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# Can we use macroinvertebrates as indicators of acidification of high-altitude Alpine lakes?

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**Riassunto:** In seguito alla riduzione delle deposizioni acide, a partire dalla metà degli anni 90, la qualità delle acque dei laghetti alpini sensibili all'acidificazione è migliorata significativamente. Nonostante ciò, sono ancora rari esempi di ripresa della biologia. Inoltre, non è ancora stato sviluppato un metodo specifico per valutare il grado di acidificazione dei laghetti alpini tramite macroinvertebrati. In questo studio abbiamo applicato diverse metriche già esistenti ai dati di macroinvertebrati di 5 laghetti alpini con pH differenti, per trovare quali riflettono meglio le differenze di acidità dei laghetti e per creare una lista di specie sensibili all'acidificazione potenzialmente presenti in questi ambienti, con lo scopo di migliorare la valutazione temporale del grado di acidificazione tramite l'analisi dei macroinvertebrati in Ticino. In generale è stato osservato che i macroinvertebrati campionati negli emissari sono migliori indicatori di quelli presenti nel litorale. In particolare, negli emissari aumentano sensibilmente con il pH il numero di taxa totale, il numero di taxa e di famiglie e la percentuale di Efemerotteri, il numero di taxa di Plecotteri, il numero di taxa, di famiglie della somma di Efemerotteri/Plecotteri/Tricotteri, il numero di taxa e la percentuale di oligocheti, il numero di taxa di chironomidi e il numero di specie sensibili all'acidificazione. Diminuiscono invece con l'aumentare del pH la percentuale di chironomidi e di predatori. Nei litorali, invece, aumentano con il pH solo il numero di taxa totale e il numero di taxa di chironomidi e oligocheti.

**Parole chiave:** pH, indice di acidificazione, chironomidi, oligocheti, litorale, emissario, Alpi

**Can we use macroinvertebrates as indicators of acidification of high-altitude Alpine lakes?**

**Abstract:** Chemical recovery of lakes in the Western Alps as a consequence of decreased acid deposition has been reported since mid 1990's. Nevertheless, examples of biological recovery are still rare. In addition, a specific method to assess acidification using benthic macroinvertebrates, for high-altitude Alpine lakes has not been developed yet. In this study we applied different already existing metrics to macroinvertebrate data from 5 high-altitude Alpine lakes with different pH, to find which reflects best differences in lake acidity and to create a list of acid sensitive species that may occur in these ecosystems, in order to improve temporal assessment of acidification through benthic macroinvertebrates in high-altitude Alpine lakes in Southern Switzerland. It resulted that in general macroinvertebrates from lake outlets are better indicators of acidity than from lake littorals. In lake outlets the following metrics increased significantly with pH: total number of taxa, number of taxa and families and relative abundance of Ephemeroptera, number of taxa of Plecoptera, number of taxa and families of the sum of Ephemeroptera/Plecoptera/Trichoptera, number of taxa and relative abundance of oligochaetes, number of taxa of chironomids, and number of acid sensitive species. Differently, the relative abundance of chironomids and predators decreased with increasing pH. In lake littorals only the total number of taxa, and the number of taxa of chironomids and oligochaetes increased with pH.

**Keywords:** pH, acidification indices, chironomids, oligochaetes, littoral, outlet, Alps

## INTRODUCTION

Acidification of acid sensitive freshwaters as a consequence of atmospheric deposition of sulphur and nitrogen compounds has been object of numerous studies during the last 30 years mainly in Europe and North America (Stoddard *et al.*, 1999; Skjelkvåle *et al.*, 2005). It has been shown that acidification of surface waters

caused substantial changes in biological communities. "Effects ranged from reductions in diversity without changes in total biomass to elimination of all organisms" (Dillon *et al.*, 1984; Schindler, 1988; Brakke *et al.* 1994).

As a consequence of reduced sulphur and nitrogen emissions during the last 20 years, chemical recovery of many surface waters has been observed (Evans *et al.*,

2001). However biological recovery, which remains the ultimate goal of emission control programmes, is still often only partial (Raddum *et al.*, 2004; Raddum *et al.*, 2007; Fjellheim & de Wit, 2011).

Benthic invertebrates have been often used as indicators for water quality. In particular the relationship between the presence or absence of certain macroinvertebrate species with surface water acidity has been used to develop several metrics to determine the status of stream ecosystems and its variation through time: the Raddum (Raddum *et al.*, 1988; Fjellheim & Raddum, 1990; Raddum, 1999) and the NIVA (Bækken & Kjellberg, 2004) indexes in Norway, the MEDIN (Henrikson & Medin, 1986) and the MISA (Johnson & Goedkoop, 2007) indexes in Sweden, the AWICfam (Davy-Bowker *et al.*, 2003; Davy-Bowker *et al.*, 2005) and AWICsp (Davy-Bowker *et al.*, 2003) indexes in UK and the Braukmann index (Braukmann & Biss, 2004) in Germany. As regard lake ecosystems, studies on benthic invertebrates as indicators of acidification were published only recently: Johnson & Goedkoop (2007) developed the MILA index, McFarland *et al.*, (2010) the LAMM index and Schartau *et al.*, (2008) tested different existing metrics on littoral data. However, all these studies are based on monitoring data from Northern Europe so that they were not generally adopted in Southern Europe due to the presence of different vicariant species.

High-altitude lakes, due to climatic factors, shallow soil cover, little vegetation, modest watershed dimension, long iced surface and rapid flushing rates, are known to be particularly sensitive to atmospheric pollution (Wathne *et al.*, 1995). In response to decreased atmospheric acid deposition (Steingruber & Colombo, 2010a), lakes in the Western Alps showed signs of chemical recovery since the mid 1990s (Rogora *et al.*, 2003; Steingruber & Colombo, 2010b). Nevertheless, examples of biological recovery are still rare (Marchetto *et al.*, 2004). Moreover, a specific metric has not yet been developed regarding macroinvertebrates and the assessment of acidification in high altitude Alpine lakes. So, the aim of this study was to apply different already existing metrics to macroinvertebrates from 5 lakes in Southern Switzerland with different pH (from acid sensitive to alkaline), with the purpose to find which reflects best differences in lake acidity and to create a list of acid-sensitive species that may occur in these ecosystems.

