 doi:10.20944/preprints202208.0408.v1

Article

Response of Chironomids to Key Environmental Factors: Perspective for Biomonitoring

Bruno Rossaro [1*], Laura Marziali [2] and Angela Boggero [3]

[1] Università degli Studi di Milano, Dipartimento Ambiente Territorio, via Celoria 2, 20133 Milano, Italy; Bruno.rossaro@unimi.it
[2] National Research Council - Water Research Institute (CNR-IRSA), Via del Mulino 19, 20861 Brugherio, Italy; laura.marziali@irsa.cnr.it
[3] National Research Council - Water Research Institute (CNR-IRSA), Corso Tonolli 50, 28922 Verbania Pallanza, Italy; angela.boggero@irsa.cnr.it
* Correspondence: Bruno.rossaro@unimi.it

Simple Summary: Benthic macroinvertebrates of inland waters are frequently used in biomonitoring. Sometime environmental data associated to species lists are not available; in this situation traits or functional adaptations of species to environment can be considered as a tool to translate the list of species into an index useful to evaluate the environmental quality of a water body.

Abstract: Chironomids are the species richest family among macroinvertebrates and are often used as indicators of ecological condition in inland waters. High taxonomic expertise is needed for identification and new species are still described even in the well-known West Palaearctic region. Data were filed in a Microsoft Access relational database and analysed using the R environment. Our database comprises data on Chironomid species collected in rivers and lakes in Italy and some other European countries over a period of about 50 years, often associated with physical-chemical data, but in some cases only benthic macroinvertebrates are available with no associated environmental data. In this case, the possibility of estimating water quality with only species composition available is discussed. Traits summarizing the species response to environmental variables were evaluated, with emphasis on natural and man influenced factors: current velocity, water temperature, conductivity, dissolved oxygen, nutrients. Traits calculations was possible using the subset of database including both environmental data and Chironomid abundances. The relations between sites, species and traits were evaluated using correspondence analysis and other multivariate methods. The response of species showed an interaction among different factors, with the possibility to order species along a single environmental gradient, extending from cold running waters to warm standing waters, with few exceptions. The utility and limits of the use of ecological traits are discussed.

Keywords: Chironomidae; freshwaters; macroinvertebrates; ecological indicators

1. Introduction

The analysis of environmental factors responsible of macroinvertebrate assemblage structure has a long history. Chironomids inside macroinvertebrates are considered a hard to identify group [1], therefore studies concerning macroinvertebrates as bioindicators have been often limited to Ephemeroptera, Plecoptera, Trichoptera (EPT). Taxonomic problems were forwarded to justify this choice, but even though Chironomids are a hard to identify group is not a valid reason to disregard them. Chironomids include species living in almost all water bodies, sometimes present with a very large number of species, so their exclusion can lead to a serious misjudgement in formulating an assessment of the ecological status of waters.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 24 August 2022 doi:10.20944/preprints202208.0408.v1

A frequently overlooked problem in the development of a biotic index is the species identification accuracy. Especially in taxonomic hard groups, frequent mistakes in species identification were observed. It must be pointed out that different species within the same genus may show a different indicator value, therefore an index based only on genus identification can lead to misleading conclusions with respect to an index based on the identification of species [2].

It is well known that different Chironomid species colonize different river reaches and lake types, suggesting the existence of krenal, rhithral and potamal species in running waters, and littoral, sublittoral and profundal species in lakes [3]. This can be easily related to few environmental factors as substrate type, water temperature, conductivity, oxygen content, current velocity. This result was evident in running waters just one century ago, with Orthocladiini and Tanytarsini dominating the upper reaches of rivers and Chironomini the lower reaches. A similar separation of tribes was observed in lakes, leading to the separation of oligotrophic *Orthocladius*/*Tanytarsus* lakes, opposed to eutrophic *Chironomus* lakes [1].

Different environmental factors were considered as responsible of Chironomids distribution. There is a huge number of contributions to this topic; temperature, salinity and oxygen [4], habitat heterogeneity and water quality [5], water temperature [6], submerged plants, sediments organic matter, distance from the mouth of river, pH [7], oxygen [8, 9], depth in lakes [10, 11, 12] were considered key factors responsible of fauna composition.

On the contrary, the attempt to use Chironomid species as indicators of toxic chemicals [13] did not make much progress, being the same tolerant/intolerant species probably tolerant/intolerant to a set of many other different factors. In contrast, studies concerning the response of Chironomid species to habitat alteration were more fruitful [14].

The use of species identification in the assessment of water quality was criticized and refined considering biological and ecological traits [15, 16, 17, 18, 19, 20], suggesting that non-taxonomic aggregation of taxa as similar as possible in their species traits could aid in interpretation of information given by taxonomic list of species. For example, biological traits were preferred to taxonomic species lists in analysing the response of multiple stressors in central European lowland rivers [21]

The problem is that the possibility to translate a list of species into biological and ecological traits needs basic research to prepare this translation.

The aim of the present paper is to discuss the advantages and limitations of the use of ecological traits respect to taxonomic approach, testing a large database with multivariate data analysis. The discussion considers the situations where environmental data associated to species lists are lacking or scanty, so the traits calculation is proposed as a method overcoming the problem of missing environmental data.

2. Materials and Methods

2.1. Chironomid database

A large database including data on Chironomid species collected in rivers and lakes in Italy and in some other European countries over a period of about 50 years was considered. Physico-chemical data associated with Chironomid samples were available, but only for a subset of data.

Data were filed in a relational database in Microsoft Access© in different **Table**s; the description of these **Table**s is here summarized.

1 **Species**: this **Table** contains a list of the variables used, including both environmental variables (morphometric, physical, chemical) and species belonging to the Chironomidae family; the species were aggregated in species groups (morphotypes), each morphotype corresponding to a genus, a subgenus, a species group or single species [3]. The **Table** contains the species name, author, year of the original description, and

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 24 August 2022 doi:10.20944/preprints202208.0408.v1

taxonomic status (senior synonym, junior synonym, new combination) as additional fields.

2 **Sites**: this **Table** contains a list of the sampling localities, and other additional fields as latitude, longitude, altitude, source distance (for running waters), depth (for lakes), habitat (krenal, kryal, rhithral, potamal, littoral, sublittoral, profundal, etc.).

3 **Conn**: this **Table** connects each environmental variable or species with the sampling station and a numerical value; for environmental variables is the value measured, for species is an index of abundance (see below); additional fields are sampling year, month, day, sampling tool, bibliographic source of information.

The samples here selected for data analysis included larvae collected with different tools, as Surber net, kick net, hand net, etc., and environmental variables measures (water temperature, conductivity, nutrients, etc.) associated to Chironomid samples, when available. The species abundance value was the number of specimens identified per unit effort, that is the number of specimens identified in the full sample, carrying out the analysis in a reasonable time, at least 15 minutes, at a stereomicroscope LEICA MZ12.5 (https://archive.epa.gov/water/archive/web/html/ch07b.html). A crosstab query was then created with sites and other variables describing the sampling site as rows, and environmental variables or species as columns.

The crosstab query created produced a matrix with 9127 sampling sites, including lentic and lotic waters, sampled in different years and months, in Italy above all, but including also data from Algeria [22] and other countries in Europe [14]. The same query included **160** columns, that is a row label, a sequence number, **6** factors, **11** environmental variables and **143** Chironomid taxa. The **11** environmental variables included were: sampling year, sampling month, altitude in m, source distance in km, O_2 content in mg l^{-1}, conductivity in µS cm^{-1}, pH, total phosphorous in µg l^{-1}, N-NO_3 in mg l^{-1}, N-NH_4 in µg l^{-1}, water temperature in °C. The **4** factors were habitat, river basin, water body, sampling station (**Table S1**).

The taxa included in the analysis were the morphotypes or species groups described in [3] (Rossaro et al., 2022); in the next part of the present work these taxa will be named "species" for simplicity, even if they are often taxa larger than species (genus, group of species.

The sites where less than 5 species were present and species present in less than 50 sites were excluded, leaving a matrix with **91** species in **2258** sites aggregated in 10 different habitats: glacial stream (k=kryal), springs (s=krenal), streams (r=rhithral), lowland rivers (p=potamal), alpine lakes (ALA), lowland large lakes (LL), small lakes (LS), volcanic lakes (V), Mediterranean lakes (ME) and brackish waters (B). These 10 habitats were further divided into 102 waterbodies. The delimitation of these habitats is described in other publications [3, 23, 24, 25].

2.2. Data analysis

The crosstab query generated a matrix with n sites as rows and p species + s environmental variables as columns (${}_n\mathbf{M}_{p+s}$), which was input in an R script (**Table S1**).

The **M** matrix was separated into an ${}_n\mathbf{L}_p$ matrix of species and in a ${}_n\mathbf{R}_s$ matrix of environmental variables. Each environmental variable was used to calculate: 1- a correlation matrix between each species and the environmental variables ${}_p\mathbf{C}_s$; 2- a weighted mean of each environmental variable for each species, i.e. means of each environmental variable weighted according to species abundances, which can be considered the optimum for each species; 3- a weighted standard deviation, which can be considered a measure of species tolerance. The weighted mean of each species with each environmental variable generated a trait matrix ${}_p\mathbf{U}_s$ with p species as rows and s environmental variables as columns [3, 26]. The presence of missing data in ${}_n\mathbf{R}_s$ matrix forced to calculate matrices ${}_p\mathbf{C}_s$ and ${}_p\mathbf{U}_s$ matrices using only the available data.

The ${}_n\mathbf{L}_p$ matrix, including the reduced n (=2258) sites and p (=91) species, and the ${}_p\mathbf{U}_s$ matrix, including the same species and s (=11) traits, were analysed with a correspondence

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 24 August 2022 doi:10.20944/preprints202208.0408.v1

analysis (unconstrained ordination) [27, 28]. The $_nL_p$ values were log(x+1) transformed before calculation. As a second step, a canonical constrained ordination was carried out using the transpose of $_nL_p$, that is $_pL'_n$, and $_pU_s$ as input matrices. As a last step the $_nL_p$ matrix was post multiplied by the $_pU_s$ matrix, submitted an unconstrained ordination and compared with the previous results. The large number of missing data in the $_nR_s$ matrix hindered to carry out a canonical constrained ordination between the $_nL_p$ and $_nR_s$ matrices.

The **sites x species** matrix $_nL_p$ was post-multiplied by **species x traits** $_pU_s$ matrix to obtain a **site x traits** matrix ($_nL\,_pU_s$), i.e., a matrix with sites as rows and species traits as columns. This $_nL\,_pU_s$ matrix was also submitted to correspondence analysis.

A discriminant analysis was carried out to test the goodness of classification in different habitats using the Chironomid taxa assemblages: both the $_nL_p$ and the $_nL\,_pU_s$ matrices were submitted to multiple discriminant analysis, using the habitats as grouping factor.

At last a cluster analysis of site x species matrix was carried out using complete linkage clustering method [28] to detect clusters of species.

3. Results

Measures of the 11 environmental variables were available for a reduced number of sites (**Table** 1), so the correlations, weighted means and standard deviations of each environmental variable with each species were calculated using sites where both species and environmental data values were available (see Methods); when less than 4 records were available for the couple environmental variable-species, correlations were not calculated and mean values and standard deviations of the environmental variable calculated over all the other species were assigned to these species.

Table 1. Number of sites available for each environmental variable: altit=altitude, dist=source distance, year, month, temp=water temperature, cond=conductivity, pH, O_2=dissolved oxygen, TP=total phosphorous, N-NO_3 =nitrate nitrogen, N-NH_4=ammonium nitrogen.

Altit	dist	Year	month	temp	pH	cond	O_2	N-NO_3	TP	N-NH_4
9127	7546	9127	9045	5951	4797	4823	5335	3530	2854	2045

Highly significant correlations ($p<0.01$) between species abundance and environmental variables were observed for a reduced number of species (**Table** 2, **Table S2**).

