Environmental factors affecting the distribution of Chironomid larvae of the Seybouse wadi, North-Eastern Algeria

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ABSTRACT

A survey of the Seybouse wadi (North-Eastern Algeria) between 2008 and 2011 was conducted in 26 sampling sites located on the main river and its tributaries using chironomids. From 3264 collected larvae, forty-five chironomid species were identified, and were correlated to 13 environmental variables to predict determinant factors affecting their distribution. Indicator value (IndVal) analysis was first performed to determine indicator chironomid species according to several factors (sites, seasons, source distance, granulometry, conductivity, water temperature, dissolved oxygen, water velocity, pollution and the abundance of filamentous algae). Co-inertia analysis (CoIA) supported the IndVal results, emphasising an upstream/downstream gradient in the first axis, while a granulometry gradient was emphasised by the second axis. A pollution gradient was also highlighted in the plane of the first two axes, separating tolerant Chironomus sp. 1, Cricotopus bicinctus and Cricotopus (Isocladius) sylvestris from intolerant species as Phaenopsectra flavipes, Rheotanytarsus sp. 1 and Cladotanytarsus sp. 1.

Key words: chironomids, environmental factors, Seybouse river, North Africa, IndVal, co-inertia analysis (CoIA).

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INTRODUCTION

North African wadis are characterised by an extensive range of physical conditions, including severe flooding and droughts (Pires et al., 1999), and by the irregularity of the flows and strong hydrological fluctuations (Giudicelli et al., 1985). The running water ecosystems of North-Eastern Algeria (Mediterranean basin) are physically very diverse, not only among geographical areas but also between upstream and downstream sections. In this area, the dominating feature of the rivers is the irregularity of the flows. Floods and low water levels are the two major events to which these lotic ecosystems are subject, thus temporary biotopes are largely dominant. In Algeria, aquatic macroinvertebrates are severely affected by aestival drought. In the summer season, portions of the channel can become partly or totally dry and lotic macroinvertebrates can survive by aestivating in cool, moist microhabitats (Samraoui et al., 1998; Samraoui, 2009), while lentic species prevail; during high flow periods, rheophilic species complete their life cycle and are dominant. The biotic components of running water systems are important components for the assessment of water quality (Stanford and Ward, 1988; Junk, 1999). One of the most basic observations in ecology is that the environment is variable in both time and space, making it difficult to predict any future environment or biota response. To assess the ecological status of water bodies, taxonomic composition, abundance and the ratio of tolerant and intolerant taxa have to be considered as biological indicators of water quality. However, knowledge of the biotic components of north African rivers or wadis is relatively poor and these hydrosystems remain some of the least-studied Mediterranean ecosystems.

Chironomidae (Diptera) are a family of small midges whose larval stage makes up over 50% of the benthic macroinvertebrate community. They are a diverse and widespread family subsisting in most climates and a wide range of water qualities (Ferrington *et al.*, 1991). These characteristics make them excellent candidates in monitoring water quality in both lotic (Coffman and Ferrington, 1996) and lentic ecosystems (Sæther, 1979).

Most early works on the hydrosystems of North Algeria were mainly devoted to the description of species, but few concerned their ecology and distribution (Reiche, 1869; Seurat, 1922; Navas, 1929; Samraoui and Menai, 1999; Annani *et al.*, 2012). More recently, some studies have been conducted on the aquatic macroinvertebrates of inland waters of this country (Gagneur *et al.*, 1986; Lounaci *et al.*, 2000b; Samraoui and Corbet, 2000a, b;





Arab et al., 2004; Belaidi et al., 2004; Chaib et al., 2011). All these studies showed the distribution patterns of aquatic macroinvertebrates in both space and time. In North Africa, chironomid species assemblages are characterised by lower species diversity than in European streams (Lounaci et al., 2000a, b), and are structured according to habitat characteristics, in particular, to the longitudinal gradient, known to be an important factor determining species assemblages. Chironomid taxocenosis in the Algerian streams is composed by opportunistic species which prevail and disappear in a short span of time (e.g. Langton and Casas, 1999).

The strong seasonal factor may hinder the effects of anthropogenic impacts on water communities. Therefore, unraveling the distinct effects of natural factors (seasonality, longitudinal gradient, and substrate composition) is complex but necessary to determine eventual anthropogenic impacts.

Contributions to the knowledge of the biological elements of inland waters which include species identification have been decreasing in the last years, some critical groups as chironomids are often included as a single family or at most as separate tribes (Gaudes et al., 2012). This hinders the possibility of an effective estimation of biodiversity. More valuable results are otherwise observed when chironomid species identification is undertaken (Marziali et al., 2010; Chaib et al., 2011; Odume and Muller, 2011; Milošević et al., 2012). Species identification is quite important for the Mediterranean area - Southern Mediterranean, in particular – an area where many species are at serious risk of extinction, because of the increasing pressure on water as a resource, with the situation being aggravated by climate warming and other global changes (Hulme et al., 2001); there is the serious risk that autochthonous and endemic species disappear, with a concomitant increase of opportunistic species.

The aims of the present paper are (i) to investigate Chironomid taxa assemblages in a Mediterranean river – the Seybouse wadi – with its tributaries, and (ii) to relate the Chironomid species to environmental factors. The Seybouse is located West of another wadi, the Kebir East, which was the focus of a previous study of its chironomid assemblages (Chaib *et al.*, 2011). The present study is a contribution to knowledge on diversity and ecology of chironomids in one of the least investigated region of the Mediterranean area.

METHODS

Study area

The Seybouse river, locally known as Seybouse wadi, has a catchment area of 6471 km² and is located in North-Eastern Algeria (Fig. 1). It originates in the high plains of the Tellian Atlas and flows to the North to reach the

Mediterranean sea, after crossing the coastal plain between Drean and Annaba. Its main tributaries are the Cherf and the Bouhamdane wadi, which join at Medjez Amar (36°26'35.82" N, 7°18'39.36" E) to form the Seybouse river.

The Seybouse basin covers 68 municipalities in seven departments (known locally as *wilayas*): Annaba, El-Tarf, Skikda, Constantine, Oum El-Bouaghi, Guelma and Souk Ahras, for a population of around 2.4 million inhabitants (2007 estimate). Thirty municipalities are entirely included in the basin and thirty-eight only partially.

The Seybouse wadi is used for irrigation of agricultural areas, but it is becoming polluted because of industrial activities, the lack of sewage treatment schemes (under implementation), and the uncontrolled discharge of industrial effluents in urban areas.

Industries in the basin are concentrated around the department of Annaba (steel at El-Hadjar and chemical fertilizers) (ASMIDAL, Annaba, Algeria). Only eight industries out of 86 in the Seybouse river basin have their own sewage treatment plant. Total flows of the wastewater rejected by the main industries in the basin is approximated to $29,152 \, \text{m}^3 \, \text{day}^{-1}$, while the total daily flows of domestic wastewater was estimated at $79,056 \, \text{m}^3 \, \text{day}^{-1}$ (915 L s⁻¹), and the measurement of BOD₅ was evaluated to 42,690.24 mg L⁻¹ O₂.

The climate of the Seybouse basin is typically Mediterranean, with dry, hot summer from June to September and a rainy period from October to May. Average yearly rainfall varies from 450 mm per year upstream to 735 mm per year downstream.

Chironomid larvae were sampled at 26 sites between summer 2008 and winter 2011 (Tab. 1). Six sites were located along the main river course of the Seybouse (sites 18, 19 and 23 to 26), and 3 along its tributaries (Fig. 1): wadi Zemzouma (20), Bradaa (21) and wadi Helia (22); 3 sites along the main course of the Bouhamdane wadi (15, 16 and 17); and 14 sites were located on the main course of the Cherf wadi and its tributaries (sites 1 to 14).

Site selection was based on land use and anthropogenic impacts (Khelifa *et al.*, 2011). Sites were subjected to non-point (agricultural runoff: sites 1 to 15) and/or point (municipal sewage and wastes: sites 16 to 26) sources of pollution (based on published technical reports of the Agence des Bassins Hydrographiques des Côtiers Contantinois-Seybouse-Mellègue).

Four sampling seasons (spring, summer, autumn and winter) from 2008 to 2011 were considered to characterise the seasonal gradient of the Seybouse wadi and its tributaries, where a total of 165 samples were collected.

