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# ECOLOGY, BEHAVIOR AND BIONOMICS

# Chironomidae Assemblage Structure in Relation to Organic Enrichment of an Aquatic Environment

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Estrutura da Assembléia de Chironomidae em Relação ao Enriquecimento Orgânico de um Ambiente Aquático

RESUMO - A diversidade taxonômica da comunidade de Chironomidae em ambientes aquáticos poluídos e não-poluídos foi estudada na área de influência do Distrito Agroindustrial de Anápolis, Anápolis, GO, avaliando-se a distribuição lognormal como indicador da integridade biótica desses sistemas. Larvas de Chironomidae foram coletadas em três pontos a montante e jusante do lançamento do efluente da estação de tratamento de esgoto desse distrito industrial. O ajuste do modelo de distribuição foi realizado utilizando-se o modelo de regressão não-linear, adotando procedimento iterativo quasi-Newton. Foram amostrados 21.498 indivíduos de 24 gêneros de Chironomidae. Chironomus, Polypedilum, Rheotanytarsus e Thienemanniella foram os mais abundantes, visto que são geralmente considerados resistentes à poluição orgânica. A assembléia do ponto a montante do lançamento foi bem descrita pelo modelo de distribuição lognormal truncada. O grande aumento da abundância de alguns gêneros no primeiro ponto a jusante impossibilitou o ajuste da distribuição lognormal truncada. O segundo a jusante, no entanto, possibilitou bom ajuste, possivelmente devido à diminuição na abundância dos grupos dominantes. Esses dados corroboram a hipótese de que as assembléias de chironomideos em ambientes não alterados são bem descritas por uma distribuição lognormal, o que não pode ser observado em ambientes degradados. Desta forma, evidenciou-se que a poluição orgânica causa desestruturação das comunidades aquáticas desse sistema, com perda das espécies sensíveis e dominância das tolerantes a esse tipo de impacto. Além disso, fica evidente que a distribuição lognormal pode ser considerada uma valiosa abordagem para avaliação de impactos ambientais.

PALAVRAS-CHAVE: Distúrbio ambiental, modelo lognormal, bioindicador

ABSTRACT - In this study we determined the taxonomic diversity of larval Chironomidae upstream and downstream to discharges of the sewage treatment plant (STP) from Agro-industrial District of Anápolis, State of Goiás, Brazil. Additionally, we evaluated the use of the lognormal distribution as a measure of biotic integrity of this system. The Chironomidae communities were sampled in three sites, upstream and downstream of the discharge of the sewage treatment plant (STP). We fitted the truncate lognormal distribution based on a nonlinear regression of the datasets using least squares as loss function in an iterative quasi-Newton procedure. The total of 21,498 individuals were sampled, from 24 Chironomidae genera, Chironomus, Polypedilum, Rheotanytarsus e Thienemanniella, with 73.9%, 14.4%, 7.1% and 4.5%, respectively, of all analyzed community; they are generally considered resistant to organic pollution. The assemblage of the site upstream was described for a truncate lognormal distribution. The extreme increase in abundance of some genera in the first site downstream produced a distribution not fitted to lognormal. The second site downstream however, permits a good fit to lognormal, probably due to the decrease in abundance of the dominant groups. These data substantiate the hypothesis that Chironomidae community is not suitably characterized by lognormal distribution at disturbed environments. Consequently, these aquatic communities were ecological disordered by organic pollution which caused the lost of sensible species and the dominance of those tolerants to this sort of impact. Additionally, the study highlighted the distribution lognormal approach as a valuable method to assess environmental impacts.

KEY WORDS: Environment disturbance, lognormal model, bioindicator

The functional diversity in ecological communities is considered one of the primary aspects that affects the resistance to disturbance (Case 1991, Hanski 1997) and determines ecological stability. Community stability has a wide range of definitions (Lewontin 1969, Holling 1973, Lawton & Brown 1993). However, if referring to its property to "maintain community elements and functionality", the concept of community stability is closest to the biotic integrity concept which has been used as a management tool for aquatic environments (Karr 1991, Angermeier & Karr 1994).

Aquatic environments in landscape areas altered by human activities accumulate a variety of disturbances that range from sewage discharges (Cairns Jr & Dickson 1971, Khan 1991, Melo *et al* 2003) to the loss of riparian cover (Ferreira-Peruquetti & De Marco 2002, Welsh *et al* 2005). All these impacts affect the species composition, the species interactions and, consequently, the structure and organization of the aquatic communities. Assuming the importance and magnitude of these impacts, a considerable effort has been done in identifying and testing ecological indicators for those impacts to be used in environmental impact assessment and monitoring programs (Walker *et al* 1991, Marques *et al* 1999, Innis *et al* 2000).

