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Chironomidae larvae as bioindicators of an acid mine drainage in Portugal

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Abstract

The abandoned São Domingos mine in South Portugal discharges acid metal pollution in the drainage system. A dynamic pH- and metal-gradient (pH 3.3–6.8) modulated by rainfall at the confluence of a tributary with the acid mine drainage (AMD)-channel, and a small reservoir contaminated with arsenic were sampled for Chironomidae in three different seasons and compared with a control river, to test whether this insect family is sensitive to AMD-pollution and arsenic. The AMD sites contained 18–22 taxa, compared to 22 taxa in the reservoir and 25 taxa in the control site. The chironomid fauna in the AMD was characterised by a high proportion of Chironominae and predatory Tanypodinae, and a low proportion of Orthocladiinae, these subfamilies being useful and easy bioindicators for AMD. The presence of morphological deformities in *Ablabesmyia monilis* and *Procladius* sp. is a potential bioindication of AMD but due to low numbers of larvae, it was of limited use. Despite high arsenic levels in the water, the small reservoir contained many taxa forming a typical lentic community.

Introduction

Abandoned mines worldwide pose a long term threat on aquatic ecosystems by continuous or intermittent flow of acidic drainage water containing high concentrations of a cocktail of heavy metals. Acidification generally induces an increase of metal bioaccumulation in insect larvae, with consequences for the trophic chain (St. Louis, 1993). Acid mine drainage (AMD) is detrimental to the aquatic fauna by its action on the physiology, such as disruptions of enzymatic processes (Farris et al., 1989). AMD represents an extremely stressful and long term pollution source due to anthropogenic disturbance of geological layers, often having a major impact on densities and taxa richness of macroinvertebrates (DeNicola & Stapleton, 2000). Small-scale gradients of pH and heavy metals within such systems can be useful as field model systems to study biological effects of acid and metal pollution. The information gained might be extrapolated with caution to develop bioassessment methods to detect pollution pulses from mining areas. Moreover, bioassessment methods are essential tools to evaluate recovery or remediation measures of deteriorated mining sites (Smith & Cranston, 1995; DeNicola & Stapleton, 2000).

Although larvae of Chironomidae often represent a significant portion of the benthic fauna in deteriorated sites (e.g. Canfield et al., 1994) and are the dominant food source for many fish (e.g. Rieradevall et al., 1995), taxonomical difficulty and lack of ecological knowledge often force the investigators to treat them at the (sub)family or tribe level (Armitage & Blackburn, 1985). However, they represent together with the Oligochaeta the major part of the sediment-dwelling fauna, which makes them especially useful for sediment quality assessment. Chironomid faunistic assemblages are for example useful to indicate the degree of trophy of Northern lakes (Saether, 1979) or as bioindication of pollution in streams (e.g. Janssens de Bisthoven & Gerhardt, 2003). Moreover, it has been experimentally demonstrated that exposure of chironomid larvae to heavy metals induces mouth part deformities (e.g. Janssens de Bisthoven et al., 2001) and more than 30 field studies indicate a causal link between metals or organic xenobiotics and deformities (e.g. Warwick, 1990, 1991; Pettigrove et al., 1995; Janssens de Bisthoven et al., 1998). In the present study, we examine the potential of Chironomidae offered by high generic and species richness and the eventual occurrence of morphological head deformities as bioindication for two sites affected by acid mine drainage and one polluted with arsenic in South Portugal.

Materials and methods

Study sites

The study sites are situated in the abandoned cupriferrous pyrite mine of São Domingos, Alentejo, S. Portugal, at the border with Spain (37 39′ 56″ N, 7 28′ 46″ W). The Acid Mine Drainage (pH = 2.3) finds its way through a series of sedimentation basins in an excavated valley and by the time it reaches a reservoir on the River Chança, it can be diluted to pH = 5 during the winter rains. The River Chança is a tributary of the River Guadiana. The annual dry period between June and October, typical for this semi-arid area, causes a gradual decrease of the dilution effect of the few tributaries. The very low pH of 2.3 in the main AMD-channel promotes the dissolution of the bedrock and mobilises heavy metals (Kang et al., 2001).

