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Surface-Floating Pupal Exuviae of Chironomidae (Diptera) in Urban Streams: is There a Best Time of Day to Sample Them?

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Abstract. The use of pupal exuviae is a method that allows evaluating the environmental condition using information provided by the diversity of Chironomidae. The exuviae can disperse over distances up to 100 m from where the adults emerged, allowing perform detailed studies on the local community. The aim of this study was verify whether there are differences in the taxonomic richness and abundance in the emergence of pupal exuviae of Chironomidae during different periods of day in three urban Cerrado streams. The samples were taken in the dry season of July 2011, in three first-order streams located nearby Anápolis municipality, Goiás State, Brazil, using drift nets for a period ranging from 8 to 20 h, with removal and replacement of the drift nets every two hours period. 6.129 exuviae were collected in the three streams and the *Onconeura* and *Polypedilum* genera totaled 70% of all individuals collected. The time at 16 h was the most representative of the number of exuviae collected, while the first time (8 h) had the lowest amount of exuviae. The genera richness and abundance of exuviae did not show changes according to the time of day on the streams, indicating that there is not a period in which there is a greater frequency of adult emergence of Chironomidae.

Keywords: Aquatic insects; Cerrado; emergence of chironomids; pupal sampling; urban streams.

Exúvias de Pupa de Chironomidae (Diptera) em Riachos Urbanos de Cerrado: Existe um Melhor Horário do Dia para Amostrar?

Resumo. O uso de exúvias de pupa é um método que permite avaliar condições ambientais utilizando informações da diversidade de Chironomidae. Exúvias de pupas podem se dispersar em distâncias de até 100 m dos locais onde os adultos emergiram, permitindo realizar estudos sobre a comunidade local. O objetivo deste estudo foi verificar se existem diferenças na riqueza taxonômica e abundância em exúvias de pupa de Chironomidae durante diferentes períodos do dia em córregos urbanos do Bioma Cerrado. As amostras foram coletadas na estação seca de julho de 2011, em três riachos de primeira ordem localizados próximos à cidade de Anápolis, Goiás, Brasil, utilizando redes de deriva por um período que variou de 8-20 h, com remoção e substituição das redes a cada duas horas. Foram coletadas 6129 exúvias nos três córregos e os gêneros *Onconeura* e *Polypedilum* totalizaram 70% de todos os indivíduos coletados. O horário de 16 h foi o mais representativo em relação ao número de exuvias coletadas, enquanto que as exúvias coletadas às 8 h foram menos abundantes. A riqueza e a abundância de gêneros de exúvias não apresentaram mudanças de acordo com o horário do dia nos córregos estudados, indicando que não existe um período no qual há uma maior frequência de aparecimento de Chironomidae adulto.

Palavras-chave: Amostragem de pupas; Cerrado; córregos urbanos; emergência de chironomídeos; insetos aquáticos.

The benthic community, although invisible to many, plays key role in maintaining and balance of aquatic ecosystems. The wide diversity of this group, with species belonging to various functional groups, enable them to act directly on nutrient cycling and energy flow in the environment. Besides that, the use of benthic macroinvertebrates is established to assess water quality, because the detection of impacts is improved due to relatively sedentary nature and the long life cycle of these organisms (Rosenberg & Resh 1993). Still, Bonada *et al.* (2006) highlight some criteria that must be met for a good monitoring using aquatic invertebrates: potential to assess ecological functions, discriminating human activities that cause environmental impacts, have low costs of sampling and identification of taxonomic groups and provide reliable information on the environment.

Bonada *et al.* (2006) point out that monitoring tools must strike a balance between its potential to achieve the proposed objectives, facilities in the implementation and ability to generate positive responses about the impacts assessed. In this sense, the monitoring of water bodies, besides contributing to the knowledge of populations and functioning of ecosystems, aims to identify the stressors that cause environmental impacts, check changes in species composition and prevent possible impacts. However, due the diversity of potential pollutants and many sources of pollution, to perform good works of monitoring, studies of various components of the environment are necessary, not only physical but also biological (Buss *et al.* 2003). Thus, combine and cross biological data with physical information is critical for accurate evaluation of the environment (Bispo *et al.* 2001).

