physical and chemical variables alone. Depth, conductivity, dissolved oxygen and trophic factors were found to be the environmental factors more responsible for influencing benthic macroinvertebrate composition in lakes in previous investigations (Free *et al.* 2009, Rossaro *et al.* 2006; Rossaro *et al.* 2007); coinertia analysis carried out on the 12 lakes confirmed that trophic and morphometric variables were the most important ones in modeling species composition.

To avoid the influence of different lake typologies on species composition and to focus on the effect of anthropogenic disturbance (eutrophication in the present case) lakes belonging to the same GIG lake type (L_AL-3) were selected, even if they belonged to different Italian lake types (AL-3, AL-6, AL-9, AL-10, Buraschi et al. 2005), but previous investigations emphasized that membership of one of these different lake types was not an important source of variance for macroinvertebrates (Rossaro et al 2007, Rossaro et al. 2011).

The calculation of the optimum response to trophic factors was the first step of the present investigation allowing the calculation of the sensitivity values for all the 177 species collected. The values were in agreement with the COI analysis results and with the species' presence – absence in reference and non-reference lakes; only two Chironomid species (*Paratrichocladius rufuventris* and *Paratanytarsus lauterborni*) and one undetermined Trichoptera species (Limnephilidae sp.) were absent in reference sites despite their high *BQIW* (> 0.6). With the exception of these 3 species all the other species exclusive to reference sites had a high *BQIW* value (> 0.5), whereas all the species exclusive to non-reference sites had a low *BQIW* (< 0.5).

These results support the good performance of the indices proposed, with emphasis on the following points: the observed benthic macroinvertebrate fauna allowed to assign all the reference Austrian lakes to the class "high", but the non-reference Anterselva and Braies lake could also be assigned to the class "high". Among the non reference lakes Como and Monate were assigned to the class moderate, Iseo, Levico and Viverone to the class "poor"; this classification was in agreement with *TSI* values, but the Monate lake gave a *BQIES* lower than expected by its *TSI* value. Anterselva was assigned to high class, but with an uncertainty greater than 20% (or with a power less than 80%); further investigation is needed to reduce uncertainty.

Future needs are the addition of new lakes to better understand the response of species to other impacts, such as hydro-morphological alteration (Solimini et al. 2006) and the response to toxic substances.

Even if data were available about the response of single taxa to heavy metal concentration in lakes (Rizzo et al. 2011) , their influence on community composition is very poorly known; often the information is based on studies with larger taxonomic resolution carried out in rivers (Masson et al 2010), little information is available about species response in lakes.

There is much debate whether species identification is really needed in comparison with a coarse taxonomic resolution (Greffard et al. 2011); our results confirm that uncertainties in taxonomic resolution may be critical in the assignment of a lake to a well- defined quality class; this is supported by two examples derived from the present dataset. The presence of an undetermined species (Limnephilidae sp.) in Anterselva and Braies lakes should be critical if an identified species of the same family would be found in other lakes; if so it should be impossible to know if the identified and the unidentified species are the same taxon. The second example is the presence of four taxa of Leptoceridae in the 5 Austrian lakes: Leptoceridae sp., *Athripsodes* sp., *A. aterrimus* and *A. cinereus* (see Table 3); the 4 taxa were identified at a different taxonomic level (family, genus, species); in the present case the slight different sensitivity value assigned to the four taxa would not be critical in assigning the lakes to the high class, but it is evident that it may not be true in other situations, so the finest taxonomic resolution is recommended. It must be emphasized that there is a greater chance to assign a lower sensitivity value to less resolved taxonomic groups, with this risk of misclassification: lower taxonomic resolution is associated with a larger risk to assign a poorer status to a water body!

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REFERENCES

Aagaard K. 1986. The chironomid fauna of north Norwegian lakes, with a discussion on methods of community classification. Holarct. Ecol. 9: 1-12.

Brinkhurst RO. 1974. The benthos of lakes. – The Macmillan Press, London Basingtoke, 190 pp.

Brundin L. 1949. Chironomiden und andere Bodentiere der südschwedischen Urgebirgseen. Ein Beitrag zur Kenntnis der bodenfaunistischen Charakterzüge schwedischer oligotropher Seen. Institute of freshwater research, Drottningholm, 30: 1-915.

Brundin L. 1974. Fifty years' limnic zoogeography. Mitteilungen Internationale Vereinigung für theoretische und angewandte Limnologie. 20: 287-300.

Buraschi A, Salerno F, Monguzzi C, Barbiero G, Tartari G. 2005. Characterization of the Italian lake-types and identification of their reference sites using anthropogenic pressure factors. Journal of Limnology, 64: 75-84.

Carstensen J. 2007. Statistical principles for ecological status classification of Water Framework Directive monitoring data. Marine Pollution Bulletin, 55: 3–15.

Dolédec S, Chessel D. 1994. Co-inertia analysis: an alternative method for studying species-environment relationships. Freshwater Biology 31: 277-94.

Dray S, Chessel D, Thioulouse J. 2003. Coinertia analysis and the linking of ecological data tables. Ecology 84: 3078-3089.

Free G, Solimini A, Rossaro B, Marziali L, Giacchini R, Paracchini B, Ghiani M, Vaccaro S, Gawlik B, Fresner R, Santner G, Schönhuber M, Cardoso AC. 2009. Modelling lake macroinvertebrate species in the shallow sublittoral: relative roles of habitat, lake morphology, aquatic chemistry and sediment composition. Hydrobiologia, 633: 123-136.