The confluence of the Mosteirao stream with the AMD creates a small-scale dynamic gradient of pH and metals. This location was chosen as model for the investigation of the biological effects of AMD. One of the two sampling sites in the Mosteirao stream was situated approximately 200 m from the confluence with the AMD-channel and had a pH varying between 4 (dry season: summer and autumn) and 6.4 (winter-spring) and consisted in the dry season of small pools fed by groundwater ('AMD-site pH 4–6'). Another site in that gradient, approximately 50 m from the AMD-channel, had a constant pH of 3.3 ('AMD-site pH

Table 1. Range of heavy metal concentrations (μg/L, *mg/L) in River Vascão (max. in October 2000–min. in May 2001), Small Reservoir, AMD-site pH 4–6 and pH 3 (general seasonal trend: min. in May or June 2000 and max. in August or October 2000)

Element (min.–max.)	Site			
	River Vascão	Small Reservoir	AMD pH 4–6	AMD pH 3
As	3.2–2.0	0.91–121.2	1.07–50.6	0.81–21.7
Cd	0.1–0.0	0.1–1.0	0.25–21.5	9.8–86.2
Co	0.3–0.1	0.2–2.6	1.0–292.1	111.0–931.8
Cu	15.2–1.6	7.2–69.5	10.3–1400^	721.0–2800
Fe	36.1–0.04	13.0–43.9	26.1–4900	225.4–7500
Mn*	14.7–7.0	0.03–0.04	0.51–16.9^	3.4–42.1
Pb	5.5–2.4	4.3–29.3	3.5–184.4^	123.0–168.5
Zn	56.0–15.0	36.0–158.0^	299.0–6400^	5900–17 200
Ca*	25.4–17.1	21.4–26.0	87.0–306.6	46.5–616.3
Cl*	109.2–32.1	23.4–25.4	19.4–113.3	25.3–147.4
K*	3.9–1.2	3.7–4.4	3.5–9.7	3.5–16.1
Mg*	42.9–13.6	7.3–10.6	123.3–127.0	28.2–333.6
Na*	82.1–30.2	22.4–27.8	29.2–96.7	32.1–166.9
S*	16.7–7.7	10.4–20.3	52.1–490.7	103.5–1050

When values were exceptionally high in May or June, details are given under ^.
^Zn in Small Reservoir was 954.0 μg/L in May/Cu in site pH 4–6 was 728.0 μg/L in May/Mn in site pH 4–6 was 98.0 mg/L in June/Pb in site pH 4–6 was 232.0 μg/L in May/Zn in site pH 4–6 was 5300 μg/L in May.

3'). A small reservoir, situated at the foot of a dam impounding the Tapada Grande reservoir near the village of São Domingos, was also sampled: its water contained high levels of As and low levels of other metals (Table 1) as it was not connected to the AMD, and it had a pH of 6.8 (site 'Small Reservoir'). A fourth site was chosen in the same Guadiana watershed, as a control: the uncontaminated River Vascão, which flows in the Guadiana National Park and contained low metal levels (Table 1), having a pH of 7–7.8. The sediments of the River Vascão and the AMD-sites pH 3 and pH 4–6 contained a mixture of sand and silt in the pools and gravel and cobbles in the riffles, while the percentage of organic matter was around 2%. The Small Reservoir contained predominantly fine sand and >2% organic matter (unpubl. data). All the AMD substrata were covered by an orange film of hydrous iron oxide. Macrophytes were absent in the AMD zone and present in the Small Reservoir and the River Vascão.

The Protoxkit F^{TM} was used to test the toxicity of the sites on the protozoan *Tetrahymena thermophila*, as external confirmation of the toxicity of the chosen study sites (e.g. Schlenk & Moore, 1994). The growth inhibition of *T. thermophila* showed a negative linear regression with the pH-values of the tested AMD-water ($r^2 = 0.85$, $p < 0.05$). The 24 h $EC_{50\ AMD}$ was at pH = 5.8 ± 0.2 (95% C.L.).

Physical and chemical characterisation

Two replicate water samples from each site were collected in May, June, August and October 2000, and in May 2001 and analysed for 14 elements with ICP-MS and ICP-AES (Table 1). In May 2001 a hail storm provoked a sudden catastrophic flood in the AMD and the Mostareio stream. One hour and 24 h after start of the flood, water samples were taken as well to illustrate the dynamism of the water chemistry during such an event. More detailed data and discussion are provided in Gerhardt et al. (2004).

