ARE CHIRONOMIDAE (DIPTERA) GOOD INDICATORS OF WATER SCARCITY? DRYLAND STREAMS AS A CASE STUDY

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ABSTRACT

Water scarcity is becoming one of the greatest challenges that human societies will face during this century. Monitoring water availability is expensive and technically challenging. In this regard, biological communities (e.g. aquatic insects) offer a cost-effective alternative, since they integrate temporal and spatial hydrological variability. Here we explore the potential of Chironomidae (Diptera), which have been usually neglected due to their complex taxonomy, as indicators of both local habitat condition and water scarcity. The study took place in 28 sites across seven dryland streams distributed within a 400 km² section of the Upper San Pedro River basin, southeastern Arizona. The selected streams covered a wide range of hydrological variability, which was continuously measured through the deployment of 15 electrical resistance (ER) sensors. Chironomidae taxa with no drought-resistance strategies were rarely found in streams that experienced frequent drying events (i.e. intermittent and ephemeral streams), suggesting that droughts have the potential to reduce species richness due to local extinctions of drought-intolerant taxa. Intermittent and ephemeral streams registered low canopy cover and a significantly higher abundance of scrapers (which mainly feed on algae) and shredders (feeding on poorly decomposed coarse organic material). This suggests that structural changes associated to drought (e.g. reduced canopy cover and decomposition rates) might lead to changes in the functional composition of the Chironomidae assemblages. We conclude that Chironomidae species can be used as indicators of hydrological variability and the impacts of drought on streams in the absence of flow gauges.

1 **1. INTRODUCTION**

2 Water scarcity, driven by our growing demand for water (FAO, 2015) and more
3 prolonged and intense droughts caused by climate change (IPCC, 2013; Trenberth et al.,
4 2014), is becoming one of the greatest challenges that human societies will face during
5 this century (UN Water, 2007). The reduction in water availability poses a risk to the
6 goods and services that we obtain from rivers and streams (Richter et al., 2003).
7 Droughts are difficult to detect and predict, since they steadily build up in
8 strength through time (Lake, 2011). Unfortunately, monitoring water availability (e.g.
9 groundwater levels, surface flows) at high spatial resolution is expensive and
10 technically challenging, especially in temporary streams. In this regard, biological
11 communities (e.g. aquatic insects) offer a good opportunity for cost-effective
12 monitoring of water availability, since these communities integrate temporal and spatial
13 hydrological variability (Prat et al. 2014).

14
15 There is a long tradition of using aquatic insects for monitoring the environmental
16 conditions (e.g. trophic status) of rivers and streams (Bonada et al., 2006). Among the
17 different groups of aquatic insects that have been used in biomonitoring, Chironomidae
18 (Diptera) have proved to be very useful as pollution or water quality indicators (Nicacio
19 and Juen, 2015; Wilson and Bright, 1973) because they are abundant, rich in species,
20 and present over a wide range of habitats and environmental conditions (Armitage et al.,
21 1995). Since Chironomidae species differ greatly in their habitat and food preferences
22 (Armitage et al., 1995), they may also be good indicators of the indirect effects of
23 droughts on stream ecosystems. For example, reductions in stream flow can lead to
24 reduced cover and species richness of riparian plants or dominance by drought-tolerant
25 shrubs (Stromberg et al., 2007, 2005). Both of these effects can reduce input of organic
26 matter into the streams, and cause the loss of some important habitats provided to
27 invertebrates by logs and branches (Lake, 2011). Moreover, loss of canopy cover may
28 reduce shading and expose the water body to greater light and heat (Lake, 2011). Given
29 their diversity and sensitivity to resource inputs and water quality conditions,
30 Chironomidae assemblages would likely respond significantly to both the direct (e.g.
31 drying) and indirect (e.g. reduced riparian leaf input) effects of drought.