Although taxon-specific indicators are very common in aquatic ecological literature, integrated indicators derived from the ecological theory are also catching the attention of many researchers in the area. A typical example is the use of lognormal distribution as a measure of terrestrial community integrity (Kevan *et al* 1997), as well as to marine systems (Gray 1981, 1987, Ugland & Gray 1982). Several authors suggested the use of species distribution pattern to verify the effect of environmental disturbances on biodiversity. Preston (1948, 1962) indicated the use of the fit to distribution model for assemblage species abundance as a tool to understand its functionality and organization. For instance, Minshall *et al* (1985) used these methods and the fit to the lognormal distribution as empirical measures of the equilibrium state in invertebrate communities from temperate ecosystem.

Chironomidae larvae assemblages can play an important role in trophic chain of aquatic ecosystems due to its numerical abundance and its importance to nutrient cycle. They can affect the composition of fine particulate organic matter and provide energetic resources to predators (Pinder 1986, Sankarperumal & Pandian 1992). Besides, they integrate several physical and biological processes in aquatic environment by colonizing different habitats with a variety of environmental conditions and showing great adaptive capacity (Seminara & Bazzanti 1988, Abílio *et al* 2005). For all these reasons they are intensely used as environmental quality indicator in lacustrine and lotic ecosystems (Kansanen *et al* 1984, Pinder 1986, Kleine & Trivinho-Strixino 2005).

However, the utilization of the Chironomidae diversity

information for these purposes are troubled by the scarce taxonomic knowledge on this group in Neotropical regions due to the shortage of specialists in this area, leading to a great number of undescribed species (Leal *et al* 2004). Under these constrains, it is usually suggested the use of the genera level to understand ecological patterns in this group (Trivinho-Strixino & Strixino 1995). Nevertheless, there are an extensive number of studies demonstrating the rationale of using a taxonomic coarse resolution in the analysis of ecological pattern in these assemblages (Guerold 2000, Waite *et al* 2004, Marshall *et al* 2006, Metzeling *et al* 2006, Heino & Soininen 2007).

The common design in environmental impact studies on habitat quality is to compare the values of environmental variables and the biological diversity in impacted and non-impacted areas. In human dominated-landscapes, and under an increase intensity of disturbance, it is harder to find control areas, i.e. not affected areas (Helson *et al* 2006). This problem motivated the use of before-after observations designs (Smith *et al* 1993) and the use of analysis concerning to community intrinsic properties (as its abundance distribution) as assessment metrics.

Especially in tropical ecosystems, there is an increasing need of rapid evaluation of human impact on the communities, both due to the information shortage and the fast damage to these systems, mainly the forest conversion for economic purposes (including energy and food production) and the direct effects of organic and industrial pollution. For instance, Hill & Hamer (1998) suggested the use of the lognormal distribution as a metric for rapid evaluation of tropical communities' disturbances.

In this study we determined the taxonomic diversity of larval Chironomidae in polluted and non-polluted sections from Abrãao stream, in the agro-industrial district of Anápolis, State of Goiás, Brazil, and evaluated the use of the lognormal distribution as a measure of biotic integrity of this system.

### **Material and Methods**

**Study area.** This study was done in the Abraão stream located in the industrial district (DAIA – Distrito Agro-Industrial de Anápolis) that extends over a 950 ha area (from 16°20' to 16°30' S and 48°50' to 49°00'W). The surrounding vegetation is the cerrado, the dominant savanna vegetation in central Brazil, and noticeably one of the world hotspots of biodiversity (Myers *et al* 2000). The Cerrado biome has been under severe pressure to land conversion to productive activities such as livestock and agriculture with high impact to aquatic systems. The region is characterized by two markedly seasons, the dry winter and the rainy summer, common in a semi-humic tropical climatic system (Nimer 1989).

The Abraão stream is located in an area between 1020 m and 1130 m altitude. The Abraão/Curado basin has 5.48 km² with 8% of mean declivity. The Agro-industrial District of Anápolis (DAIA) has 67 pharmacy-chemical and transformation industries. The organic load in the crude sewage of 90% of the industries of DAIA, had 1.4 ton/day. After sewage treatment at the sewage treatment plant (STP), the final load discharged into the Abraão stream has undetermined proportion of zinc, ammoniac surfactants, phosphate, benzene, lead, chloride and nitrogen compounds. The sewage treatment is efficient in minimizing the amount of biochemical and chemical demand of oxygen, however, values of pH, electric conductivity and total dissolved solids are higher than the values in the stream's water *in natura* (Nascimento-de-Jesus - unpublished).