Chironomidae

Chironomidae were collected with a handnet (250 μm mesh size) during 15 min in all habitats, which include sand, mud, gravel, pebbles and fine detritus (stratified sampling) at the different sites, in June 2000, October 2000 and in May 2001 just before the catastrophic flood. After the flood in May 2001, no chironomid sample was taken, as it was clear that no resident larvae would have been found at all due to the extreme water velocity. In June 2000 no larvae were found in River Vascão and Small Reservoir, while in October 2000, no larvae were found in AMD pH 4–6 and AMD pH 3. If cobbles were present, about 10 cobbles were scratched with brushes within a bucket to collect epilithic Chironomidae. The larvae were prepared for light microscopy according to Janssens de Bisthoven et al. (1998). Identification of the larvae was according to the keys in Wiederholm (1983). Pupal skins, present in small numbers in the samples, were identified according to Langton (1991), as they could confirm the presence of taxa found with larval identifications, and add more taxa to the checklist. Screening of deformities was done according to Janssens de Bisthoven et al. (1998) and Janssens de Bisthoven & Gerhardt (2003). Damage caused by breakage and mechanical abrasion was not considered as deformity. In the guts of larger chironomid larvae, chironomid head capsules from larvae taken as prey were recorded, since it could provide useful information about trophic relationships in this extreme environment and eventually confirm the predatory status of Tanypodinae, as mentioned in the literature.

Data analysis

The following metrics were calculated to characterise the taxa richness and the dissimilarities amongst the sites: Shannon diversity index $H = -\sum P_i \ln P_i$ ($i = 1$ to S), Shannon equitability index $J = H/\ln S$, with P the relative abundance of taxon i in the whole sample and S the number of taxa in the sample (Begon et al., 1987) and the Bray–Curtis dissimilarity Index $BC_{jk} = \sum|x_{ij} - x_{ik}|/\sum(x_{ij} + x_{ik})$ (for site j and site k, $i = 1$ to n) (Podani, 2000). All metrics were calculated on the basis of larvae. Additional information provided by sporadically found pupal exuviae is provided in Appendix A. In order to give a graphical overview of the seasonal shifts and other disturbances of the water quality, a factor analysis was applied on the chemical data. All analyses were performed with Statistica 4.5.

Results

Chemical and physical characterisation of the sampling sites

Heavy metals in the AMD sites were generally 10–100 times higher as in the River Vascão and the Small Reservoir (Table 1). During the summer dry season the concentrations of metals increased at the sites 'AMD pH 3' and 'AMD pH 4–6' more than hundred to thousand times. Salinisation generally increased during the summer dry season at all sites, except for the Small Reservoir. Arsenic was the highest in the small reservoir. A catastrophic flood registered in May 2001 provoked intensive physical stress and a short temporal increase of Fe- and Al- concentrations (Gerhardt et al., 2004), while other metals were diluted (Fig. 1). The water chemistry is summarised in Table 1 and in a factor analysis biplot (Fig. 1).

Chironomid community

Only *Ablabesmyia monilis* occurred at all four sites with more than 10 larvae, being by far dominant with over 100 larvae in the AMD-site pH 4–6. The next dominant species were *Cladotanytarsus mancus* gr. in River Vascão and Small Reservoir, *Cricotopus trifascia* gr. in River Vascão and *Microchironomus* sp. in Small Reservoir. The most species-rich genera were *Tanytarsus* and *Cricotopus* (Appendix A). In the River Vascão, about 46% of the larvae belonged to the subfamily of the Chironominae, and another 44% to the Orthocladiinae (Table 2). In the Small Reservoir, 80% of the larvae belonged to the Chironominae. In the two AMD-sites, 43–70% of the larvae belonged to the Tanypodinae, and the majority of other larvae to the Chironominae (Table 2). The Shannon H index reflected the pollution gradient. The Bray–Curtis index (BC), showed highest dissimilarities between River Vascão and the two AMD-sites (BC = 0.81) and lowest between River Vascão and small reservoir (BC = 0.59). Identification of pupal exuviae provided additional taxa. About 40% of the *Ablabesmyia* larvae in the AMD-site pH 4–6 contained 1 or more chironomid larvae in their gut, mostly small Tanytarsini (*Tanytarsus* sp. and *Paratanytarsus* sp.), but also a few Tanypodinae and Orthocladiinae.