32
33 In spite of this sensitivity to drought and changing abiotic conditions, Chironomidae
34 tend to be neglected (i.e. only identified to family or tribe level) in most ecological

1 studies, probably due to their taxonomic complexity (Bazzanti et al., 2008). This scarcity
 2 of information is especially true regarding the ecological traits of Chironomidae species,
 3 including knowledge of life history traits that confer resistance to drought and habitat
 4 drying. It is known that some species like *Hydrobaenus aetheri* can aestivate (Benigno
 5 and Sommer 2008; Cranston and Dominguez, 2007) using a desiccation-resistant
 6 resting stage. However the literature on the tolerance of other Chironomidae species to
 7 drought is incomplete, and real data about resistance to desiccation for most of the
 8 Chironomidae (and many other aquatic insects) is scarce (Moller-Pillot, 2013, 2009).
 9

10 In discussing drought resistance, we adopt the viewpoints of Cranston & Nolte (1996).
 11 These authors suggest that for most of the desiccation-tolerant Chironomidae the resting
 12 stage does not include a true cryptobiose (or diapause). Concordantly, the word
 13 “diapause” is not mentioned in the recently published monograph on the larvae of
 14 Holarctic Chironomidae (Andersen et al., 2013). The ecological strategies of
 15 Chironomidae inhabiting temporary streams are poorly known, but many species might
 16 be able to survive in different refuges (e.g. hyporheic habitats, rain pools) during
 17 drought, from which they can quickly colonize streams when surface flow
 18 returns. Chironomidae, for instance, often are the first taxa to recolonize ephemeral
 19 streams (i.e. those that flow only briefly after heavy precipitation events) after flow
 20 resumption (Bogan et al., 2015; Boulton et al., 1992; Chester and Robson, 2011; Frouz
 21 and Matěna, 2015; Frouz et al., 2003). For example, Montalto and Paggi (2009) found that
 22 chironomid larvae were capable of staying completely dry for periods of more than 28
 23 days in a marginal fluvial wetland of the Middle Paraná River floodplain. The authors
 24 observed that most species built tubes or used macrophyte cover to avoid drying, and
 25 some species were capable of digging 15 cm deep into sediments as a strategy to
 26 survive surface drying, and this capacity seems to occur among many species of midges.
 27 Larvae can also survive the loss of body water. For example, Frouz and Matěna (2015)
 28 reported that *Chironomus stercorarius*, *Macropelopia martini* and *Chironomus dorsalis*
 29 may survive losses of 84.2, 72.6 and 67.4% of their original water content, respectively.
 30 Extreme drought resistance has been observed in *Polypedilum vanderplanki*, which can
 31 enter anhydrobiosis multiple times during its life cycle, but this case seems to be the
 32 exception more than the rule in midges (Andersen et al, 2013). Given this complexity, we
 33 use the general term “drought adaptation” to refer to any morphology, behavior or life-
 34 history strategy that confers resistance or resilience to drought (Lytle and Poff, 2004).

1
2 In this paper, we compile information on the drought adaptations of Chironomidae
3 species inhabiting dryland streams in Arizona (USA). Our intent is to determine the
4 potential of Chironomidae as indicators of the direct and indirect effects of water
5 scarcity along a gradient of stream flow permanence. We selected this region due to the
6 wide temporal and spatial hydrological variation that it covers. Some streams have
7 water the entire year (permanent streams), others dry for several months each year
8 (intermittent streams) and others flow only briefly during heavy rain episodes
9 (ephemeral streams) (Levick *et al.* 2008; Jaeger & Olden 2012). This hydrological
10 variability is known to shape the composition (Bogan *et al.* 2013; Bogan and Lytle 2007;
11 Canedo-Arguelles *et al.* 2015) and the functional structure (Schriever *et al.*, 2015) of
12 invertebrate assemblages. Additionally, dryland regions are among the most vulnerable
13 to further reductions in water availability. Some dryland streams are already
14 experiencing drying trends, including transitions from perennial to intermittent or
15 ephemeral flow (Bogan *et al.*, 2015; Webb *et al.*, 2014). Thus, there is an urgent need for
16 new tools to quickly characterize local flow regimes in the face of intensifying
17 droughts. Such information could facilitate proper management of water resources in
18 these areas (Cook *et al.*, 2004; Gao and Giorgi, 2008).