Three sampling sites were established in the Abraão stream which presents leaf and sandy as predominant substrates and is 2.3 m wide, with a 0.2 m³/s mean flow. The first site was located 500 m upstream of the discharges from the DAIA sewage treatment plant (STP), and the remaining sampling sites were at 200 m and 500 m downstream the STP discharge.

**Data collection.** Samples were obtained during the dry season, considered ideal for ecological studies in streams due to the high abundance and species richness of benthonic animals in stream of the Central Brazil (Bispo *et al* 2001). The physical and chemical variables as pH, water temperature, the concentration of dissolved oxygen by the Winkler method, as well as the total nitrogen and phosphorus according the Standard Methods (Clescerl *et al* 2004), were determined for each sampling site.

Sampling of Chironomidae larvae was carried out by 10 sub-samples arbitrarily taken using Surber sampler with 0.225 mm of mesh size and an area of 0.1 m<sup>2</sup> (total of 1m<sup>2</sup> sampled by site). Subsequent to a preliminary classification in the field, the material was fixed in 5% formalin. Returning to the laboratory, the larvae were sorted, identified at a stereomicroscope and preserved in 70% ethanol. The taxonomic identification was performed at the genera level according to Trivinho-Strixino & Strixino (1995), Merrit & Cummins (1984) and Cranston (2000).

**Data analysis.** We used the truncate lognormal distribution based on the general equation described below (Preston 1948).

Equation 1 
$$S(R) = S_o \exp(-a^2 R^2)$$
;

where S(R) = number of species on the r<sup>th</sup> class.

 $S_0 =$  number of species on the modal class,

 $a = (2\sigma^2)^{1/2}$  = inverse amplitude of the distribution.

*So* is the frequency of species on the modal class and *a* is inversely related to the dispersion of the distribution.

This approach allowed us to compare equity since it uses the taxa equity in the final estimate. In this study we used the number of genera instead of species due to the uncertainties in taxonomic identification when using the finest resolution at species level.

Originally, studies as Magurran (2003) fitted this distribution using the class distribution based on a 2<sup>R</sup> sequence (or octaves). But recently, others as Williamson & Gaston (2005) used another class division (2<sup>R</sup>/2<sup>1/2</sup>) which allows comparisons among studies which try to fit this distribution as a test to the unified neutral theory of Hubbell (2001), as used here. We fitted the lognormal distribution to the equation 1 based on a nonlinear regression of the datasets, and using least squares as loss function in an iterative quasi-Newton procedure. The model allowed an enlargement of the sample size and a widen inference since it uses the genera as sample unit.

#### Results

Characterization of the water quality. No differences in the values of water temperature from up to downstream were observed. An increase in nitrogen phosphorus and pH was also evident, as well as a decrease in dissolved oxygen, which were consistent with the enrichment of stream's water caused by the DAIA wastewater (Table 1). Values of dissolved oxygen had been within the limits specified by the Brazilian Environmental National Council to maintain aquatic wildlife (6.7 mg/l) (CONAMA resolution 357). However, values of nitrogen and phosphorus were much higher than those allowed by CONAMA even subsequent to the sewage treatment load at the sewage treatment plant (STP) of DAIA. Higher concentrations of these nutrients very likely are consequence of the organic matter load in the sites under the influence of the sewage treatment station of DAIA.

**Chironomidae assemblage composition.** A total of 21,498 specimens from 24 Chironomidae genera were sampled (Table 2). *Chironomus, Polypedilum, Thienemanniella* and

Table 1 Values of the physical-chemical variables and physics parameters measured in the Abraão Stream, Anápolis, Goiás, Brazil.

	Upstream	Downstream 1	Downstream 2
pН	6.36	7.33	7.65
Water temperature (°C)	21.10	22.90	22.8
Dissolved oxygen (mg.l <sup>-1</sup> )	7.50	6.40	6.40
Total nitrogen (mg.l <sup>-1</sup> )	0.75	2.36	2.48
Phosphorus (mg.l <sup>-1</sup> )	1.44	2.70	3.60
Discharge (m <sup>3</sup> .s <sup>-1</sup> )	0.21	0.26	0.17

Table 2 Chironomidae assemblage composition groups recorded in Abraão Stream, Anápolis, Goiás, Brazil, upstream and downstream to the sewage discharges of Distrito Agroindustrial de Anápolis (DAIA).