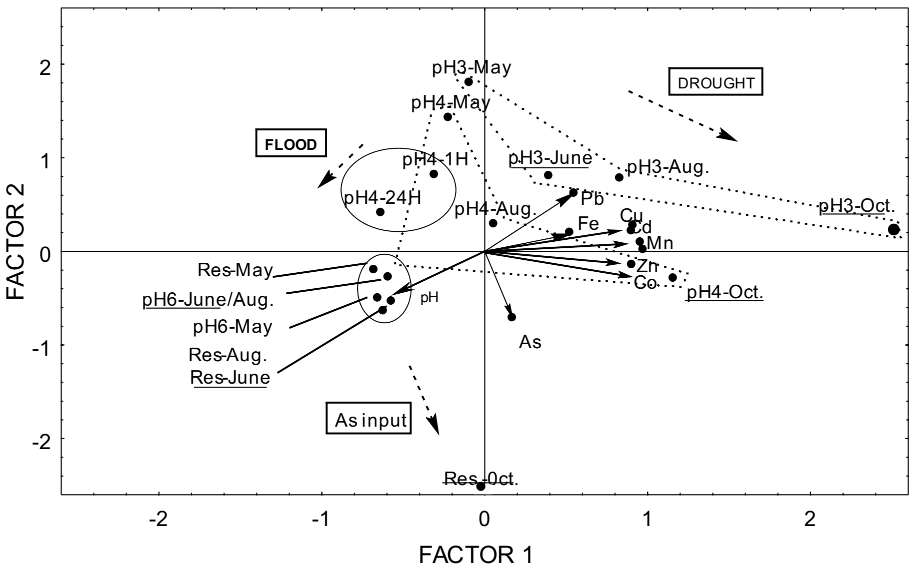

Figure 1. Factor analysis (% of variability: 1st factor, 63%; 2nd factor, 13%) on the metal concentrations in the AMD study sites and the Small Reservoir, situated in the S. Domingos mine, S. Portugal. The River Vascão (low metal values) was excluded for seek of clarity. Several sampling dates are given to illustrate: (1) the seasonal shifts in function of dry periods, (2) the As-input in the Small Reservoir and (3) the sporadical shifts in function of flooding due to thunderstorms. Underlined sites correspond to chironomid samples in the present study (AMD pH 3 = pH 3, AMD pH 4–6 = pH 4 and pH 6).

Table 2. Community metrics for Chironomidae from pooled samples of June–October 2000 and May 2001

Sites	River Vascão	Small Reservoir	AMD pH 4–6	AMD pH 3.3
% nr. of Chironomidae larvae/macrobenthos	18.8	57.3	11.3	15.0
Chironomidae				
Total no. of analysed chironomid larvae	140	135	183	87
Number of taxa (S)	25	22	22	18
Shannon index H	2.56	2.47	1.82	1.74
Equitability index $J = H/\ln S$	0.79	0.79	0.59	0.60
No. of larvae				
% Tanypodinae (mostly predatory)	8.6	9	70	46
% Chironominae (filter-, collector-feeding)	46	80	27	43
% Orthocladiinae (grazers, mixed)	44	11	2	11
No. of taxa				
% Tanypodinae	8	9	23	32
% Chironominae	44	61	68	54
% Orthocladiinae	48	27	9	17

Chironomid deformities

Of the 47 taxa, 7 had larvae with weak morphological deformities (Table 3). From the 7 taxa, only one occurred at all 4 sites, *A. monilis*. Although its numbers of larvae in the River Vascão and the Small Reservoir were not satisfactory, it showed increasing deformity rates with increasing

Table 3. Morphological deformities of the head capsule in chironomid larvae of the four study sites

Taxon	Site	Structure deformed	Nr. def./total larvae	% Deformed
Ablabesmyia monilis	River Vascão	–	0/11	–
	Small Reservoir	–	0/11	–
	AMD pH 4–6	Ligula, paraligula	4/106	3.8
	AMD pH 3.3	Paraligula	2/21	9.5
Procladius sp.	River Vascão/Small Reservoir	–	Not found	–
	AMD pH 4–6	Pecten, ligula	2/17	11.8
	AMD pH 3	–	0/7	–
Cryptochironomus sp.	River Vascão/Small Reservoir	–	Not found	–
	AMD pH 4–6	–	0/4	–
	AMD pH 3	Premandible	1/20	5.0
Endochironomus sp.	River Vascão	–	Not found	–
	Small Reservoir	Mandible	1/2	–
	AMD pH 4–6	Mentum	1/1	–
	AMD pH 3	Mentum	1/2	–
Polypedilum sp. B	River Vascão	Mandible	1/4	–
Cricotopus trifascia gr.	River Vascão	Mentum	1/22	4.5
Psectrocladius sordidellus gr.	Small Reservoir	Premandible	1/8	–