19 20 **2. METHODS**

21 *2.1 Sampling*

22 Chironomidae were sampled at 28 sites across seven dryland streams distributed within
23 a 400 km² section of the Upper San Pedro River basin, southeastern Arizona, USA as
24 part of a larger study focusing on taxonomic and functional community diversity
25 (Bogan *et al.* 2013, Canedo-Arguelles *et al.* 2015, Schriever *et al.* 2015) (Figure 1).
26 Streams in the area generally have perennial flow in montane headwaters, intermittent
27 flow in upper alluvial fan reaches, ephemeral flow lower on alluvial fans, and then
28 alternating perennial and intermittent reaches in valley rivers like the San Pedro (Bogan
29 *et al.*, 2013). We distributed our sample sites among perennial, intermittent and
30 ephemeral reaches (classification follows (Levick *et al.*, 2008) but used a continuous
31 flow metric to quantify permanence (see below). Sites were sampled three times a year
32 (March/April, August/September, and November/December) between 2009 and 2011.
33 The number of sites and samples collected differed among streams because not all sites
34 had flow during each sampling event. This time period spanned numerous dry seasons,

several periods of ephemeral flows from summer monsoon rains, and one period of intermittent flows resulting from a wet winter. The majority of the sampling occurred during the fall (Nov/Dec) and winter (Mar/Apr) seasons for a total of 144 site x sampling event combinations.

As reported in Schriever *et al.* (2015) both riffle and pool microhabitats were sampled at each site, when present. For riffle samples we disturbed 0.33 m² of stream substrate to a depth of 5 cm while capturing invertebrates immediately downstream with a D-net (500-µm mesh). Pool samples consisted of sweeping the entire pool area including water column, surface, and pool benthos with a D-net at an effort of 10 seconds for every 1 m² of pool habitat (following Bogan and Lytle 2007). Samples were preserved in 95% ethanol and invertebrates were identified in the laboratory to the finest taxonomic level practical. Abundances from replicate microhabitat samples collected from the same site during the same sampling event (e.g., three riffles in November) were summed for each taxon and divided by the number of replicates to acquire relative abundances.

During each visit, water temperature, pH (Whatman pH Indicators, Whatman International, Maidstone, England), and conductivity (Milwaukee waterproof EC meter C65; Milwaukee Instruments, Rocky Mount, NC, U.S.A.) were measured. Canopy cover and benthic substrate were visually estimated on a percent cover scale (0 - 100%; substrate categories: silt, sand, gravel, cobble and bedrock). The timing and duration of streamflow was monitored through the deployment of 15 electrical resistance (ER) sensors (Jaeger and Olden, 2012), each representing the hydrologic conditions at the nearest location of invertebrate sampling. The sensors logged the presence or absence of water in the stream channel at 15 minute intervals from April 2010 to December 2011. From the sensor data, four hydrologic metrics were calculated for each site: (1) % flow permanence by year, (2) % flow permanence by season (spring = March-June; summer = July-September; fall = October – November; winter = December-April), (3) mean duration (number of days) of zero flow periods (ZFP) each year, and (4) total number of ZFP each year. Flow permanence is defined as the percentage of time a given reach is wetted or flowing, while the duration of ZFP indicates how long (in days) a given reach is dry during each drying event. For example, a site with permanent streamflow would have a flow permanence of 100% and would receive a value of 0 for ZFP duration. In

calculating the seasonal and annual flow permanence metrics and the duration of ZFP, 15-minute time periods of both wet and dry conditions for the sampling period and for individual zero flow periods were summed, converting the time unit to either days or years as appropriate for the individual metric. An average of 2010 and 2011 flow data was used to estimate flow conditions for fall 2009 sampling period (16 samples) that occurred prior to the deployment of flow sensors. We calculated annual and seasonal flow permanence percentages because both may influence reach-scale biological communities; % flow permanence by year characterizes the average flow conditions in a given reach, while % flow permanence by season describes the flow regime experienced by local biological communities for the exact season when each sample was collected. While these metrics were all calculated from the two years of flow sensor records, they are intended to characterize longer-term attributes of the hydrologic regime that may influence chironomid larvae occurrence and abundance.

2.2 Ecological traits

As explained in Schriever et al. (2015) we developed a trait database using publications from primary literature (Table 1), previously published databases, and available specialist knowledge to define trait states for the Chironomidae species registered in this study. We compiled information on seven functional traits (body size, voltinism, respiration, functional feeding group, dispersal capability, drought adaptation, and habit or primary locomotion), with 30 modalities across the seven traits. However, only functional feeding group, drought adaptation and habit or primary locomotion differed among Chironomidae species.

