

INSECT ECOLOGY

Chironomidae as indicators of water pollution in Pesquería River (México)

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Despite their potential as indicators of water quality and their key role in river ecosystems, Chironomidae is still poorly studied in Neotropical rivers. This lack of knowledge is especially rele-

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vant for rivers subjected to intense human activities, such as many rivers in Mexico. The aim of this investigation is to contribute to the knowledge of the midges of the Pesquería River (Mexico) along its main courses and relate the composition and abundance to river health. Thirty samples were collected during two different periods (August 2015 and February 2016) using a D-frame and kick sampling. Thirty-five taxa were found in total, with four taxa found in more than 50% of the sites and 19 only found once. Midges accounted for more than 50% of the total macroinvertebrate abundance. *Chironomus* gr. *plumosus*, *Rheotanytarsus* spp. and *Cricotopus* gr. *bicinctus* were the most abundant species. Collector-gatherers dominated in August (71% of individuals), whereas collector-filterers dominated in February (43,2%). The major factor explaining the midge distribution and abundance is pollution, while the structure of riparian area does not explain much of the midge richness. This is most likely related to the organic pollution coming from untreated or poorly treated sewage in the city of Monterrey and its surroundings. Three main sectors are distinguished along the river: i) the upper part section with higher biodiversity and presence of intolerant taxa; ii) the middle sewage polluted area with the presence of large red midges very tolerant to pollution (*Chironomus*, *Dicrotendipes*); iii) the lower section in the agricultural zone where the community is dominated by red, small midges (*Rheotanytarsus*). Overall, our study shows that Chironomidae can be useful as better indicators of water quality when *genera* or species levels are used instead of family or subfamily, as is usually found in most papers on river pollution.

Introduction

Midges are an important component of river life. At some sites Chironomidae larvae comprise an important percentage of the total density and biomass of aquatic macroinvertebrates and they can be an important component of the diet of fishes and other predators (Armitage *et al.*, 1995). They can even significantly contribute to the terrestrial organic matter budget of riparian areas by exporting flying adults (Soininen *et al.*, 2015). However, the taxonomic classification of midges is difficult and time-consuming (Andersen *et al.*, 2013). Thereby, midges are usually identified only at the family level in ecological studies and water quality assessments and are considered to be pollution-resistant taxa and collector-gatherers as a trophic group (Armitage *et al.*, 1995). However, they comprise a great variety of species covering a wide range of pollution tolerance. Thus, the taxonomic level at which midges are identified is very important (Edward *et al.*, 2000; Molineri *et al.*, 2020). The use of genera, species groups, or species (when possible) can help to reveal ecological patterns and

processes (e.g., Puntí *et al.*, 2009; Cañedo-Argüelles *et al.*, 2016; González-Trujillo *et al.*, 2019).

Usually, environmental factors explain most of the differences in the chironomid assemblage composition (e.g., Nicacio & Juen, 2018), although dispersal limitations might be important depending on the network connectivity (Cañedo-Argüelles *et al.*, 2015) and the temporal (Cañedo-Argüelles *et al.*, 2020) and spatial scales considered (Viana & Chase, 2020). Micro-scale study of substrate composition and temporal changes between seasons and years may explain the coexistence of many species in the same site, but species level is necessary to detect such differences (Prat & Garcia-Roger, 2018).

The Chironomidae of Mexico are still poorly known (Bello-González *et al.*, 2019); a taxa list is available by Spies & Reiss, 1996, mostly from old papers. As Mexico shares part of its territory with Neotropical and Nearctic regions, the study of midges is more complex in this region. No specific keys exist for midges of Mexico; larvae are classified using the keys of Ferrington *et al.*, (2008) and Epler (2001) for Nearctic fauna. For the Neotropical region, the key of Da Silva *et al.*, (2018) is useful. But there is still a long way to arrive at a good understanding of the taxonomy, biogeography, richness, and ecology of Mexican Chironomidae.