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 24 August 2022 doi:10.20944/preprints202208.0408.v1

Table 2. Highly significant correlations (* = p<0.01) between species and environmental variables, + = positive correlations, - =negative correlations. Detailed results in Table S2.

	alt		dist		y		m		T		pH		cond		O_2		$N\text{-}NO_3$		TP		$N\text{-}NH_4$	
Ablabesmyia	+	*	-	*	+	*	+		-	*	+	*	-	*	+	*	-		-	*	-	
Brillia	-		-		-		+		-		-	*	-	*	-		+		-		-	
C.anthracinus	+	*	-		+		+		-		+	*	-		+		-		-		-	
C.bicinctus	-		-		+		-		-	*	+		+		+	*	+		-		-	
C.fuscus	+	*	-		+		-		-	*	+		-		+		-		-		-	*
C.plumosus	+	*	-	*	-	*	-	*	-	*	+	*	-		+	*	+		-	*	-	
C.thummi	+		-		+		+		-		+	*	-		-		+		+		-	
C.tremulus	-		-		-		-		-		-		+		+		-		-		-	
C.trifascia	-		+		-		-		-		+		+		+		+		-		-	
Chaetocladius	+	*	-	*	+	*	+		-		-	*	-		+		-		+		-	
Cladopelma	+		-	*	+		+	*	+		+		-		+		-		-		-	
Cladotanytarsus	+	*	-	*	-		-		-	*	+	*	-		+	*	+		-		-	*
Conchapelopia	+		-		-	*	+		-	*	+	*	+		+	*	-		-	*	-	*
Corynoneura	+	*	-		+	*	+	*	-	*	-		-	*	+		-	*	-	*	-	*
Cryptochironomus	+	*	-	*	-	*	-		-	*	+	*	-	*	+	*	-		-	*	-	*
D.aberrata	+		+		+		+		-		-		+		+		+		-		-	
D.cinerella	-		+		+		-		+		+		-		-		-		-		-	
D.dampfi	+		-		+		+		-		+		-		+		-		-		-	
D.latitarsis	+	*	-	*	+	*	+		-	*	-	*	-	*	+		-		-		-	
D.tonsa	+	*	-		+		+		-	*	-	*	-	*	+		-		-		-	*
D.zernyi	+	*	-		+	*	+		-	*	-	*	-	*	+		-	*	-		-	
Demicryptochironomus	-		-		-	*	+		-		-		-		+		-		+		+	
Diamesa	+	*	-		+	*	+		-	*	-		-	*	+		-		-		-	
Dicrotendipes	+	*	-	*	+		-		-	*	-		-		+	*	-	*	-	*	-	*
E.claripennis	+	*	-	*	+		-		-		-	*	-		-		-		-		-	
E.devonica	-		+		+	*	+		+		+		-		+		-		+		-	
E.minor	+	*	-		+	*	+		-	*	-	*	-	*	-		-	*	-		-	
Endochironomus	+	*	-	*	-		+		-		+		-		+		-		-	*	-	
Eudactylocladius	+		+	*	+	*	-		-		-		-		+		-		-		-	
Eukiefferiella	+		+		+		-		+		+		-		-		+		-		-	
Euorthocladius	+	*	-	*	+	*	-		-		-	*	+		-		-		-	*	-	*
Glyptotendipes	+		-		+		+		-		+	*	-	*	-		-		-		-	
Harnischia	+	*	-	*	+	*	-		-	*	+	*	-		+	*	-		-	*	-	
Heleniella	+	*	-		+	*	+		-		-		-	*	+		-		-		-	
Heterotrissocladius	+	*	-		+	*	+	*	-	*	-		-	*	+	*	-	*	-		-	*
Holotanypus	+		-	*	-		-		-	*	+	*	-	*	+	*	-		-	*	-	*
I.sylvestris	-		-		-	*	-		-		+		-		+		-		-		-	
M.atrofasciata	+	*	-	*	+	*	-		-	*	-	*	-	*	-		-	*	-	*	-	
M.notescens	+		+		+		-		+		+		+		-		+		+		+	
M.radialis	+		+		-		-		-		+		-	*	+	*	-		-		-	
Macropelopia	+	*	+		+	*	+		-	*	-		-	*	+		-		-		-	
Mesorthocladius	+	*	-		+	*	+		-	*	-		-	*	-		-		-		-	
Microchironomus	-		+		-		-		-		+		-		+	*	-		-		-	*
Microtendipes	+		-		-	*	-		-		+	*	-		+	*	-		-		-	
Nanocladius	-		-		+		+		+		-	*	+		-		-		-		-	
O.decoratus	+		-	*	+	*	-		-		-		+		+		+		-		-	
O.oblidens	+		-		-	*	-		-	*	-		-		+		-		-	*	-	
Orthocladius	-		-		+	*	-	*	+		+		+		-		-		-		-	
P.austriacus	+		-		+		+		-		+		-		+		-		-		+	
P.laetum	+		-		+		+		-		+		+		+		+		-		-	
P.limbatellus	+		-	*	-	*	-		-	*	+		-	*	+	*	-		-		-	
P.nubeculosum	+		-	*	-	*	-		-		+	*	-	*	+	*	-		-		-	*

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 24 August 2022 doi:10.20944/preprints202208.0408.v1

P.rufiventris	-		-		+	*	-		+		+		-		-		+		+		+				
P.skirwithensis	-		+		+		-		-		+		-		-		-		-		-				
P.sordens	-		+		-		-		-		+		+		-		-		-		+				
P.sordidellus	+		-		+		+		-		+		-		+		-	*	-		-				
Pagastiella	+		-	*	+		-		-		+		+	*	+		-		-		-				
Parachironomus	-		-		-	*	-		-		+		+		+		+		-		-				
Paracladius	+		-		+		+		-		+		-		+		-		-		+				
Paracladopelma	+	*	-	*	+	*	-	*	-		+		-		+		+	*	+		-				
Paracricotopus	-		-		+		-		+		-		+		-		-		+		+				
Parakiefferiella	-		+		+		-		+		+		-		+	*	+		-		-				
Paralauterborniella	+	*	-	*	+	*	-		-	*	+	*	+		+		+		-		-				
Parametriocnemus	+	*	-		+	*	+		-	*	-	*	-		+		-		-		-				
Paratanytarsus	+	*	-	*	-		-		-		+		-		+	*	-		-		-				
Paratendipes	-	*	-	*	-	*	-	*	-		+	*	-		+	*	-		-	*	-				
Paratrissocladius	+		+		+		-		+		-		+		-		+		-		-				
Parorthocladius	+	*	-		+	*	+		-	*	-		-	*	+		-		-		-				
Phaenopsectra	+	*	-		+		-		-		+		-		+	*	-		-	*	-				
Potthastia	-		-		+	*	-		+		+		-		-		-		-		-				
Prodiamesa	+		+	*	+		+		-	*	+	*	-	*	+		-		-		-				
Pseudochironomus	+		-	*	-		-		-		+		-		+	*	-		-	*	-				
Pseudodiamesa	+	*	+		+	*	+		-	*	-	*	-	*	-		-		-		-				
Psilocricotopus	-		-		+		+		+		+		-		-		-		-		-				
Rheocricotopus	-		-		+		-		+		+		-		-		-		+		+				
Rheopelopia	+		+	*	+		+		-	*	+		-		-		+		-		-				
Rheotanytarsus	+		-		+		+		-		+		-		+		-		+		-				
Stempellina	+	*	-	*	+		+		-		+		+		+	*	+		+		-				
Sympotthastia	+		-		+	*	-		-		+		-		-		-		-		-				
Synorthocladius	+	*	-		+	*	-		-		-		-		-		-		-		-				
Tegarius	+	*	-	*	+		+		-		-		-		+	*	-		-		-	*			
Tanypus	-		+		-		+		+		+		-		-		-		+		+				
Tanytarsus	+	*	-	*	+	*	-		-	*	+	*	-		+	*	-		-	*	-	*			
Thienemannimyia	+	*	-		+		+		-		+		-		+		-		-		-				
Tripodura	+	*	-	*	+	*	+		-		+		+		+		-		-		-				
Trissopelopia	+		+		-	*	-		-		-		-		-		-		-		-				
Tvetenia	+		+		+	*	-		+		+		-		-		-		-		-				
Uresipedilum	+		-		-		-		-		+		+		+		-		-		-				
Virgatanytarsus	+		-		+		+		+		+		+		-		-		-		-				
Xenochironomus	+		-	*	-	*	-		-		+	*	-		+	*	-		-		-				
Zavrelimyia	+	*	-		+	*	+		-	*	-		-		+		-		-		-				

The weighted means, considered the optimum values for all species [27], were used to create a **species x traits** matrix ${}_{p}\mathbf{U}_{s}$ with p species as rows and s environmental variables as columns (**Table** 3). Weighted standard deviation as a measure of species tolerance and the number of observations available are in **Table S3**.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 24 August 2022 doi:10.20944/preprints202208.0408.v1

Table 3. Matrix of traits: weighted mean of each environmental variable for each species. Standard deviations and number of sites used in Table S3.

Traits	altit	dist	year	month	temp	pH	cond	O_2	$N\text{-}NO_3$	TP	$N\text{-}NH_4$
Ablabesmyia	516	41	1999	6	18	7	273	7	1	69	239
Brillia	522	19	1988	6	17	7	328	5	2	207	973
C.anthracinus	319	55	1981	6	17	8	239	8	1	53	119
C.bicinctus	220	109	1993	6	21	7	775	5	3	212	885
C.fuscus	809	28	1992	6	16	7	415	4	2	34	502
C.plumosus	212	95	1981	6	20	7	349	6	1	127	469
C.thummi	223	122	1993	6	22	7	669	4	2	335	920
C.tremulus	354	94	1990	6	20	7	455	4	1	143	372
C.trifascia	269	65	1996	6	21	7	569	3	3	141	560
Chaetocladius	1872	4	1996	8	8	6	75	7	1	80	241
Cladopelma	284	40	1983	7	19	7	281	7	1	98	410
Cladotanytarsus	361	24	1989	6	17	8	318	8	1	57	101
Conchapelopia	414	53	1987	6	18	7	632	5	2	99	696
Corynoneura	1229	33	1995	7	15	7	180	6	0	26	84
Cryptochironomus	269	54	1982	6	18	7	281	8	1	67	218
D.aberrata	1329	15	1985	6	12	7	279	4	2	246	457
D.cinerella	1272	35	1998	6	11	7	218	4	1	178	626
D.dampfi	1384	7	1990	6	11	7	147	8	1	114	428
D.latitarsis	2089	4	1999	8	7	6	108	8	1	61	90
D.tonsa	1051	22	1991	6	15	7	253	5	2	124	509
D.zernyi	1917	6	1996	8	8	6	127	7	0	53	153
Demicryptochironomus	217	62	1977	7	20	7	314	8	1	116	298
Diamesa	1735	6	1992	8	8	6	158	6	0	50	118
Dicrotendipes	254	74	1987	6	20	7	513	6	1	72	278
E.claripennis	830	33	1994	6	17	7	433	5	1	101	606
E.devonica	305	79	1998	6	21	7	441	4	2	154	505
E.minor	1433	15	1993	7	11	7	219	5	1	76	486
Endochironomus	215	90	1981	7	19	8	352	4	1	64	165
Eudactylocladius	1144	39	1995	7	15	7	248	6	1	100	596
Eukiefferiella	511	81	2000	6	19	7	352	3	1	190	509
Euorthocladius	702	59	1994	6	18	7	394	4	1	218	467
Glyptotendipes	164	176	1985	7	21	7	264	4	1	86	297
Harnischia	205	155	1991	6	21	8	535	5	1	73	397
Heleniella	1978	6	1998	8	8	6	62	7	0	29	59
Heterotrissocladius	1604	14	1995	7	11	7	69	7	0	13	28
Holotanypus	367	48	1985	6	17	7	300	8	1	67	216
I.sylvestris	280	118	1990	6	22	7	649	4	1	126	543
M.atrofasciata	807	35	1994	6	17	7	382	4	1	105	559
M.notescens	651	45	1989	6	19	7	600	2	4	180	1107
M.radialis	743	62	1996	7	16	7	187	8	1	81	414
Macropelopia	1081	32	1998	7	13	7	180	7	1	61	219
Mesorthocladius	1354	16	1994	6	13	7	224	5	1	64	381
Microchironomus	233	64	1988	6	16	8	296	7	1	38	49
Microtendipes	341	48	1990	6	20	7	459	6	2	80	409
Nanocladius	252	108	1990	7	24	7	497	4	1	267	616
O.decoratus	425	107	1997	5	21	7	528	5	2	242	886
O.oblidens	261	66	1987	5	19	7	422	6	1	87	186
Orthocladius.sstr	331	69	1994	5	20	7	560	4	2	199	663
P.austriacus	1899	3	2000	8	10	7	81	8	0	5	51
P.laetum	312	111	1990	6	23	7	681	4	3	283	974
P.limbatellus	497	29	1980	6	17	7	207	7	1	66	194
P.nubeculosum	302	72	1983	6	19	7	357	6	1	78	334
P.rufiventris	432	42	1996	6	20	7	678	4	2	176	778
P.skirwithensis	1193	20	1994	7	11	7	249	5	1	70	200