acidity and metals (observed versus expected $\chi^2 = 18.3$, df = 3, $p = 0.0004$). *Procladius* sp. and *Cryptochironomus* sp. larvae were absent in the control site and Small Reservoir, but present in the AMD-sites, however in low numbers. Three species containing one deformed larva were only found in one site, and therefore of no use for comparison. Other more abundant taxa (*Cladotanytarsus mancus* gr., *Rheocricotopus* sp. *Microchironomus* sp., *Rheotanytarsus* sp. and *Tanytarsus* spp.) had no deformities.

Discussion

Chironomid community

The present study confirmed ecological response patterns to AMD compared to the control site as found and described for the macrobenthos in the same mining area (Gerhardt et al., 2004): (1) loss of acid-sensitive species, compensated by addition of more acid-tolerant species, which results in only a slight decrease of α-diversity, and (2) more predatory taxa and higher abundance of predators. Comparison of the three sampling seasons showed a high variation in larval numbers (high β-diversity), most probably due to sporadic floods which wash away the whole aquatic fauna.

Concerning the taxa commonly found in our study and the study of Orendt (1999) on chironomids in acidic streams in Germany, his classification according to acid-sensitivity was in good agreement. The presence of many chironomid species in the AMD-sites is surprising, given the extremely high levels of heavy metals, compared with the control site River Vascão. It is either the result of a high acidity-metal tolerance, or it is the result of drift from upstream reaches which are not under the influence of AMD, or a combination of both. However, the drift hypothesis is very unlikely, as 8 of the 18 taxa from the AMD pH 3 site were not found upstream in the AMD pH 4–6 site. The Small Reservoir, a lentic environment characterised by higher organic content and finer substrate, had a dominance of *Microchironomus* sp. and other Chironominae, considered as collector-gatherers or collector-filterers (Berg, 1995), typical for lentic conditions (Pinder, 1995). Shannon Index, Equitability Index, number of taxa and percentage of Tanypodinae were very similar to the values found for River Vascão, suggesting that the high As levels in the water were not toxic to the chironomid fauna. Most larvae belonging to the subfamily of the Chironominae contain haemoglobin. Jernelöv et al. (1981) mentioned the buffering capacity of haemoglobin in 'red larvae' enabling these larvae to tolerate high acidity. However, the many colourless larvae found in the AMD sites must have other detoxifying mechanisms. Armitage & Blackburn (1985) found two tolerant taxa (*Pseudokiefferiella* and *Limnophyes*) in an acidic stream draining an old coal mine, while their least polluted site in Northern England contained as many taxa (21) as the AMD-site pH 4–6 in the present study. Larvae of *Limnophyes* sp. appear to be very acidophilic (Armitage & Blackburn, 1985, this study; Orendt, 1999). Smith & Cranston (1995) and Cranston et al. (1997) also found a high chironomid richness in their AMD-study in Australia and stressed that different species of the same genus may have different sensitivities. This could apply for the very species-rich *Tanytarsus* spp., from which 23 species are recorded for the Iberian peninsula (Soriano et al., 1997). A behavioural assay with the 'Multispecies Freshwater Biomonitor®' using allochtonous *Chironomus* larvae and combined with bioaccumulation measurements on the same São Domingos sites and acid-only exposures, has indicated that at a pH of 3.3 acidic stress predominates over metal stress, because of competition of the hydroxonium ions with the metal ions (Janssens de Bisthoven et al., 2004). This could explain why acid-tolerant chironomids are able to live in AMD containing such high metal levels which would otherwise be lethal in circumneutral pH (Timmermans et al., 1992). A recent study of 5 circumneutral mine drainages in Canada (Swansburg et al., 2002) concluded that Orthocladiinae were relatively more abundant at sites receiving mine drainage, while Tanytarsini larvae were metal-sensitive. This is in clear contrast to our study and this difference is probably explained by the acidity of the AMD in São Domingos mine.