2.3 Statistical analyses

All statistical analyses were performed in R version 3.2.0 (R Core Team, 2015). We tested the correlation of the hydrological variables with the rest of environmental variables through Spearman correlation tests, considering any variable with a correlation coefficient ≥ 0.90 as highly correlated. We characterized environmental gradients across the study streams with a principal components analysis (PCA), using the function “prcomp”. The PCA served to group all the environmental variables (e.g. substrate, canopy, flow metrics) into two main environmental gradients and arrange all sites along such gradients. The sites were subsequently grouped in two groups according to K-means analysis using the function “kmeans” and using the PCA axes as

inputs. The K-means method aims to partition the points (i.e. sites) into k groups (two in this case) such that the sum of squares from points to the assigned cluster centers is minimized. We used the algorithm of Hartigan and Wong (1979). Previous to K-means and PCA analyses all the environmental variables were normalized by subtracting the mean and dividing by the standard deviation. Indicator species analyses (IndVal) (Dufrêne and Legendre, 1997) was used to find the species significantly associated to each of the groups resulting from K-means analysis. IndVal analysis was performed using “multipatt” function in the R package “indicspecies” (Cáceres and Legendre, 2009). This function chooses the group with a highest association value to each species according to the abundance and the frequency of the species in each group. The calculated indicator value of a given taxon for its preferred group ranges from zero (worst) to one (best). Best matching patterns are tested for statistical significance of the associations. Finally, the differences in the relative abundance of each trait (e.g. scraper/grazer) in each group (i.e. relative abundances were calculated for each trait category separately, since each category contains the total of individuals in each site) were assessed through an analysis of variance (ANOVA) using the function “aov”. Normality was checked performing the Shapiro–Wilk test of normality. In the cases where normality was not met, data were transformed using square root.

3. RESULTS

The PCA (Figure 2) resulted in two main components explaining 30.3% and 22.9% of the total variance in measured environmental factors. The first component was related to hydrological variability; positive values were associated with high flow and dense canopy cover and negative values were associated with long, recurring drying events and low canopy cover. The second component was related to variation in habitat conditions; positive values were associated with coarse substrate (i.e. gravel and cobbles) and negative values were associated with fine sediment, bedrock, and high pH. The K-means analysis resulted in two groups of streams that are clearly separated along the first component of the PCA (Figure 2), and exhibit distinct flow regime and habitat characteristics. The first group consisted of perennial sites with dense canopy cover and the second group consisted of intermittent and ephemeral streams with low canopy cover. Hydrological variables were not highly correlated with any habitat or water chemistry variables (i.e. Spearman correlation ≤ 0.90).

Most Chironomidae life history traits were significantly more abundant in one of these two distinct stream types. Burrowers, climbers, clingers, filter-feeders, engulfer-predators and species with no drought adaptation were significantly more abundant in perennial streams; sprawlers, scrapers, shredders and species with possible drought adaptation were significantly more abundant in ephemeral and intermittent streams (Figure 3). According to IndVal analyses, four taxa were significant indicators of intermittent and ephemeral streams (all of them showing drought adaptation) and 16 taxa were significant indicators of perennial streams (Table 2).

4. DISCUSSION

Based on habitat characteristics and flow regime, we found two distinct groups of streams (perennial with dense canopy and temporary with sparse canopy), each of which were significantly associated with distinct Chironomidae taxa and ecological traits. Substrate and flow conditions are tightly linked to community structure and biological traits in many regions (e.g. Mediterranean: Bonada et al., 2007). Surprisingly, the presence of drought adaptations was not significantly associated with intermittent and ephemeral streams, but the absence of drought adaptations was significantly associated with perennial streams. This suggests that species lacking drought adaptations are much more likely to be found in perennial than temporary streams, but that species with drought adaptations do occur in perennial habitats. However, this does not mean that species from temporary streams lack the capacity to withstand drought. In fact, all four of the significant indicator taxa for ephemeral and intermittent sites were adapted to drought. Therefore, drought seems to act like an environmental filter that prevents maladapted species from successfully colonizing and persisting in intermittent and ephemeral streams (Poff, 1997; Schriever et al., 2015). Persistence in temporary streams can result from a combination of finding drought refugia (e.g. hyporheic zone: Boulton et al., 1992; del Rosario and Resh, 2000), developing resistance strategies (e.g. absorbing water directly from air in high humidity conditions: Frouz and Matěna, 2015), or seeking cover in moist microhabitats (e.g. organic matter cover: Montalto and Paggi, 2009).