## METHODS

The study area is located in the Lepontine Alps in the northern part of Canton Ticino, Switzerland (fig. 1). Location and morphometric parameters of the 5 studied lakes are described in tab. 1. The catchments of all lakes, with exception of Lago Bianco, are dominated by crystalline rocks (especially gneiss). The latter receives waters from a reservoir located in a larger basin with a broader calcareous area. Particular are also the cases of lakes Laghetto, cascade lakes connected through the outflow of Laghetto Superiore, and Lago del Starlaresc da Sgiof, a shallow lake with a maximum depth of around 6 m surrounded by marshes (part of it can be considered a transition to a marsh). The lake's watersheds are covered mainly by bare rocks with vegetation often confined to small areas of alpine meadows. The selected Alpine lakes are situated between an altitude of 1690 m and 2080 m far from local pollution sources and are characterized by intensive irradiation, short vegetation period, long period of ice coverage and by low nutrient concentrations (phosphorus  $< 5 \mu\text{g P l}^{-1}$ ).

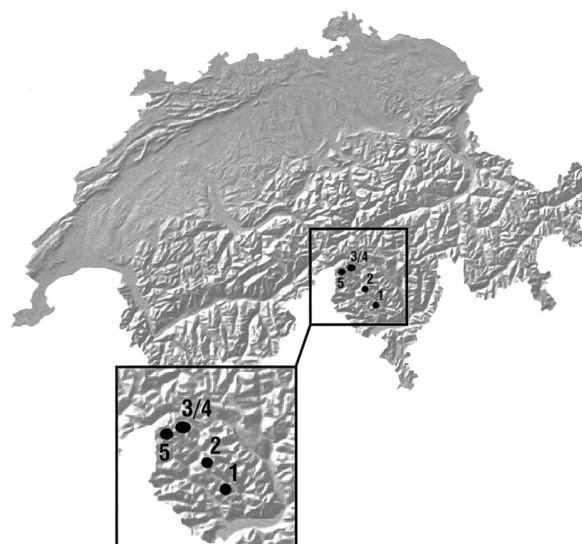


Figure 1: 1 Lago del Starlaresc da Sgiof (STA)  
2 Lago di Tomè (TOM)  
3 Laghetto Superiore (SUP)  
4 Laghetto Inferiore (INF)  
5 Lago Bianco (BIA)

Table 1: Main geographic, morphometric and geologic parameters of the monitored lakes.

Lake name	Long E CH m	Lat N CH m	Long E	Lat N	Altitude m a.s.l.	Catchment area ha	Lake area ha	Max depth m	Characterization watershed surface*		
									% rock	vegetation	% water
Lago del Starlaresc da Sgiof	702°905	125°605	8.777	46.276	1875	23.0	1.1	6	29	67	5
Lago di Tomè	696°280	135°398	8.691	46.364	1692	294.0	5.8	38	57	41	2
Laghetto Superiore	688°020	147°835	8.587	46.477	2128	125.0	8.3	29	65	29	7
Laghetto Inferiore	688°627	147°855	8.594	46.477	2074	182.0	5.6	33	65	27	8
Lago Bianco	683°030	145°330	8.519	46.454	2077	-	ca. 4.0	-	-	-	-

\* personal communication from Meteotest

Since 2000 up to now lake surface waters were sampled from 2 to 3 times per year (1 at the beginning of summer and 1-2 in autumn) directly from an helicopter, while before 2000 the same were sampled only irregularly. Waters were analyzed for the main chemical parameters (pH, conductivity, alkalinity, sulphate, nitrate, chloride, ammonium, calcium, magnesium, sodium, potassium, aluminium, zinc, copper). Macroinvertebrates have been collected by “kicking” the littoral and the outlet of each lake with a 250 µm mesh size net according to the ICP Waters Programme Centre Protocol (2010) usually simultaneously with chemical sampling during the ice free period. In order to compare the macroinvertebrate population of acid and acid-sensitive lakes with an alkaline one, Lago Bianco was added to the monitoring list in 2006. Samples have been preserved in 70% ethanol in the field, then sorted, identified to species or to higher taxonomic level and then counted in the lab. Usually, oligochaetes and chironomids, were neglected but because of their relative importance in lakes (Boggero *et al.*, in press), we concentrated our first attempt of analysis on 2007 data to test their influence on the acidification assessment. Identification of oligochaetes and chironomids occurred according to Ferrarese & Rossaro (1981), Rossaro (1982), Ferrarese (1983), Wiederholm (1983), Nocentini (1985), Wiederholm (1986), Janeczek (1998), Timm (2009), Schmelz *et al.* (2010), while for all other taxa the following literature was used: Aubert (1959), Castagnolo *et al.* (1980), Consiglio (1980), Belfiore (1983), Carchini (1983), Moretti (1983), Sansoni (1988), Studelmann *et al.* (1992), Campaioli *et al.* (1994), Waringer & Graf (1997), Campaioli *et al.* (1999), Sartori and Landolt (1999), Wildermuth *et al.* (2005). The quality of the taxonomic work was assured by participating annually at an international intercalibration exercise with region specific samples to be identified (Fjellheim & Raddum, 2008). The biological data are regularly transmitted to the Swiss Biological Records Center. Number of taxa refers to both sampling periods and only the deepest identification level has been considered (e.g. if both *Baetis* sp. and *Baetis alpinus* were found only the second was counted). Finally, the mean annual relative abundances were calculated.

The applied acidification metrics can be divided in:

- general metrics (total number of taxa, number of taxa, families and relative abundance of Ephemeroptera, Plecoptera, Trichoptera, number of taxa and relative abundance of Diptera (Chironomidae), Oligochaeta, relative abundance of predators).
- indexes based on presence/absence or relative abundance of acid sensitive taxa (Raddum index, NIVA index, AWICfam index, AWICsp index, Braukmann index and LAMM index).
- and classification systems based on both previous type of metrics (MEDIN and MILA indexes).

## RESULTS

### Chemical characterization of the lakes

Water chemistry of the studied lakes is presented in tab. 2.

Considering their alkalinities, the lakes can be divided in **sensitive to acidification** ( $0 \text{ meq l}^{-1} < \text{Alk} < 50 \text{ meq l}^{-1}$ ), **slightly sensitive or intermediate** ( $50 \text{ meq l}^{-1} < \text{Alk} < 200 \text{ meq l}^{-1}$ ) and **well buffered** ( $> 200 \text{ meq l}^{-1}$ ).