Mexican rivers are subjected to great pressure. Despite that CONABIO (National Commission for the Understanding and Usage of Biodiversity) implemented the Priority Hydrological Regions (RHP) program in 1998 (Arriaga *et al.*, 2009), their ecological degradation is increasing year after year. The program considers the Pesquería River (Nuevo León, México) as a priority case for ecological evaluation. Recent studies on this river showed that it is heavily impacted along most of its catchment, and a few sites are in good ecological status (Castro-López *et al.*, 2019a, b).

Despite recent efforts to consider biological communities (Torres-Olmera *et al.*, 2018), water quality assessment currently relies exclusively on physicochemical parameters in Mexico. However there are several macroinvertebrate-based biological indices in Mexico (e.g., Perez-Munguia & Pineda-López, 2005), but most of them use a limited taxonomical knowledge (Family) (e.g., Carmona-Jiménez & Caro-Borrero, 2017), also in biomonitoring studies (Castillo *et al.*, 2018). Only a few studies regarding Paleolimnology (Pérez *et al.*, 2013) or focusing on epiphytic midges (Caro-Borrero, A. & Carmona-Jiménez, J. 2018) have explored the use of *genera* or species-level.

The aim of this investigation was to contribute to the knowledge of midges in the Pesquería River (Mexico) along its main course and to assess their potential as indicators of river health. The specific objectives of the study were to: i) characterise the biodiversity of Chironomidae in the Pesquería River; ii) assess the relationship of Chironomidae communities with river health; iii) discuss the potential of Chironomidae to be used as bioindicators in Mexican rivers and streams.

Materials and Methods

Study area

This study was conducted in The Pesquería River located in Northeastern Mexico (San Juan River Basin, at the coordinates 26°38'24"-25°26'24" latitude N and 100°54'00"-98°56'24" longitude W). The main stem flows through Monterrey city metropolitan area. The river has an average gradient of 0.4%, a length of 288.22 km and an area of 5255.56 km², with an annual average flow of 2,04 m³/s (Ferriño, 2016). The section studied consisted of the last 108 km of the main course and comprised altitudes ranging

between 104 and 542 m. a.s.l. The climate in the area is semi-arid to arid. The wet season occurs during the months of May to October, while the dry season occurs during the remaining months (November to April) (Castro-López *et al.*, 2019)

Sampling

We sampled Chironomidae on two occasions (August 2015 and February 2016) at 15 sites. The location of stations along the river is in Figure 1 from Castro-López *et al.*, (2019b). Macroinvertebrate samples were taken using a D-frame net (250 um mesh), in a 100 m reach. 20 kick samples were taken proportionally to the habitats present and combined in a unique sample. We preserved samples in 10% formalin and transported them to the laboratory. Initially, we identified macroinvertebrates at different taxonomic levels but midges only at the family level (Castro-López *et al.*, 2019b). During the sorting, we pooled midges together in a tube and preserved them in 70% ethanol until the moment of their examination for taxonomical purposes. We studied a total number of 1670 and 1723 individuals for each season.

The midges were classified using a two-step process. First, we classified midges into morphotypes under a stereoscope at x20. We used color, the presence of body setae, and others for distinguishing morphotypes following the criteria of Prat *et al.* (2012) and Rossaro *et al.*, 2022a. With this approach, several *genera* or even species groups are easily recognized. When the morphotypes were not enough for identifying a *genera* or species group, we mounted 10 individuals of each morphotype to be identified using a microscope and following Epler (2001). Instead of Euparal, we use DMXF as mounting media because no dehydration was necessary. We identified the larvae using the keys of Epler (2001), Andersen *et al.* (2013), Ferrington *et al.* (2008), and Prat *et al.* (2012). We used the material from the collection of the senior author (N. Prat) for comparison. *Genera* or species groups are the result of such work, which is enough for the purposes of this paper and relevant for ecological studies (Molineri *et al.*, 2020).

We measured the status of riparian areas with the index QBR adapted for the Pesquería River (QBR-RNMx in Castro-López *et al.*, 2019a) from the original index described in Munné *et al.* (2003).