Nonetheless, caution should be exercised when using our classification of drought adaptation for Chironomidae (Table 1). This classification has been mainly developed at the genus level. For example, we classify *Cricotopus* as a genus with drought resistance capacity because it was reported to aestivate and overwinter in the dry

1 temporary pools by Wiggins et al. (1980). Similarly, this genus has been recorded in
2 temporary pools with short wet phase in central Italy (Bazzanti et al., 1997). However,
3 *Cricotopus* includes a great variety of species which occur across wide environmental
4 gradients (Armitage et al. 1995, Andersen et al., 2013, Moller-Pitot, 2013, 2009). Some
5 of these species might be able to truly aestivate, while others might seek moist
6 microhabitats, and some might simply be very resilient, colonizing habitats very
7 quickly after flow resumption. In fact, one study from western Australia suggests that
8 aestivation beneath stones and the use of desiccation-resistant life stages were far less
9 important drought refuges for macroinvertebrates than perennial surface waters (Chester
10 and Robson, 2011). Regardless, our results suggest that the ability of Chironomidae to
11 survive drought varies markedly among taxa, and that taxa intolerant of drying tend to
12 be absent from intermittent and ephemeral streams. Therefore, as suggested by
13 Schriever et al. (2015), drying events in perennial streams have the potential to reduce
14 species richness due to local extinctions of drought-intolerant taxa. Loss of perennial
15 refugia can also reduce the source of colonists to intermittent and ephemeral streams
16 after flow resumes (Cañedo-Argüelles et al., 2015; Frouz and Kindlmann, 2015). In any
17 case, drought tolerance seems to be relatively rare in Chironomidae, and resilience may
18 be the major pathway to restoring populations when flow returns.

19
20 Regarding functional feeding groups, the relative abundance of scrapers and shredders
21 was significantly higher in intermittent and ephemeral streams than in perennial
22 streams. Scrapers remove algae from benthic substrates. The scarcity of water at
23 intermittent and ephemeral sites offers little opportunity for trees to grow, resulting in
24 low canopy cover (Stromberg et al., 2007, 2005). Therefore, increased light intensity in
25 temporary streams favours algal growth, which may promote the scraper/grazer taxa that
26 feed on them. The same situation has been reported for naturally saline streams (Millán
27 et al., 2011) where salt accumulation prevents riparian vegetation from growing and
28 scrapers dominate the aquatic invertebrate assemblages. Shredders are adapted to chew
29 coarse plant material. Their higher relative abundance in intermittent and ephemeral
30 streams may be related to the substantial input of litter from riparian plants that results
31 from water stress as streams dry (Steward et al., 2012). Furthermore, the accumulation of
32 coarse detritus during long dry periods, and its later incorporation into the ecosystem
33 during flow resumption, can provide excellent food sources for shredders (Acuña et al.,
34 2007; Dance et al., 1979).

1
2 In perennial streams, we found a higher relative abundance of Chironomidae
3 predators. Predators have been reported to be among the later colonizers of newly created
4 ponds (Cañedo-Argüelles and Rieradevall, 2011) and rewetted temporary ponds (Boix
5 et al., 2000; Lake et al., 1989; Williams, 1997). Thus, the short duration of flow in
6 intermittent and ephemeral streams could prevent the establishment of predator
7 populations, as has been previously observed in studies from the region (Bogan et al.,
8 2013). Filter-feeders were also more abundant in perennial streams. This could be
9 related to the fact that permanent flow favours the decomposition of coarse materials
10 such as leaves (Corti et al., 2011; Datry et al., 2011; Dieter et al., 2011), increasing the
11 availability of fine particulate organic matter.
12
13 Among locomotive traits, we found that burrowers, clingers and climbers were
14 significantly more abundant in perennial than temporary streams. The presence of sandy
15 sediments in our perennial streams may favour species that can burrow deep into the
16 sediment (e.g. *Brillia*) or cling to the substrate to avoid being carried away by the
17 current (e.g. *Cricotopus*). Climbers were most likely associated with aquatic vegetation
18 and submerged roots that are found mainly in perennial streams in the region (Bogan et
19 al., 2015). Sprawlers tended to dominate in intermittent and ephemeral streams, where
20 riffles are rare or absent in the dry summer period and no adaptations are needed to
21 withstanding high current velocities. These taxa are adapted to staying on top of the
22 substrate and moving around to look for suitable habitats (i.e. food and water
23 availability) (Tachet et al., 2000).
24
25 Our study shows that Chironomidae assemblages clearly differ between perennial and
26 intermittent and ephemeral sites, reflecting changes in water availability and habitat
27 characteristics, as is the case in Mediterranean streams (Puntíet al., 2009). Thus, they
28 have good potential to be used as indicators of water scarcity in streams. For example, in
29 our study region (southwestern USA) the increase in *Chaetocladius*, *Hydrobaenus* and
30 *Krenosmittia* in a stream would mean that this stream is probably threatened by water
31 scarcity (i.e. undergoing periods of drought). In the same way, a shift from predator
32 towards scraper dominance, as well as an increase in drought-tolerant taxa could be an
33 early warning of water scarcity. Biological traits have already been proven to be useful
34 in predicting flow connectivity in Mediterranean rivers (Cid et al., 2015), especially