The Seybouse wadi presents a typical Mediterranean character of intermittent streams: eleven studied sites were dry during the seasons of summer and autumn, and the accessibility to the selected sites located on the main

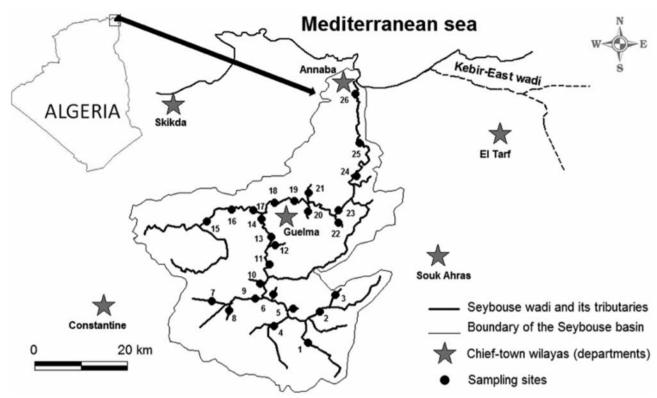


Fig. 1. Map of the sampling sites location along the main course of the Seybouse wadi and its tributaries (North-Eastern Algeria). See Tab. 1 for site names.

Tab. 1. List of the sampling sites with geographical and typological information and number of chironomid samples per site.

N°	Names of wadis	Codes	Sampling sites	Latitude (N)	Longitude (E)	Altitude (m)	Hydoperiod	Number of sample
1	Cherf	BSD	Barrage Sedrata	36°3'30.96"	7°27'12.54"	744	Permanent	6
2	Cherf	CPS	Cherf à Sedrata	36°4'28.74"	7°29'38.40"	747	Permanent	9
3	Cherf	OKR	Wadi Krab – effluent	36°7'12.60"	7°32'46.80"	778	Temporary	3
4	Cherf	CKS	Cherf à Ksar Sbahi – effluent	36°3'12.42"	7°19'33.42"	751	Permanent	10
5	Cherf	ONL	Wadi El Nil – effluent	36°8'22.80"	7°26'43.86"	775	Temporary	3
6	Cherf	ODB	Wadi Dbabcha – effluent	36°16'13.58"	7°25'53.92"	609	Temporary	8
7	Cherf	OML	Wadi el Maleh – effluent	36°14'23.16"	7° 8'45.96"	742	Temporary	4
8	Cherf	OBM	Wadi Beni Mheni – effluent	36°10'43.42"	7°12'5.67"	668	Temporary	2
9	Cherf	BMK	Barrage Ain Makhlouf	36°13'31.68"	7°17'46.98"	643	Permanent	6
10	Cherf	OAR	Wadi El Aare – effluent	36°18'29.74"	7°19'54.63"	609	Temporary	3
11	Cherf	CMK	Cherf à Ain Makhlouf	36°21'40.05"	7°20'12.64"	600	Permanent	12
12	Cherf	OCH	Wadi Cheniour – effluent	36°23'11.82"	7°22'43.38"	742	Temporary	11
13	Cherf	CHS	Cherf à Ain Hsainia	36°23'11.13"	7°19'24.96"	270	Permanent	6
14	Cherf	CMA	Cherf à Medjez Amar	36°26'31.56"	7°18'40.62"	273	Permanent	7
15	Bouhamdane	BHD	Bouhamdane à Hammam Debagh	36°29'30.07"	7°11'18.31"	305	Permanent	2
16	Bouhamdane	BMR	Bouhamdane à Mermoura	36°29'30.97"	7°14'27.85"	480	Permanent	4
17	Bouhamdane	BMA	Bouhamdane à Medjez Amar	36°30'2.12"	7°17'8.83"	274	Permanent	7
18	Seybouse	SSS	Seybouse à Salah Salah Salah	36°27'41.82"	7°20'22.92"	251	Permanent	8
19	Seybouse	SFJ	Seybouse à El –Fedjouj	36°28'53.58"	7°24'55.56"	222	Permanent	9
20	Seybouse-effluent	OZM	Wadi Zimba – effluent	36°25'45.43"	7°30'35.41"	291	Temporary	8
21	Seybouse-effluent	OBR	Wadi Bradâa – effluent	36°30'48.18"	7°27'2.22"	285	Temporary	3
22	Seybouse-effluent	OHL	Wadi Helia – effluent	36°20'41.48"	7°39'55.03"	144	Temporary	5
23	Seybouse	SZM	Seybouse à Zemzouma	36°24'47.70"	7°36'40.56"	143	Permanent	9
24	Seybouse	SBD	Seybouse à Boudaroua	36°29'43.26"	7°41'11.19"	100	Temporary	3
25	Seybouse	SCH	Seybouse à Chihani	36°37'29.38"	7°42'27.02"	12	Permanent	9
26	Seybouse	SDR	Seybouse à Dreân	36°41'0.12"	7°45'31.62"	18	Permanent	7

course of the Seybouse wadi was not always secured in winter and spring. This explains why only 165 samples were collected.

Environmental data

The main environmental variables were measured *in situ* using field multi-probes at mid-water depth: water temperature (°C), conductivity (µS cm⁻¹), dissolved oxygen (mg L⁻¹), pH and turbidity (NTU). For each sampling site, current velocity (cm s⁻¹) was also measured using a Global Flow Probe [FP101-FP201] (Supplementary Table 1). Geographical and typological information were collected for each site. GPS coordinates were measured using a Garmin GPS. Site source distance was measured from the high plains of the Tellian Atlas into the mouth of the Seybouse river in the Mediterranean sea using Quantum Gis Development Team (2012).

Substrate composition was determined as percentage of silt, sand, gravel and cobble (Supplementary Table 1). Particle size distribution was estimated using the Wentworth scale (Wentworth, 1922) but approximated in 9 classes: cobble, pebbles, coarse gravel, medium gravel, fine gravel, coarse sand, medium sand, fine sand and silt. Substrate size was ranged from cobble (100%) to silt (<25%). Higher values mean higher percentage of large sized particles (Supplementary Table 2).

Contamination level was ranged into five classes from unpolluted sites (class 1) to heavily polluted sites (class 5) (Supplementary Table 1, 2). The five classes were based on the presence and/or abundance of filamentous algae associated to the low concentrations of dissolved oxygen and the high conductivities.

Chironomid data

All chironomid samples were collected with a Surber net (300 µm mesh size, 50 cm width). Ten hauls were made in the opposite sense of the current along the sampling station, in the middle of the current and near the banks and we randomly scrutinised by hands 5 submerged cobbles with a total surface area of 1 m², dislodging any hidden larvae (Chaib et al., 2011). Samples were preserved in 5% formaldehyde (larvae and pupae). then examined under a stereo-microscope in the laboratory. Subsequently, permanent slides were prepared in Faure or Balsam mounting media, to allow taxonomic identification of specimens. Identification to species level was based on the presence of some pre-pupae, where the pupal characters were visible, and of mature pupae. Italian keys for larvae determination were used (Ferrarese, 1983; Ferrarese and Rossaro, 1981; Nocentini, 1985; Rossaro, 1982), along with keys for Palaearctic larvae (Wiederholm, 1983) and pupae (Langton and Visser, 2003).

Data analysis

Indicator value (IndVal) analysis was first performed using R environment (Dufrêne and Legendre, 1997; R Development Core Team, 2009) to determine indicator chironomid species according to several ecological factors (granulometry, conductivity, temperature, dissolved oxygen, current velocity, pollution and source distance). The environmental factors (except granulometry) were ranged into 5 classes [where the highest values represent the fifth class, the lowest values are grouped in the first class, and granulometry is ranged into 9 classes (Supplementary Table 2)]. The choice of the class limits was based on the observation of ranges in the distribution of values in each class.

Indicator species were also determined for each site and for each season (winter, *i.e.* December to February; spring, *i.e.* March to May; summer, *i.e.* June to September; and autumn, *i.e.* October and November) between 2008 and 2011.

IndVal analysis combines information on the abundances of single species in a particular group (specificity) and the occurrence in a particular group (fidelity). Species with a high specificity and high fidelity within a habitat will have a high indicator value. Good indicator species are then those that prefer sites in a given group and avoid other groups (Dufrêne and Legendre, 1997; McCune and Grace, 2002).

Indicator values were tested for statistical significance using a randomisation (Monte Carlo) technique (Dufrêne and Legendre, 1997).

To investigate the environmental factors determining species distribution along the Seybouse wadi and its tributaries, co-inertia analysis (CoIA) – a two-table ordination method – was performed using the Ade-4 package in the R environment (Dray *et al.*, 2007; Thioulouse *et al.*, 1997). This multivariate analysis tool is used to ordinate samples by searching for a co-structure maximising the covariance between 2 matrices prepared as sites x environmental variables and sites x species. The CoIA factorial map explains the part of variability similar to each separate analysis, and the 2 coordinate systems are superimposed to emphasise the relationship between the 2 matrices. In the present case, 13 environmental variables were related to 45 chironomid species.