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 24 August 2022 doi:10.20944/preprints202208.0408.v1

P.sordens	123	179	1990	6	22	7	596	2	1	116	718
P.sordidellus	693	35	2001	6	19	7	214	6	1	92	283
Pagastiella	294	50	1979	6	20	7	263	9	1	60	54
Parachironomus	136	143	1986	6	23	7	466	4	2	161	409
Paracladius	607	54	1992	6	17	7	291	6	1	105	772
Paracladopelma	530	54	1997	5	17	8	213	8	1	57	50
Paracricotopus	391	38	1996	6	19	7	506	5	1	225	1292
Parakiefferiella	426	36	1987	5	16	7	208	9	1	47	51
Paralauterborniella	378	48	1992	6	13	7	236	9	1	16	31
Parametriocnemus	896	19	1994	6	15	7	360	5	1	73	382
Paratanytarsus	408	85	1988	6	21	7	427	5	2	60	391
Paratendipes	370	33	1979	6	16	8	308	7	1	45	102
Paratrissocladius	535	17	1996	6	16	7	623	7	1	75	428
Parorthocladius	1806	4	1997	8	10	6	187	6	0	55	122
Phaenopsectra	335	69	1995	6	21	7	450	6	1	127	812
Potthastia	211	107	1996	5	22	7	349	4	2	140	448
Prodiamesa	557	53	1990	6	16	7	263	7	1	92	389
Pseudochironomus	290	36	1981	6	20	7	273	8	1	55	123
Pseudodiamesa	1733	16	1993	7	9	7	109	5	0	28	135
Psilocricotopus	237	90	1994	7	22	7	503	4	2	265	457
Rheocricotopus	501	46	1992	6	18	7	698	4	3	225	1300
Rheopelopia	226	249	1993	6	18	7	395	5	3	201	573
Rheotanytarsus	225	152	1992	6	20	7	452	4	2	456	393
Stempellina	322	51	1984	6	16	7	215	9	1	55	52
Sympotthastia	246	88	2003	4	21	7	365	4	1	122	94
Synorthocladius	313	81	1996	6	21	7	444	4	1	188	764
Tegarius	339	50	1981	6	17	7	228	8	1	42	74
Tanypus.sstr	170	133	1990	7	24	7	763	4	2	283	1448
Tanytarsus	573	65	1996	6	19	7	480	6	2	67	320
Thienemannimyia	599	55	2002	6	17	7	492	6	1	16	38
Tripodura	214	156	1995	7	23	7	853	5	1	202	459
Trissopelopia	680	38	1992	7	16	7	438	3	0	22	428
Tvetenia	668	38	1995	6	18	7	429	5	2	93	387
Uresipedilum	249	129	1990	7	22	7	998	5	4	191	909
Virgatanytarsus	375	49	1995	7	22	7	951	3	5	114	1417
Xenochironomus	224	56	1976	6	20	7	387	7	1	59	174
Zavrelimyia	1480	11	1997	7	12	7	176	7	0	29	41

The **sites x species** matrix ${}_{n}\mathbf{L}_{p}$ was submitted to a correspondence analysis; three major gradients (**Figs. 1, 2, Figs. S1, S2, Table 4**) were evidenced, the former accounting for 7.6 % of total variance, the second 5.1 %, the third 3.8 % of the total variance, with eigenvalues equal to 0.71, 0.48, 0.35 respectively; the species and sites ordered in the plane of the two axes showed the typical horseshow or arch effect [28]. The first gradient separated running waters from standing waters, the second separated upstream stations from downstream stations in running waters, with the following sequence (**Figs. 1, 2, Figs. S1, S2**): 1- frigo-stenothermal species living in kryal were plotted in the bottom left of the graph; 2- rhithral species living in streams were plotted above the former; 3- eurithermal species, living in potamal, were plotted at the apex of the arch, extending from the top to the right part of the plot; 4- species living preferably in lentic waters, were plotted on the right part of the graph; 5- species living in springs were plotted in the central part of the area. A further separation was of species from small alpine lakes as *Paratanytarsus austriacus*, *Heterotrissocladius*, *Corynoneura* and *Zavrelimyia* plotted in the centre of the area, species characterizing profundal zone of lowland large lakes as *Micropsectra radialis*, *Paracladopelma* also plotted closer to the centre of the area at right of alpine lakes species, small prealpine and volcanic lakes were grouped on the right of the plot. This separation was

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 24 August 2022 doi:10.20944/preprints202208.0408.v1

still better emphasized in a 3D plot (**Fig.** 1, **Fig. S1**), where kryal, rhithral, krenal, potamal and lentic species was evident.

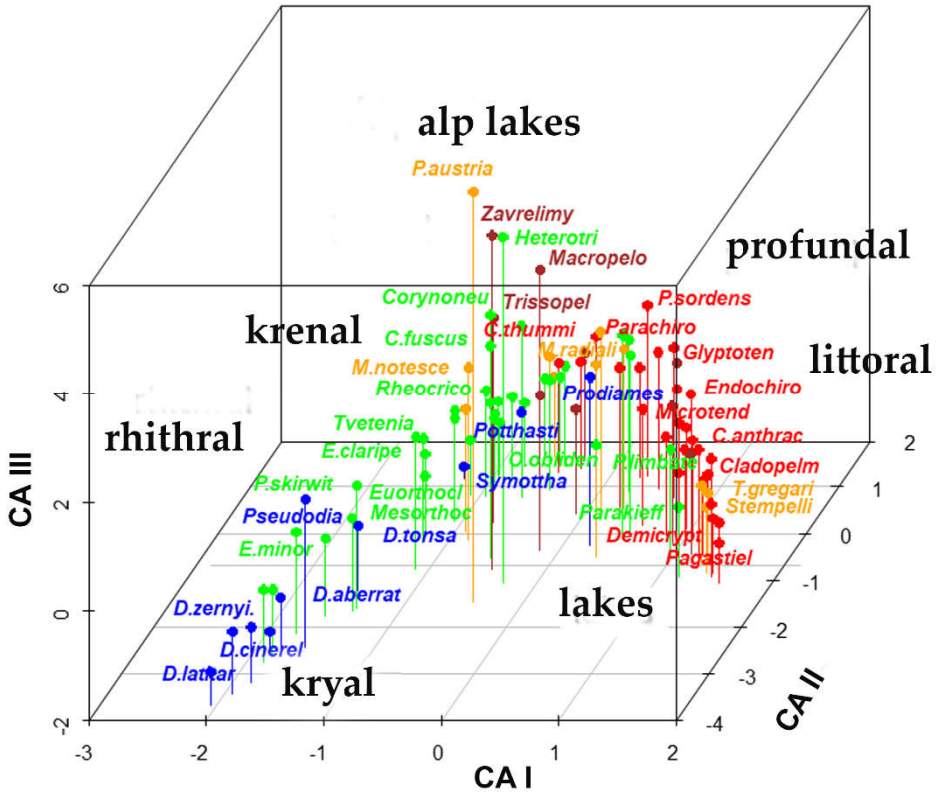

Figure 1. plot of the species scores in the first 3 axes resulting from CA carried out from sites x species (${}_{n}\mathbf{L}_{p}$) matrix (the full set of species names is in **Fig. S**1).

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 24 August 2022 doi:10.20944/preprints202208.0408.v1

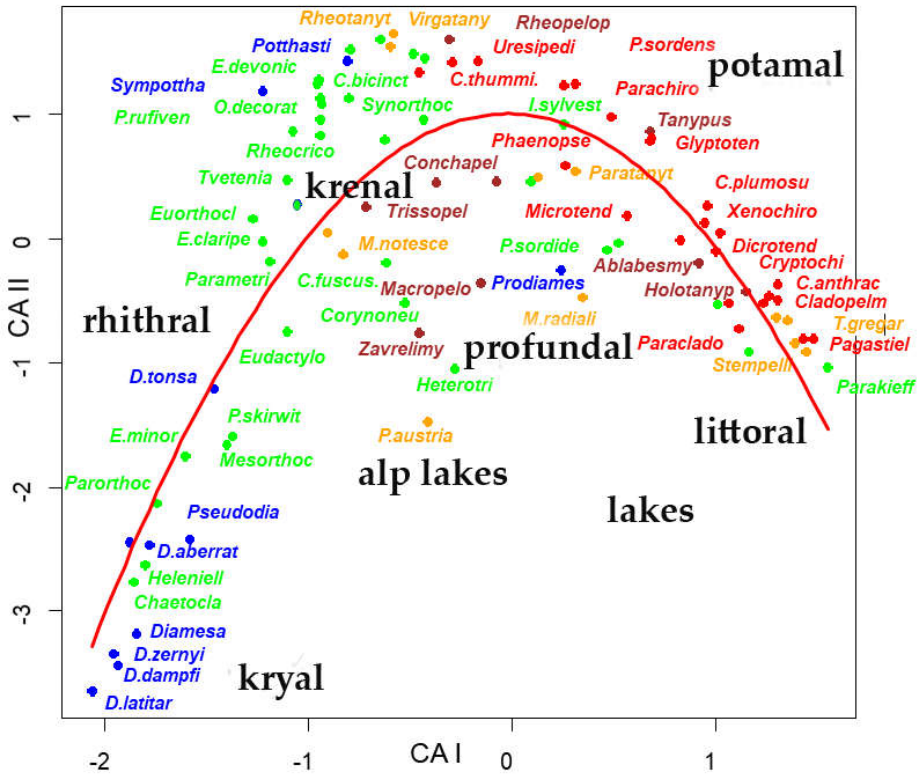

Figure 2. plot of the species scores in the first 2 axes resulting from CA carried out from sites x species ($_{n}\mathbf{L}_{p}$) matrix and the fitted second degree polynomial (the full set of species names is in **Fig. S2**).

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 24 August 2022 doi:10.20944/preprints202208.0408.v1

Table 4. Correspondence analysis (CA) results of sites x species matrix; eigenvalues, proportion of variance explained, factor loadings of species. Results of other multivariate analysis in Tables S4, S5, S6.

	CA1	CA2	CA3
Eigenvalue	0.715	0.480	0.353
Proportion Explained	7.6 %	5.1 %	3.8 %

species	CA1	CA2	CA3	CA4	CA5
Ablabesmyia	0.919	-0.194	0.514	-0.087	0.548
Brillia	-1.057	0.277	0.025	0.692	-2.146
C.anthracinus	1.237	-0.522	-0.010	0.028	0.863
C.bicinctus	-0.431	1.451	-0.135	-0.312	0.055
C.fuscus	-0.616	-0.201	1.636	0.596	-1.708
C.plumosus	0.949	0.129	-0.162	-0.940	0.818
C.thummi	-0.291	1.422	-0.023	-0.720	0.161
C.tremulus	-0.434	0.957	0.032	-0.587	-0.194
C.trifascia	-0.796	1.521	-0.882	0.865	1.516
Chaetocladius	-1.857	-2.763	-0.646	-1.242	-0.403
Cladopelma	1.309	-0.493	-0.477	-0.072	0.295
Cladotanytarsus	1.358	-0.655	-0.696	0.586	-0.098
Conchapelopia	-0.074	0.459	-0.097	0.514	-1.571
Corynoneura	-0.529	-0.524	2.502	0.065	0.669
Cryptochironomus	1.263	-0.465	-0.630	0.280	0.059
D.aberrata	-1.781	-2.463	-1.056	-1.494	-0.697
D.cinerella	-1.878	-2.447	-1.693	-1.271	2.449
D.dampfi	-1.940	-3.429	-0.850	-1.640	-0.151
D.latitarsis	-2.065	-3.645	-1.417	-2.169	0.678
D.tonsa	-1.465	-1.214	-0.817	-0.296	-0.145
D.zernyi	-1.959	-3.345	-0.939	-1.806	0.568
Demicryptochironomus	1.434	-0.812	-0.996	1.185	-0.417
Diamesa	-1.845	-3.178	-0.972	-2.015	0.141
Dicrotendipes	1.006	-0.101	-0.356	-0.262	-0.112
E.claripennis	-1.225	-0.020	-0.501	0.222	-0.099
E.devonica	-0.949	1.278	-1.057	1.190	2.353
E.minor	-1.603	-1.747	-0.569	-0.480	-0.716
Endochironomus	0.958	0.265	0.350	-2.380	0.031
Eudactylocladius	-1.104	-0.755	0.436	-0.058	0.438
Eukiefferiella	-1.079	0.865	-1.019	1.134	2.492
Euorthocladius	-1.274	0.159	-1.063	0.386	1.313
Glyptotendipes	0.676	0.780	0.775	-3.588	-0.147
Harnischia	0.688	0.808	-0.011	-1.820	0.997
Heleniella	-1.804	-2.633	-0.773	-1.272	0.180
Heterotrissocladius	-0.280	-1.046	4.377	1.436	1.791
Holotanypus	1.153	-0.430	-0.172	0.144	0.055
I.sylvestris	0.254	0.924	0.785	-2.154	0.230
M.atrofasciata	-0.905	0.051	0.245	0.412	-0.693
M.notescens	-0.829	-0.132	1.177	0.706	-4.154
M.radialis	0.347	-0.475	1.536	1.026	1.007
Macropelopia	-0.153	-0.352	3.178	1.196	-0.538
Mesorthocladius	-1.403	-1.659	-0.277	-0.325	-0.007
Microchironomus	1.306	-0.371	-0.285	-0.938	2.111
Microtendipes	0.567	0.188	0.125	-0.238	-0.445
Nanocladius	-0.484	1.488	-0.374	-0.262	0.037
O.decoratus	-0.942	1.133	-0.757	0.681	2.077
O.oblidens	0.094	0.460	-0.754	0.992	0.153
Orthocladius	-0.938	1.087	-0.805	0.841	1.154