Table 2: Water chemistry of the sampled lakes: 2007 average values. Lake code as in fig. 1.

Water chemistry	Lake				
	STA	TOM	SUP	INF	BIA
Conductivity 20°C ( $\mu\text{S cm}^{-1}$ )	8.5	8.6	8.8	10.1	64.0
pH	5.5	5.7	6.7	6.7	7.6
Alkalinity ( $\text{meq m}^{-3}$ )	3	5	33	36	441
$\text{Ca}^{2+}$ ( $\text{meq m}^{-3}$ )	25	42	51	59	579
$\text{Mg}^{2+}$ ( $\text{meq m}^{-3}$ )	8	6	8	9	59
$\text{Na}^{+}$ ( $\text{meq m}^{-3}$ )	15	14	12	14	15
$\text{K}^{+}$ ( $\text{meq m}^{-3}$ )	4	4	8	11	20
$\text{NH}_4^{+}$ ( $\text{meq m}^{-3}$ )	2	1	0	0	0
$\text{SO}_4^{2-}$ ( $\text{meq m}^{-3}$ )	31	32	28	33	210
$\text{NO}_3^{-}$ ( $\text{meq m}^{-3}$ )	22	28	16	19	11
$\text{Cl}^{-}$ ( $\text{meq m}^{-3}$ )	3	3	2	2	3
DOC ( $\text{mg C l}^{-1}$ )	0.78	0.25	0.42	0.34	0.26
$\text{SiO}_2$ ( $\text{mg l}^{-1}$ )	1.4	1.8	1.2	1.4	1.6
Al dissolved ( $\mu\text{g l}^{-1}$ )	65	26	6	6	6
Al tot ( $\mu\text{g l}^{-1}$ )	79	33	15	22	19
Cu dissolved ( $\mu\text{g l}^{-1}$ )	<0.2	<0.2	<0.2	<0.2	<0.2
Cu tot ( $\mu\text{g l}^{-1}$ )	<0.4	<0.2	<0.2	<0.2	<0.2
Zn dissolved ( $\mu\text{g l}^{-1}$ )	4.3	1.7	2.0	1.1	<0.3
Zn total ( $\mu\text{g l}^{-1}$ )	4.6	1.8	1.4	1.3	<1.0

### General characteristics of the macroinvertebrate population

Relative abundances of the main taxonomic groups are presented in tab. 3, while the detailed taxonomical entities are listed in tab. 8.

As suggested by the position of the five studied lakes (high altitude, far from local pollution sources), many taxa typical of oligotrophic and of high altitude and cold waters were found (*Diamesinae*, *Krenosmittia boreoalpina*, *Parorthocladius nudipennis*, *Stilocladius montanus*, *Apsectrotanytus trifascipennis*, *Macropelopia nebulosa* gr., *Zavrelimyia melanura* gr., *Baetis alpinus*, *Ecdyonurus frigidus* gr., *Ecdyonurus venosus* gr., *Nemoura mortoni*, *Drusus discolor*, *Philopotamus ludificatus*, *Crenobia alpina*, ...).

In the littoral samples a total of 14373 individuals were collected and 70 taxa were identified, while in the outlets the number of individuals and of taxa were 20298 and 99, respectively. Twenty taxa were exclusive to the lentic, 44 to the lotic habitats and 42 were common to both sampling zones. In general Diptera was the dominant order regarding both abundance and diversity. With exception of the outlets of both Laghetto lakes, where Simuliidae were also abundant, chironomids prevailed in all samples. Oligochaetes were also highly abundant

(mainly Naididae in the outlets), excluding the outlets of lakes Tomè and Lago del Starlaresc da Sgiòf and the littoral of the latter. In most littorals nematods were numerous, as well.

Table 3: Annual average relative abundances (%) of families or higher taxonomic levels in the outlets and the littorals of the monitored lakes. Lake code as in fig. 1. 0 stays for percentage values > 0 and < 0.5

TAXA	OUTLET					LITTORAL				
	STA	TOM	SUP	INF	BIA	STA	TOM	SUP	INF	BIA
OLIGOCHAETA	1	0	41	32	46	3	21	8	12	30
HYDRACARINA	0	0	0	0	0	0	7	1	3	6
INSECTA										
COLEOPTERA	0	0			0		2	1	1	
COLLEMBOLA		1	0	0	0		0		0	0
DIPTERA	77	61	47	56	40	89	62	45	75	55
Chironomidae	68	52	28	35	40	72	62	44	73	55
Simuliidae	1	9	19	20	0					
Other DIPTERA	8	0	0	1	0	16	0	1	2	0
EPHEMEROPTERA			0	0	1					
HETEROPTERA	0					2				
MEGALOPTERA		0				0				
ODONATA	5					2				
PLECOPTERA	14	36	6	6	11		0	4	1	0
TRICHOPTERA	2	1	1	0	1	1	7	5	2	
BIVALVIA									1	0
GASTROPODA									0	
NEMATODA	2	0		0	0	4	1	34	6	10
TURBELLARIA		0	4	6	1					

The greater taxa number in the outlets belongs mainly to species typical of flowing waters. A list of species found in the studied lakes that according to literature are typical for flowing and standing or slow flowing waters is shown in tab. 4. Lago del Starlaresc da Sgiòf is characteristically represented by the same community along the littoral and the outlet, because of the gentle slope of the outlet maintaining a weak current.

However, next to flow velocity and chemistry also other factors determine differences in macroinvertebrate population among habitats. For example, because lakes littorals have in general more areas with fine substrates quite rich in organic matter, they were inhabited by higher abundances of Lumbriculidae and Tubificidae, particularly Lago Bianco where *Tubifex tubifex*, *Microtendipes pedellus*, *Prodiamesa olivacea*, *Paracrepidius conversus* were easily found. In addition, Lago Bianco being a glacier-fed lake maintains many cold-stenothermal species. On the other hand, the shallow Lago del Starlaresc da Sgiòf reduced to a marsh in part of its body showed a macroinvertebrate community highly differing from the other lakes characterized by the presence of species like dragonflies, water beetles, water boatmen, the caddisflies, *Limnephilus coenosus*, *Oligotrichia striata* more typical of still waters and the chironomids *Cladotanytarsus mancus* gr. and *Pagastiella orophila* common in organic mud bottoms.