Genera	Upstream	Downstream 1	Downstream 2	Total
Ablabesmyia	-	-	3	3
Apedilum	-	1	-	1
Beardius	3	3	-	6
Chironomus	1	8,280	7,523	15,804
Caladomyia	-	-	2	2
Corynoneura	5	5	-	10
Cricotopus	4	33	32	69
Cryptochironomus	1	1	-	2
Dicrotendipes	-	8	-	8
Djalmabatista	-	3	-	3
Endotribelos	-	-	6	6
Fissimentum	-	2	1	3
Gymnometriocnemus	-	1	-	1
Harnischia (?)	3	3	-	6
Larsia	1	1	-	2
Parachironomus	-	-	3	3
Parametriocnemus	-	-	2	2
Polypedilum	11	670	294	975
Rheotanytarsus	361	813	343	1,517
Stenochironomus	1	1	2	4
Tanytarsus	1	1	-	2
Thienemanniella	21	1,940	1,108	3,069

Rheotanytarsus were the most abundant groups, with 73.9%, 14.4%, 7.1% and 4.5% of the whole assemblage, respectively. These genera are usually considered resistant to organic pollution.

The abundance of the most frequent genera was highest at the first sample site downstream the effluent discharge. In the upstream site, the dominant group was *Rheotanytarsus* with 84.4% (361 specimens). At the first downstream site there was a change of dominance for *Chironomus* with 70.4% (8,279 specimens), followed by *Thienemanniella* which represented 16.5% (1,940 specimens). A decrease in total abundance of these groups was observed in the second downstream site.

Analysis of abundance distribution. The changes in composition from the upstream to the first downstream were also followed by a change in the abundance distribution. The site upstream was explained for a truncate lognormal distribution. The severe increase in abundance of some genera produced a distribution not fitted to truncate lognormal in the first site downstream. The second site downstream fitted to truncate lognormal as well, possibly due to the decrease in abundance of the dominant groups (Fig 1).

The So parameter, which represents the class with peak of abundance, clearly followed the changes in the total abundance

among the sites. The parameter a is the most interesting measure of assemblage structure since it retains information on the inverse of the standard deviation of the distribution, denoting the dominance in species abundance distribution. It also increased towards the first site downstream, suggesting the increase in dominance. The fitted distribution for the second site downstream was even more uniform (and possibly more "diverse") than the site upstream (Table 3).

#### **Discussion**

Changes in community composition. Organic pollution usually decreases dissolved oxygen concentrations affecting the persistence of many aquatic groups and decreasing the taxonomic richness (Bachmann 1995, Roque *et al* 2003). Most Chironomidae groups are resistant to environmental disturbances due to their ability to live in conditions of low oxygen concentrations and high organic content (Sanseverino *et al* 1998, Roque *et al* 2004, de Bisthoven *et al* 2005, Cranston 2007). Barbour *et al* (1996) claimed that *Chironomus* is the genera with the highest tolerance to organic pollution due to its ability to use hemoglobin for oxygen transportation, helping to sustain aerobic metabolism even under low oxygen situations. This ability has an important role in determining

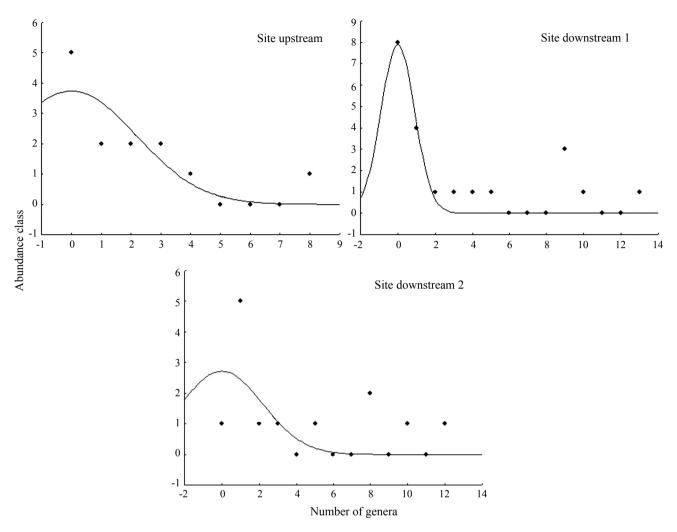


Fig 1 Distribution of abundance of the genera of Chironomidae in sites upstream, downstream 1 e downstream 2 to the sewage discharges of Distrito Agroindustrial de Anápolis (DAIA).