Among the groups of benthic macroinvertebrates, Chironomidae represents the most abundant taxon in lentic and lotic systems because they colonize a wide variety of biotopes and live under different environmental conditions (Sublette *et al.* 1998). Because of this tremendous diversity, Chironomidae has been a key group for aquatic environment studies, both for purely ecological studies, as to matters involving environmental impacts. The life cycle of Chironomidae involves a pupal period, an intermediate stage between larval and adult phases, commonly this period contains adult characters, even if they still have some larval features (Wiederholm 1986; Cranston 1995; Jacobsen 2008). When the adult emerges, a kind of carapace is released, the exuviae, this structure stay drifting, floating in the environment, composing part of the suspended biomass (Borror & Delong 1988).

Biomonitoring using exuviae of Chironomidae is a method that allows evaluating the environmental condition using information provided by the diversity of chironomids, so that the specificities of each genera or species indicate particular levels of environmental impacts. The collection method was first proposed by Thienemann (1910) and since then, has been widely used as a supplement in studies with larvae (Wiederholm 1986; Wilson 1992; Bouchard Jr. & Ferrington 2011). This methodology allows us to estimate the diversity and frequency of emergence of individuals who pass the pupal stage to adult in a wide range of microhabitats (Wilson & Bright 1973; Ferrington *et al.* 1991).

The exuviae lasts about 48 hours until they are damaged by the environmental conditions, mainly due to the temperature, which has the potential to accelerate its degradation and stimulates the decomposition process (Ferrington *et al.* 1991). From the moment they are released, the exuviae can disperse over distances up to 100 meters, but in this way they can be attached to any material that is in the water body or else in backwater areas (Wilson & Bright 1973). The use of exuviae brings some advantages, since this method does not violate ethical issues by not result in the death of individuals, has a low cost and requires less time and more efficient identification of species (Coffman & De la Rosa 1998; Kranzfelder & Ferrington 2015). In contrast, the lack or unknown of emergency patterns can cause major biases in the sample, mainly leading to an underestimation of abundance and species richness of the studied site.

Technically, the best time to collect is one that offers a greater diversity of organisms, as it provides better conditions for analysis of the environment (Wilson 1996). Ferrington *et al.* (1991) reported that the number of samples with more than 200 exuviae is sufficient to describe the community of chironomids present at the collection site. However, studies that use drift nets do not specify the best hour to collecting exuviae, implying that the time of day it is indifferent to obtain a good sampling.

On the other hand, studies on emergence of aquatic insects claim that the changes occurring in the environment throughout the day can interfere with the emergence of these organisms (Ivkovic *et al.* 2013). But, there are still no clear conclusions about these patterns, since there are evidence of strong temporal and seasonal patterns in adult emergence for certain regions, while other studies have reported weak associations between adult emergence and environmental variables (but see Coffman & De la Rosa 1998; Ubero-Pascal *et al.* 2000; Siqueira *et al.* 2008; Wright & Burgin 2010). Furthermore, the identification of emergence patterns can help a lot in biomonitoring programs, since many species emerge at dusk and others early in the morning. The knowledge of this information is essential to save effort and money in the sampling programs, but it needs to be further tested for tropical regions. Thus, the aim of this study was determine whether there are differences in the richness and abundance in the emergence of pupal exuviae of Chironomidae during different times of day in urban Cerrado streams.

MATERIAL AND METHODS

Study Area. This study was conducted in the dry season of July 2011, in three first-order streams located in the Cerrado Biome, near the city of Anápolis, Goiás, Brazil. The Barreiro stream passes through the *campus* of the Universidade Estadual de Goiás, in the Unidade de Ciências Exatas e Tecnológicas (UNUCET) and is located between the coordinates 16º23'78" S and 48º56'37" O, at 1041 m. of altitude. The substrate is very heterogeneous with predominance of rocky, leafy and sandy types. The Jurubatuba Stream is located between the coordinates 16º18'36" S and 49º04'18" O, at 840 m. above sea level and has a leafy, sandy and rocky substrates. The Quicé Stream is between the coordinates 16º08'13" S and 48º59'32" O, the 1063 m. of altitude, with substrates composed of stones, weeds, leaves, sand and pebbles. This season of year is characterized by lower flow streams, a fact that favors the increase of the heterogeneity of habitats and the diversity of species in the environment, providing adequate time for ecological studies (Bispo *et al.* 2001).