Table 4: Species typical for flowing and standing waters found at the study sites.

FLOWING WATERS	STANDING WATERS
INSECTA	INSECTA
DIPTERA	DIPTERA
<i>Parorthocladus nudipennis</i>	<i>Microtendipes pedellus</i>
<i>Rheocricotopus effusus</i>	<i>Pagastiella orophila</i>
Clinocerinae	<i>Paracrepidius camptolabis</i> gr.
Simuliidae	<i>Prodiamesa olivacea</i>
EPHEMEROPTERA	HETEROPTERA
<i>Baetis alpinus</i>	Corixidae
<i>Ecdyonurus helveticus</i> gr.	ODONATA
PLECOPTERA	<i>Aeshna caerulea</i>
<i>Leuctra</i> sp.	<i>Aeshna juncea</i>
<i>Perlodes intricatus</i>	<i>Somatochlora alpestris</i>
TRICHOPTERA	PLECOPTERA
<i>Drusus discolor</i>	<i>Nemurella pictetii</i>
<i>Odontocerum albicorne</i>	TRICHOPTERA
<i>Philopotamus ludificatus</i>	<i>Allogamus uncatus</i>
<i>Rhyacophila tristis</i>	<i>Limnephilus coenosus</i>
TURBELLARIA	<i>Oligotricha striata</i>
<i>Crenobia alpina</i>	<i>Plectrocnemia conspersa</i>
	BIVALVIA
	<i>Pisidium casertanum</i>
	GASTROPODA

#### Application of acidification metrics to stream macroinvertebrates

Results from the application of different metrics are shown in tab. 5. The total number of taxa increased with pH. Also metrics related to Ephemeroptera (number of families, taxa and relative abundance) and to Oligochaeta (number of taxa and relative abundance) increased with pH. For Plecoptera only the number of taxa clearly increased with pH. For Trichoptera any metric correlated significantly with pH. However, a clear increase with pH could be observed for the number of families and taxa of the sum of the orders Ephemeroptera, Plecoptera and Trichoptera (EPT). The number of taxa of Diptera and Chironomidae also increased in lakes with pH. Furthermore the relative abundance of Diptera and predators were higher in more acidic waters. On the contrary, for most of the specific acidification indexes tested no correlation was observed between indexes and pH with the exception of the Raddum index, although it seems not to be very sensitive toward changes in pH, and the MILA index. Finally, the number of acid sensitive species (tab. 6) increased in lakes with higher pH, while differences in the relative abundance of acid sensitive taxa can be observed only between acid sensitive (Lago del Starlaresc da Sgiòf, Lago di Tomè) and slightly acid lakes (Laghetto Superiore, Laghetto Inferiore, Lago Bianco).

Table 5: Application of existing metrics regarding macroinvertebrates and acidity. For each classification system the possible ranges are indicated in brackets from the less acidic toward the most acidic sites. Lake codes as in fig. 1.

METRICS	OUTLET					LITTORAL				
	STA	TOM	SUP	INF	BIA	STA	TOM	SUP	INF	BIA
<b>General metrics</b>										
Total N° of taxa	35	40	41	46	55	27	28	41	38	31
<b>EPHEMEROPTERA (E)</b>										
N° of families	0	0	1	1	2	0	0	0	0	0
N° of taxa	0	0	1	2	2	0	0	0	0	0
Rel. Abundance %	0	0	0.3	0.9	1.3	0	0	0	0	0
<b>PLECOPTERA (P)</b>										
N° of families	2	3	3	3	3	0	1	1	1	1
N° of taxa	1	3	5	6	6	0	1	1	1	1
Rel. Abundance %	14.2	36.0	5.9	6.0	10.7	0	0.4	4.1	1.1	0.1
<b>TRICHOPTERA (T)</b>										
N° of families	3	3	3	3	4	1	2	2	2	0
N° of taxa	4	4	4	3	5	1	2	3	2	0
Rel. Abundance %	1.5	1.1	1.2	0.4	0.6	0.6	6.7	4.9	2.4	0
<b>EPT</b>										
N° of families	5	6	7	7	9	1	3	3	3	1
N° of taxa	5	7	10	11	13	1	3	4	3	1
Rel. Abundance %	15.7	37.2	7.3	7.2	12.6	0.6	7.1	9.0	3.5	0.1
<b>DIPTERA</b>										
N° of taxa	19	23	23	27	30	15	13	22	21	17
Rel. Abundance %	76.7	60.7	47.5	55.6	40.4	88.5	61.6	45.1	74.5	55.1
<b>CHIRONOMIDAE</b>										
N° of taxa	16	21	21	24	27	13	12	19	19	15
Rel. Abundance %	67.7	52.1	27.9	35.0	39.8	72.3	61.6	44.2	73.0	55.0
<b>OLIGOCHAETA</b>										
N° of taxa	3	3	4	4	7	4	7	10	9	10
Rel. Abundance %	0.7	0.3	40.7	31.5	45.8	3.0	21.4	8.2	12.3	29.6
<b>Predators</b>										
Rel. Abundance %	11.3	10.5	6.1	7.5	2.7	13.8	52.7	21.5	30.3	9.9
<b>Acidification metrics</b>										
Braukmann index (1-5)	4	5	5	5	5	4	4	4	4	4
Braukmann modified index (1-5)	4	4	2	2	2	4	4	4	4	4
Raddum index (1-0)	0	0.5	0.5	0.5	1	0	0	0.5	0.25	0
NIVA index (1-4)	4	4	4	4	4	4	4	3	3	4
AWIClam index (6-0)	3.9	4.0	3.5	3.5	3.8	5.5	3.7	4.0	4.0	3.7
AWICsp index (10-0)	5.7	5.3	5.2	5.2	5.3	6.0	5.1	5.5	5.4	5.0
MEDIN index (1-5)	5	5	5	5	5	5	5	4	5	5
MILA index (100-1)	15	21	28	29	33	25	7	13	3	23
LAMM index (6-0)	4	4	4	4	4	6	2	4	3	4
Relative abundance of acid sensitive taxa according to Table 6	0.0	0.3	5.6	9.1	3.6	0.1	0.0	0.1	0.0	6.7
Number of acid sensitive taxa according to Table 6	0	3	4	7	8	1	0	2	0	2

### Application of acidification metrics to littoral macroinvertebrates

Interestingly, most of the existing metrics applied to littoral samples seemed not to correlate with pH. Considering that the number of taxa in Lago Bianco is probably reduced compared to the other two slightly acid lakes (Laghetto Superiore and Laghetto Inferiore), because of its more monotone substrate consisting mainly of fine material and its extreme water temperature conditions, only the number of total taxa, and the number of taxa

of Diptera, Chironomidae and Oligochaeta seemed to slightly increase with pH. All other general metrics, the specific acidification assessment indexes and the number of acid sensitive taxa did not seem to correlate with lake acidity.