Greffard MH, Saulnier-Talbot E, Gregory-Eaves I. 2011. A comparative analysis of fine versus coarse taxonomic resolution in benthic chironomid community analyses. Ecological Indicators 11: 1541–1551

Johnson RK, Wiederholm T, Rosenberg M. 1993. Freshwater biomonitoring using individual organisms, populations and species assemblages of benthic macroinvertebrates. In:

Rosenberg, DM, Resh VH. (eds.). Freshwater biomonitoring and benthic macroinvertebrates. Chapman & Hall, New York, London.

Kansanen PH, Aho J, Paasivirta L. 1984. Testing the benthic lake type concept based on chironomid associations in some Finnish lakes using multivariate statistical methods. Annales Zoologici Fennici, 21: 55-76.

Lang C. 1990. Quantitative relationships between oligochaete communities and phosphorus concentrations in lakes. Freshwater Biology, 24: 327-334.

Lencioni V, Marziali L, Rossaro B. 2011. Diversity and distribution of chironomids (Diptera, Chironomidae) in pristine Alpine and pre-Alpine springs (Northern Italy) Journal of Limnology, 2011: 70 (Suppl. 1): 106-121.

Lenz F. 1925. Chironomiden und Seetypenlehre. Die Naturwissenschaften, 13: 5-10.

Leonardsson K., Blomqvist M., Rosenberg R. 2009. Theoretical and practical aspects on benthic quality assessment according to the EU-Water Framework Directive – examples from Swedish waters. Marine Pollution Bulletin, 58: 1286-1296.

Lods-Crozet B, Reymond O. 2005. Ten years trends in the oligochaete and chironomid fauna of Lake Neuchâtel (Switzerland). Revue Suisse Zoologie, 112: 543-558.

Lundbeck 1936. Untersuchungen über die Bodenbesiedlung der Alpenrandseen. Arch. Hydrobiol. 10: 207-358

Magurran AE. 1988. Ecological diversity and its measurement. Croom Helm, London.

Masson S, Desrosiers M, Pinel-Alloul B, Martel L. 2010. Relating macroinvertebrate community structure to environmental characteristics and sediment contamination at the scale of the St. Lawrence River. Hydrobiologia 647: 35–50

Naumann E. 1921. Einige Grundlinien der regionalen Limnologie. Lunds Univ. Arsskr. N. F. (2) 17, 8: 1-21.

Reiss F. 1968. Verbreitung lakustrischer Chironomiden (Diptera) des Alpengebietes. - Ann. zool. fenn. 5: 119-125.

Rizzo A, Arcagni M, Arribere MA, Bubach D, Ribeiro Guevara S. 2011. Mercury in the biotic compartments of Northwest Patagonia lakes, Argentina. Chemosphere 84 : 70–79

Rosenberg R, Blomqvist M, Nilsson HC, Cederwall H, Dimming A. 2004. Marine quality assessment by use of benthic species-abundance distributions: a proposed new protocol within the European Union Water Framework Directive. Marine Pollution Bulletin, 49: 728-739.

Rossaro B, Boggero A, Lods-Crozet B, Free G, Lencioni V, Marziali L. 2011. A comparison of different biotic indices based on benthic macro-invertebrates in italian lakes Journal of Limnology., 70(1): 109-122.

Rossaro B, Boggero A, Lencioni V, Marziali L, Solimini A. 2006. A Benthic Quality Index for Italian Lakes. Journal of Limnology, 65: 41-51.

Rossaro B, Marziali L, Cardoso AC, Solimini A, Free G, Giacchini R. 2007. A biotic index using benthic macroinvertebrates for Italian lakes. Ecological Indicators, 7: 412-429.

Rossaro B, Mietto S, Lencioni V. 2000. The Chironomid species of the Italian lakes: a review. In: Offrichter O. (ed.), Late 20° Century Research on Chironomidae. An Anthology from the XIII° Int. Symp. Chironomidae. Shaker Verlag (Germany), Freiburg 5-9 September 1997: 549–564.

Rossaro B, Lencioni V, Casalegno C. 2001. Relazioni tra specie di Ditteri Chironomidi e fattori ambientali esaminate con un data base relazionale. Atti Congresso Gadio 5-7 maggio 2001. Studi Trentini di Scienze Naturali Acta Biologica, 78: 201-206.

Sæther OA. 1979. Chironomid communities as water quality indicators. Holarctic Ecology, 2: 65-74.

Solimini A, Free G, Donohue I, Irvine K, Pusch M, Rossaro B, Sandin L, Cardoso AC. 2006. Using benthic macroinvertebrates to assess ecological status of lakes current knowledge and way forward to support WFD Implementation. Technical Report EUR 22347, European Commission, J.R.C., Ispra: 48 pp.

Tartari G, Buraschi E, Legnani E, Previtali L, Pagnotta R, Marchetto A. 2006. Tipizzazione dei laghi italiani secondo il Sistema B della Direttiva 2000/60/CE. CNR: 1-20.

Thienemann A. 1953. Chironomus Leben Verbreitung und wirtschaftliche Bedeutung der Chironomiden. Die Binnengewässer, Bd. 20, Schweizerbart'sche Verlagsbuchhandlung, Stuttgart, 834 pp.

Wiederholm T. 1980. Use of benthos in lake monitoring. Journal of the Water Pollution Control Federation, 52: 537-547.

Winer BJ. 1962. Statistical principles in experimental design. McGraw-Hill Co., New York: 907 pp.

Wolfram G, Argillier C, Bortoli J de, Buzzi F, Dalmiglio A, Dokulil MT, Hoehn E, Marchetto A, Martinez PJ, Morabito G, Reichmann M, Remec-Rekar S, Riedmüller U, Rioury C, Schaumburg J, Schulz L, Urbanic G. 2009. Reference conditions and WFD compliant class boundaries for phytoplankton biomass and chlorophyll-*a* in Alpine lakes. Hydrobiologia 633:45-58