The water velocity was measured by the float method, estimated by the required time that a floating object travels a predetermined stretch of the stream. The discharge was calculated by estimating the product of the average velocity of water by a sectional area of the stream (Bispo & Oliveira 1998). The characterization of each stream, as well as its geographical location, is described in Table 1.

Exuviae Sampling. Sampling of pupal exuviae was carried out using drift nets (250 µm). The nets were placed in the channel of streams in areas of fast discharge to intercept exuviae in drift at a single point (Wilson & Bright 1973; Siqueira & Trivinho-Strixino 2005). A "*Chironomid Pupal Exuviae Technique*" (CPET) is a technique to collect exuviae developed by Wilson & Mcgill (1979). The main advantages of using CPET concern the comparability of sampling conducted over several months and the simplicity of continued monitoring. The field samples are free of interference from the operator and can be performed quickly, even by semi-skilled staff (Wilson 1996).

Sampling began at 6 h and lasted until 20 h, with removal and replacement of the drift nets every two hours. Thus, withdrawals were taken at 8, 10, 12, 14, 16, 18 and 20 h. Each removal was followed by a wash of the drift nets and the material was packed in pots properly labeled according to the time of collection and the sampled stream. The samples were fixed in 70% alcohol for better conservation of exuviae.

In the laboratory, the Chironomidae exuviae were separated from the other materials, such as pieces of leaves, algae, exuviae of other insects, macrophytes and wood fragments. After separated by morphotypes, the exuviae were fixed in blades in Euparal® for taxonomic identification at genera level. For the identification process were used the following reference manuals Wiederholm (1986), Epler (2001), Bouchard Jr. & Ferrington (2008), Rufer & Ferrington (2007), Jacobsen (2008), as well as consultations with specialists.

Data Analysis. The expected richness was calculated using the "rarefy" function implemented in the Vegan package on R software (R Core Team 2014). This function calculates the expected species richness based on random sampling of each community. Thus, we obtained a value for each sampled time in each stream.

RESULTS

In total 3,161 exuviae were collected and identified in the Barreiro stream; 1,931 in Jurubatuba and 1,037 in Quicé, distributed within 24 different genera of Chironomidae (Table 2). The most

Table 1. Physical characterization (depth, wide, discharge, altitude and geographic coordinates) of the three Cerrado streams sampled nearby Anápolis, Goiás, Brazil.

Stream	Depth Mean (m)	Wide (m)	Discharge (m/s)	Altitude	Geographic Coordinates
Barreiro	0.15	4.3	3.2	1041	16º 23' 78"S 48º 56' 37"O
Jurubatuba	0.56	5	6	840	16º 18' 36"S 49º 04' 18"O
Quicé	0.19	3.5	1.5	1063	16º 08' 13"S 48º 59' 32"O

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abundant genus was the orthocladiine *Onconeura* (n = 2,569), followed by chironomine *Polypedilum* (n = 1,641).

The *Onconeura, Polypedilum* and *Asheum* genera were the ones that had occurred at all sampling times. In contrast, *Monopelopia, Xenopelopia, Zavrelimya, Tanytarsus, Goeldichironomus, Apedilum, Harnischia, Paramerina* and *Telmatopelopia* genera occurred in only one of the hours sampled (Table 3). The three most abundant genera of each subfamily are shown in Figure 1 where it is possible verify that only Chironominae is distributed more evenly over the sampling periods.

Table 2. Abundance of the genera of surface-floating exuviae of Chironomidae identified in three urban Cerrado streams (Barreiro, Jurubatuba and Quicé).