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 24 August 2022 doi:10.20944/preprints202208.0408.v1

P.austriacus	-0.416	-1.470	5.558	1.585	3.067
P.laetum	-0.455	1.332	0.003	-0.501	-0.761
P.limbatellus	1.007	-0.534	0.015	0.717	-0.108
P.nubeculosum	0.825	-0.007	-0.203	-0.619	-0.422
P.rufiventris	-0.942	0.961	-0.420	0.628	-0.254
P.skirwithensis	-1.373	-1.588	0.248	-0.165	-0.518
P.sordens	0.314	1.250	1.147	-4.057	-0.605
P.sordidellus	0.468	-0.087	1.744	0.222	1.588
Pagastiella	1.551	-1.038	-1.272	1.861	-0.875
Parachironomus	0.488	0.986	0.499	-2.691	0.481
Paracladius	0.525	-0.032	1.335	0.378	1.521
Paracladopelma	1.115	-0.726	0.669	1.077	1.159
Paracricotopus	-0.955	1.250	-0.629	1.008	0.242
Parakiefferiella	1.169	-0.910	-0.724	1.579	-0.538
Paralauterborniella	1.448	-0.908	-0.672	1.340	0.008
Parametriocnemus	-1.192	-0.185	-0.090	0.465	-1.188
Paratanytarsus	0.310	0.541	0.964	-1.438	0.061
Paratendipes	1.069	-0.517	-0.422	-0.069	-0.062
Paratrissocladius	-0.628	0.793	1.167	0.954	-4.519
Parorthocladius	-1.743	-2.135	-0.132	-0.590	0.310
Phaenopsectra	0.264	0.587	0.561	-0.492	-0.300
Potthastia	-0.808	1.435	-0.994	1.057	2.775
Prodiamesa	0.244	-0.253	1.121	0.684	-0.459
Pseudochironomus	1.482	-0.807	-1.096	0.999	-0.632
Pseudodiamesa	-1.583	-2.418	0.699	-0.648	-0.671
Psilocricotopus	-0.645	1.603	-0.508	0.297	0.286
Rheocricotopus	-0.943	0.828	-0.053	0.621	-1.605
Rheopelopia	-0.308	1.607	-0.017	-0.522	-0.639
Rheotanytarsus	-0.582	1.653	-0.516	0.271	0.875
Stempellina	1.393	-0.837	-0.799	1.436	-0.372
Sympotthastia	-1.227	1.186	-1.761	1.595	5.236
Synorthocladius	-0.800	1.135	-0.447	0.917	0.710
Tanypus	0.680	0.868	0.408	-2.783	0.910
Tanytarsus	0.132	0.499	1.312	-0.202	0.524
Tegarius	1.298	-0.639	-0.542	0.752	-0.063
Thienemannimyia	-0.375	0.449	0.184	1.309	0.383
Tripodura	0.253	1.230	0.003	-1.328	0.792
Trissopelopia	-0.715	0.249	1.784	1.108	-5.422
Tvetenia	-1.105	0.468	-0.284	0.878	0.223
Uresipedilum	-0.166	1.434	0.423	-1.454	-1.375
Virgatanytarsus	-0.595	1.541	-0.046	0.412	-2.829
Xenochironomus	1.025	0.052	-0.318	-0.857	-0.021
Zavrelimyia	-0.459	-0.756	4.151	1.508	0.321

A polynomial of second degree was fitted to species scores of the two first axes (**Fig.** 2), resulting in a multiple R-squared 0.6845, adjusted R-squared 0.6773, F-statistic: 95.47 with 2 and 88 degrees of freedom (D.F.), p-value 2.2e-16, residual standard error 0.7344 with 88 D.F. The species more distant from the parabolic curve are visible in **Fig.** 2 and are also evident in **Fig. S2**, where all species names are plotted. Species from small Alpine lakes and from profundal areas of large lakes are the ones more deviating from parabolic curve.

The environmental variables were included as passive variables in the map and were converted into factors with 6 different levels; when missing data were present a level, plotted as void circles, grouped these data. The factors included were: habitat, station (**Fig.** 3), altitude, source distance (**Fig.** 4), temperature, conductivity (**Fig.** 5), oxygen, total phosphorous (**Fig.** 6), nitrate and ammonium nitrogen (**Fig.** 7).

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 24 August 2022 doi:10.20944/preprints202208.0408.v1

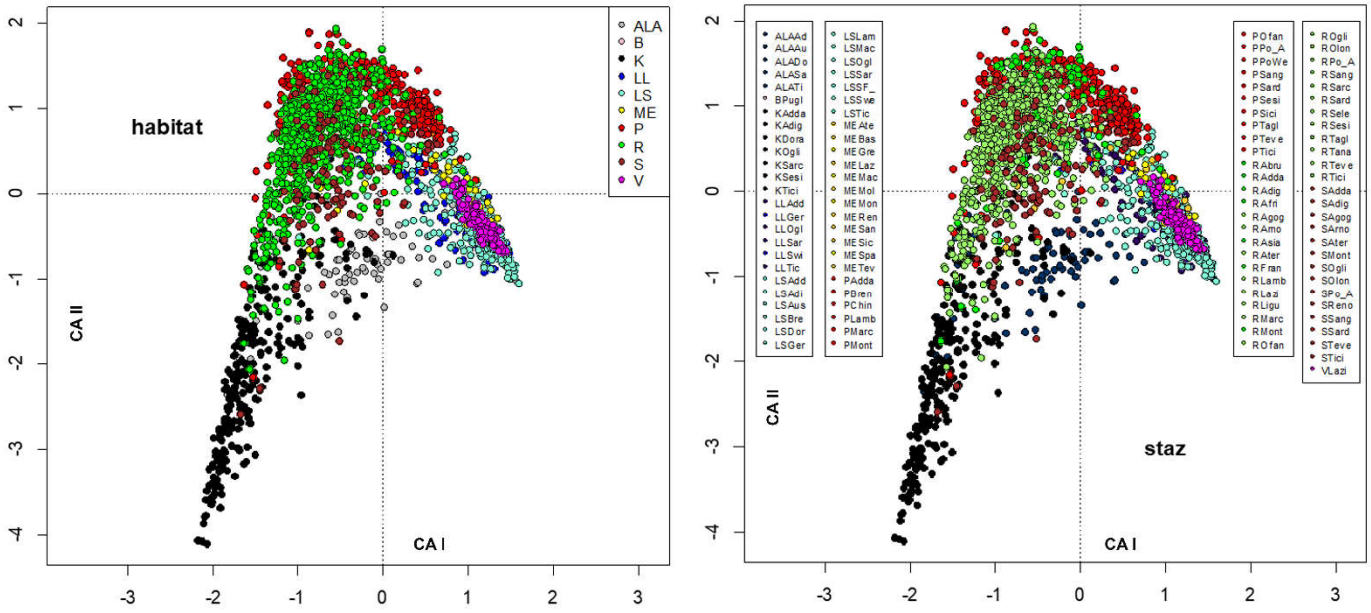

Figure 3. plot of sites scores in the first 2 axes resulting from CA of sites x species ($_nL_p$) matrix, by marking sites with different colours according to habitat (left) and to sampling station (right).

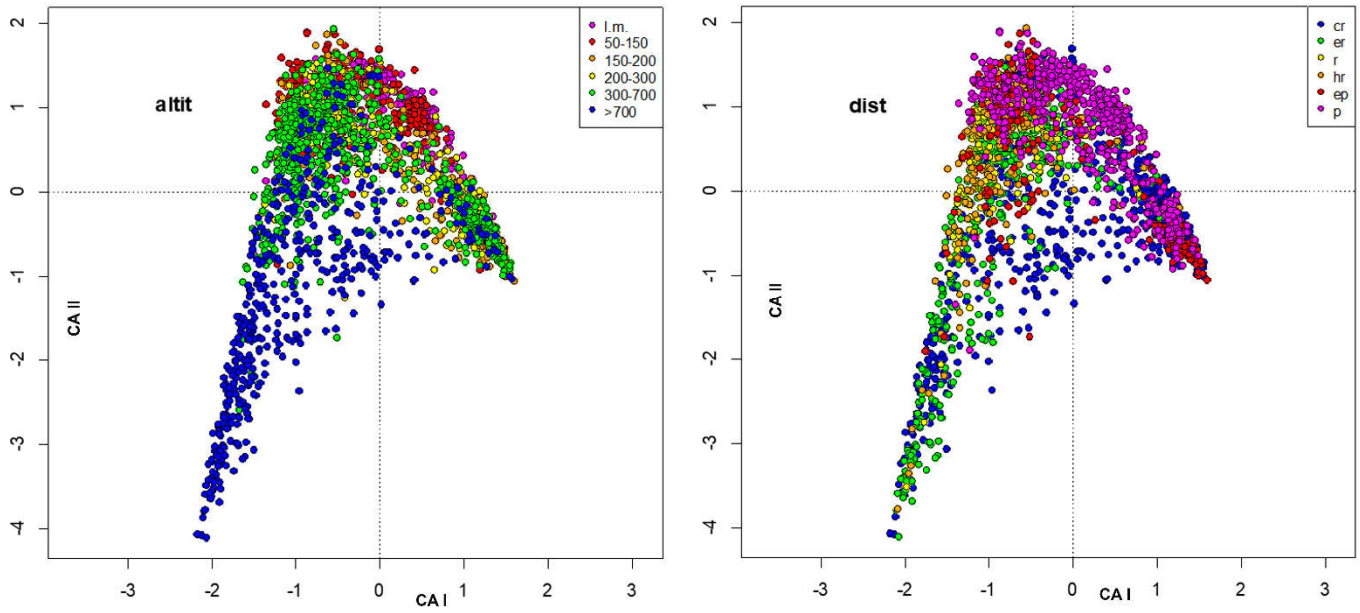

Figure 4. plot of sites scores in the first 2 axes resulting from CA of sites x species ($_nL_p$) matrix, by marking sites with different colours according to altitude (left) and to source distance (right); cr: crenal, er: epirhithral, r: rhithral, hp: hyporhithral, ep: epipotamal, p: potamal.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 24 August 2022 doi:10.20944/preprints202208.0408.v1