Different randomisation methods [procrustes randomisation test (PROTEST) and RV test] were used to test the association between the environmental and the faunal matrices. In these procedures, the rows of a matrix are randomly permuted, a parameter measuring the association between the original and the permuted matrix is calculated, and the frequency distribution of the parameter plotted from simulated data is compared with the observed value of the parameter calculated from the original matrix.

RESULTS

Forty-five chironomid species (Tab. 2) were collected. *Cricotopus bicinctus* showed the highest abundance (63 ind m⁻² for BMA) and was widespread in almost all the sampling sites with a total abundance of 488 ind m⁻² (Tab. 2). *Polypedilum nubifer* was the most abundant in BSD with 301 ind m⁻², and *Polypedilum cultellatum* in OCH and SDR with a maximum abundance of 109 and 103 ind m⁻², respectively. The sampling site BSD showed the lowest diversity, while the sites ODB,

CMK, SFJ, CMA and CKS presented the highest diversity (Chaib *et al.*, in press).

The IndVal analysis allowed us to identify species preferring specific environmental conditions (Tab. 3). Some species were significantly related to environmental variables, other species, even if responding to environmental factors, did not show a significant relation.

Few species were indicators of single stations: *Corynoneura scutellata* in a non-impacted station upstream of the Bouhamdane wadi at an altitude of 305 m a.s.l.

Tab. 2. Total abundance (*i.e.* total number of specimens) of chironomid species collected in the 26 sampling sites of Seybouse wadi and its tributaries between 2008 and 2011.

Chironomid species	Code species	Total abundance (ind m ⁻²)
Tanypus punctipennis (Meigen, 1818)	T. punctipennis	103
Procladius choreus (Meigen, 1804)	P. choreus	30
avrelimyia punctatissima* (Goetghebuer, 1934)	Z. punctatissima	17
Conchapelopia pallidula* (Meigen, 1818)	C. pallidula	103
Prodiamesa olivacea (Meigen, 1818)	P. olivacea	2
ukiefferiella sp. 1 (Thienemann, A., 1926)	Eukiefferiella sp.1	2
ukiefferiella bedmari* (Vilchez-Quero & Laville, 1987)	E. bedmari	22
ukiefferiella hospita* (Edwards, 1929)	E. hospita	8
ukiefferiella gracei* (Edwards, 1929)	E. gracei	1
ukiefferiella claripennis (Lundbeck, 1890)	E. claripennis	38
ardiocladius fuscus (Card Kieffer, 1924)	C. fuscus	1
sectrocladius (Psectrocladius) psilopterus* (Kieffer, 1906)	P. (P.) psilopterus	10
heocricotopus fuscipes* (Kieffer, 1909)	R. fuscipes	141
rthocladius (Orthocladius) excavatus* (Brundin, 1947)	O. (O.) excavatus	120
rthocladius (Orthocladius) rubicundus* (Meigen, 1818)	O. (O.) rubicundus	54
orthocladius (Euorthocladius) ashei* (Soponis, 1990)	O. (E.) ashei	22
aratrichocladius rufiventris* (Meigen, 1830)	P. rufiventris	210
ricotopus (Cricotopus) bicinctus (Meigen, 1818)	C. (C.) bicinctus	488
ricotopus (Isocladius) sylvestris (Fabricius, 1974)	C. (L.) sylvestris	263
(ydrobaenus sp. 1* (Fries, 1830)	Hydrobaenus sp.1	203
	Metriocnemus sp.1	3
Metriocnemus sp. 1* (Van Der Wulp, 1874)	1	9
aratrissocladius excerptus* (Gouin In Gouin & Thienemann, 1942)	P. excerptus	7
arametriocnemus stylatus (Kieffer, 1924)	P. stylatus	
imnophyes minimus* (Meigen, 1818)	L. minimus	1
araphaenocladius sp. 1*	Paraphaenocladius sp.1	1
orynoneura scutellata (Winnertz, 1846)	C. scutellata	8
unytarsus sp. 1	Tanytarsus sp.1	79
ladotanytarsus sp. 1* (Kieffer, 1921)	Cladotanytarsus sp.1	31
heotanytarsus sp. 1* (Thienemann & Bause, 1913)	Rheotanytarsus sp.1	45
aratanytarsus sp. 1* (Thienemann & Bause, 1913)	Paratanytarsus sp.1	108
ficrotendipes pedellus (De Geer, 1776)	M. pedellus	42
haenopsectra flavipes* (Meigen, 1818)	P. flavipes	2
olypedilum nubifer* (Skuse, 1889)	P. nubifer	418
olypedilum laetum* (Meigen, 1818)	P. laetum	3
olypedilum cultellatum (Goetghebuer, 1931)	P. cultellatum	374
olypedilum (Tripodura) scalaenum (Schrank, 1803)	P. (T.) scalaenum	158
enendotendipes impar* (Walker, 1856)	S. impar	1
enus sp. near <i>Tribelos</i> *	Genus sp. near Tribelos	20
icrotendipes nervosus (Stäger, 1839)	D. nervosus	36
hironomus sp. 1* (Meigen, 1803)	Chironomus sp.1	238
hironomus plumosus* (Linnæus, 1758)	C. plumosus	6
ryptotendipes sp. 1* (Lenz, 1941)	Cryptotendipes sp.1	1
Jarnischia fuscimana* (Kieffer, 1921)	H. fuscimana	6
Cryptochironomus defectus* (Kieffer, 1913)	Č. defectus	28
Pobackia sp. 1* (Sæther, 1977)	Robackia sp.1	2

Species are in phylogenetic order. *, species recordedin Seybouse wadi and not in Kebir-East wadi.

Tab. 3. Indicator chironomid species according to the classes of the environmental factors (see Supplementary Table 2).