Data treatment

The assignment of Chironomidae to a trophic group was made according to the categories used in Castro-López *et al.* (2019): predators, shredders, herbivores, collector-gatherers, and collector-filterers. Usually, midges were considered as collectors gatherers

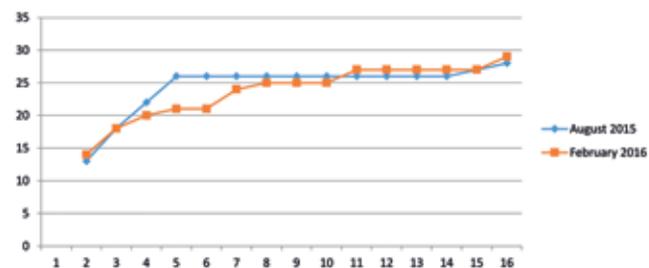


Figure 1. Relationship between QBR and number of taxa of midges.

in many studies. However, there are different feeding strategies within the Chironomidae genera or species (Serra *et al.*, 2015, Caleño-Ruiz, 2015; Rodríguez-Lozano *et al.*, 2015). We used our knowledge and personal observations on gut contents of midges from Pesqueria, to assign to each genus a trophic category.

Results

Environmental data

Environmental data, the extent, and characteristics of the land cover are provided in previous papers (Castro-López *et al.*, 2019a, 2019b). We distinguish three areas along the river (Table 1). The upper part area, before the urban metropolitan area of Monterrey (sites 1-3). Here, most of the basin cover is a mixed forest or shrubland that extends until the riparian area, while water is mesohaline according to the geology of the area. In the middle area, the urban sprawl of Monterrey dominates, and the riparian area is mostly devoid of vegetation. In this section, six sewage plants and several unthreatened discharges discharge along the river, with a very large increase in values of conductivity and lower values of oxygen. Finally, in the final part of the river, after Monterrey, orchards dominated, both in the basin and in the riparian area. Conductivity is lower, and oxygen recovers, but pollution from sewage plants remains. We will use this river delimitation, previously defined by Castro-López *et al.*, 2019a, to compare changes in the composition and abundance of midges.

Number of taxa

35 Chironomidae taxa were found, 31 in August 2015 and 29 in February 2016 (*Supplementary Material*). The number of midge taxa per site ranged between 1 and 16 in August and between 2 and 14 in February. Nineteen taxa in August and 17 in February were

rare (recorded in less than 2 samples). Only 7 taxa in August and 3 in February were present at more than 50% of the sites. Seven of the taxa were found only in August, and four only in February. In August, the headwater sites (1-4) accounted for most of the species richness, whereas on February 6 sites captured most of the taxa (Figure 2).

Composition and relative abundance

Chironomidae was present in all samples, and its total abundance was close to 50% of the macroinvertebrates found in both seasons (Table 2). The most frequent and abundant taxa were the same on both dates (Table 3): *Rheotanytarsus* spp., *Chironomus* gr. *plumosus*, *Cricotopus* gr. *bicintus* and *Thienemannimyia* sp. While *Ch. gr plumosus* dominated in August (up to 50% of the individuals), *Rheotanytarsus* (42%) was in February.

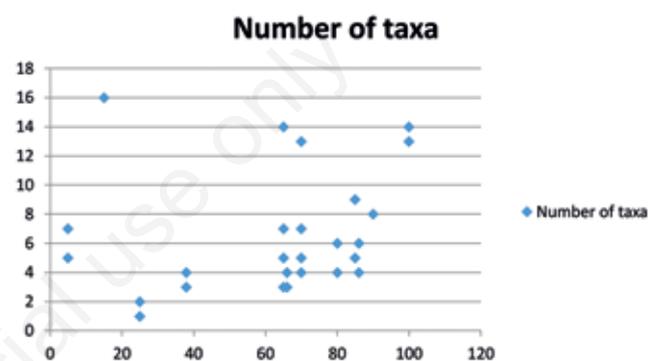


Figure 2. Accumulated number of taxa of Chironomidae in the two seasons studied in the Pesquería River (Mexico).

Table 1. Riparian uses and values of conductivity in the three main sections of the Pesqueria-River (data from Castro-López *et al.*, 2019a).