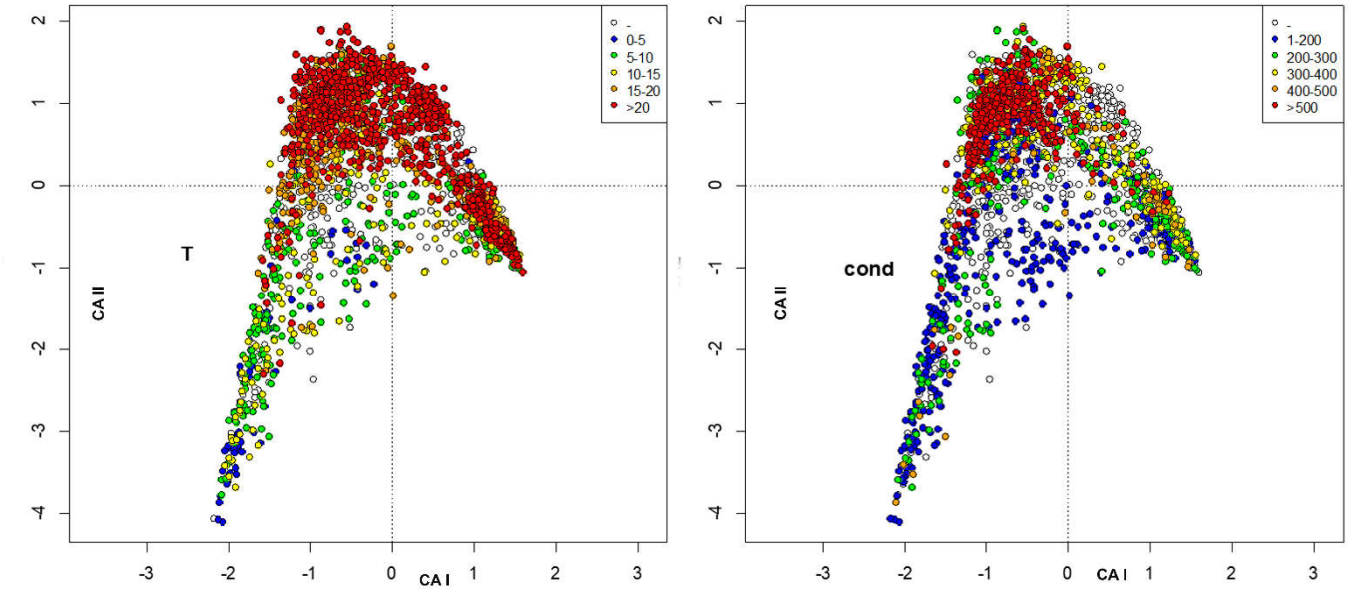

Figure 5. plot of sites scores in the first 2 axes resulting from CA of sites x species ($_nL_p$) matrix, by marking sites with different colours according to water temperature (°C) (left) and to water conductivity (μS cm^{-1}) (right).

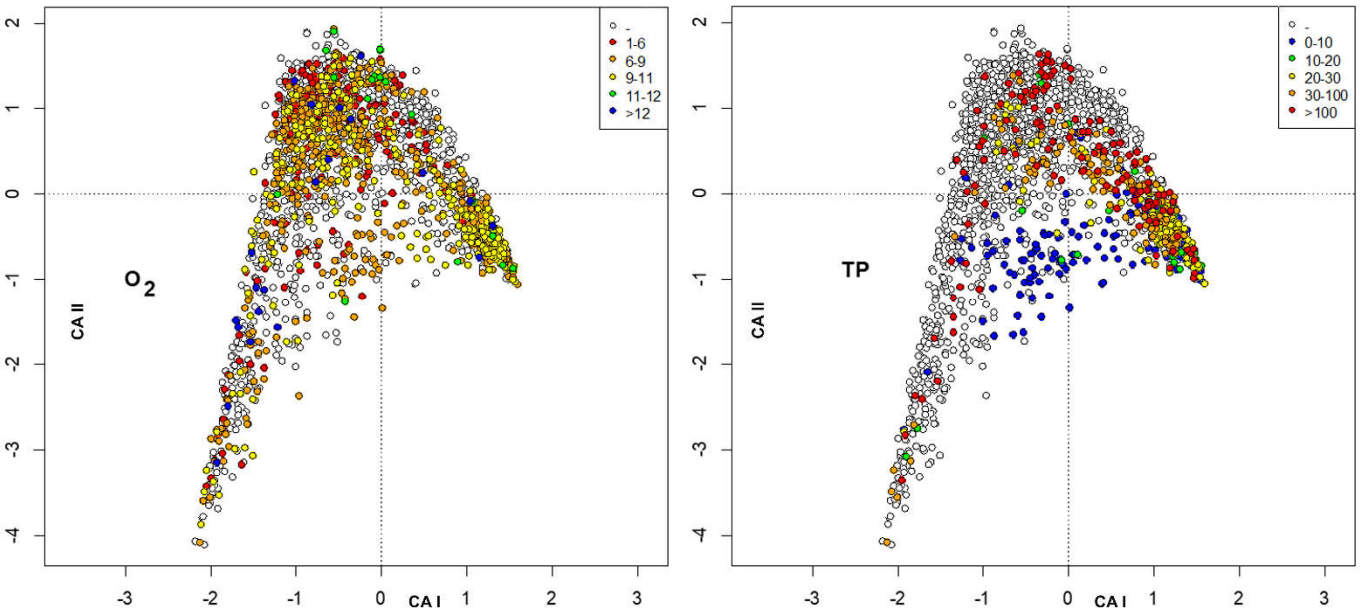

Figure 6. plot of sites scores in the first 2 axes resulting from CA of sites x species ($_nL_p$) matrix, by marking sites with different colours according to dissolved oxygen (mg l^{-1}) (left) and total phosphorous (TP) (μg P l^{-1}) (right).

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 24 August 2022 doi:10.20944/preprints202208.0408.v1

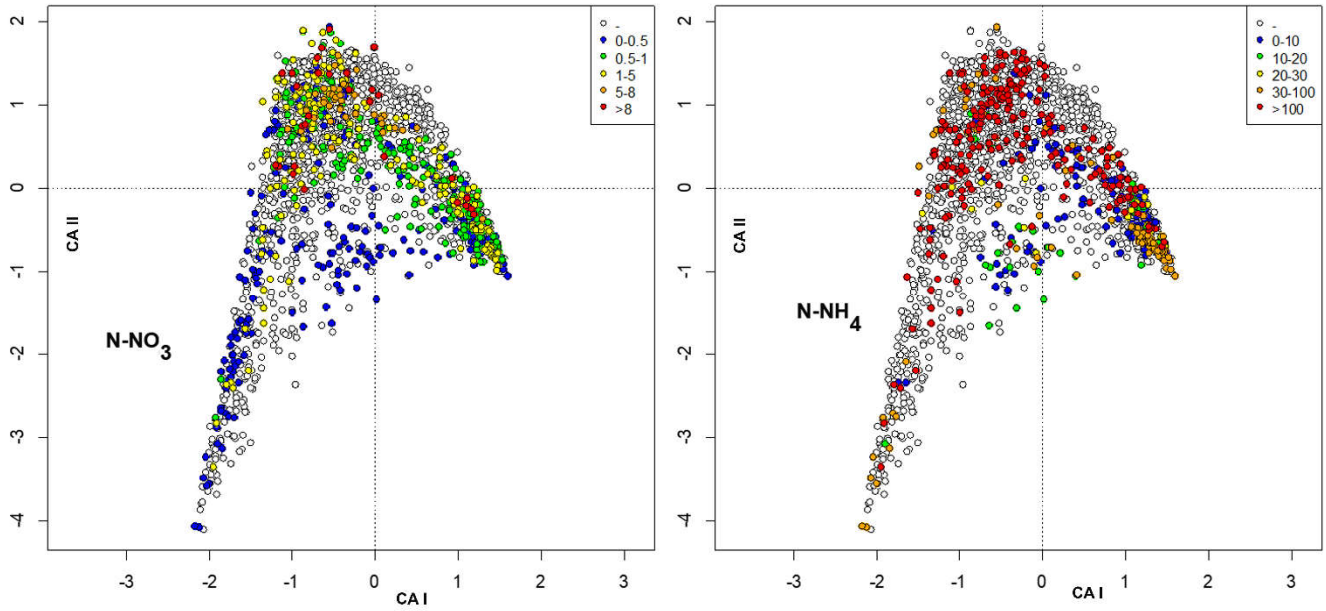

Figure 7. plot of sites scores in the first 2 axes resulting from CA of sites x species (${}_{n}\mathbf{L}_{p}$) matrix, by marking sites with different colours according to N-NO_3 (mg N l^{-1}) (left) and to N-NH_4 (µg N l^{-1}) (right).

The **species x traits** matrix ${}_{p}\mathbf{U}_{s}$ was also submitted to a correspondence analysis (**Fig.** 8, **Table S4**). The first 2 axes accounted for 70 and 21 % of the total variance, eigenvalues were 0.14 and 0.04 respectively. The first gradient separated species according to an upstream-downstream gradient, with the extreme scores assigned to altitude, source distance and conductivity, the second gradient separated species according to a trophic gradient, with the extreme scores assigned to oxygen, and N-NH_4, with *Tanypus* and *Chironomus thummi* plotted in the bottom left area as other tolerant species, *Diamesa* species were plotted in the bottom right area. Species requiring high O_2 content as *Paralauterborniella, Pagastiella, Stempellina* were plotted at the top of the graph.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 24 August 2022 doi:10.20944/preprints202208.0408.v1

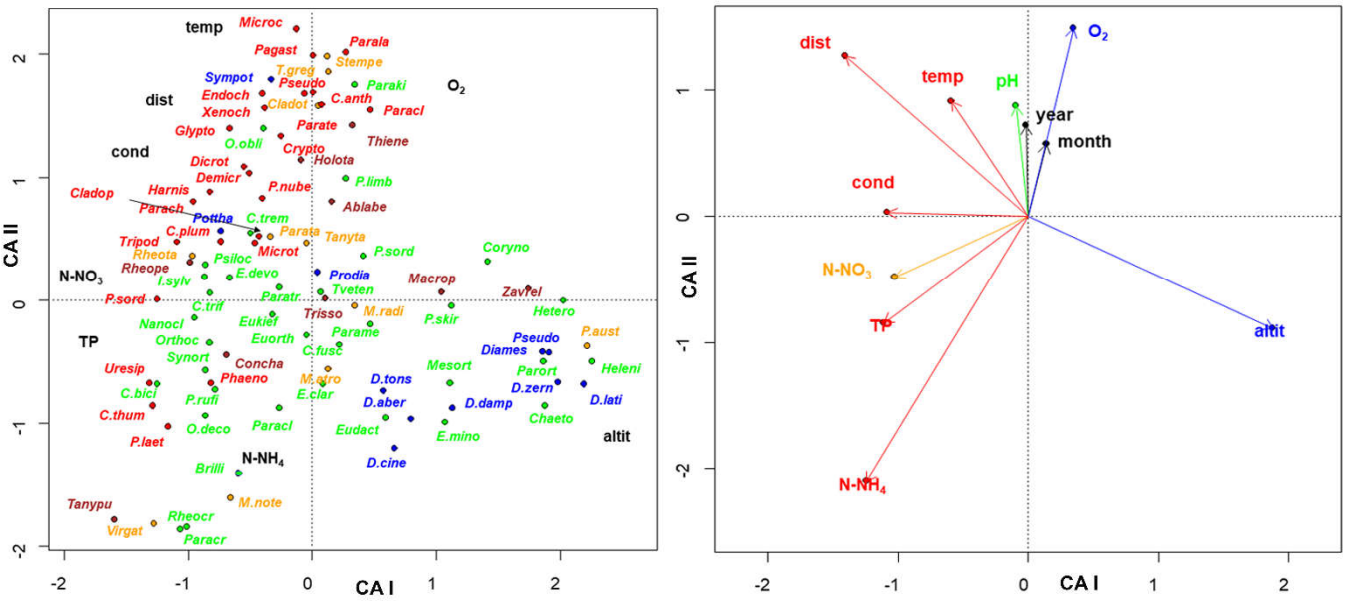

Figure 8. plot of the species scores (left), and of the trait scores (right) in the first 2 axes resulting from CA carried out from species x traits ($_{p}\mathbf{U}_{s}$) matrix.