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sissima 13 0.235 4 mis 2 0.055 1 1 0.055 1 1 0.055 1 1 0.055 1 1 0.055 1 1 0.055 1 1 0.024* 3 1 0.051 1 1 0.051 2 1 0.052 1 1 0.054 3 1 0.151 2 1 0.180 4 1 0.180 4 1 0.180 4 1 0.180 4 1 0.180 4 1 0.180 4 1 0.180 4 1 0.180 4 1 0.180 4 1 0.180 4 1 0.180 4 1 0.180 4 1 0.180 3 1 0.180 4 1 0.180 4 1 0.014 3 1 0.014 3 1 0.018 3 1 0.018 3 1 0.018 3 1 0.018 3 1 0.018 3 1 0.018 3 1 0.018 3 1 0.018 3 1 0.018 3 1 0.018 3 1 0.018 3 1 0.018 3 1 0.018 3 1 0.018 3 1 0.058 3 1 0.058 3 1 0.058 3 1 0.058 3 1 0.058 3 1 0.058 3 1 0.058 3 1 0.058 3 1 0.058 3 1 0.058 3 1 0.058 3 1 0.058 3 2 0.106 3 2 0.106 3 2 0.106 3 2 0.116 1 2 0.110 1 2 0.110 1 2 0.110 1 3 0.110 1 4 0.110 1 4 0.110 1 5 0.110	- 1 2 8 5 % 9 9 7 % 4 4 0 % & & & & & & & & & & & & & & & & & &	0.205 1.000 1.000 0.486 0.162 0.717 0.873 0.023* 0.023* 0.045 0.007* 0.642	3 5 -	699.0	5 0.085	5 7	7 0.023*	23*	3 0.	0.117	5	0.175		*	0.290
iii 1 0.055 1 iii 25 0.356 2 ii 25 0.356 2 ii 6 0.024* 3 ii 8 0.428 4 iii 9 0.151 2 ii 25 0.119 2 iii 3 0.465 3 iiii 13 0.465 3 iiii 13 0.427 4 iiii 13 0.427 4 iiii 13 0.427 4 iiiii 13 0.427 4 iiii 12 0.124 3 iiii 12 0.124 3 iiii 12 0.124 3 iiii 12 0.045 1 iii 12 0.124 3 iiii 13 0.423 4 iiii 13 0.423 4 iiiii 13 0.423 4 iiii 13 0.423 4 iiiii 13 0.423 4 iiiii 13 0.423 4 iiiiii 13 0.423 4 iiiiiii 13 0.423 4 iiiiiii 13 0.423 4 iiiiiiii 13 0.423 4 iiiiiiiii 13 0.423 4 iiiiiiiiii 13 0.423 4 iiiiiiiiiiii 13 0.423 4 iiiiiiiiiiiiii 13 0.423 4 iiiiiiiiiiiiiiii 13 0.423 4 iiiiiiiiiiiiiiiiiiiiiiiiiiiiiiiiiiii	2 8 9 % 9 9 2 % 4 4 0 % & & & & & & & & & & & & & & & & & &	1.000 1.000 0.486 0.162 0.129 0.717 0.873 0.023* 0.023* 0.045 0.007* 0.642	wω	0.062	3 0.508		_	ž5*	3 0.	0.147	3 (0.012^{*}	2 0.252	3	0.160
i 25 0.356 2 i 16 0.024* 3 i 16 0.024* 3 i 16 0.024* 3 i 17 0.024* 3 i 18 0.024* 3 i 19 0.024* 3 i 19 0.024* 3 i 19 0.024* 3 i 19 0.015 2 i 19 0.151 2 i 19 0.151 2 i 19 0.169 3 i 19 0.178 4 i 10 0.18 4 i 10 0.18 4 i 10 0.18 4 i 10 0.18 3 i 10 0.01 3 i 10 0.01 3 i 10 0.01 3 i 10 0.01 3 i 10 0.028* 4 i 10 0.01 3 i 10 0.028* 4 i	8 9 ° 8 9 9 7 ° 8 4 4 0 ° 8 ° 8 ° 8 ° 8 ° 8 ° 8 ° 8 ° 8 ° 8 °	1.000 0.486 0.162 0.129 0.717 0.023* 0.023* 0.045 0.075 0.007* 0.642	Э	1.000	4 0.757	_		00	3 0.	0.588	4	0.338	1 0.100	4	0.776
i 25 0.356 2 la sp.1 8 0.024* 3 8 0.428 4 8 0.428 4 8 0.428 4 9028 1 18 0.151 2 25 0.119 2 18 0.151 2 19 0.151 2 10 0.180 4 20 0.247 3 21 0.180 4 22 0.247 3 23 0.178 3 23 0.178 3 24 0.169 3 25 0.190 3 26 0.190 3 27 0.247 3 28 13 0.427 4 29 0.247 3 20 0.247 3 20 0.247 3 21 0.124 3 22 0.124 3 23 0.178 3 24 0.060 3 25 0.163 3 27 0.028* 4 27 0.028* 4 27 0.028* 4 28 0.001* 3 28 0.058 3 29 0.153 3 20 0.153 3 20 0.153 1 20 0.297 4 21 0.097 4 22 0.106 3 23 0.106 3 24 0.400 4 25 0.106 3 26 0.400 4 27 0.110 0.110	6 % 6 9 2 C 4 4 4 0 % 8 8 8 8 8 9 9 4 4 4 1 2 2 4 2 4 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8	0.486 0.162 0.129 0.717 0.873 0.023* 0.445 0.857 0.007* 0.642 0.007*		1.000	3 0.706	-		00	4 0.	0.244	3	0.461	5 0.142	4	1.000
16 0.024* 3 8 0.428 4 9 9 9 9 9 9 9 9 9	**************************************	0.162 0.129 0.717 0.873 0.023* 0.445 0.857 0.007* 0.642 0.088	5	0.527	5 0.161	1 9		35	3 0.	0.131	4	0.011^{*}	2 0.256	4	0.165
8 0.428 4 stilla sp.1 8 0.683 1 18 0.151 2 18 0.151 2 25 0.119 2 opterus 5 0.465 3 uus sp.1 13 0.169 3 inctus 21 0.138 4 swantas 20 0.247 3 ris 13 0.427 4 ris 13 0.427 4 inctus 1 0.316 4 sestris 23 0.178 3 ts 12 0.124 3 mus sp.1 20 0.945 1 s 12 0.124 3 mus sp.1 20 0.945 1 s 12 0.028 4 arrsus sp.1 12 0.028 3 ris p.1 12 0.028 3 ris p.1 17 0.058 3 ris p.1 17 0.110 1 ric Trirbelos 17 0.110 1 ric Trirbelos 17 0.110 1	6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6	0.129 0.717 0.873 0.023* 0.445 0.857 0.007* 0.642 0.088	α	0.428	5 0.128			22	3 0.	0.436	4	0.722	3 0.042*	*	0.759
ella sp.1 8 0.683 1 18 0.151 2 25 0.119 2 opterus 5 0.465 3 uus sp.1 13 0.169 3 tei 7 0.138 4 bicundus 21 0.180 4 cavatus 20 0.247 3 ris 13 0.427 4 inctus 1 0.316 4 inctus 1 0.316 4 inctus 20 0.247 3 ris 13 0.427 4 inctus 1 0.316 4 occladius sp.1 20 0.945 1 s nocladius sp.1 9 1.000 3 ta arsus sp.1 12 0.028* 4 tarsus sp.1 12 0.028* 4 tarsus sp.1 12 0.028* 4 um 21 0.297 4	0 7 6 4 4 0 % 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6	0.717 0.873 0.023* 0.445 0.857 0.075 0.007* 0.0642	7	0.387	1 0.139	9 6	5 0.642	45	2 0.	0.410	3	0.472	5 0.143	2	0.071
18 0.151 2 25 0.119 2 25 0.119 2 25 0.119 2 25 0.119 2 25 2.119 2 25 2.119 2 25 2.119 2 25 2.119 2 25 2.119 2 25 2.119 2 2 2.119 2 2 2 2 2 2 2 2 2	2 w 4 4 0 *	0.873 0.023* 0.445 0.857 0.975 0.007* 0.070	3	0.393	4 0.863	3 6	5 1.000	00	2 0.	0.785	3	0.675	5 0.386	2	0.104
sin sp. 1 special sp. 1	€ 4 4 0 * ∞ € € € €€ 4 4 € € € €€ 4 6 € € €€ 5 € € €€ 6 €<td>0.023* 0.445 0.857 0.975 0.007* 0.042 0.070</td><td>S</td><td>0.158</td><td>1 0.945</td><td>5 3</td><td>3 0.065</td><td>55</td><td>1 0.</td><td>0.822</td><td>3</td><td>0.751</td><td>1 0.913</td><td>3</td><td>0.355</td>	0.023* 0.445 0.857 0.975 0.007* 0.042 0.070	S	0.158	1 0.945	5 3	3 0.065	55	1 0.	0.822	3	0.751	1 0.913	3	0.355
between 5 0.465 3 us sp.1 13 0.169 3 ei 7 0.138 4 cicundus 21 0.180 4 avanus 20 0.247 3 is 0.427 4 arctus 1 0.316 4 servis 23 0.178 4 arctus 1 0.316 4 ocladius sp.1 20 0.945 1 ocladius sp.1 9 0.001 3 arctus sp.1 12 0.001 3 arctus sp.1 17 0.008 3 arctus sp.1 10 0.318 11 arctus sp.1 10 0.110 0	4 4 0 **	0.445 0.857 0.975 0.007* 0.642 0.070	S	0.273	3 0.311	1 8	3 0.537	37	3 0.	0.473	4	0.328	3 0.925	4	0.549
ei 7 0.169 3 ei 7 0.138 4 icundus 21 0.180 4 avanus 20 0.247 3 is 13 0.427 4 inctus 1 0.316 4 syris 23 0.178 3 avanus sp.1 20 0.945 1 7 0.860 4 ocladius sp.1 9 1.000 3 avanus sp.1 12 0.028* 4 avanus sp.1 12 0.038* 1 iv 6 0.400 4 iv 7 0.058 3 iv 7 0.0	4 0 ** & & & & & & & & & & & & & & & & &	0.857 0.975 0.007* 0.642 0.070	3	0.627	3 0.725	5 3	985.0	. 68	2 0.	0.502	_	0.113	4 0.370	5	0.476
ei 7 0.138 4 evanus 21 0.180 4 avatus 20 0.247 3 is 13 0.427 4 is 13 0.427 4 avatus 1 0.316 4 stris 23 0.178 3 stris 16 0.945 1 ocladius sp.1 20 0.945 1 avas sp.1 20 0.945 1 ocladius sp.1 9 1.000 3 a rsus sp.1 12 0.028 4 arsus sp.1 12 0.028 4 arsus sp.1 12 0.028 4 is 1 0.001 3 c 2 0.153 3 is 2 0.153 3 is 2 0.153 3 is 3 0.153 3 is 3 0.153 3 is 4 0.406 3 is 6 0.400 4 is 6 0.400 3 is 6 0.400 3 is 6 0.400 3 is 6 0.400 3 is 7 0.106 3 is 6 0.406 3 is 7 0.106 3 is 7 0.106 3 is 7 0.101 1	8.8 8.8 8.8 8.8 8.8 8.8 8.8 8.8 8.8 8.8	0.975 0.007* 0.642 0.070	4	0.264	4 0.784	7	0.070	70	3 0.	0.559	3	0.063	2 0.218	4	0.750