Family	Genera	Barreiro	Jurubatuba	Quicé
Tanypodinae				
	Larsia	22	2	7
	Pentaneura	13	5	67
	Ablabesmyia	112	0	9
	Fittkauimya	3	0	0
	Monopelopia	8	0	0
	Xenopelopia	0	0	5
	Zavrelimya	0	0	2
Chironominae				
	Polypedilum	1003	320	318
	Cryptochironomus	10	8	45
	Chironomus	101	250	0
	Asheum	578	210	19
	Goeldichironomus	0	0	2
	Apedilum	0	0	2
	Cladopelma	0	0	15
	Endotribelos	0	0	15
	Lauterborniella	0	150	6
	Tanytarsus	0	0	7
	Harnischia	0	0	8
Orthocladiinae				
	Onconeura	1304	843	422
	Parametriochnemus	2	2	7
	Cricotopus	0	141	53
	Lopescladius	5	0	23
	Paramerina	0	0	4
	Telmatopelopia	0	0	1
TOTAL		3161	1931	1037

Table 3. Abundance of surface-floating exuviae of Chironomidae identified in three urban Cerrado streams (pooled) in each sampled time.

Taxa	8 h	10 h	12 h	14 h	16 h	18 h	20 h
Tanypodinae							
Larsia	6	6	5	4	0	2	2
Pentaneura	4	7	6	0	24	32	12
Ablabesmya	1	8	0	112	0	0	0
Fittkauimya	0	0	0	1	0	0	1
Monopelopia	0	0	0	8	0	0	0
Xenopelopia	0	0	5	0	0	0	0
Zavrelimya	0	0	2	0	0	0	0
Chironominae							
Polypedilum	271	266	207	196	235	63	403
Cryptochironomus	5	4	1	13	0	17	21
Chironomus	8	118	5	111	4	0	105
Asheum	40	211	176	114	38	14	214
Goeldichironomus	2	0	0	0	0	0	0
Apedilum	0	2	0	0	0	0	0
Cladopelma	0	0	15	0	0	0	0
Endotribelos	0	0	15	0	0	0	0
Lauterborniella	0	150	0	0	6	0	0
Tanytarsus	0	0	0	0	7	0	0
Harnischia	0	0	0	0	0	8	0
Orthocladiinae							
Onconeura	165	131	310	305	961	523	174
Parametriochnemus	0	2	0	0	2	7	0
Cricotopus	93	18	5	13	15	41	0
Lopescladius	0	0	10	0	12	0	0
Paramerina	0	4	0	0	0	0	0
Telmatopelopia	0	0	1	0	0	0	0

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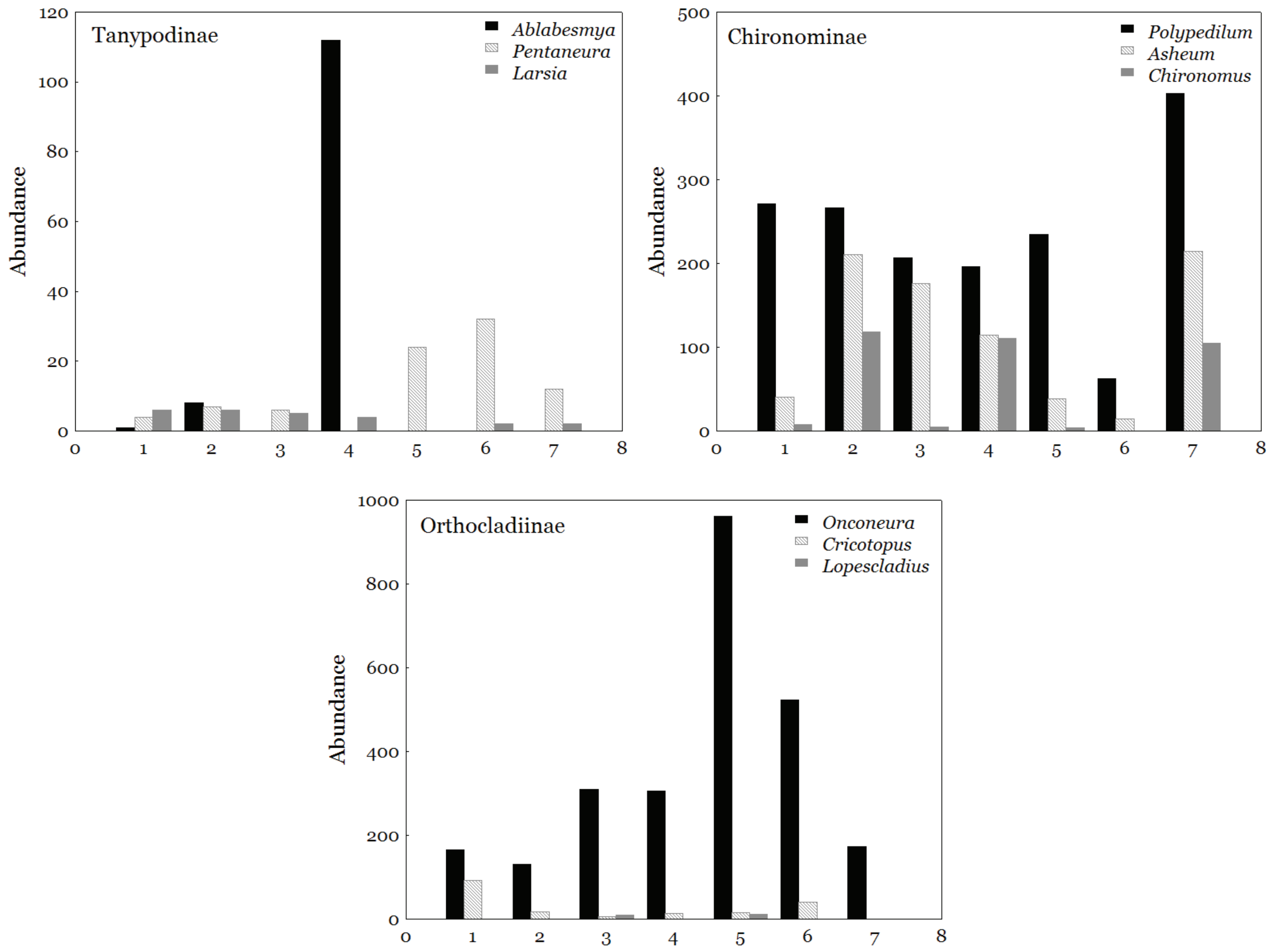