ecological characteristics in this group, and Lee *et al* (2006) considered that the expression levels of this protein in *Chironomus* should be used as an indicator to evaluate the health of an aquatic system. The presence of hemoglobin and the wide tolerance to environmental conditions seemed to explain the increase of *Chironomus*, *Thienemanniella*, *Rheotanytarsus* and *Polypedilum*, immediately after the sewage discharge (Day *et al* 2006, Helson *et al* 2006).

There is one general problem in this approach to Chironomidae related to the degree of impacts that these areas have suffered at large temporal scales. The whole river system is in an area in which the original riparian cover was removed, allowing other sources of organic pollution to enter the system (this could also explain the higher nitrogen and phosphorus concentrations even before sewage discharges). The community composition in this area is possibly the result of the intense loss of biodiversity at a greater time scale, and the more specialized and restricted species are expected to have been locally extinct. Actual composition was biased by this process and represents a

Table 3 Truncate lognormal fit for the Chironomidae assemblage at three stream sites. So = number of species on the modal class. a = equitability in species abundance distribution. SE = standard error)

	S	So		a		
	Estimate	SE	Estimate	SE	χ	Р
Upstream	3.843	1.406	0.067	0.050	5.641	< 0.001
Downstream 1	8.514	2.006	0.198	0.087	79.921	>0.066
Downstream 2	1.728	0.526	0.017	0.011	3.389	< 0.001

limited variation in response to other disturbances.

#### Lognormal fit and unstable community structure.

The effect of the effluent discharge on the Chironomidae community structure was remarkable, changing the abundance distribution and corroborating Day *et al* (2006) data, which also demonstrated the impact of the increase of organic concentration upon the benthic community structure of a tidal freshwater forested wetland in Louisiana. Henrique-Oliveira *et al* (2003) considered that the organic content represents the main food source for organisms of these systems, but its increase can destabilize the community, resulting in both an extreme increase in abundance of some groups and elimination of others.

Rabeni & Wang (2001) suggested that Chironomidae should be excluded from monitoring studies due to their high tolerance to environmental disturbances. Our data showed otherwise, since the changes in abundance distribution and composition, even at the genera level, was useful to indicate disturbances. This study shows the feasibility of using the fit of the lognormal distribution to study the changes in assemblage structure in relation to environmental disturbances. This approach has been followed to understand assemblages of pollinators in ecosystem stressed for pesticide (Kevan et al 1997), and to understand the effects of habitat disturbance upon tropical forests (Hill & Hamer 1998) and upon invertebrate communities (Minshall et al 1985). This is especially interesting because the interpretation of the results based on the fit to lognormal distribution can be used even under the constraint of the small sample size with few sites, given that in this analysis the species or taxa are considered the sample unit. Nevertheless, the use of lognormal has a clear weakness. The test has low power at low species richness. In case of small number of species or taxonomic groups, it is hard to demonstrate a non-fit to the lognormal distribution. The increase in sample size would increase the number of species (or groups), and consequently would allow a better description of the community (Nee et al 1991).

We were successful in fitting the lognormal distribution to an assemblage classified at the genus level. This is important because mostly of the studies on macroinvertebrate community structure use this level of taxonomic resolution. A better taxonomic knowledge in this family obviously would generate a more precise identification of disturbance (Kleine & Trivinho-Strixino 2005). However, some authors suggested that in rapid biodiversity assessments studies the taxonomic resolution at the higher *taxa* is adequate to macroinvertebrates (Marshall *et al* 2006, Metzeling *et al* 2006, Heino & Soininen 2007), and the genus level is appropriate for Chironomidae (Waite *et al* 2004).

Environmental impact assessment is an activity severely constrained by time. Usually, the calls to evaluate the alterations in an environment are simultaneous to the installation of possible sources of disturbances and habitat destruction, and of urgent need for information to determine which action should be taken. In these scenarios, the choice of rapid assessment procedures to produce the best reliable data under a shortage of time and effort expenditure, is generally accepted (Jones & Eggleton 2000, Clark & Richardson 2002). As showed in this study, the fit to lognormal distribution should be considered a

valuable approach to understand patterns in reasonable rapid biodiversity assessment studies.

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