Table 6: Presence of acid sensitive species. Lake codes as in fig. 1.

ACID SENSITIVE TAXA	REF	OUTLET					LITTORAL				
		STA	TOM	SUP	INF	BIA	STA	TOM	SUP	INF	BIA
<b>INSECTA</b>											
<b>DIPTERA</b>											
Athericidae	1				x						
Chironomidae											
<i>Tanytarsus lugens</i> gr.	2								x		x
Empididae	1, 3		x		x	x	x		x		x
<b>EPHEMEROPTERA</b>											
<i>Baetis alpinus</i>	4								x		
<i>Baetis</i> sp.	1, 5								x		
<i>Ecdyonurus helveticus</i> gr.				x	x						
<i>Ecdyonurus</i> sp.	3, 4, 6			x	x						
<i>Rhithrogena</i> sp.	1, 3, 4, 6								x		
<b>PLECOPTERA</b>											
<i>Protonemoura nimborum</i>	4			x	x	x					
<i>Isoperla</i> sp.	7								x		
<i>Perlodes intricatus</i>				x	x	x					
<i>Perlodes</i> sp.	4					x	x				
<b>TRICHOPTERA</b>											
<i>Philopotamus ludificatus</i>	4								x		
<i>Rhyacophila tristis</i>	4		x								
<b>GASTROPODA</b>											
	8										x
<b>TURBELLARIA</b>											
<i>Crenobia alpina</i>	5		x	x	x	x					

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1. Lepori *et al.*, 2003
2. Schnell & Raddum, 1995
3. Mc Farland *et al.*, 2010
4. Braukmann & Biss, 2004
5. Fjellheim & Raddum, 1990
6. Davy-Bowker *et al.*, 2003
7. Raddum, 1999
8. Raddum *et al.*, 1988

## DISCUSSION

The study revealed a typical community of high mountain lakes of the Pennine-Leptine Alps, with cold-stenothermal species adapted to live in oligotrophic waters (Boggero & Nobili, 1998; Boggero & Lencioni, 2006; Boggero *et al.*, 2006; Maiolini *et al.*, 2006; Dumnicka & Boggero, 2007). Macroinvertebrate community structure along the littorals showed the dominance of chironomids and oligochaetes. This is in agreement with results from other studies of Alpine lakes (Boggero & Lencioni, 2006; Boggero *et al.*, 2006; Maiolini *et al.*, 2006; Fjellheim *et al.*, 2009; de Mendoza & Catalan, 2010). Also in lake outlets chironomids and oligochaetes were abundant. Interestingly, in most monitoring programmes using macroinvertebrates as indicators for acidification, chironomids and oligochaetes are rarely determined to species, because their studies requires a high taxonomic and ecological knowledge and because generally they are not considered to be very sensitive to acidification (Wiederholm & Eriksson, 1977; Brodin & Gransberg, 1993; Wathne *et al.*, 1997).

Comparing the different habitats, various environmental factors may determine their macroinvertebrate populations like lake acidity, water flow velocity, substrate type, presence of littoral vegetation and temperature (Boggero *et al.*, 2005; Hieber *et al.*, 2005; Boggero *et al.*, 2006; Füreder *et al.*, 2006; Marchetto *et al.*, 2009). However, notwithstanding the presence of a high number of factors influencing presence and distribution of macroinvertebrates, many general metrics seem to well describe differences in pH in outlet samples. However, the tendency of the here studied lake outlets to have a more heterogeneous morphology at higher pH may also be partly responsible for these results. The total number of taxa, (often used to compare community diversity) and the number of EPT taxa (recognized as the most sensitive to pollution; Weber, 1973), are known to decrease with water acidity. Not surprisingly in the two acid sensitive lakes any Ephemeroptera, highly susceptible to acidification (Raddum *et al.*, 1988), have been found. The number of EPT families also increased with pH but less significantly than the number of EPT taxa. The relative abundance of Diptera (mainly Chironomidae) and of predators, usually showed a reverse trend increasing with acidification (Johnson & Goedkoop, 2007). Interestingly, the number of taxa of the numerically important chironomids and oligochaetes, increased with pH, suggesting that an improvement of lake pH is also reflected in their taxa richness. Although chironomids have not generally been regarded as sensitive indicators for acidity (Wiederholm & Eriksson, 1977; Mossberg & Nyberg, 1979; Meriläinen & Hynynen, 1990; Schnell, 2001; Olander, 2002), results from other studies were consistent to ours (Raddum & Sæther, 1981; Allard & Moreau, 1987; Halvorsen *et al.*, 2001). As regards oligochaetes, next to the taxa richness also the relative abundance showed a sort of increase with lake pH. Oligochaetes, are generally known to be tolerant to acidification (Wathne *et al.*, 1997), or even preferring acidified environments (Rota, 1995), however some studies observed that, although the diversity of oligochaetes does not change significantly with pH, their total abundance may decrease with decreasing pH (Keller *et al.*, 1990; Lonergan & Rasmussen, 1996; Sommer & Horwith, 2001). Regarding the acidification metrics only the number of sensitive taxa and the MILA index correlated with pH, which is not surprising. In fact, the number of acid sensitive taxa is based on a list of species occurring at the study site and the MILA index is a multimetric index based on general metrics, most of them just shown to correlate positively with lake pH in this study (relative abundance of Ephemeroptera, Diptera and predators, number of taxa of Ephemeroptera and Gastropoda, AWICfam index). The lack of correlation of the other metrics also could be expected: the Raddum and the NIVA indexes are based on the presence/absence of Norwegian acid sensitive species, the AWICfam and AWICsp indexes on the average sensitivity score of families or species from the UK. Both the MILA and the LAMM index were developed specifically for lake ecosystems. However, the first is based on the presence/absence of many