Figure 1. Abundance by period of the three genera with higher incidence in Tanypodinae, Chironominae and Orthocladiinae subfamilies.

The stream with greater abundance of exuviae was Barreiro, with peak of abundance at 20 h (n = 734). The lowest abundance was recorded in the Quicé stream 8am (n = 17). Considering the times in aggregate, 1,309 exuviae were collected at 16 h and 934 at 20 h, these were the two most abundant times (Figure 2).

Similarly to patterns of abundance, the Barreiro stream was richer in number of genera (Figure 3). However, despite not having the greatest abundance, the 14 h time had the highest richness among all streams (n = 9). The Quicé stream, despite having a lower abundance, obtained higher richness values than Jurubatuba stream (Figure 3). Note that despite the time range from 16:00 to have been the most abundant, was the least diverse (Figure 3 and Figure 4), this occurred because of the dominance of *Onconeura* at this time (see Tables 2 and 3).

The highest expected richness values were to Barreiro stream (Figure 4), however, the most similar time between the three streams were at 10 am and 18 pm. While the Barreiro stream has a tendency of increasing richness in the early evening, the Jurubatuba stream presented a decay and the Quicé stream showed no tendencies with their expected richness. Thus,

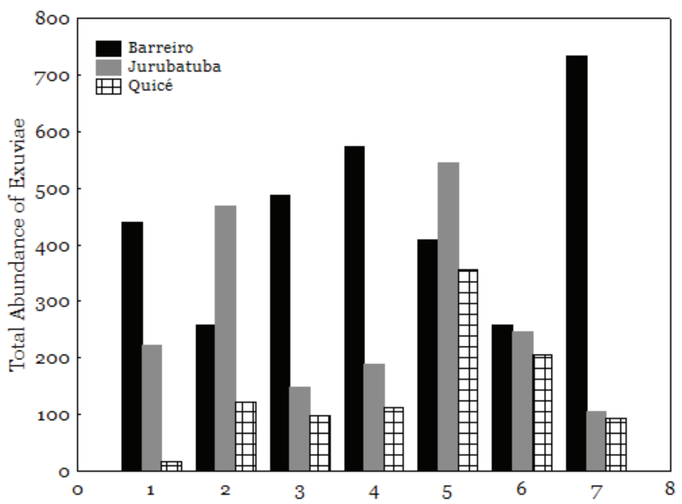

Figure 2. Total Abundance of exuviae in each sampled period for Barreiro, Jurubatuba and Quicé streams.

