



## The drift effect on nestedness of Ephemeroptera, Trichoptera and Plecoptera orders in the Xingu River

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LEAL, T.B., OLIVEIRA, R.S., GIARRIZZO, T., GODOY, B.S. **The drift effect on nestedness of ephemeroptera, trichoptera and plecoptera orders in the Xingu River.** *Biota Neotropica* 23(1): e20221354. <https://doi.org/10.1590/1676-0611-BN-2022-1354>

**Abstract:** The drift movement consists of the displacement of the organisms inside the water column which allows its passive locomotion. This movement will result in a variation of the communities of organisms along the river, generating spatial patterns. Based on this, we tested the hypotheses a) the drift of individuals in an upstream-downstream direction creates a nestedness pattern, when the upstream is a subset of downstream communities of aquatic insects; b) there will be an increase in the number of individuals and genera as we approach the most downstream point. The present study was carried out in seven sampling points distributed along the Xingu River. The sampling occurred at night in the central area of the river. The number of genera along the river remained constant, and the nestedness distribution of the communities in the upstream-downstream gradient was not observed. Based on the results, it is possible to visualize a turnover of genera in the longitudinal gradient of the river, but with an accumulation of genera in the downstream region. Organisms that are transported by the flow of the water current respond to the characteristics of the body of water by adapting to the type of environment in which they are found. □

**Keywords:** *Dispersion; Aquatic Insects; Amazonian River; Upstream-downstream movement.*

### O efeito da deriva no aninhamento das ordens Ephemeroptera, Trichoptera e Plecoptera no Rio Xingu

**Resumo:** O movimento de deriva consiste no desprendimento dos organismos dentro da coluna de água, o que permite a sua locomoção passiva. Este movimento resultará numa variação das comunidades de organismos ao longo do rio, gerando padrões espaciais. Com base nisto, testamos as hipóteses a) o movimento de indivíduos em direção montante-jusante criará um padrão aninhado, no qual as comunidades de insetos aquáticos a montante são um subconjunto das comunidades a jusante; b) haverá um aumento no número de indivíduos e gêneros à medida que nos aproximamos do ponto mais a jusante. O presente estudo foi realizado em sete pontos de amostragem distribuídos ao longo do rio Xingu. A amostragem ocorreu durante a noite no canal central do rio. O número de gêneros ao longo do rio se manteve constante, e não observamos uma distribuição de aninhamento das comunidades no gradiente ascendente e descendente do rio. Com base nos resultados, é possível visualizar uma substituição dos gêneros no gradiente longitudinal do rio, porém ocorrendo um acúmulo de gêneros na região mais a jusante. Os organismos que são transportados pelo fluxo da corrente de água respondem as características do corpo de água adaptando-se ao tipo de ambiente em que se encontram.

**Palavras-chave:** *Dispersão; Insetos Aquáticos; Rio Amazônico; Movimento montante-jusante.*

### Introduction

Water flow plays a major role in the dynamics of lotic environments (e.g., rivers and streams) and is related to the stability of biological populations (Poff & Ward 1991). The movement of water promotes the drift of organisms, which consists of their transport using the water flow. The drift behavior is related to several factors, such as current velocity, water chemistry, period of the year and photoperiod (Fierro et al. 2015). The drift can be classified as active, when the organism is cast into the water column in order to escape from predation, competition, or seek food; or passive when the organism is involuntarily

thrown into the water column (Brittain & Eikeland 1988, Poff & Ward 1991, Castro et al. 2013a).

The study of the drift movement is fundamental to understand the transport of these organisms through the water flow, to understand the process of colonization and recolonization of habitats, as well as to identify the functional ecosystem role of different species. The drift movement of aquatic insects can be responsible for the stability and structure of the communities existing in each environment through the process of repopulating localities (Vellend 2010). There is this dependence because the distribution of the species is also linked to

the environmental variables and to the physiological tolerance of each organism (Godoy, Queiroz, et al. 2022, Vellend 2010). Thus, studies on drift movement are being used to understand the distribution of organisms in order to determine the connection between localities (Poff & Ward 1991, Anholt 1995, Covich 2006).