% Riparian Use	Natural	Urban	Agricultural
Sites 1-3	73-100	0-27	0
Sites 4-9	0	100	0
Sites 10-16	0-39	0	41-100
Conductivity	1758 (44)	5272 (1008)	2187 (116)

Table 2. Most frequent and relatively abundant taxa of macroinvertebrates in Pesquería River (México) (data from Castro-López *et al.*, 2019).

Macroinvertebrates	August		February	
	Sites (n=16)	%	Sites (n=16)	%
<i>Chironomidae</i> (cg)	16	48.38	15	54.53
<i>Hyaella</i> (cg)	7	18.78	8	12.06
Oligochaeta (cg)	14	14.71	16	15.96
Notodromidae (cg)	10	6.13	15	3.87
<i>Smicridea</i> (cf)	11	3.17	1	1.69
<i>Simulini</i> (cf)	6	0.27	11	4.06
<i>Nehalennia</i> (p)	11	0.18	10	0.11
<i>Enallagama</i> (p)	11	0.15	11	0.11
Ceratopogonidae (p)	11	1.6	6	0.16
<i>Argia</i> (p)	11	0.27	12	0.37
<i>Agraylea</i> (h)	10	0.65	10	0.36

p, predator; h, herbivore; cg, collector gatherer; cf, collector-filterer.

In polluted parts (below sewage plants, sites 9 and 10) *Chironomus* were present and abundant together with *Polypedilum gr. halterale* (see Annex 1). Only in sites 1 to 3 (upstream) and 16 and 17 (downstream), the four most abundant taxa were not dominant in both sampling periods. Most of the “rare species” are present in these 4 sites, while impacted sites usually have lower richness and higher abundances of common species with red color (with hemoglobin). However, large community differences exist between sites 1-3 (upstream) and 16-17. The last ones have dominance of some pollution-tolerant species (like *Dicrotendipes gr fumidus* or *Rheotanytarsus*), while these are scarce in less disturbed sites of the headwaters of the river.

Functional feeding groups

According to the previous studies (Castro-López *et al.*, 2019a) and personal observations from gut contents, the most common midges in Pesquería River are collector-gatherers and collector filterers (Tables 3, 4). As we can see in Table 3, the collector-gatherers dominate in the first sampling season and collector filterers in February.

This is coincident with the dominance of the different subfam-

ilies of Chironomidae, as can be seen in Table 5, where Chironominae (*Chironomus* and *Dicrotendipes*) dominated in the first season and Tanytarsini (*Rheotanytarsus*) in the second. The predators are members of the subfamily Tanypodinae and represent close to 10% of the individuals usually. Orthocladiinae are the more abundant subfamily within the herbivores.

The importance of riparian area for midge composition and abundance

In the upper part of the river, QBR-RNMx is high or very high (70-100). Values may be very low (sites 4-5-6) or high (up to 70 in sites 7-8). In the most polluted sites, QBR is lower than 50 (sites 9-10), with some recovery afterward in the agricultural zone, where the index is closer to or higher than 70. More details about the QBR and its importance in the Pesquería River are in Castro-López *et al.* (2019a).

There is no direct relationship between the values of QBR-RNMx and the number of taxa of midges (Fig. 2). High values of both indexes upstream, while in the urban area taxa richness is low, but QBR may be variable because, although the river flows into

Table 3. Most common and abundant Chironomidae of Pesquería River in the two seasons studied.

Chironomidae	August		February	
	Sites (n=16)	%	Sites (n=16)	%
<i>Larsia</i> (p)	8	2.8	6	2.6
<i>Thienemanninia</i> (p)	9	4.6	10	5.3
<i>Thienemanniella</i> (h)	6	1.9	5	1.6
<i>Cricotopus gr. bicinctus</i> (h)	8	6.3	7	13
<i>Chironomus gr. plumosus</i> (cg)	10	56	6	19
<i>Dicrotendipes gr. fumidus</i> (cg)	9	5.7	5	7.4
<i>Polypedilum gr. halterale</i> (cg)	9	8.9	3	0.8
<i>Rheotanytarsus</i> (cf)	9	2.4	10	42

p, predator; h, herbivore; cg, collector gatherer; cf, collector-filterer.