taxonomic groups, that are rare or absent in the Alps even at high pH (Amphipoda, Hirudinea, Elmidae, Gastropoda, Bivalvia) and on the presence/absence of acid sensitive species from Sweden. The second is based on the relative abundance of acid sensitive species common in the UK, and their sensitivity score. Finally, the Braukmann index, although developed for German river ecosystems, is based on a list of acid indicator taxa, where most species found during this study (except chironomids and oligochaetae) are considered. However, the calculated indexes were also not sensitive to pH, because it considers also the relative abundances of sensitive indicator taxa, that are higher in lowland river ecosystems compared to high-altitude lake outlets. This problem can be overcome by slightly modifying the method proposed by Braukmann & Biss (2004) to determine the degree of acidification of a stream. Normally, the relative abundances of the taxa with the same acidification index are added and the so obtained total relative abundances are added cumulatively from the lowest (1 = acid sensitive organisms) to the highest acidification index (5 = very acid-resistant organism) until a minimum threshold of 10% is obtained. The acidification index so reached can be related to the degree of acidification of the water course (i.e. index 1 → not acidified, index 2 → slightly acidified, ...). For the here studied lakes better results are obtained by using a threshold value of 1%.

Regarding lake littoral samples, results from the application of general and specific metrics are less significant because most of the taxa known to be acid sensitive are at the same time rheophil or rheobiotic preferring fast flowing waters (as many Ephemeroptera, Plecoptera and Trichoptera) and are therefore absent in the littorals. Another reason may be the fact that other environmental factors than lake acidity, mainly substrate, tend to overhang the effect of pH variability on macroinvertebrate composition. From this study, it seems that the total number of taxa and the relative abundance and number of taxa of Diptera, Chironomidae and Oligochaeta (i.e. the most important groups in lakes) were the only metrics, effective in structuring the macroinvertebrate community as a result of variation in lake acidity.

Because of the presence of only few acid sensitive species according to tab. 6 also in slightly acidic lakes, it would be interesting to have more information on the acid sensitivity of chironomids and oligochaetes, that are generally not considered in existing acidification metrics. Particularly interesting in this respect are species that are absent in acid sensitive lakes (Lago del Starlaresc da Sgiòf and Lago Tomè) but present in all other lakes. Examples are the chironomids *Paracladopelma camptolabis* gr. and *Macropelopia nebulosa* gr. in the littorals and *Pseudodiamesa branickii*, *Eukiefferiella claripennis*, *Paratrichocladius rufiventris*, *P. skirwithensis*, *Rheocricotopus effusus* in the outlets. Signs of acid sensitivity of some species of the genus *Paracladopelma* sp., *Macropelopia* sp., *Eukiefferiella* sp. are given in Raddum & Sæther (1981), of *Paracladopelma* sp., *Pseudodiamesa* sp. in Schnell &

Raddum (1993) and of *Macropelopia nebulosa* in Ruse (2011). Other examples of chironomid species that are absent in the acid sensitive lakes but present in at least one of the other lakes are shown in tab. 7. However, it must be considered that many of the species present only in Lago Bianco may be related more to low temperatures and to fine substrate than to alkaline water. Nevertheless, some of these species are also reported elsewhere being absent or rare in acid waters: *Cricotopus fuscus* (Ruse, 2011), *Eukiefferiella brevicar* (Orendt, 1999), *Microtendipes pedellus* (Schnell & Raddum, 1993), *Microspectra* sp. (Raddum & Sæther, 1981), *Microspectra atrofasciata* (Ruse, 2011), *Paracladius conversus* (Ruse, 2011), *Parametrioctenemus stylatus* (Ruse, 2011), *Tanytarsus lugens* gr. (Schnell & Raddum, 1993), *Pseudosmittia* sp. (Raddum & Sæther, 1981).

Table 7: List of chironomid species present in the slightly sensitive lakes INF and SUP and in the well buffered lake BIA. More abundant species are written in bold letters and the lake codes (according to fig. 1) in brackets refer to the lakes where individuals have been found.

OUTLET	LITTORAL
<i>Cricotopus fuscus</i> (BIA)	<i>Macropelopia nebulosa</i> gr. (SUP, INF, BIA)
<i>Diamesa bertrami</i> (INF)	<i>Microspectra atrofasciata</i> (SUP, INF)
<i>Diamesa cinerella</i> gr. (INF)	<i>Microtendipes pedellus</i> (BIA)
<i>Diamesa starmachi</i> (BIA)	<i>Paracladius conversus</i> (BIA)
<i>Eukiefferiella brevicar</i> (INF, BIA)	<i>Paracladopelma camptolabis</i> gr. (SUP, INF, BIA)
<i>E. claripennis</i> (SUP, INF, BIA)	<i>Paratrichocladius rufiventris</i> (BIA)
<i>E. minor/fittkai</i> (SUP, INF)	<i>P. skirwithensis</i> (SUP, INF)
<i>E. tirolensis</i> (SUP)	<i>Stilocladius montanus</i> (BIA)
<i>Krenosmittia boreoalpina</i> (BIA)	<i>Tanytarsus lugens</i> gr. (SUP, BIA)
<i>Microspectra atrofasciata</i> (SUP, INF)	<i>Tvetenia bavarica/calvescens</i> (SUP, INF)
<i>Parametrioctenemus stylatus</i> (BIA)	
<i>Paratrichocladius rufiventris</i> (SUP, INF, BIA)	
<i>P. skirwithensis</i> (SUP, INF, BIA)	
<i>Paratrichocladius nudipennis</i> (BIA)	
<i>Pseudodiamesa branickii</i> (SUP, INF, BIA)	
<i>P. nivosa</i> (BIA)	
<i>Rheocricotopus effusus</i> (SUP, INF, BIA)	
<i>Stilocladius montanus</i> (BIA)	
<i>Synothocladius semivirens</i> (INF)	
<i>Tanytarsus lugens</i> gr. (BIA)	
<i>Thienemanniella clavicornis</i> (SUP, INF)	
<i>Tvetenia bavarica</i> (SUP)	
<i>T. calvescens</i> (SUP)	
<i>T. discoloripes/verralli</i> (BIA)	

To conclude, according to this study, stream macroinvertebrates seem to be better indicators of acidity than those belonging to lake littorals. In addition, because of the high abundance and richness, mainly of chironomids, but also of oligochaetes, and their ability to respond to changes in pH in high-altitude Alpine lakes, identification to species should be recommended. Moreover, more studies on the acid sensitivity and autoecology of chironomids and oligochaetes species are necessary to deepen our knowledge on the studied habitats and metrics to be used. However, before any generalisation, it is necessary to apply the here described metrics to a much larger number of high-altitude Alpine lakes in order to reduce the influence of other environmental parameters than pH.