The **sites x species** matrix was transposed ($_{p}\mathbf{L'}_{n}$) and a canonical constrained ordination (CCA) was carried out relating this matrix with the species x traits matrix $_{p}\mathbf{U}_{s}$ ($_{p}\mathbf{L'}_{n}$~ $_{p}\mathbf{U}_{s}$) (**Fig.** 9, **Fig. S3, Table S5**,). The first and second axis accounted for 7 % and 5 % of the total variance and eigenvalues 0.69, 0.46 respectively. The scores of each species calculated according to the left (sites) and right (traits) set were joined by a line in the figure. The species showing preferences for the cold sites at high altitude were plotted in the bottom right of the graphs, the ones present sites with high oxygen content in the bottom left, tolerant species as *Chironomus thummi*, *Cricotopus (Cricotopus) trifascia* and *Virgatanytarsus* present in high $N\text{-}NO_3$, TP, $N\text{-}NH_4$ and low oxygen content waters were plotted in the top part of the graph, *Rheopelopia*, *Uresipedilum*, *Tanypus* from sites with high temperature and conductivity were mapped in the top left part. An arch/horseshoe effect was also visible here, with species preferring lentic waters plotted on the left, kryal and cold spring species on the bottom right and species characterizing potamon in the top right.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 24 August 2022 doi:10.20944/preprints202208.0408.v1

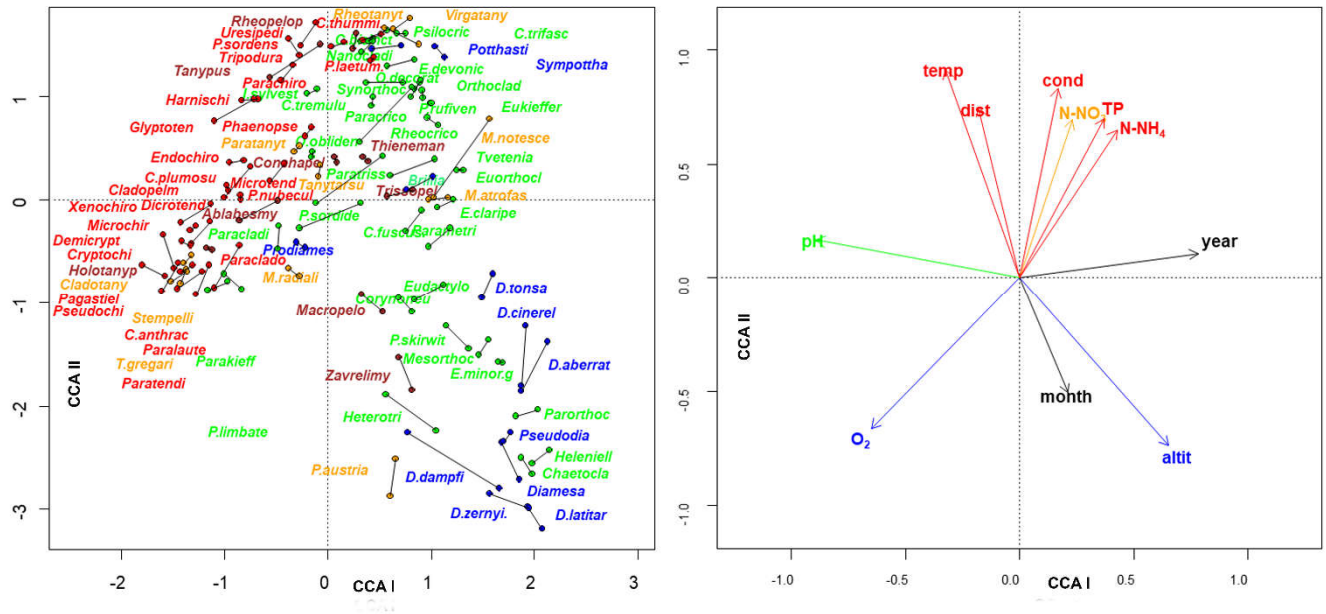

Figure 9. plot of the species scores (left) of the trait scores (right) in the first 2 axes resulting from CCA analysis carried out from ${}_{p}\mathbf{L'}_{n} \sim {}_{p}\mathbf{U}_{s}$ matrices; the scores of the same species obtained with the first and second matrix are joined with a line (see **Fig. S**4 for the full set of species names).

A comparison between factor loadings of species in canonical constrained and unconstrained ordination showed a good agreement in the species ordination, except for a few species such as *Diamesa dampfi*, *Micropsectra notescens*, *Paratrissocladius*, *Paracricotopus*, *Psectrocladius sordidellus*, *Heterotrissocladius*, which showed different scores in the CA first axis (calculated from ${}_{n}\mathbf{L}_{p}$ matrix) and in the CCA first axis (calculated from ${}_{p}\mathbf{L'}_{n} \sim {}_{p}\mathbf{U}_{s}$ matrices) (**Fig. S4, Table S5**) and as a consequence were plotted at some distance from the regression line.

The **sites x species** matrix ${}_{n}\mathbf{L}_{p}$ was post-multiplied by **species x traits** ${}_{p}\mathbf{U}_{s}$ matrix to obtain a **site x traits** matrix (${}_{n}\mathbf{L}_{p}\mathbf{U}_{s}$). This ${}_{n}\mathbf{L}_{p}\mathbf{U}_{s}$ was also submitted to correspondence analysis (**Fig.** 10, **Table S6**). In this case sites were rows and traits were columns. The first two axes accounted for 72 % and 24 % of total variance with eigenvalues 0.05, 0.02. The first axis reproduced an upstream downstream and a water temperature gradient, the second axis a water quality gradient (**Fig.** 10). This analysis does not allow to map species, because the species (columns of the first matrix and rows of the second) do not appear in the product matrix.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 24 August 2022 doi:10.20944/preprints202208.0408.v1

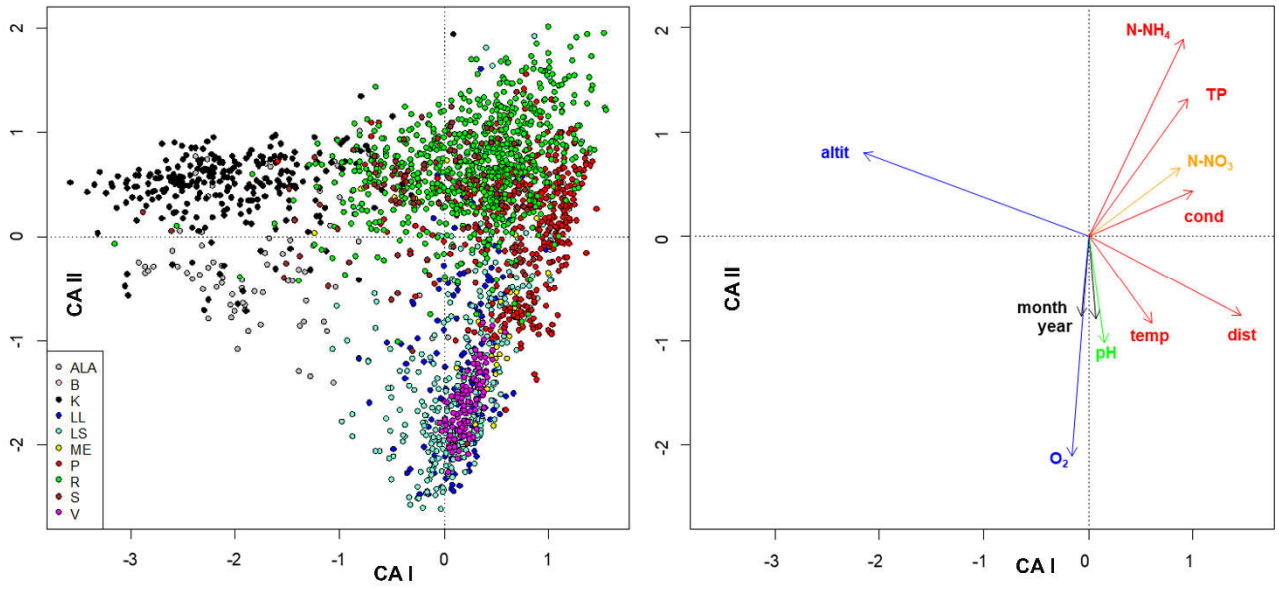

Figure 10. sites scores (left) traits scores (right) from the first 2 axes of the site x traits ($_{n}\mathbf{L}_{P}\mathbf{U}_{S}$) matrix.

A discriminant analysis was carried out to test the goodness of classification of sites in different habitats when Chironomid taxa assemblages are used to discriminate among habitats (**Table** 5, **Table S7**). Both $_{n}\mathbf{L}_{P}$ and $_{n}\mathbf{L}_{P}\mathbf{U}_{S}$ matrices were submitted to multiple discriminant analysis, using habitat as grouping factor; the % of correct classifications was 46 % for the $_{n}\mathbf{L}_{P}$ matrix and 47 % for the $_{n}\mathbf{L}_{P}\mathbf{U}_{S}$, emphasizing that the addition of the trait matrix does not improve the classification significantly, in any case the result is that Chironomid assemblages are good discriminators of the different habitats.

A cluster analysis of species confirmed that separation of species clusters is in agreement with different habitats (**Fig.** 11).

Table 5. Results of discriminant analysis: hits and misses in samples classification according to taxonomic and traits analysis. ALA: alpine lakes, B: brackish waters, K: kryal, LL: large lakes, LS: small lakes, ME: Mediterranean lakes, P: potamal, R: rhithral, S: krenal, V: volcanic lakes. Detailed results of Discriminant Analysis in Table S7.

		ALA	B	K	LL	LS	ME	P	R	S	V
nLp	hits	56	100	79	9	11	28	40	38	33	68
	misses	44	0	21	91	89	72	60	62	68	32
nLpUs	Hits	59	100	83	14	10	28	36	34	38	72
	misses	41	0	17	86	90	72	64	66	63	28

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 24 August 2022 doi:10.20944/preprints202208.0408.v1

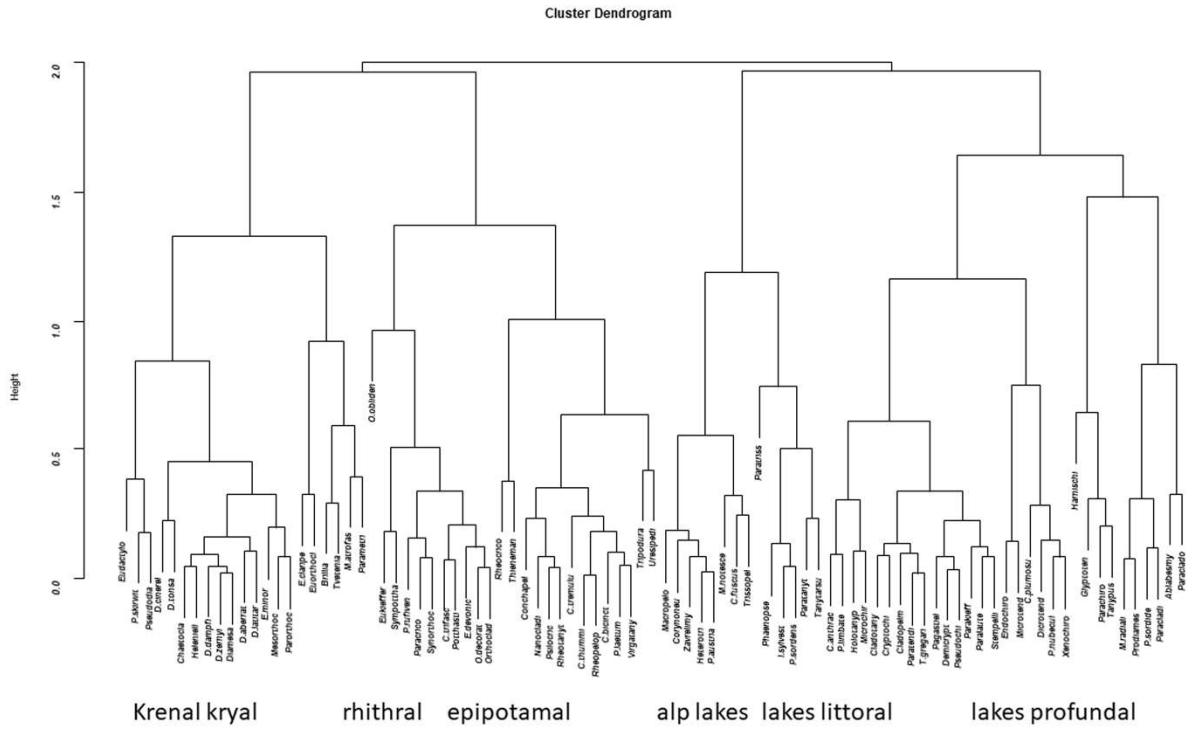

Figure 11. cluster analysis of species from sites x species ($_nL_p$) matrix.