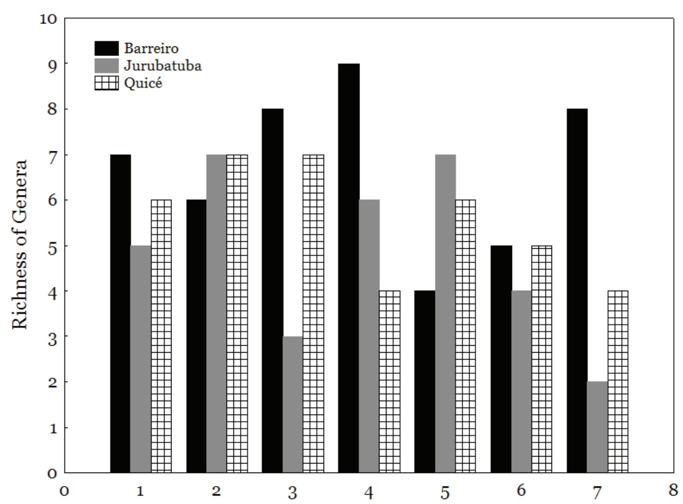

Figure 3. Richness of genera in each sampled period for Barreiro, Jurubatuba and Quicé streams.

there is no clear pattern of emergency Chironomidae, since the environmental condition of each stream can drive different groups to emerge at different times of the day.

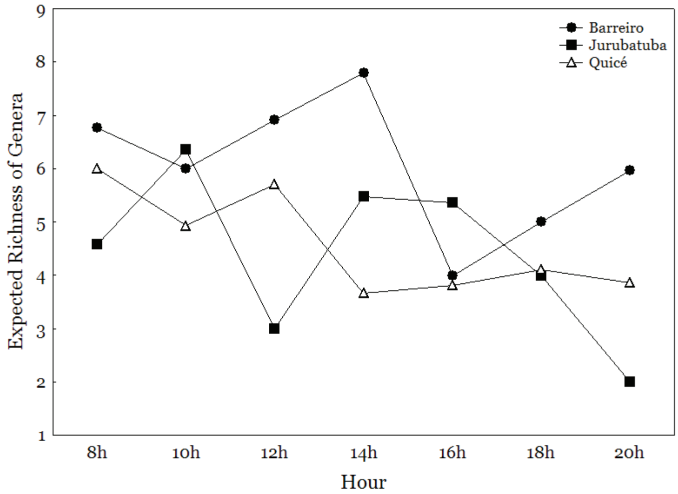

Figure 4. Expected richness of genera in each sampled period for Barreiro, Jurubatuba and Quicé streams.

DISCUSSION

The richness and abundance of exuviae did not show changes according to the time of day, indicating that there is not a period in which there is a greater frequency of adult emergence of chironomids, contradicting several previous studies that have detected clear emergency patterns (Gendron & Laville 1992; Wright & Burgin 2010; Ivkovic *et al.* 2013). Although the lack of big differences between the hours, the abundance of exuviae sampled in this study (n = 6,129), although differences in sampling effort difficult direct comparisons, is superior to several other studies, performed in different environments such as in streams of Southeast Australia (n = 1,813; Wright & Burgin 2010), streams in the central region of São Paulo state (n = 1,264; Siqueira & Trivinho-Strixino 2005) and also in Northern Australia (n = 969; Hardwick *et al.* 1995).

The higher abundance in this work in relation to others may be related to the characteristic of the streams, because they are characterized as urban streams having a high degree of anthropogenic activities. These characteristics are responsible for eliminating sensitive species and provide conditions for the dominance of groups that support lower oxygen conditions and high organic matter content (Hsu & Yang 2005; Almeida *et al.* 2006). This justification also applies to reduced diversity in the collection sites, whereas in other environments, despite their lower abundance, there is superior richness (Hardwick *et al.* 1995; Siqueira & Trivinho-Strixino 2005). Nevertheless, Siqueira *et al.* (2008) working on a stream in southeastern Brazil sampled 48 weekly samples and collected 16,054 individuals belonging to 71 species of chironomid.