Table 4. Percentage of individuals of midges in each functional feeding group, in Pesquería River (categories of each taxon in Table 2).

Functional feeding groups	August	February
Predators	11.3	10
Herbivores	12.2	15.8
Collectors-gatherers	71.8	31
Collectors-filterers	4.6	43.2

Table 5. Changes in the frequency of appearance and dominance in the subfamilies of Chironomidae in the Pesquería River in two different seasons.

Subfamilies of Chironomidae	August		February	
	Sites	%	Sites	%
Tanypodinae	14	9.58	14	9.29
Orthocladiinae	12	10.90	9	15.79
Chironominae	15	79.52	15	74.93
Chironomini	15	73.35	14	31.40
Pseudochironomini	3	1.80	4	0.70
Tanytarsini	11	4.37	12	42.83
Total	15	100	15	100

densely populated area, some stretches still have patches of remnant riparian forests, where QBR-RNMX may be high. In the agricultural zones (sites 13-15), the values of QBR recover (up to 85) but not the richness of midges. No relationships exist with the midge trophic group or the QBR-RNMX (data not shown). These results are in concordance with those of Castro-López *et al.* (2019a), which also conclude that no relationships existed between the landscape, riparian characteristics, and freshwater macroinvertebrates.

Relationships with pollution

We use the three-river sectors from Table 1 to explore the relationships between pollution and midges. The upper less polluted part had lower values of conductivity (below 2000 uS/cm), high oxygen content (>8mg/l), and no inputs from sewage plants or urban effluents and is the more biodiverse area. High-polluted sites are in the Monterrey urban area, especially after the inputs of the 6 sewage plants, where oxygen can be extremely low, salinities higher, and plenty of organic matter. The river's lower part, surrounded by agricultural fields, has middle values of conductivity with variable oxygen contents but not direct sewage inputs. We have made some statistical treatment of the data (InVal, for example), but no clear pattern emerged from this treatment due to the high variability between sites and the low number of taxa. Any other statistical data treatment provides relevant information.

Discussion

The limited information about midges and their importance for environmental studies

There is a very limited information on midges in Mexico. Even less from the Pesquería River where only unpublished degree thesis of the engineering faculty of the Universidad Nuevo León provide some information. Thus Bermejo (2003) reports some *genera* from two sites of Pesquería River and Torres (2013) studies the water quality and some biological indicators but identifies only *Chironomus gr. plumosus* from all the midges collected and uses the family Chironomidae in the biological indices he proposed.

If we classify Chironomidae (Diptera) only at family level, their relative importance in taxa richness is not evident. In many cases, the larvae of midges are a major component in taxa number and abundance, especially when some nutrient enrichment or pollution is present. It is the case of Pesquería River, where 1/3 of the taxa and close to 50% of the total individuals are Chironomidae (Castro-López *et al.*, 2019b). The study of larvae of Chironomidae is not easy, and it usually takes some time to identify *genera* or species groups, and the separation of species is not possible in many genera; this is the main reason why such studies are unusual. In addition, several species of the same genera may coexist in a site or even in the same stone (Prat & Garcia-Roger, 2018). The coexistence of several species may be explained by differences in substrate composition or life-cycle characteristics as in Prat & Garcia-Roger, (loc. cit.), but this requires such a work that studies on this topic are the exception in the literature as are those related to functional feeding groups, and even more in the Neotropical region (Caleño-Ruiz *et al.*, 2018). For these reasons, it is usual that most of the studies in macroinvertebrates do not use midges at the *genera* level, and few ones use the subfamily level. The lack of keys to classify the midges made more difficult the study of midge larvae in Mexico and Latin America in general. Even the most recent keys (Da Silva *et al.*, 2018) are difficult for a non-specialist. Our study reports the actual fauna of midges in the Pesquería River and

its relationship with major river basin changes. The results can be used as an indicator of recovery or degradation in further studies.