Table 8: Macroinvertebrate taxonomical entities found in the study lakes. Lake codes as in fig. 1.

O stays for outlet and L for littoral.

Taxa	Lake				
	STA	TOM	SUP	INF	BIA
<b>OLIGOCHAETA</b>					
<b>Enchytraeidae</b>			L		
<i>Cernosvitoviella atrata</i> Bretscher 1903	0		O, L	L	L
<i>Cernosvitoviella goodhui</i> Healy 1975				L	
<i>Cernosvitoviella immota</i> Knöllner 1935	0				
<i>Cernosvitoviella</i> sp.	O, L	O, L	O, L	O, L	O, L
<i>Cognettia glandulosa</i> Michaelsen 1888		L	L	L	L
<i>Cognettia</i> sp.	O, L	O, L	L	L	0
<i>Cognettia sphagnetorum</i> Vejdovsky 1878	O, L	O, L	L	L	
<i>Henlea perpusilla</i> Friend 1911			L		
<i>Mesenchytraeus armatus</i> Levinsen 1884			L		L
<b>Lumbricidae</b>					
<i>Eiseniella tetraedra</i> Savigny 1826			L		
<b>Lumbriculidae</b>		0			
<i>Stylogdrilus heringianus</i> Claparède 1862	L	L		O, L	L
<i>Stylogdrilus</i> sp.	L	O, L	O, L	0	O, L
<b>Naididae</b>			0		
<i>Nais bretscheri</i> Michaelsen 1899					O, L
<i>Nais communis</i> Piguët 1906		L	O, L	O, L	O, L
<i>Nais communis / variabilis</i>			O, L	O, L	O, L
<i>Nais pardalis</i> Piguët 1906					L
<i>Nais</i> sp.		L		L	
<i>Nais variabilis</i> Piguët 1906		L	O, L	O, L	O, L
<i>Pristina</i> sp.				L	L
<b>Tubificidae</b>	L	L	L	L	O, L
<i>Tubifex tubifex</i> Müller 1974	L	L	L	L	L
<b>HYDRACARINA</b>	O, L	O, L	O, L	O, L	O, L
<b>COLEOPTERA</b>					
<b>Dytiscidae</b>	0				
Agabinae			L		
Dytiscinae			0		
Hydroporinae	0	0			
Lacophilinae	0	L	L	L	
Hydrophilidae					0
<b>COLLEMBOLA</b>		O, L	0	O, L	O, L
<b>DIPTERA</b>			L		
<b>Athericidae</b>				0	
<b>Ceratopogonidae</b>	O, L	L	L	L	
<b>Chironomidae</b>	O, L	O, L			O, L
Chironominae					
<b>CHIRONOMINI</b>					
<i>Endochironomus dispar</i> gr.	O, L			L	
<i>Microtendipes pedellus</i> de Geer 1776					L
<i>Pagastiella orophila</i> Edwards 1929	L				
<i>Paracladopelma camptolabis</i> gr.				L	L
<i>Polypedilum laetum</i> gr.			0		
<i>Polypedilum nubeculosum</i> gr.		O, L			
<b>TANYTARSINI</b>					
<i>Cladotanytarsus mancus</i> gr.	L				
<i>Cladotanytarsus</i> sp.	0				
<i>Microspectra atrofasciata</i> Kieffer 1911				O, L	O, L
<i>Microspectra</i> sp.	O, L	O, L		O, L	0
<i>Paratanytarsus austriacus</i> Kieffer 1924	O, L	O, L	O, L	O, L	O, L