when detailed hydrological information (e.g. from flow gauges) is missing, as it is the case for most streams around the world. Additionally, hydrological variability was previously found to be the strongest predictor of aquatic invertebrate functional diversity in our study area (Schriever et al., 2015). The information provided here could be useful to further studies estimating hydrological variability from biological data. Although identifying Chironomidae larvae can be difficult, the use of Chironomidae pupal exuviae (i.e. the floating molt that the adults leave behind when they emerge from the water) may be a cost-effective solution. Exuviae can be easily and rapidly collected, are easier to identify than larvae, and integrate information from multiple habitats (Cañedo-Argüelles et al., 2012; Wilson and Ruse, 2005). Thus, we conclude that Chironomidae species can be used as indicators to monitor hydrological variability and the impacts of water scarcity on streams.

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7
8

1 **FIGURE LEGENDS**

2 **Figure 1:**Map of sampling localities for Chironomidae in southeastern Arizona (USA). Inset map shows
3 the location of the study area. Continuous blue line = perennial streams. Short-dashed green line =
4 intermittent streams. Long-dashed red line = ephemeral streams. Black circles represent sampling
5 localities.

6
7 **Figure 2:**Principal component analyses of the environmental variables (i.e. hydrology, substrate type,
8 canopy, and water chemistry) recorded in this study. The percentage of total variance explained by each
9 principal component is shown in brackets. The blue and orange ellipses correspond to the clusters of
10 perennial and intermittent sites, respectively. The clusters were estimated using K-means analysis using
11 the algorithm of Hartigan and Wong(Hartigan and Wong, 1979) and specifying 2 cluster centers ($k = 2$).
12 Flow year = % of flow permanence along the year. Flow season = % of flow permanence by season.
13 NumZFP = number of zero flow periods per year. MdZFP = duration of zero flow periods per year.

14
15 **Figure 3:** Trait relative abundance (i.e. proportion of individuals showing a specific trait) for perennial
16 and intermittent/ephemeral streams. Only those traits showing a significant difference between groups
17 according to ANOVA are shown here. Note that y-axis scales differ among graphs. Statistical
18 significance is shown in the title of each graph as follows: * = $0.05 > p > 0.01$; ** = $0.01 > p > 0.001$; ***
19 = $p < 0.001$.

Figure 1

Figure 2

Figure 3

Table 1: List of drought-adapted Chironomidae (i.e. those that have been reported to persist in dry or ephemeral streams). The reliability of this table as been classified as follows: Certain = resting stages collected and rehydrated in laboratory experiments; Likely = very closely related taxa can aestivate or some species in the genera are only known from intermittent streams. Possible¹: when larvae of some species are predominantly known from intermittent streams; Possible²: when other species in the genus or species groups are known from terrestrial habitats; Possible³: when larvae are known from ephemeral streams (i.e. those that flow only briefly after heavy precipitation events). It should be noted that in many cases drought adaptation comes from observations or comparisons between temporary and permanent streams and could be related to the ability of organisms to rapidly recolonize streams after flow resumption.