icumdus 21 0.180 4 avatus 20 0.247 3 is 13 0.427 4 netus 1 0.316 4 stris 23 0.178 3 stris 12 0.124 3 mus sp.1 20 0.945 1 cocladius sp.1 9 0.000 3 arsus sp.1 12 0.028* 4 mn 21 0.028* 3 sp.1 17 0.058 3 c 6 0.400 4 mn 21 0.297 4 mn 21 0.297 4 mn 21 0.297 4 mn 21 0.318 1 renum 1 0.318 1 Tribelos 17 0.058	** © © & & & & & & & & & & & & & & & & &	0.007* 0.642 0.070 0.088	4	0.145	2 0.521	1 7	0.119	. 61	2 0.	0.382	2	0.099	2 0.134	_	0.648
is nectus 20 0.247 3 is nectus 13 0.427 4 nectus 1 0.316 4 stris 23 0.178 3 s 16 0.318 4 is nectus 21 0.124 3 nus sp.1 20 0.945 1 is nectus 20 0.945 1 is nectus 20 0.945 1 is nectus 20 0.0045 1 is nectus 20 0.001 3 is nectus 20 0.001 3 is nectus 20 0.0028 3 is nectus 20 0.0029 4 is nectus 20 0.0029 4 is nectus 20 0.003 3 is nectus 20	ωωνωων4	0.642 0.070 0.088	_	0.157	5 0.009*	6 *¢	0.773	73	4 0.	0.526	5	0.318	$2 0.012^*$	*	0.648
is 13 0.427 4 netus 1 0.316 4 stris 23 0.178 3 s 16 0.318 4 12 0.124 3 nus sp.1 20 0.945 1 7 0.860 4 ocladius sp.1 9 0.001 3 arsus sp.1 13 0.423 4 arsus sp.1 12 0.028* 4 arsus sp.1 12 0.028* 4 is 10 0.028* 3 sp.1 17 0.058 3 sp.1 17 0.058 3 sp.1 17 0.058 3 c 0.400 4 m 21 0.297 4	ω v v ω v 4	0.070	3	0.486	3 0.272	2 2	9.441	41	2 0.	0.484	3	0.278	2 0.675	2	0.684
retuss 1 0.316 4 stris 23 0.178 3 s 16 0.318 4 12 0.124 3 nus sp.1 20 0.945 1 cocladius sp.1 9 0.001 3 arsus sp.1 13 0.028 4 arsus sp.1 12 0.028 4 arsus sp.1 17 0.058 3 sp.1 17 0.058 3 sp.1 17 0.058 3 return 21 0.297 4 m 21 0.297 4	δ δ δ 4	0.088	_	0.018^{*}	4 0.298	7	7 0.125	25	5 0.	0.224	3	0.448	1 0.877	2	0.503
serris 23 0.178 3 s 16 0.318 4 12 0.124 3 12 0.124 3 12 0.124 3 13 0.945 1 1 0.860 4 1 0.001 3 1 0.001 3 1 0.001 3 1 0.001 3 1 0.001 3 1 0.001 3 1 0.001 3 1 0.0028 4 1 0.008 3 1 0.028 4 1 0.028 4 1 0.058 3 1 0.059 4 1 0.059 4 1 0.059 1 1 0.059 1 1 0.059 1 1 0.059 1 1 0.059 1 1 0.059 1 1 0.059 1	5 4	000.0	7	0.929	3 0.111	1 4	1 0.427	27	3 0.0	0.023*	4	0.154	1 0.415	4	0.170
s 16 0.318 4 uus sp.1 20 0.945 1 7 0.860 4 ocladius sp.1 9 1.000 3 rsus sp.1 12 0.011 3 rsus sp.1 12 0.028 4 rsus sp.1 17 0.058 3 rsus sp.1 17 0.059 4 rsus sp.1 17 0.059 4 rsus sp.1 10 0.297 4 rsus sp.1 10 0.29		0.608	1	0.623	3 0.969	9 6	5 0.48	80	5 0.0	0.027^{*}	5 (0.016^{*}	3 0.373	S	0.518
nus sp.1 20 0.124 3 nus sp.1 20 0.945 1 7 0.860 4 0 0.945 1 7 0.860 4 1 0.001* 3 1 0.001* 3 1 0.028* 4 1 0.028* 4 1 0.028* 4 1 0.028* 4 1 0.028* 4 1 0.028* 4 1 0.028* 4 1 0.028* 4 1 0.028* 4 1 0.028* 4 1 0.058* 1 2 0.028* 4 2 0.058* 3 3 0.15 3 4 0.400 4 1 0.297 4 1 0.297 4 1 0.297 4 1 0.297 4 1 0.297 4 1 0.297 4 1 0.297 4 1 0.297 4 1 0.297 4 1 0.297 4 1 0.297 4 2 0.406 3 2 0.406 3 3 0.406 3 4 0.406 3	5 2	0.278	4	0.504	1 0.233	3 9	0.830	30	1 0.	0.985	3	3.958	5 0.723	3	0.607
mus sp.1 20 0.945 1 7 0.860 4 0 0.01 1 0.001 3 a 1 0.001 3 rsus sp.1 13 0.423 4 arsus sp.1 12 0.028 4 rsus sp.1 12 0.028 4 rsus sp.1 12 0.038 1 sp.1 17 0.058 3 sp.1 17 0.058 1 m 21 0.297 4	5 3	0.089	-	0.309	4 0.852	2 3	3 0.374	74	1 0.	434	3	0.780	4 0.590	3	0.395
ocladius sp.1 9 1.000 3 a rsus sp.1 1 0.001° 3 rsus sp.1 13 0.423 4 arsus sp.1 12 0.028° 4 rsus sp.1 23 0.153 3 sp.1 17 0.058 3 sp.1 17 0.058 3 c 0.400 4 m 21 0.297 4 m 21 0.297 4 m 21 0.297 4 renum 1 0.318 1 Tribelos 17 0.110	8	0.691	4	0.354	3 0.359	9	0.628	28	5 0.	0.536	2	0.884	5 0.775	5	0.202
a rsus sp.1 9 1.000 3 a rsus sp.1 13 0.423 4 arsus sp.1 12 0.028* 4 rsus sp.1 12 0.028* 4 rsus sp.1 23 0.153 3 sp.1 17 0.058 3 i	7 5	0.762	_	0.228	5 0.287	7	1.0	00	1 0.	0.611	7	0.292	2 1.000	_	0.340
arsus sp.1 13 0.423 4 arsus sp.1 13 0.423 4 rsus sp.1 12 0.028* 4 rsus sp.1 23 0.153 3 sp.1 17 0.058 3 i	5 5	0.717	Э	1.000	5 0.280	6 0	1.000	00	4 0.	0.215	4	1.000	3 0.774	4	1.000
rsus sp.1 13 0.423 4 arsus sp.1 12 0.028* 4 rsus sp.1 23 0.153 3 sp.1 17 0.058 3 i	0 4	0.323	3	0.795	4 0.140	9 0	5 0.307	. 26	2 0.	0.867	5	0.130	3 0.861	2	0.105
arsus sp.1 12 0.028* 4 rsus sp.1 23 0.153 3 sp.1 17 0.058 3 i 15 0.373 1 i 15 0.373 1 i 15 0.373 1 i 17 0.058 3 i 18 0.373 1 i 19 0.571	2 3	0.209	_	0.025^{*}	5 0.755	5 7	7 0.083	83	4 0.	0.070	3 (0.023*	2 0.490	3	0.079
sp.1 23 0.153 3 sp.1 17 0.058 3 i 15 0.373 1 i 6 0.400 4 iiiiiiiiiiiiiiiiiiiiiiiiiiiiiiiiii	5 5	0.874	-	0.046^{*}	5 0.148	8 3	3 0.301	. 10	4 0.	0.313	4	0.768	5 0.903	4	0.711
sp.1 17 0.058 3 15 0.373 1 16 0.400 4 17 0.297 4 18 0.406 3 18 0.406 3 19 0.406 3 10 0.318 1 11 0.318 1 11 0.318 1 11 0.318 1 11 0.318 1 11 0.318 1	6* 4	0.294	3	0.487	5 0.056	6 4	1 0.401	10	3 0.	0.087	5 (0.029*	3 0.671	4	0.211
15 0.373 1 6 0.400 4 m 21 0.297 4 4 0.406 3 5 0.106 3 renum 1 0.318 1 Tribelos 17 0.110	1 3	0.250	3	0.727	4 0.341	1 7	7 0.257	57	1 0.	0.167	3	0.226	3 0.605	3	0.160
m 21 0.297 4 4 0.406 3 5 0.106 3 remm 1 0.318 1 Tribelos 10 0.541 1	2 2	0.069	_	0.947	2 0.448	8 5	5 0.045*	. 15*	4 0.	0.191	2 (0.017^{*}	4 0.269	3	0.123
m 21 0.297 4 4 0.406 3 5 0.106 3 remm 1 0.318 1 Tribelos 17 0.110	0 1	0.278	_	0.582	1 0.370	0 1	0.865		2 0.0	0.047*	7	0.548	5 0.462	5	0.176
5 0.106 3 1 0.318 1 17 0.110 4	9 3	0.492	_	0.046^{*}	5 0.033*	3* 6	5 0.276	92	3 0.	0.496	2	0.377	2 0.209	Э	0.151
5 0.106 3 1 0.318 1 17 0.110 4	2 4	0.216	n	1.000	3 0.784	2	0.683	83	3 0.	0.536	4	0.314	3 0.743	4	0.786
1 0.318 1 17 0.110 4	2 2	0.288	33	0.330	2 0.443	3 1	0.304	40	2 0.	0.104	1	0.011^{*}	4 0.041*	*	0.471
17 0.110 4	5 5	0.198	_	0.305	5 0.463	3 6	0.846	46	1 0.	0.899	4	0.588	1 0.004*		0.403
10 05/11 1	3 2	0.469	7	0.375	3 0.724	4	0.423	23	1 0.	0.590	_	0.132	5 0.166	_	0.333
1 1+0.0 (1	9 5	0.720	4	0.985	5 0.706	8 9	3 0.124	24	2 0.	0.823	2	0.489	5 0.675	5	0.037
2 0.432 2	0 3	0.290	5	0.654	2 1.000	9 0	0.627	27	3 1.	1.000	4	1.000	2 1.000	4	1.000
0.353 2	3 2	0.294	5	0.203	1 0.267	7 1	0.313	13	5 0.0	*800.0	7	0.442	4 0.217	5	0.013^{*}
0.821 2	5 1	0.598	S	0.207	3 0.749	9 5	0.360	09	5 0.	0.246	5	0.893	2 1.000	3	0.887
0.803 4	0 5	0.720	7	0.383	4 0.484	4	0.289	68	5 0.	0.126	2	0.636	1 0.556	2	0.554
16 0.826 4	6 4	0.909	-	809.0	5 0.139	5 6	0.795	95	4 0.	0.473	4	0.888	2 0.078	2	0.522
23 0.331 3	6 4	0.317	-	0.891	5 0.023	3*	0.080	80	3 0.1	0.045*	3	0.411	1 0.277	4	0.315
Robackia sp.1 19 0.248 3 0.838	8 1	0.275	4	0.278	3 1.000	8 0	3 0.050*	ž0*	2 1.	1.000	_	0.171	5 0.442	2	0.197