Few papers in Latin America examined the importance of identifying midges at the *genera* level for a better interpretation of the biodiversity of streams and its relationships with environmental factors (González-Trujillo *et al.*, 2019). The protection of headwaters may be the key to the recovery of downstream waters, due to the facility for the drift of insects, including the midges (González-Trujillo, *et al.*, loc. cit.).

The use of midges in biomonitoring

Most of the literature on bioindicators uses midges at the family level (or at most at the subfamily). Many researchers as Edwards *et al.* (2000) and Rossaro *et al.* (2022b), pointed out the need to use midges at the *genera* or species level for biomonitoring studies. The revision on the topic (chironomids as bioindicators) made by Nicacio and Juin (2015), does not seem to have contributed to an increase of taxonomic studies in midges related to biomonitoring in Mexico or elsewhere in Latin America (except one paper by Molineri *et al.*, 2020).

Many of the water quality metrics used for biomonitoring use family or even order as taxonomic level. This is for most of Mexican rivers where people use as the main pollution index the BMWP system (*e.g.*, Pérez-Munguia and López-Pinedo, 2005), including the Pesquería River (Torres-Muñoz, 2013; Castro-López *et al.*, 2019b). Usually this may be enough to characterize the importance of pollution for macroinvertebrates, but doing this (and forgetting the Chironomidae), some important information on richness, diversity, feeding strategies, or pollution tolerance of macroinvertebrate assemblages is missed.

Midges are not used at a low taxonomic level for biomonitoring to the difficulty and time-consuming task of classifying larvae at *genera* or species level (even more in Mexico), unless molecular methods may be applied. For example, Ekrem (2019) pointed out that the use of barcoding may improve the usefulness of midge species as bioindicators, but still much work is required to associate a large number of OTUS of midges found in streams to actual species (Failla *et al.*, 2015). For example, in Spain, more than 50% of the taxa of midges present a lack of barcoding results (Murria *et al.*, 2020). The task appears even more difficult in Latin America, where most of the Chironomidae species remain undescribed.

Midges, pollution, and landscape

The use of midges as indicators of water quality, including eutrophication (mostly in lakes), is common, mostly for their ability to cope with low oxygen water due to the presence of haemoglobin in their body (reviewed by Nicacio & Juen, 2015). In Pesquería River, the red-blood midges are very abundant in both periods (66-71% of individuals). In August, the large *Chironomus plumosus* (Chironomini) are the dominant species. But this taxa is replaced in February by small *Rheotanytarsus* (Tanytarsini), less suited for strong anoxia but still with haemoglobin. This indicated the predominance of organic matter in water and the pollution coming mainly from the non-effective sewage plants or raw sewage in the waters when the river encounters the metropolitan area. In the less polluted sites, several taxa from different subfamilies appear as representatives. Our results also suggest that the use of subfamilies and tribes may be, sometimes, useful for biomonitoring purposes, as did Molineri *et al.* (2020).

We found that the riparian environment is not important, in Pesquería River, for the composition of midges as was in Carro-Borrero and Carmona-Jimenéz (2019), because no correlation

between the values of QBR and the species richness exists. As the river water pollution is so high, the contribution of riparian forests to increase the biodiversity (shade, leaf litter inputs, nutrient filtering...) is overpassed by the intensity of river pollution, which only diminishes at the very end of the river after several sites with moderate to the low influence of human population in the riparian quality (Castro-López *et al.*, 2019a).

Regarding the question of the relevance of landscape in midge composition, we found that only sites with non-modified uses have some characteristic midges, but no differences exist between agricultural and urban landscapes, probably because agricultural landscapes are downstream of the urban sites and part of the pollution coming from urban areas remains in the agricultural landscapes. The change is limited to dominant species, from large red *Chironomus* to small red *Rheotanytarsus*, a temporal change related to the season (August *vs.* February, dry *vs.* wet season). This paper is relevant because it characterizes the actual situation of the Pesquería River and may be used as control when further implementation of water purification is done.

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Online supplementary material:

Table 1.