Taxon	Reliability	References
<i>Acricotopus</i>	Certain	(Wiggins, Mackay & Smith 1980)
<i>Apedilum</i>	Likely	(Thomas & Ferrington 1997)
<i>Bryophaenocladus</i>	Possible ²	(Cranston, Oliver & Saether 1983)
<i>Chaetocladus</i>	Likely	(Caldwell 1997; Chou <i>et al.</i> 1999)
<i>Chironomus</i>	Certain, Likely, Possible ³	(Williams & Hynes 1976; Wiggins <i>et al.</i> 1980; Paltridge <i>et al.</i> 1997; Thomas & Ferrington 1997; Doran 1999; Balaraman 2008)
<i>Corynoneura</i>	Likely, Possible ¹ , Possible ³	(Chou <i>et al.</i> 1999; Doran 1999; Rüegg & Robinson 2004)
<i>Cricotopus</i>	Certain	(Wiggins <i>et al.</i> 1980)
<i>Cricotopus/ Orthocladus</i>	Likely, Possible ¹	(Langton & Casas 1998; Chou <i>et al.</i> 1999)
<i>Crictotopus Pe16</i>	Possible ¹	(Langton & Casas 1998)
<i>Demicryptochironomus</i>	Possible ¹	(Langton & Casas 1998)
<i>Diamesa</i>	Possible ¹	(Rüegg & Robinson 2004)

<i>Diamesa aberrata</i>	Possible ¹	(Langton & Casas 1998)
<i>Dicrotendipes</i>	Likely	(Paltridge <i>et al.</i> 1997)
<i>Diplocladius</i>	Likely	(Chou <i>et al.</i> 1999)
<i>Eukiefferiella</i>	Certain, Possible ¹	(Wiggins <i>et al.</i> 1980; Rüeegg & Robinson 2004)
<i>Eukiefferiella coerulescens</i>	Possible ¹	(Langton & Casas 1998)
<i>Eukiefferiella claripennis</i>	Likely	(Madder, Rosenberg & Wiens 1977; Chou <i>et al.</i> 1999)
<i>Hydrobaenus</i>	Certain, Likely, Possible ³	(Wiggins <i>et al.</i> 1980; Doran 1999; Tronstand, Tronstand & Benke 2005; Benigno & Sommer 2008)
<i>Hydrobaenus knodoi</i>	Certain	(Kondo 1996)
<i>Hydrobaenus saetheri</i>	Certain	(Cranston & Dominguez 2007; Benigno & Sommer 2008)
<i>Krenosmittia camptophelps</i>	Possible ¹	(Langton & Casas 1998)
<i>Labrundinia</i>	Possible ¹	(Griswold <i>et al.</i> 2008)
<i>Larsia</i>	Likely	(Chou <i>et al.</i> 1999)
<i>Limnophyes</i>	Certain, Likely, Possible ¹ , Possible ³	(Wiggins <i>et al.</i> 1980; Langton & Casas 1998; Chou <i>et al.</i> 1999; Doran 1999; Benigno & Sommer 2008)
<i>Mesosmittia</i>	Possible ²	(Cranston <i>et al.</i> 1983)
<i>Micropsectra</i>	Certain, Likely, Possible ¹	(Williams & Hynes 1976, 1977; Chou <i>et al.</i> 1999; Rüeegg & Robinson 2004)
<i>Orthocladius calva</i>	Possible ¹	(Langton & Casas 1998)
<i>Orthocladius spp.</i>	Likely	(Chou <i>et al.</i> 1999)
<i>Orthocladius thienemanni</i>	Possible ¹	(Langton & Casas 1998)
<i>Parakiefferiella</i>	Likely	(Chou <i>et al.</i> 1999)