4. Discussion

Chironomids species distribution in the environment is confirmed to be related to ecological conditions. Distribution of Chironomids linked to biogeographic factors were never observed within the western Palearctic area, except for the species linked to glacial areas [29], so biogeographic factors are not considered in the present discussion.

Chironomids have been frequently used as indicators of past climatic change [30], while it is impossible to establish the occurrence of alien species [31], even if it is expected. Some species like *Polypedilum nubifer* are probably invaders [32], but it is impossible to state if and when they reached the West Palaearctic region. It is well known that Chironomid distribution is related to ecological factors, such as water temperature [33,34], so an extension or reduction of the home range of a species is expected in relation to global warming [35].

Being the ecological niche known, it is possible to translate the information given by each species into information about habitat. From a mathematical point of view, the ecological niche can be expressed as a vector whose elements are the optimum values of the species for each factor, expressed as weighted mean, while the measure of niche extension can be expressed as a weighted standard deviation. [27]. The vectors can be aggregated to create a trait matrix $_pU_s$ with p species as rows and s traits as columns. This $_pU_s$ matrix was firstly proposed calculating aquatic beetle traits and a fuzzy coding analysis was suggested to allow the inclusion of diverse kinds of biological information [36]. Species abundances can be expressed as a matrix $_nL_p$ with n samples as rows and p species as columns. A matrix multiplication of the matrix $_nL_p$ by the $_pU_s$ matrix generate a $_nL_pU_s$ product matrix, with n sites as rows and s traits as columns; this approach was proposed for vegetation studies [37], it was used for invertebrates living in running waters [38] and extended to Chironomids [19, 20, 39]:

$$_nM_s = {}_nL_p * {}_pU_s$$

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 24 August 2022 doi:10.20944/preprints202208.0408.v1

This approach allows to translate the information given by a species list in ecological traits, allowing the construction of an index of environmental quality. Attempts to create the ${}_{p}U_{s}$ matrix for Chironomids and other benthic invertebrates were matter of many efforts [3, 39, 40, 41, 42], but the results were obviously dependent by the database used for calculations. In the present paper, we tried to develop a new trait matrix considering the largest database available from collections of larval samples from both lotic and lentic habitats. Indeed, traits of chironomids were often assigned without a well-founded support of information, this was underlined in estimating recovery of lakes after measures of restoration from acidification [43]. Significant differences were observed between traits developed for North American and European species [44] and between Scandinavian and Mediterranean species [45]. Lack of information may lead to apparently contradicting results. For example, the haemoglobin content, tube building ability, feeding habit, voltinism and body size of Chironomid larvae suggested that haemoglobin-rich species, with tube building capacity and short generation time be dominant in disturbed sites, the reverse should be in less disturbed sites. But this approach gave some unexpected results, such as the presence of: 1- haemoglobin-rich species also in less disturbed sites; 2- species with long generation time in disturbed sites [38], and/or 3- small body sized species in less disturbed habitats [20]. These apparently conflicting results were explained supposing that oxygen deficit was not the only factor determining disturbed conditions. It was supposed that not all haemoglobin-rich species are tolerant to low oxygen levels [8]. For example, species belonging to *Polypedilum* may be responsible of this conflicting result, because this haemoglobin-rich genus is often present in undisturbed sites, possibly due to the presence of small oxygen-poor microhabitats included in large oxygen-rich habitats. Chironomini genera (*Chironomus, Glyptotendipes, Polypedilum, Paratendipes, Microtendipes* etc.) are all haemoglobin-rich, but have very different response to pollution. The same is true for body size: the large *Chironomus* and *Propsilocerus* often prevail in disturbed sites, while it is expected that the small body sized trait prevail in disturbed sites [46].

Another attractive approach is the so-called 4th corner solution problem [47, 48], where the sites x species matrix ${}_{n}\mathbf{L}_{p}$, the species x traits matrix ${}_{p}\mathbf{U}_{s}$ and the sites x environmental variables matrix ${}_{n}\mathbf{R}_{q}$ are combined to produce a ${}_{q}\mathbf{D}_{s} = {}_{q}\mathbf{R}'{}_{n}\mathbf{L}_{p}\mathbf{U}_{s}$ matrix, which allows a comparison between an expected and an observed community [49]. In the present case, the ${}_{n}\mathbf{R}_{q}$ matrix presents a lot of missing data, so this analysis was not performed. It is suggested to be cautious in using this matrix approach to evaluate the ecological status, because an incomplete information available about the ecology of single taxa can conduce to misleading results or false representations. This approach could be useful in the future when more accurate information will be available about different Chironomid species.

In the present study, as in many others [3, 40, 50], it is evident that Chironomid species respond to a limited number of factors, so they can be ordered according to few gradients. We preferred to start the analysis ordering taxa with an unconstrained ordination method [27], because environmental data supporting the description of sampled sites were incomplete. Moreover, it is well known that the presence-absence of a species is not bound to the point instantaneous water condition, but the result of an integration of factors over a relatively long time period, information that cannot be given by physico-chemical analysis.

Despite these limitations, the ordination of sites, based only on Chironomid species assemblages available in the present database, emphasized few major gradients responsible of the observed distributions: 1- a gradient separating lotic from lentic habitats, with species living in fast running waters separated from species living in standing waters; 2- a gradient emphasizing an upstream-downstream gradient in running waters, separating: a) intolerant species living at high altitudes, low water temperatures, high oxygen concentrations, low conductivity, from b) tolerant species living downstream, at higher temperatures, lower oxygen concentrations, higher conductivity and salinity; 3- a trophic gradient separating species living in oligotrophic nutrient-poor waters from species living in organic-rich or eutrophic waters. Each of these gradients does not necessarily coincide with the principal axes resulting from canonical ordination. In the present case, the first

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 24 August 2022 doi:10.20944/preprints202208.0408.v1

axis separates lotic from lentic habitats, the second axis is explained as an oxygen-temperature gradient, and the ordering of sites resulted in the classic arch or horseshoe effect [27, 28]. This effect observed in the correspondence analysis [27] is generated by species data having unimodal distribution along a single gradient [28]; in the present case, it is a gradient from high altitude, cold, oxygen-rich, fast flowing running waters observed in glacial streams, toward lowland, warmer, oxygen-poor, slow flowing waters observed in lowland rivers, and continuing in still slow flowing, but cooling down and oxygen enriching waters, as observed in large lakes with increasing depth. Conductivity and nutrients are often included in this principal gradient, in several possible interactions. In relation to this principal gradient, each species can adjust with its own peculiarities, moving more or less far from this gradient. For example, species living in small-sized cold waters lakes at high altitude (*Zavrelimyia, Heterotrissocladius, Corynoneura, P. austriacus*) and species living at high depth in large lakes (*M. radialis, Paracladopelma*) appear displaced toward the centre of the plot (**Fig.** 2, **Fig. S2**).

Species cannot be clustered in well-defined groups, because only few species are restricted to well defined habitats, most species are opportunistic. For example, few species belonging to *Diamesa* are restricted to kryal (*Diamesa laticauda*), but most (*Diamesa tonsa, Diamesa zernyi*) colonize different types of cold waters, some Orthocladiinae genera (*Eukiefferiella, Rheocricotopus, Euorthocladius, Orthocladius, Cricotopus*) characterize rhithral streams with moderate or fast current, but can be collected also in slow flowing waters, many Tanytarsini are typical of oligotrophic lakes, but are also common in spring and streams, many Chironomini genera (e.g. *Dicrotendipes, Chironomus)* characterize eutrophic lakes, but many of them live also in potamal and in littoral of lakes associated to vegetation (*Endochironomus, Glyptotendipes)* or to sand banks (*Cryptochironomus, Harnischia*).

In conclusion, the key factors separating Chironomid species are confirmed to be substrate, current velocity, water temperature, dissolved oxygen, conductivity, nutrients, but these factors are differently related in various situations and anthropogenic stress can contribute in creating other more complex interactions [9].

The advantage of having a matrix of ecological traits available ($_pU_s$) is the possibility to use only assemblage structure information to evaluate the ecological status of an ecosystem, without the support of environmental data, this is a necessity when sampling campaigns include only the monitoring of macrobenthos; in this case, be a trait matrix available, taxonomic information can be translated into water quality assessment.

5. Conclusions

It is often stated that functional traits analysis is better than taxonomic composition analysis [20]. Indeed, this statement stresses the obvious, because the use of functional traits requires to have a traits matrix available, and the development of a traits matrix implies to have a sound taxonomic knowledge, needed to create the traits matrix. It is more appropriate to state that when a trait matrix is available, a less thorough taxonomic knowledge is sufficient to evaluate the ecological status of a water body. In other words, a species groups list, instead of a more thorough species list, can be sufficient to analyse the system. The traits matrix approach has the advantage that a taxonomic species list can provide information comparable with the one given by a physical-chemical analysis, when a trait matrix is available. If both a traits matrix $_p\mathbf{U}_s$ and an environmental variables matrix $_n\mathbf{R}_q$ are available, you can go a further step, calculating an expected ecological status and comparing with an observed one [49] (Brown et al., 2014).

Author Contributions: "Conceptualization, B. Rossaro; investigation, B. Rossaro, L. Marziali and A. Boggero; methodology, software, data curation, B. Rossaro; writing – Original Draft Preparation, B. Rossaro; Writing – Review & Editing, L. Marziali and A. Boggero. All authors have read and agreed to the published version of the manuscript.".

Funding: "This research received no external funding".

Data Availability Statement: The data presented in this study are openly available in Supplementary materials.

Conflicts of Interest: "The authors declare no conflict of interest."

Supplementary Materials: Fig. S1 plot of the species scores in the first 3 axes resulting from CA carried out from sites x species matrix. As **Fig.** 1, but with all species plotted; **Fig. S2** plot of the species scores in the first 2 axes resulting from CA carried out from sites x species matrix and the fitted second degree polynomial. As **Fig.** 2, but with all species plotted; **Fig. S3** plot of the species scores in the first 2 axes resulting from CCA analysis carried out from **pL'n** and **pUs** matrices; the scores of the same species obtained with the first and second matrix are joined with a line. As **Fig.** 9 (left), but with all species plotted; **Fig. S4** plot of the species scores in the first axis resulting from CA analysis of **nLp** (abscissa) against species scores in the first axis resulting from CCA analysis of **pL'n ~ pUs** (ordinate) with the fitted regression line; **Table S1** input data matrix; **Table S2** correlations between species and environmental variables, p values and number of samples; **Table S3** Matrix of traits: weighted standard deviation of each environmental variable for each species; **Table S4** correspondence analysis (CA) results of species x traits matrix $_{p}\mathbf{U}_{s}$; **Table S5** canonical constrained ordination (CCA) results between species x sites $_{p}\mathbf{L'}_{n}$ matrix and species x traits $_{p}\mathbf{U}_{s}$ matrix = $_{p}\mathbf{L'}_{n}$~ $_{p}\mathbf{U}_{s}$; **Table S6** correspondence analysis (CA) results of sites x traits $_{n}\mathbf{L}_{p}\mathbf{U}_{s}$, matrix = sites x species multiplied by species x traits); **Table S7** discriminant analysis results using habitat as grouping factor of the first two CA scores, calculated from from $_{n}\mathbf{L}_{p}$ and $_{n}\mathbf{L}_{p}\mathbf{U}_{s}$ matrices