Generally, the patterns of emergency and release of exuviae are dependent on the time of day due to environmental variations that occur in water and also in the external environment with emphasis on the fluctuations of temperature and light availability (Gendro & Laville 1992; Füreder *et al.* 2005). Some authors point out another reason for the differences in time of emergencies, the escape of predators, that lead the adults to emerge at times when predators are less active (Wright & Burgin 2010). Aspects such as vegetation cover of the stream, light incidence, foraging for some fish species and, especially the temperature variation may interfere in the strategy of emergence (Wright & Burgin 2010).

The *Onconeura* taxon was the most abundant in this study. Individuals of this genus are tolerant to high temperatures, high conductivity and sediments in the water column. Described species in Brazil were reported in both living conditions freestyle, as on the surface of stones (Wiedenbrug *et al.* 2009). *Polypedilum*, the second most abundant in this work, presents a very wide distribution throughout the world, except Antarctica (Maschwitz & Cook 2000). According to Pinder & Reiss (1983) larvae of *Polypedilum* occupy a large variety of aquatic environments, using different substrates. *Cricotopus*, which was representative of Jurubatuba stream, colonizes various types of water bodies, and live mainly associated with macrophytes (Wilson 1996), besides they are tolerant to pollution and organic enrichment (Anjos & Takeda 2010).

The high plasticity of these groups justify their abundance in the sampled streams, since all are urban streams and have a high degradation, under these conditions tolerant species tend to increase their abundance significantly. They are widely distributed and withstand various environmental conditions, *Onconeura* and *Polypedilum* genera had much occurring in all streams and also in all sampling times and, together totaled almost 70% of all sampled exuviae. Furthermore, at 16h there was a peak of occurrence of *Onconeura*, which has led to dominance at this time.

Wright & Burgin (2010) working in the southeastern of Australia found that richness and abundance of Chironomidae exhibit a strong variation throughout the day, with the greatest abundance occurring at night, especially during dawn. Several studies have a sample design for collections for a period of 24 hours, and in this work we collect during the entire day until the 20 h (no samples during the night). We believe that has not affected the results, since, even after sunset, the diversity and abundance did not change and, theoretically, the variation in light availability should induce the emergence of other groups, which did not occur because there was no increase in diversity and abundance after sundown.

In addition to the variation of the photoperiod, the physical and chemical parameters can also be critical in determining the emergency patterns of exuviae. Everaert *et al.* (2014) show that there are specific abiotic preferences for certain families of macroinvertebrates, including chironomids. Siqueira *et al.* (2008) studied the phenology of chironomids in tropical streams and found no associations with environmental variables, however, they worked with weekly samples and not assessed daily patterns. We did not find large daily variations in exuviae richness, but among the streams there was a big difference in relation to abundance. These variations in abundance can be explained by limnological and physical differences between the streams that are responsible for generating adequate conditions for the development of the species that live there. Also, many species of Chironomidae are tolerant to low oxygen conditions and high concentrations of organic matter, enabling them to having high abundances and dominate environments with these characteristics.

A possible explanation for the lack of temporal patterns is the limited number of samples, since the identification of temporal patterns is not always easy in biological communities, given that certain cycles may last from days to years. Thus, we understand that the samples taken in this study have limitations and that the results obtained should be analyzed and interpreted within the conditions under which the data were collected.

These results are important and fundamental to direct new research that take into account the emergence patterns of exuviae of Chironomidae because, unlike other works, we demonstrated that there was no distinct pattern by time for the release of exuviae, in other words, there is still controversy over what time and how long the sampling should be done (Bourchard Jr. & Ferrington 2011) and it is clear that there is not a general pattern, since several studies have shown different factors and specific conditions for the emergence of exuviae. In terms of biomonitoring, the use of exuviae can contribute greatly, as samples not imply high investment and reliable responses can be obtained from the collected taxa. Furthermore, since all Chironomidae species pass through this stage of life, this method can contribute significantly to biodiversity studies. Based on our results, it is not possible to define a best time for sampling, because there were not many differences throughout the day, however, the use of exuviae was effective to study the local chironomid assembly, characterized by predominance of tolerant genera and low diversity, typical of impacted sites.

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