The shift in the AMD sites towards predatory Tanypodinae within the Chironomidae, compared with the control site, supports the findings for the macrobenthos (Gerhardt et al., 2004), where high densities of predatory Corixidae and Coleoptera were found in the AMD-sites. Other studies (Winner et al., 1980; McKay & Kersey, 1985; Canfield et al., 1994; Janssens de Bisthoven & Gerhardt, 2003) also reported high numbers of Tanypodinae in polluted sites. Most larvae of the subfamily of the Tanypodinae are considered as predatory 'engulfers' and 'piercers' and/or opportunistic omnivores (Berg, 1995). The occurrence of chironomid head capsules in the guts of the large *Ablabesmyia* sp. found in AMD pH 4–6 confirms their predatory status. Apparently, stressed conditions favour an increase of secundary consumers, changing considerably the shape of the food pyramide. This phenomenon has also been reported for the macroinvertebrates of the AMD (Gerhardt et al., 2004) and for fish in stressed lakes (Goldschmidt et al., 1993), and corroborates with major shifts in ressource utilisation, possibly including a decrease of the number of trophic levels, implying a simplification of the trophic web. These ecological processes need further analysis in AMD-environments, but could explain why Tanypodinae can be used as bioindicators.

Most Orthocladiinae on the other hand are considered to be scrapers and shredders (Berg, 1995) and were almost absent from the AMD sites. However, some species of the Orthocladiinae genera *Cricotopus* and *Eukiefferiella* are tolerant to heavy metals (Winner et al., 1980; Yasuno et al., 1985). Most taxa within the Chironominae are collector-filterers and collector-gatherers, but some (e.g. *Cryptochironomus* sp., *Endochironomus* spp., *Glyptotendipes* spp., *Polypedilum* spp. and *Chironomus* spp.) are also predatory on oligochaetes. All these potentially predatory Chironominae were found in the AMD-sites, where Oligochaeta were present too (Gerhardt et al., 2004). A plausible mechanism for the observed difference in trophic groups between the AMD-sites and the River Vascão can be found in the hypothesis formulated by Lindegaard (1995): "the effect of low pH is surpassed by the blanketing effect of precipitation of hydrous metal oxides, which greatly decreases the abundance of periphyton, and consequently grazers such as Diamesinae and Orthocladiinae disappear (e.g. McKnight & Feder, 1984; Rasmussen & Lindegaard, 1988)."

Deformities

Tennessen & Gottfried (1983) reported deformities in *Ablabesmyia annulata*, however without knowing their causes. In our study, *A. monilis* seemed to be a useful species in the deformity screening method. Its ubiquitous distribution, large size and easy identification are great advantages and merit further research. The next most important deformity indicator was *Procladius* sp., which unfortunately did not occur in the control site and Small Reservoir. Many studies use *Procladius* as a deformity indicator for environmental pollution as it is one of the most tolerant species to anoxia and heavy metals (e.g. Warwick, 1991; Diggins & Stewart, 1993, 1998; Pettigrove et al., 1995; Janssens de Bisthoven & Gerhardt, 2003). All genera exhibiting deformities in the present study were reported in the literature as exhibiting deformities in polluted areas (e.g. Wiederholm, 1984; Warwick, 1990; Bird, 1994). The robustness of the deformity field screening method however stands and falls with the number of larvae in the sample and the presence of genetical adaptation (called 'tolerance' by Swansburg et al., 2002) with suppression of morphological responses. The possibility of adaptation is high because of the historical character of the mine. The low rates of deformities in the As polluted Small Reservoir should be further investigated. Janssens de Bisthoven & Gerhardt (2003) and Martinez et al. (2002) advocated for considering deformity rates at the individual taxon level and not pooled over all taxa of a given site, because not enough is known about interspecific differences in deformity responses and pollution tolerances. However, despite this scientifical uncertainty, some authors (e.g. Bird, 1994; Swansburg et al., 2002) favour the use of deformity rates of pooled taxa, for the obvious reason that (1) the confidence limits decrease with increasing number of larvae, resulting in increasing inferring power and (2) it seems to work well in differentiating polluted sites from clean ones.

We conclude that the acid mine drainage induced a significant shift within the community structure of chironomid larvae, as compared to an unaffected site. Especially the predatory Tanypodinae and the detritus-eating Chironominae appear to be useful as bioindicators for acid mine drainage. Taxonomical richness within the Chironomidae remains considerable in acid mine drainage, because loss of sensitive species is compensated by addition of tolerant species. The use of deformities, especially in *Ablabesmyia* sp. and *Procladius* sp., adds a bonus of information about the toxicity, but was limited by the low number of larvae per taxon.