<i>Paramectriocnemus</i> Pe4	Possible ¹	(Langton & Casas 1998)
<i>Parametriocnemus</i>	Possible ¹	(Rüegg & Robinson 2004; Griswold <i>et al.</i> 2008)
<i>Paraphaenocladus</i>	Certain, Likely	(Wiggins <i>et al.</i> 1980; Chou <i>et al.</i> 1999)
<i>Paratanytarsus</i>	Certain, Likely	(Wiggins <i>et al.</i> 1980; Paltridge <i>et al.</i> 1997; Benigno & Sommer 2008)
<i>Paratendipes</i>	Likely	(Chou <i>et al.</i> 1999)
<i>Parorthocladus</i>	Possible ¹	(Rüegg & Robinson 2004)
<i>Phaenopsectra</i>	Likely, Possible ¹ , Possible ³	(Grodhaus 1980; Langton & Casas 1998; Doran 1999)
<i>Polypedilum</i>	Certain, Likely, Possible ¹	(Hinton 1951; Wiggins <i>et al.</i> 1980; Thomas & Ferrington 1997; Tronstand <i>et al.</i> 2005; Griswold <i>et al.</i> 2008)
<i>Psectrocladius</i>	Certain	(Wiggins <i>et al.</i> 1980)
<i>Pseudosmittia</i>	Likely	(Chou <i>et al.</i> 1999)
<i>Pseudodiamesa</i>	Possible ¹	(Rüegg & Robinson 2004)
<i>Smittia</i>	Certain, Possible ³	(Wiggins <i>et al.</i> 1980; Cranston <i>et al.</i> 1983)
<i>Stictochironomus</i>	Likely	(Chou <i>et al.</i> 1999)
<i>Rheotanytarsus</i>	Likely	(Paltridge <i>et al.</i> 1997)
<i>Stenochironomus</i>	Likely	(Paltridge <i>et al.</i> 1997)
<i>Stictochironomus</i>	Likely	(Paltridge <i>et al.</i> 1997)
<i>Tanytarsus</i>	Certain, Likely, Possible ¹	(Grodhaus 1980; Wiggins <i>et al.</i> 1980; Langton & Casas 1998; Tronstand <i>et al.</i> 2005)
<i>Thienemanniella</i> Pe1b	Possible ¹	(Langton & Casas 1998)
<i>Tvetenia</i>	Possible ¹	(Rüegg & Robinson 2004)

<i>Zavrelimyia</i>	Possible ¹ ,Possible ³	(Doran 1999; Rüegg & Robinson 2004)
<i>Thienemanniella</i>	Certain/ Possible ¹	(Langton & Casas 1998; Tronstand <i>et al.</i> 2005)

Table 2: List of Chironomidae species significantly associated to each group of sites (intermittent, in orange, and perennial, in blue) according to Indicator Species Analysis. Species are arranged according to their indicator value (IV). * = 0.05 < p-value < 0.01. ** = 0.01 > p-value. It should be noted that in many cases drought adaptation comes from observations or comparisons between temporary and permanent streams and could be related to the ability of organisms to rapidly recolonize streams after flow resumption.

Species	IV	p.value		Ecological traits
<i>Hydrobaenus</i>	0.836	0.005	**	Scraper/grazer; Aestivation possible; Sprawler
<i>Eukiefferiella rectangularis</i>	0.798	0.010	**	Scraper/grazer; Drought adaptation possible; Sprawler
<i>Chaetocladius</i>	0.748	0.020	*	Scraper/grazer; Drought adaptation possible; Sprawler Collector-gatherer; Drought adaptation possible;
<i>Krenosmittia</i>	0.655	0.020	*	Sprawler
<i>Tvetenia bavarica</i> grp.	0.961	0.005	**	Collector-gatherer; No drought adaptation; Climber
<i>Thienemannimyia</i> grp.	0.956	0.005	**	Collector-gatherer; No drought adaptation; Burrower
<i>Tanytarsus</i>	0.946	0.005	**	Collector-gatherer; Aestivation possible; Clinger
<i>Micropsectra</i>	0.932	0.005	**	Collector-gatherer; No drought adaptation; Climber
<i>Cricotopus/ Orthocladius</i>	0.909	0.050	*	Collector-gatherer; Drought adaptation possible; Clinger
<i>Polypedilum</i>	0.906	0.010	**	Collector-gatherer; Drought adaptation possible; Clinger
<i>Ablabesmyia</i>	0.882	0.005	**	Engulfer predator; No drought adaptation; Sprawler
<i>Brillia</i>	0.845	0.005	**	Shredder; No drought adaptation; Burrower
<i>Paramerina</i>	0.825	0.020	*	Shredder; No drought adaptation; Sprawler
<i>Apsectroptanypus</i>	0.819	0.030	*	Engulfer predator; No drought adaptation; Burrower
<i>Larsia</i>	0.792	0.015	*	Engulfer predator; No drought adaptation; Sprawler
<i>Stempellinella</i>	0.791	0.005	**	Collector-gatherer; No drought adaptation; Burrower
<i>Microtendipes pedellus</i> grp.	0.789	0.010	**	Filter-feeder; No drought adaptation; Clinger
<i>Pentaneura</i>	0.753	0.015	*	Engulfer predator; No drought adaptation; Sprawler
<i>Phaenopsectra</i>	0.739	0.015	*	Scraper/grazer; Drought adaptation possible; Clinger Collector-gatherer; Drought adaptation possible;
<i>Parakiefferella</i>	0.593	0.050	*	Sprawler