CI, class number in which the species have the highest indicator value; P values, probability of obtaining as high an indicator values as observed over 999 iterations. See Tab. 2 for species names and codes. Each site was considered as one class in IndVal analysis. *, significant levels of the permutation test.

(BHD), *Cladotanytarsus* sp.1 in a tributary of Cherf wadi (668 m a.s.l.) (OBM), *Eukiefferiella hospita* in a tributary of the Seybouse wadi downstream at 144 m a.s.l. (OHL).

Many species were indicators of source distance: *P. nubifer* of upstream reaches, *Cricotopus (Isocladius) sylvestris* and *Paratanytarsus* sp. of downstream reaches, *Microtendipes pedellus, Rheotanytarsus* sp., *Zavrelimyia punctatissima* and *Eukiefferiella bedmari* of intermediate reaches.

Few species were indicators of low temperature (<6°C): *P. cultellatum, Paratrissoeladius, rufiventris, Rheotanytarsus* sp. 1, *Cladotanytarsus* sp. 1. None was indicator of high temperature. The following species, instead, prevailed at high temperatures, but not significantly (*Cardiocladius fuscus, Chironomus* sp. 1, *Chironomus plumosus* gr., *Rheocricotopus fuscipes, E. bedmari, Synendotendipes impar, Prodiamesa olivacea*).

Polypedilum (Tripodura) scalaenum was related to low current velocity, while Conchapelopia pallidula, Orthocladius rubicundus and E. hospita were linked to moderate current velocity. The result of Tanypus punctipennis and P. nubifer as indicators of high current velocity is questionable, because other factors correlated with current velocity can be responsible of the high densities observed of these two species.

M. pedellus was associated with sandy substrates, C. pallidula and Z. punctatissima with gravel substrates. Robackia sp. was indicator of stony substrates, but this result will be further discussed. No species was indicator of silt substrates.

Paratanytarsus sp. 1 and E. hospita were indicators of spring samples, whereas O. rubicundus was indicator of winter season. Summer and autumn samples were not characterised by any indicator species.

Among the species significantly correlated with environmental factors, *Chironomus* sp.1 and *C.(I) sylvestris* showed a preference for polluted stations (pollution class 5). *Phaenopsectra flavipes* was associated with low impacted sites. No species was indicator of unimpacted stations, even if the following species emphasised preferences for non-polluted stations, though the IndVal values were not significant: *Tanytarsus sp.*, *Procladius choreus*, *Parametriocmenus stylatus*, Genus sp. near *Tribelos*, *C. fuscus*, *P. (T.) scalaenum*, *Paratrissocladius excerptus*.

P. cultellatum, Cryptochironomus defectus and O. rubicundus were indicators of high oxygen content; the very high measured values of dissolved oxygen (well above saturation values) probably results from a very high photosynthetic activity, as supported by the presence of filamentous algae and was visualised on the CoIA analysis (Fig. 2a).

O. rubicundus and R. fuscipes were associated with conductivity above 900 μS cm⁻¹.

The CoIA carried out with 13 environmental variables

and 45 chironomid species ordered the 26 sampling sites. The first axis accounted for 56% of total variance and the second axis for 23%.

Co-inertia analysis emphasised an upstream-downstream gradient in the first axis of environmental variables, directly correlated with current velocity and inversely with source distance (Fig. 2a). A granulometry gradient was emphasised by the second axis, with samples with stony substrates prevailing having negative values, and samples with silt prevailing having positive values (Fig. 2a).

Species were related to the environmental gradients and their response confirmed IndVal analysis results (Fig. 2b). *Chironomus* sp.1, *C. (I.) sylvestris* and *C. bicinctus* indicators of pollution were mapped in the top left, *Rheotanytarsus* sp.1 and *Cladotanytarsus* sp.1 were located in the bottom right (Fig. 2b), suggesting these species as indicators of good water quality.

The first two axes showed also a pollution gradient, with polluted sites (CPS, SZM) mapped in the top left, and non-polluted sites in the bottom right (OCH, ONL, OAR) (Fig. 2c).

There was a significant agreement between the environmental and the biological systems of coordinates. The PROTEST and RV tests emphasised that the environmental and faunistic tables were significantly correlated (Fig. 3), the correlation between the two sets was 0.776 for the first axis and 0.767 for the second axis (Tab. 4).

The inertia of each separate analysis and the projections of inertia on co-inertia axes are given in Tab. 4.

Of interest is the presence of an undescribed species belonging to a genus near *Tribelos* in the non-polluted station ONL; this station is characterised by a prevailing stony substrate, relatively low temperature and high current velocity.

DISCUSSION

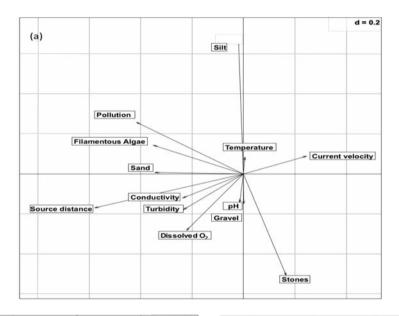
The analysis of chironomid fauna from the Seybouse basin indicated, as expected, that species respond both to natural and to anthropogenic factors. Hot dry summer deeply influences the fauna composition and only few thermal tolerant species as *T. punctipennis* and *C. (I.) sylvestris* are able to tolerate these extreme conditions. Water uptake and pollution aggravate the situation, making the permanence of many Chironomid species critical. Fortunately, the improved conditions observed in winter and spring allow the presence of a more diversified fauna, and in these still poorly studied areas some undescribed taxa are still waiting to be described. In the present investigation an uncommon larva belonging to a Genus sp. near *Tribelos* was captured, confirming the interest of this area from both a taxonomic and ecological point of view.