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Appendix A. Number of Chironomidae larvae per taxa found in the River Vascão and the São Domingos mine at different sampling sites and dates

Site	River Vascão		Small Reservoir		AMD pH 4–6		AMD pH 3.3	
Taxon	Oct. 00	May 01	Oct. 00	May 01	June 00	May 01	June 00	May 01
Tanypodinae								
Tanypodinae unidentified						2		
Ablabesmyia monilis		11	1	10	32	74		21
Conchapelopia sp.								3
Larsia sp.					1			
Thienemannimyia sp.								2
Paramerina sp.		1						4
Procladius sp.					5	12	1	6
Zavrelimyia sp.					3			
Chironominae								
Chironomini unidentified[a]		2			2	1		
Tanytarsini unidentified[a]					1			
Chironomus sp. A[a]							1	
Chironomus sp. B[a]						1		
Cladotanytarsus mancus group	1	25	10	19	1			
Cryptochironomus sp.					1	3		20
Demicryptochironomus sp.								2
Dicrotendipes cf. *notatus*					6		1	
Endochironomus sp.		1		1		1		2
Endochironomus cf. *tendens*			1		3			
Kiefferulus sp.[b]					1			
Microchironomus sp.			23					
Micropsectra sp.[a]				1	2			
Microtendipes pedellus gr.				3				
Nilothauma cf. brayi[b]				1				
Paratanytarsus sp.		2	2	1	9			
Polypedilum (*P.*) cf. *nubeculosum*	1	7	1	2	2	2		
Polypedilum sp. B		4						1
Rheotanytarsus sp.		11			1			
Stictochironomus sp.			2			1		1
Tanytarsus sp. A[a]		2	6	12	6	3	7	1
Tanytarsus sp. B[a]		1		7	1	1	1	
Tanytarsus sp. C		2		9				
Tanytarsus sp. D		3		7				
Virgatanytarsus sp.	1	3						
Orthocladiinae								
Orthocladiinae unidentified		1			1			1
Cardiocladius sp.		2						
Chaetocladius sp.[a]		2						
Cricotopus sp.	3			1				
Cricotopus bicinctus		3						
Cricotopus (*Isocladius*) *sylvestris* gr.				1				

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Appendix A. (*Continued*)

Site	River Vascão		Small Reservoir		AMD pH 4–6		AMD pH 3.3	
Taxon	Oct. 00	May 01	Oct. 00	May 01	June 00	May 01	June 00	May 01
Cricotopus trifascia gr.		22						
Eukiefferiella sp.[c]				1				
Eukiefferiella gracei gr.[c]		1						
Limnophyes sp.[a]							6	
Nanocladius sp.		1						
Orthocladius sp.		3		1				
Parakiefferiella sp.						3		3
Psectrocladius sordidellus gr.				8				
Psectrocladius limbatellus gr.		4						
Rheocricotopus sp.[c]		13						
Orthocladiinae								
Cf. *Stilocladius* sp.		6	3					
Synorthocladius sp.		1						

Pupal exuviae found in the respective samples were identified to provide further support to the larval identifications and eventual additional taxa to the list of larvae (*): River Vascão; May 2001: 1 *Orthocladius* sp., 1 *Rheotanytarsus curtistylus* (*), 6 *Rheotanytarsus* sp., 1 Rheotanytarsus Pe 2 (*), 1 *Paratanytarsus* sp., 1 *Paratanytarsus* Pe 1 (*^), 4 *Paratanytarsus* cf. *dimorphis* (*^), 1 *Paramerina*, 1 *Polypedilum* sp., 1 *Polypedilum cultellatum* (*), 3 *Tanytarsus* sp., 3 *Virgatanytarsus* sp., Diamesinae: 1 *Diamesa* sp. (*). Small Reservoir, Oct. 2000: 5 *Glyptotendipes pallens* (*). AMD pH 4, Oct. 2000: 2 *Psectrocladius* sp.

[a] Acid-tolerant taxa according to Orendt (1991).

[b] Only 1 species known for the Iberian peninsula (Soriano et al., 1997).

[c] Acid-sensitive taxa according to Orendt (1991).

Not found in the checklist of Chironomidae of the Iberian peninsula (Soriano et al., 1997).