References

1. Armitage, P.; Cranston, P.; Pinder, C. The Chironomidae. Biology and ecology of non-biting midges. 1st ed., Chapman Hall, Lond., Glasgow, Weinheim, N. Y., Melbourne, Madras. 1995 pp. 1–572.
2. Fornaroli, R.; Cabrini, R.; Zaupa, S.; Bettinetti, R.; Ciampittiello, M.; Boggero, A. Quantile regression analysis as a predictive tool for lake macroinvertebrate biodiversity. *Ecological Indicators*, **2016,** 61, 728-738.
3. Rossaro, B.; Marziali, L.; Montagna, M.; Magoga, G.; Zaupa, S.; Boggero, A. Factors controlling morphotaxa distributions of Diptera Chironomidae in freshwaters. *Water* [Basle], **2022,** *14(7, item 1014),* 1–23 + online suppl. material. [publ. online 23.iii.2022]
4. Ni, Zh.; Zhang, E.; Herzschuh, U.; Mischke, S.; Chang, J.; Sun, W.; Ning, D. Taxonomic and functional diversity differentiation of chironomid communities in northern Mongolian Plateau under complex environmental Impacts. *Hydrobiologia*, **2020**, *847(9)*, 2155–2167.
5. Sartori L.; Canobbio, S.; Cabrini, R.; Fornaroli, R.; Mezzanotte, V. Macroinvertebrate assemblages and biodiversity levels: ecological role of constructed wetlands and artificial ponds in a natural park *J. Limnol.,* **2015,** *74(2),* 335–345 *DOI: 10.4081/jlimnol.2014.1018*
6. Tixier, G.; Wilson, K.P.; Williams, D.D. Exploration of the influence of global warming on the chironomid community in a manipulated shallowoundwater system *Hydrobiologia,* **2009**, *624,* 13–27.
7. Takamura, N.; Ito, T.; Ueno, R.; Ohtaka, A.; Wakana, I.; Nakagawa, M.; Ueno, Y.; Nakajima, H. Environmental gradients determining the distribution of benthic macroinvertebrates in Lake Takkobu, Kushiro wetland, northern *Japan Ecol. Res.*, **2009,** *24,* 371–381.
8. Int Panis, L.; Goddeeris, B.; Verheyen, R. On the relationship between vertical microdistribution and adaptations to oxygen stress in littoral Chironomidae (Diptera) *Hydrobiologia,* **1996**, *318*, 61–67.
9. Boggero, A.; Zaupa, S.; Bettinetti, R.; Ciampittiello, M.; Fontaneto, D. The benthic quality index to assess water quality of lakes may be affected by confounding environmental features. Water**, 2020**, 12.2519, 1-17.
10. Moore, J.W. Factors influencing the species composition, distribution and abundance of benthic invertebrates in the profundal zone of a eutrophic northern lake *Hydrobiologia*, **1981**, *83*, 505–510.
11. Verneaux, J.; Verneaux, V.; Guyard, A. Classification biologique des lacs jurassiens à l'aide d'une nouvelle méthode d'analyse des peuplements benthiques. I. Variété et densité de la faune *Annls Limnol.,* **1993**, *29*, 59–77.
12. Verneaux, V.; Verneaux, J.; Guyard, A. Classification biologique des lacs jurassiens à l'aide d'une nouvelle méthode d'analyse des peuplements benthiques. II. Nature de la faune. *Annls Limnol.,* **1993**, *29*, 383–393.
13. Al-Shami, S.A.; Rawi, C.S.M.; HassanAhmad, A.; Nor, S.A.M. Distribution of Chironomidae (Insecta: Diptera) in polluted rivers of the Juru River Basin, Penang, Malaysia Journal of Environmental Sciences, 2010, 22(11), 1718–1727
14. Free G.; Solimini, A.; Rossaro, B.; Marziali, L.; Giacchini, R.; Paracchini, B.; Ghiani, M.; Vaccaro, S.; Gawlik, B.; Fresner, R.; Santner, G.; Schoenhuber,M.; Cardoso, A.C. Modelling lake macroinvertebrate species in the shallow sublittoral, relative roles of habitat, lake morphology, aquatic chemistry and sediment composition. *Hydrobiologia*, **2009**, *633*, 123–136.
15. Resh, V.H.; Hildrew, A.G.; Statzner, B; Townsend, C. Theoretical habitat templets, species traits, and species richness - a synthesis of long-term ecological research on the upper Rhone river in the context of concurrently developed ecological theory. *Freshwat. Biol*., **1994**, *31*, 539–554
16. Dolédec, S.; Statzner, B. Theoretical habitat templets, species traits, and species richness - 548 plant and animal species in the upper Rhone river and its floodplain *Freshwat. Biol.,* **1994**, *31*, 523–538

17. Statzner, B; Hoppenhaus, K; Arens, MF; et al. Reproductive traits, habitat use and templet theory: a synthesis of world-wide data on aquatic insects. *Freshwat. Biol.*, **1997**, *38*, 109–135
18. Usseglio-Polatera, P; Bournaud, M; Richoux, P; Tachet, H. Biological and ecological traits of benthic freshwater macroinvertebrates: relationships and definition ofoups with similar traits *Freshwat. Biol.*, **2000** *43*, 175, 205
19. Serra, S.R.Q.; Cobo, F.;aça, M.A.S.; Dolédec, S.; Feio, M.J. Synthesising the trait information of European Chironomidae (Insecta, Diptera): toward a new database. *Ecol. Indic.*, **2016**, *61(2),* 282–292.
20. Jiang, X.; Pan, B.; Song, Zh.; Xie, Zh. Do functional traits of chironomid assemblages respond more readily to eutrophication than taxonomic composition in Chinese floodplain lakes? *Ecol. Indic.*, **2019**, *103,* 355–362 + online suppl. material.
21. Lemm, J.U.; Feld, C.K. Identification and interaction of multiple stressors in central European lowland rivers. *Sc. Tot. Environ.*, **2017**, 603–604, 148–154
22. Chaib, N.; Fouzari, A.; Bouhala, Z.; Samraoui, B.; Rossaro, B. Chironomid (Diptera, Chironomidae) species assemblages in northeastern Algerian hydrosystems. *J. ent. acarol. Res.*, **2013**, *45(1),* 4–11.
23. Thienemann, A. Chironomus *Leben Verbreitung und wirtschaftliche Bedeutung der Chironomiden. Die Binnengewässer Bd. 20*. Schweizerbart'sche Verlagsbuchhandlung, Stuttgart, 1954, pp. 834.
24. Buraschi, E.F.; Salerno, C.; Monguzzi, G.; Barbiero, G.; Tartari, G. Characterization of the Italian lake-types and identification of their reference sites using anthropogenic pressure factors. *J. Limnol.*, **2005**, *64(1),* 75–84.
25. Tartari, G.; Buraschi, E. ; Legnani, E.; Previtali, L.; Pagnotta, R.; Marchetto, A. *Tipizzazione dei laghi italiani secondo il Sistema B della Direttiva 2000/60/CE*. Documento presentato al Ministero dell'Ambiente e della Tutela del Territorio e del Mare. 2006, CNR: 1–20.
26. Rossaro, B.; Marziali, L.; Cardoso, A.C.; Solimini, A.; Free, G.; Giacchini, R. A biotic index using benthic macroinvertebrates for Italian lakes. *Ecol. Indicators,* **2007**, *7,* 412–429.
27. Ter Braak, C.J.F.; Prentice, I.C. A Theory of gradient Analysis *Adv. Ecol. Res.*, **1988**, *18,* 271–317, (18) (PDF) A Theory of Gradient Analysis (researchgate.net)
28. Legendre P.; Legendre, L. *Numerical Ecology Development in Environmental modelling 20*. Elsevier Amsterdam 1998; p. 853.
29. Serra-Tosio, B. Ecologie et biogéographie des Diamesini d'Europe (Diptera, Chironomidae) *Trav. Lab. Hydrobiol. Piscic. Univ. Grenoble* **1973**, *63*, 5–175.
30. Larocque, I.; Hall, R.I.; Grahn, E. Chironomids as indicators of climate change: a 100-lake training set from a subarctic region of northern Sweden (Lapland) *J. Paleolimnol.*, **2001**, *26,* 307–322.
31. Failla, A.J.; Vasquez, A.A.; Fujimoto, M.; Ram, J.L. The ecological, economic and public health impacts of nuisance chironomids and their potential as aquatic invaders. *Aquatic Invasions,* **2015**, *10(1),* 1–15.
32. Cranston, P.S.; Martin, J.; Spies, M. Cryptic species in the nuisance midge *Polypedilum nubifer* (Skuse) (Diptera, Chironomidae) and the status of *Tripedilum* Kieffer. *Zootaxa*, **2016**, *4079(4),* 429–447.
33. Rossaro, B. Chironomids and water temperature. *Aq. Ins.*, **1991**a, *13(2)*, 87–98.
34. Marziali, L.; Rossaro, B. Response of chironomid species (Diptera, Chironomidae) to water temperature: effects on species distribution in specific habitats. *J. ent. acarol. Res.*, **2013**, *45(2)*, 73–89 + 6 pp. .
35. Brown, L.E.; Khamis, K; Wilkes, M; Blaen, P; Brittain, J.E.; Carrivick, J.L.; Fell, S.; Friberg, N.; Füreder, L.; Gislason, G.M.; Hainie, S.; Hannah, D.M.; James, W.H.M.; Lencioni, V.; Olafsson, J.S.; Robinson, C.T.; Saltveit, S.J.; Thompson, C.; Milner, A.M.. Functional diversity and community assembly of river invertebrates show globally consistent responses to decreasing glacier cover *Nature Ecology & Evolution,* **2018,** *2,* 325–333
36. Chevenet, F.; Dolédec, S.; Chessel, D. A fuzzy coding approach for the analysis of long term ecological data. *Freshwat. Biol.*, **1994**, *31*, 295–309
37. Mabry, C.; Ackerly, D.; Gerhardt, F. Landscape and species-level distribution of morphological andlife history traits in a temperate woodland flora *Journal of Vegetation Science* **2000,** *11,* 213-224.
38. Gayraud, S., Statzner, B.; Bady, P.; Haybachp, A.; Scholl, F.; Usseglio-Polatera, P.; Bacchi, M. Invertebrate traits for the biomonitoring of large European rivers, an initial assessment of alternative metrics *Freshwat. Biol.*, **2003**, *48*, 2045–2064
39. Serra, S.R.Q.; Graça, M.A.S.; Dolédec, S.; Feio, M.J. Chironomidae traits and life history strategies as indicators of anthropogenic disturbance *Environ. Monit. Assess.*, **2017***a, 189,* 326, 1–16
40. Rossaro, B. Factors that determine Chironomidae species distribution in fresh waters *Boll. Zool.*, **1991b**, 58, 281–286.
41. Rossaro, B.; Boggero, A.; Lods Crozet, B.; Free, G.; Lencioni, V.; Marziali, L. A comparison of different biotic indices based on benthic macro-invertebrates in italian lakes. *J. Limnol.*, **2011**, *70(1),* 109–122.
42. Serra, S.R.Q.; Graça, M.A.S.; Dolédec, S.; Feio M.J. 2017b Discriminating permanent from temporary rivers with traits of chironomid genera *Ann. Limnol. - Int. J. Lim.*, **2017b,** *53*, 161–174
43. Van Kleef H, Verberk WCEP, Kimenai FFP, van der Velde G. & Leuven RSEW Natural recovery and restoration of acidified shallow softwater lakes: successes and bottleneck revealed by assessing life history strategies of Chironomid larvae *Basic and Applied Ecology*, **2015**, *16,* 325–334.
44. Serra, S.R.Q.; Graça, M.A.S.; Dolédec, S.; Feio M.J. Chironomidae of the Holarctic region: a comparison of ecological and functional traits between North America and Europe *Hydrobiologia*, **2017**c,*794*, 273–285
45. Bonada, N.; Dolédec, S. Does the Tachet trait database report voltinism variability of aquatic insects between Mediterranean and Scandinavian regions? *Aquatic Sciences,* **2018**, *80,7*, 1–11.
46. Feio, M.J.; Dolédec, S. Integration of invertebrate traits into predictive models for indirect assessment of stream functional integrity, a case study in Portugal. *Ecol. Indic.*, **2012**, *15*, 236–247.

47. Legendre, P.; Galzin, R.; Harmelin-Vivien, M.L. Relating behavior to habitat: solutions to the fourth-corner problem. *Ecology*, **1997,** *78*, 547–562.
48. Dray, S. & Legendre, P. (2008) Testing the species traits-environment relationships: the fourth-corner problem revisited. Ecology, 89, 3400–3412.
49. Brown, A.M.; Warton, D.; Andrew, N.R.; Binns, M.; Cassis, G.; Gibb, H. The fourth-corner solution – using predictive models to understand how species traits interact with the environment. Meth. Ecol. Evol., **2014**, *5*, 344–352. Doi: 10.1111/2041-210X.12163.
50. Lindegaard, C. Classification of water-bodies and pollution. In, *The Chironomidae. Biology and ecology of non-biting midges;* Armitage, P.D.; Cranston, P.S.; Pinder, L.C.V. Eds.. Chapman and Hall, London, etc. 1995, pp. 385–404.