In the Seybouse river an upstream-downstream gradient related to current velocity and conductivity appeared

to be the major factor driving chironomid species composition and it was estimated by CoIA as a first axis. A granulometry gradient was correlated with the second CoIA axis. An upstream-downstream gradient was related to the first CoIA axis also in the Kebir-East (Chaib *et al.*, 2011), but water temperature was more correlated with the second CoIA axis in Kebir-East. In Seybouse basin water temperature does not account for a large source of variation, and source distance is not related to granulometry, as resulted for the Kebir-East sites. In Seybouse there are

some upstream stations with fine sediments prevailing and some downstream station with large sediment size. The variable more correlated with source distance is current velocity in Seybouse (r=-0.445, with 163 df, P<0.001): current velocity decreases downstream, while temperature is not correlated (r=0.017, with 163 df, P=0.60) with source distance (Supplementary Table 3).

IndVal results are in close agreement with the CoIA; thus, the grouping of the coding environmental variables in few classes, as required by IndVal, for highlighted re-



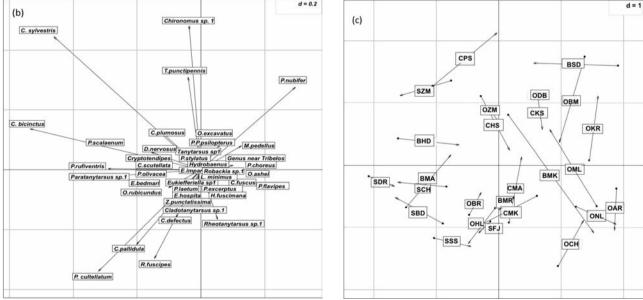


Fig. 2. Co-inertia analysis of 165 samples from 26 sampling sites located along the Seybouse wadi and its effluents (2008-2011), including 45 chironomid species and 13 environmental variables: (a) environmental variables scores; (b) species scores (see Tab. 2 for species names); (c) site scores (see Tab. 1 for site names).

lations between environmental factors and chironomid species, but the present statistical analysis results must be critically examined in comparison with the results found in other river basins.

For example, *P. nubifer* here was indicator of upstream sites characterised by fine substrate and turbulent flow, but the species is considered invasive and can be found in very different habitats (Pinder and Reiss, 1983; Jacobsen and Perry, 2007). The interaction of different factors can explain some apparently anomalous results given by

IndVal: for instance, *T. punctipennis* was found to be an indicator of high current velocity, contradicting the common knowledge about this species which is considered thermal tolerant, preferring fine sediments, associated to low current velocity (Pillot, 2009). The result can be explained if it is observed that *T. punctipennis* is abundant in upstream sites rich in silt in Seybouse, so the relation with current velocity is indirect.

The Indval analysis results showed that *Robackia* sp. was an indicator taxon of stony substrates (Tab. 3), but

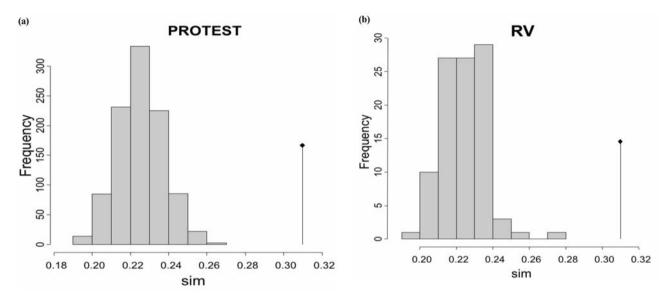


Fig. 3. Procrustes randomisation (PROTEST) (a) and RV (b) test results from the co-inertia analysis showing histograms of simulated values. The vertical line shows the observed value.

Tab. 4. Results of co-inertia analysis.

Co-inertia axes	Eigenvalues	Covariance	Variance 1	Variance 2	Correlation
1	0.08	0.28	1.60	0.23	0.78
2	0.05	0.23	1.42	0.22	0.77
Inertia and co-inertia X					
	Inertia	Max	Ratio		
1	2.56	2.59	0.98		
2	4.59	4.77	0.96		
Inertia and co-inertia Y					
	Inertia	Max	Ratio		
1	0.05	0.06	0.85		
2	0.10	0.12	0.87		

RV=0.441

Covariance, covariance between both systems of coordinates of CoIA (maximised by the analysis); CoIA, co-inertia analysis; Variance 1, inertia of the environmental variables data projected onto co-inertia axes; Variance 2, inertia of the chironomid species data projected onto co-inertia axes; Correlation, correlation between both systems of coordinates of CoIA; RV, coefficient of correlation between both systems of coordinates of CoIA.

this species is generally known to be indicator of sandy substrates (Pillot, 2009).

As far as the response to pollution is concerned, *T. punctipennis*, *P. nubifer*, *Chironomus* sp.1 and *C.(I)* sylvestris were abundant in the polluted stations of the Seybouse wadi. These species are known as tolerant (Hare and Shooner, 1995), or invasive species (Jacobsen and Perry, 2007). *P. choreus* was also found in unpolluted stations, even if this species was reported to be an indicator of polluted lakes (Arslan *et al.*, 2010) or ubiquitous (Vallenduuk and Pillot, 2007).

A comparison of the Seybouse wadi with the recently studied Kebir-East emphasises some similarity and some differences in species composition and responses to environmental factors. In both rivers, an upstream-downstream gradient is dominant, but morphological factors (granulometry) are prevalent in Seybouse, while seasonal factors (water temperature) are more influent in the Kebir-East. Sixteen species are common between both areas, while 29 species were exclusive to the Seybouse wadi (Tab. 3) and 21 were exclusive to the Kebir-East wadi.

There is partial agreement in the distribution of species along a longitudinal gradient between the two river systems. *Orthocladius (Euorthocladius)* sp. was present in both wadis in the upstream sites and *P. (T.) scalaenum* in downstream sites, but differences were observed in the response of other species. *C. scutellata*, for example, prevailed in the downstream stations of the Kebir-East, while it was indicator of upstream reaches in the Seybouse wadi (Tab. 3). The species was captured in different habitats in Europe, but it is known to be relatively cold stenothermal, being abundant at high altitude alpine lakes (*e.g.* Marziali *et al.*, 2009).

CONCLUSIONS

Results confirm that the analysis of chironomid species assemblages is an invaluable tool to uncover the response of benthic macroinvertebrate communities to anthropogenic factors in running waters. In fact, previous studies on quality assessment carried out in lotic ecosystems (Armitage and Blackburn, 1985; Marziali et al., 2010) showed that the response obtained analysing the chironomid assemblages (at genus/species level) was similar to the results obtained considering the whole macroinvertebrate community (at family/genus level), showing the potential of chironomids in biomonitoring. A comparison with biomonitoring programmes in Europe (Pecher et al. 2010) is premature as our knowledge of the freshwater macroinvertebrate fauna of the region is still limited. Larval and adult stages of the three insect orders Ephemeroptera, Plecoptera and Trichoptera, collectively known as EPT and commonly used in biomonitoring programmes elsewhere, are poorly known in Algeria despite pioneering studies (Malicky and Lounaci, 1987; Gagneur and Thomas, 1988; Lounaci and Vinçon, 2005).

There are areas in the world where water demand is critical and this challenge is seriously aggravated by climate change, so it is urgent to deepen our knowledge of these areas; the southern Mediterranean region must surely qualify as a region of high interest and the findings of new taxa, unknown to science, confirm the persistent lack of knowledge of such areas.

ACKNOWLEDGMENTS

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Environmental factors affecting the distribution of Chironomid larvae of the Seybouse wadi, North-Eastern Algeria

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Supplementary Table 1. Mean environmental data with standard error for the 26 sampling sites from the Seybouse wadi and its tributaries (2008-2011). See Supplementary Table 2 for classes of pollution and filamentous algae, and Tab. 1 for site codes.