Taxa	Lake					Taxa	Lake				
	STA	TOM	SUP	INF	BIA		STA	TOM	SUP	INF	BIA
<i>Tanytarsus lugens</i> gr.			L		0, L	<i>Tvetenia bavarica</i> / <i>calvescens</i>	0		0, L	0, L	0
<i>Tanytarsus</i> sp.	L					<i>Tvetenia calvescens</i> Edwards 1929			0		
Diamesinae						<i>Tvetenia discoloripes</i> / <i>verralli</i>					0
<i>Diamesa bertrami</i> Edwards 1935				0		Tanypodinae	0, L	0	L		0, L
<i>Diamesa cinerella</i> gr.				0		MACROPELOPIINI					
<i>Diamesa starmachi</i> Kownacki & Kownacka 1970					0	<i>Apsectrotanypus</i> sp.	0, L				
<i>Pseudodiamesa branickii</i> Nowicki 1873			0	0	0	<i>Apsectrotanypus trifascipennis</i> Zetterstedt 1838	L			0	0
<i>Pseudodiamesa nivos</i> a Goetghebuer 1928					0	<i>Macropelopia nebulosa</i> gr.			L	L	L
<i>Pseudodiamesa</i> sp.		0			L	<i>Macropelopia</i> sp.		L			L
<i>Syndiamesa</i> sp.				L		PENTANEURINI					
Prodiamesinae						<i>Trissopelopia</i> sp.			0		
<i>Prodiamesa olivacea</i> Meigen 1818		L	L	L	L	<i>Zavrelimyia barbatipes</i> Kieffer 1911		0	0, L	0, L	L
Orthoclaadiinae						<i>Zavrelimyia melanura</i> gr.	0, L	0, L			0
<i>Campocladius</i> sp.				L		<i>Zavrelimyia punctatissima</i> Goetghebuer 1934	0, L	L			0
<i>Cardiocladius</i> sp.					0	<i>Zavrelimyia</i> sp.		0			0, L
<i>Chaetocladius</i> sp.	0		0	0	0	PROCLADIINI					
<i>Coryoneura coronata</i> Edwards 1924		0				<i>Procladius choreus</i> Meigen 1804		0			
<i>Coryoneura lobata</i> Edwards 1924		0	0	0, L	0, L	<i>Procladius</i> sp.		L			
<i>Coryoneura scutellata</i> Winnertz 1846	0, L	0, L	0, L	0, L	0, L	<b>Empididae</b>	L		L	0	0, L
<i>Cricotopus anulator</i> / <i>tibialis</i>		0				Clinocerinae			0		0
<i>Cricotopus fuscus</i> Kieffer 1909					0	<b>Limoniidae</b>	0		0, L	L	0, L
<i>Cricotopus</i> sp.					0	<b>Simuliidae</b>	0	0	0	0	0
<i>Cricotopus</i> / <i>Orthocladus</i> sp.					0, L	<b>EPHEMEROPETERA</b>					
<i>Diplocladius</i> sp.					0, L	<b>Baetidae</b>					
<i>Eukiefferiella bavarica</i> / <i>calvescens</i>		0				<i>Baetis alpinus</i> Pictet 1843					0
<i>Eukiefferiella brevicar</i> Kieffer 1911				0	0	<i>Baetis</i> sp.					0
<i>Eukiefferiella claripennis</i> Lundbeck 1898			0	0	0	<b>Heptageniidae</b>					
<i>Eukiefferiella discoloripes</i> / <i>verralli</i>		0				<i>Ecdyonurus helveticus</i> gr.				0	0
<i>Eukiefferiella minor</i> / <i>fittkai</i>			0	0		<i>Ecdyonurus</i> sp.				0	0
<i>Eukiefferiella tirolensis</i> Goetghebuer 1938			0			<i>Epeorus</i> sp.				0	
<i>Heterotrissocladius marcidus</i> Walker 1856	0, L	0, L	L	0, L	0, L	<i>Rhithrogena</i> sp.					0
<i>Krenosmittia borealpina</i> Goetghebuer 1944					0	HETEROPTERA					
<i>Limnophyes</i> sp.	0, L	0, L	0, L	0, L		Corixidae	0, L				
<i>Metriocnemus</i> / <i>Thienemannia</i> sp.			L			MEGALOPTERA					
<i>Neozavrelia</i> sp.			L			Sialidae					
<i>Orthocladus frigidus</i> Zetterstedt 1838		0				<i>Sialis fuliginosa</i> Pictet 1836	L	0			
<i>Orthocladus</i> sp.			0, L	0	0	ODONATA					
<i>Orthocladus</i> ( <i>Eudactylocladius</i> ) sp.		0	0, L	0	0	Aeshnidae	0, L				
<i>Orthocladus</i> ( <i>Euorthocladus</i> ) sp.			0			<i>Aeshna caerulea</i> Ström 1783	0, L				
<i>Orthocladus</i> ( <i>Orthocladus</i> ) sp.		0	0			<i>Aeshna juncea</i> Linnaeus 1758	0, L				
<i>Paracladius conversus</i> Walker 1856					L	<i>Aeshna</i> sp.	0, L				
<i>Parametriocnemus</i> sp.	0				0	Corduliidae					
<i>Parametriocnemus stylatus</i> Kieffer 1924					0	<i>Somatochlora alpestris</i> Sélys 1840	0, L				
<i>Paratrachocladius rufiventris</i> Meigen 1830			0	0	0, L	PLECOPTERA					
<i>Paratrachocladius skirwithensis</i> Edwards 1929			0, L	0, L	0	Leuctridae				0	0
<i>Paratrachocladius rufiventris/skirwithensis</i>				0		<i>Leuctra</i> sp.			0	0	0
<i>Parorthocladus nudipennis</i> Kieffer 1908					0	Nemouridae	0	0	0	0	0
<i>Psectrocladius limbatellus</i> gr.		0, L	0, L	0, L		<i>Nemoura mortoni</i> Ris 1902				0	0
<i>Psectrocladius sordidellus</i> gr.	0	0, L	L	0, L		<i>Nemurella pictetii</i> Klapalek 1900		0, L	0, L	0, L	0, L
<i>Pseudosmittia</i> sp.			L	L		<i>Protonemura nimborum</i> Ris 1902				0	0
<i>Rheocricotopus effusus</i> Walker 1856			0	0	0	<i>Protonemura nitida</i> Pictet 1835					0
<i>Smittia</i> sp.				L		<i>Protonemura</i> sp.		0	0	0	0
<i>Stilocladius montanus</i> Rossaro 1979					0, L	Perlodidae					
<i>Synorthocladus semivirens</i> Kieffer 1909				0		<i>Isoperla</i> sp.					0
<i>Thienemanniella clavicornis</i> Kieffer 1911			0	0		<i>Perlodes intricatus</i> Pictet 1841			0	0	0
<i>Tvetenia bavarica</i> Goetghebuer 1934			0			<i>Perlodes</i> sp.					0

Taxa	Lake				
	STA	TOM	SUP	INF	BIA
TRICHOPTERA		L			
Limnephilidae					
Apataniinae		L	0, L	0, L	0
<i>Allogamus uncatatus</i> Brauer 1857	0				
<i>Drusus discolor</i> Rambur 1842			0		
<i>Drusus</i> sp.			L		0
<i>Limnephilus coenosus</i> Curtis 1834	0				
Odontoceridae					
<i>Odontocerum albicorne</i> Scopoli 1763		0			
Philopotamidae					
<i>Philopotamus ludificatus</i> McLachlan 1878					0
Phryganeidae					
<i>Oligotricha striata</i> Linnaeus 1758	0, L				
Polycentropodidae	0	0, L	0, L	0, L	
<i>Plectrocnemia conspersa</i> Curtis 1834		0, L	0, L	0, L	0
<i>Plectrocnemia</i> sp.	0	0, L	0, L	L	
Rhyacophilidae			0		
<i>Rhyacophila (Rhyacophila)</i> sp.		0	0	0	0
<i>Rhyacophila (Rhyacophila) dorsalis</i> gr.			0	0	
<i>Rhyacophila (Hyporhyacophila) tristis</i> Pictet 1834		0			
<i>Rhyacophila</i> sp.		0	0	0	0
BIVALVIA					
Pisidiidae					
<i>Pisidium casertanum</i> Poli 1791			L		
<i>Pisidium</i> sp.				L	
GASTROPODA				L	
NEMATODA	0, L	0, L	L	0, L	0, L
TURBELLARIA					
Planariidae			0		
<i>Crenobia alpina</i> Dana 1766		0	0	0	0

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