Site codes	Temperature (°C)	Conductivity (µS cm ⁻¹)	Dissolved O ₂ (mg l ⁻¹ O ₂)	Turbidity (NTU)	рН	Current velocity (cm s ⁻¹)	Source distance (km)			Sand (%)	Gravel (%)	Cobble (%)	Filamentous algae
BHD	10.4±0.28	880±173.94	9.85±1.62	10.75±0.36	6.70±1.07	0.075±0.007	7 81	3	40	40	0	20	4
BMA	14.1±6.54	1286.57±503.42	9.11±2.58	71.15±10.93	7.55±0.81	0.32 ± 0.13	18	3	40	40	0	20	4
BMK	12.41±5.31	1357.5±320.42	2.93±0.39	5.73±2.89	7.49 ± 0.35	0.94 ± 0.65	1	1	0	0	0	100	1
BMR	23.92±2.38	1588.25±650.33	5.57±1.81	26.68±12.15	7.31 ± 0.73	0.2 ± 0.26	90	3	0	0	0	100	4
BSD	15.08±7.17	367.67±117.87	3.59 ± 0.94	14.38 ± 8.12	6.31 ± 0.48	1.84±1.17	1	2	100	0	0	0	5
CKS	13.46±7.28	1634.6±163.06	6.75 ± 8.55	59.95±33.25	7.45±0.69	11.76±9.97	2	2	100	0	0	0	5
CMA	13.27±6.44	812.71±644.68	7.35 ± 5.73	116.76±16.08	7.52 ± 1.09	20.27±25.03	9	2	40	40	0	20	2
CMK	13.28±8.28	2071.17±897.37	10.83±6.51	168.11±93.33	7.63 ± 0.47	6.56±12.61	5	4	0	0	0	100	4
OAR	10.93±2.56	678.67±320.27	4.6±1.55	5.62±2.64	7.87 ± 0.20	18.91±4.82	1	1	0	0	0	100	1
OBM	13.45±0.49	627.5±342.94	6.5±1.83	42.5±5.93	7.63 ± 0.26	17.89±11.44	1 2	1	50	50	0	0	1
OBR	9.9 ± 5.89	771.67±23.45	7.5±3.06	83.17±45.90	7.67±0.28	0.57±0.21	3	3	0	50	50	0	4
OCH	13.52±6.12	660.91 ± 175.73	5.24±3.23	27.33±26.38	7.41 ± 0.69	11.08±13.7	5	1	0	0	0	100	3
ODB	19.53±8.88	608.25±226.25	3.64±1.09	3.85 ± 2.31	7.67 ± 0.52	18.08±13.97	7 2	4	70	0	0	30	3
OHL	15.96±8.20	1191.6±271.23	9.84±5.31	510.2±401.01	7.51±0.54	0.43 ± 0.37	81	3	0	0	0	100	4
OKR	12.56±3.70	441.67±78.38	3.84 ± 3.04	28.35±41.36	7.55 ± 0.37	14.78±10.48	3 1	1	100	0	0	0	1
OML	11.08±6.74	417.00±231.27	5.53 ± 0.92	20.66±14.63	7.57±0.13	16.29±10.93	3 1	2	50	50	0	0	1
ONL	14.1±1.8	294.00±139.67	4.13±0.92	8.14±4.65	7.43 ± 0.47	23.90±5.11	1	1	0	0	60	40	1
OZM	12.1±5.23	923.50±316.03	8.87 ± 3.27	35.82 ± 19.30	7.77 ± 0.43	0.21 ± 0.11	5	3	70	30	0	0	4
SBD	10.13±4.27	2248.67±874.52	16.03±8.35	199.53±79.70	7.95 ± 1.00	0.55 ± 0.36	130	3	40	40	0	20	4
SCH	14.77±5.03	1787.67±557.69	10.32±4.85	279.72±153.18	7.65±0.79	0.44 ± 0.15	146	3	40	40	20	0	4
SDR	12.34±5.74	1615.86±567.33	10.55±4.89	382.10 ± 213.31	7.68 ± 0.88	0.58 ± 0.36	154	3	40	40	0	20	4
SFJ	14.92±6.78	1285.35±407.31	10.25±3.46	162.37±86.99	7.41 ± 0.81	0.55 ± 0.28	87	3	0	0	0	100	4
CHS	16.78±7.21	763.17±174.12	4.22 ± 1.52	22.88±8.17	7.4 ± 0.44	13.41±5.03	81	2	40	40	0	20	2
CPS	14.68±7.87	642.44±112.68	2.55±1.65	22.04±14.83	7.03 ± 0.72	19.19±8.00	21	5	100	0	0	0	5
SSS	13.75±7.04	1412.88±417.45	12.03±5.72	281.61 ± 124.47	7.83 ± 0.72	0.42 ± 0.15	81	3	0	0	0	100	4
SZM	16.37 ± 6.87	1383.33±315.62	6.50 ± 2.10	155.54±49.10	7.16 ± 0.73	0.41 ± 0.07	110	5	70	30	0	0	5





Supplementary Table 2. Classes of the environmental factors.

Environmental factors	Classes	Intervals
Seasons	Class 1	Autumn
	Class 2	Summer
	Class 3	Spring
	Class 4	Winter
Granulometry	Class 1	<25%
(particle size distribution)	Class 2	25<32.5%
	Class 3	32.5<37.5%
	Class 4	37<45%
	Class 5	45<47.5%
	Class 6	47.5<50%
	Class 7	50<62.5%
	Class 8	62.5<85%
	Class 9	85<100%
Water conductivity	Class 1	<400 μs cm ⁻¹
•	Class 2	400<700 μs cm ⁻¹
	Class 3	700<900 μs cm ⁻¹
	Class 4	900<1500 μs cm ⁻¹
	Class 5	>1500 μs cm ⁻¹
Water temperature	Class 1	<6°C
F	Class 2	6<9°C
	Class 3	9<14°C
	Class 4	14<19°C
	Class 5	>19°C
Pollution	Class 1	Unpolluted
	Class 2	Slightly polluted
	Class 3	Moderately polluted
	Class 4	Very polluted
	Class 5	Heavily polluted
Dissolved O ₂	Class 1	<3 mg L ⁻¹
	Class 2	3<5 mg L ⁻¹
	Class 3	5<8 mg L ⁻¹
	Class 4	8<13 mg L ⁻¹
	Class 5	$>13~{ m mg}~{ m L}^{-1}$
Current velocity	Class 1	<0.3 cm s ⁻¹
	Class 2	0.3<0.5 cm s ⁻¹
	Class 3	0.5<3 cm s ⁻¹
	Class 4	3<20 cm s ⁻¹
	Class 5	$>20~{\rm cm}~{\rm s}^{-1}$
Source distance	Class 1	<1 km
	Class 2	1<2 km
	Class 3	3<9 km
	Class 4	9<90 km
	Class 5	>90 km
Filamentous algae	Class 1	Little or no growth observed
	Class 2	Thin layer present
	Class 3	Crusts or coatings of diatoms
	Class 4	Abundant floating algae
	Class 5	Carpets or blankets of algae
	Ciuss J	Carpets of blankets of algae

Higher values of particle size distribution meaning higher percentage of large sized particles.

Supplementary Table 3. Correlation matrix of the environmental factors.

	Temp.	Cond.	O_2 dis.	Turb	Hd	Current vel.	Source dist.	Pollution	Silt	Sand	Gravel	Cobble	Fil. algae
Temp.	1	-0.134	-0.438	-0.086	-0.120	-0.130	0.017*	0.122	0.036*	-0.102	-0.048*	0.027*	0.074
Cond.	-0.134	-	0.642	0.079	0.160	-0.458	0.448	0.176	-0.382	0.044^{*}	-0.065	0.309	0.057
O ₂ dis.	-0.438	0.642	1	0.315	0.233	-0.438	0.428	0.102	-0.326	0.165	-0.002*	0.195	0.094
Turb.	980.0-	0.079	0.315	-	0.316	-0.265	0.448	0.148	-0.188	0.087	0.013*	0.113	0.162
F	-0.120	0.160	0.233	0.316	_	-0.116	0.098	-0.074	-0.181	0.068	0.034^{*}	0.110	-0.157
Current vel.	-0.130	-0.458	-0.438	-0.265	-0.116	-	-0.445	-0.115	0.217	-0.112	0.038*	-0.138	-0.262
Source dist.	0.017*	0.448	0.428	0.448	0.098	-0.445	_	0.367	-0.137	0.443	0.019*	-0.085	0.346
Pollution	0.122	0.176	0.102	0.148	-0.074	-0.115	0.367	-	0.305	0.056	-0.110	-0.247	0.689
Silt	0.036^{*}	-0.382	-0.326	-0.188	-0.181	0.217	-0.137	0.305	1	0.160	-0.178	-0.843	0.347
Sand	-0.102	0.044^{*}	0.165	0.087	0.068	-0.112	0.443	0.056	0.160	1	0.187	-0.612	-0.072
Gravel	-0.048*	-0.065	-0.002*	0.013*	0.034	0.038*	0.019^{*}	-0.110	-0.178	0.187	1	-0.184	-0.115
Cobble	0.027*	0.306	0.195	0.113	0.110	-0.138	-0.085	-0.247	-0.843	-0.612	-0.184	_	-0.223
Fil. algae	0.074	0.057	0.094	0.162	-0.157	-0.262	0.346	689.0	0.347	-0.072	-0.115	-0.223	-

Temp., water temperature; Cond., water conductivity; O2 dis., dissolved oxygen; Turb., water turbidity; Current vel., current velocity; Source dist., source distance; Fil. algae, filamentous algae. *=significant values (P<0.05).