The flow of water into a river is unidirectional, so it is expected in an upstream-downstream direction for the transport of organisms carried by the stream. This will generate a pattern of species distribution, of species addition in the upstream-downstream gradient. The pattern generated may also be of the nestedness type, in which the upstream communities are a subset of those found downstream (Covich 2006, Almeida-Neto et al. 2008). The concept of nestedness was created to explain the communities of island colonized by species from mainland, wherein the better disperses colonize the great portion of islands, independent of the distance (Darlington 1966). In other hand, the poor disperses occur only in the more connected island, creating a nested pattern of species occurrences, based in the species incidence. The knowledge about nestedness distribution is important to understand the patterns of community composition in ecosystems and based on this knowledge create strategies for preservation and conservation of the environment (Ulrich 2009). However, most of the studies on drift movement are concentrated in streams, resulting in a knowledge gap in relation to large rivers that present a differentiated dynamic, with a more intense water flow and variation between environments.

The aquatic insects are strongly influenced by the water dynamics of the water bodies and, in order to establish themselves in the environment, use adapted mechanisms to the conditions to which they are submitted (Mazzucco et al. 2015). In addition to the water flow, these organisms suffer interference from the substrate type, and rocky substrates have a differentiated community when compared to sandy environments (Bispo et al. 2004). The availability of food and predation will also be limiting factors and may define the population density of each locality (Ciborowski 1983, Hay et al. 2008, Godoy et al. 2016). Environments with high population density show an increase in biotic interactions,

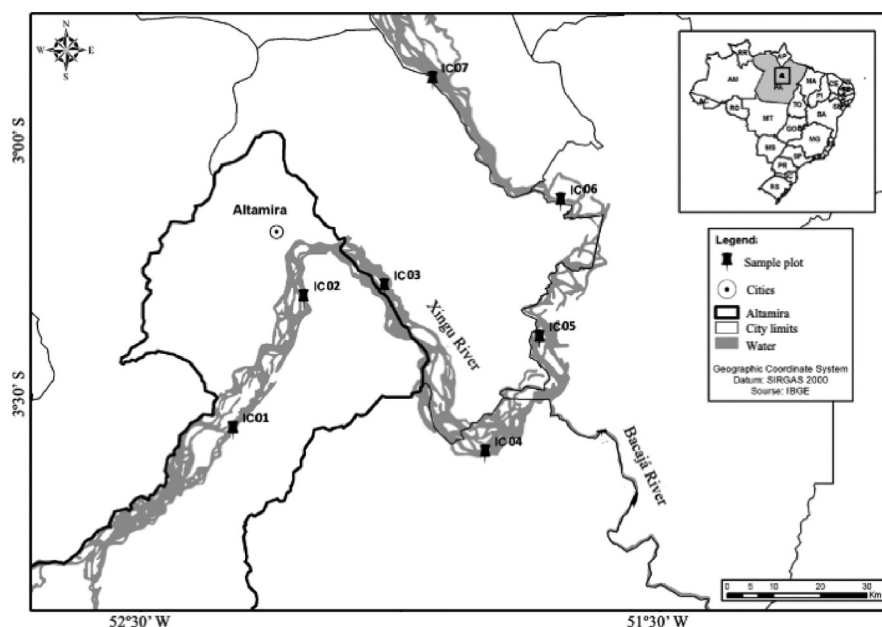
causing the escape of individuals who are forced into the water column. The active dispersal movement occurs more intensely during the night period, where the lack of luminosity provides a protection against the predators, guaranteeing a greater success during the displacement in the water column (Bishop 1969, Koetsier 2005). There are many studies with the orders Ephemeroptera, Plecoptera and Trichoptera (EPT) about their distribution and life cycle (Godoy, Valente-Neto, et al. 2022, Godoy, Queiroz, et al. 2022, Merritt et al. 2008, Sarremejane et al. 2020). In addition, they are orders with high environmental sensitivity, wide distribution within the lotic environments, high abundance and each order presents high richness and complexity (Godoy et al. 2019), which allows its use as model organisms for studies of the drift process.

The Xingu River is characterized as a large river, presenting along its length a high environmental heterogeneity, characterized as a river of high complexity. Its landscapes are composed by waterfalls and rapids, where in these localities the speed of the current appears very varied. In addition to this variation of the current, there will be a variation of the biological communities within its course, resulting in a distribution of varied species within each environment. The lack of studies in large-scale rivers undermines understanding of ecosystem dynamics and how changes in water flow alter the structure of aquatic insect communities. Thus, in this study we tested the following hypotheses: 1) there will be a nestedness distribution of the genera Ephemeroptera and Trichoptera in an upstream-downstream gradient along the Xingu River; 2) there will be an increase in the abundance and richness of genera as we approach the most downstream point on the Xingu River, and c) the community of aquatic insects moves upstream.

## Material and Methods

### 1. Study area

The study was carried out on the Xingu River (02°51'33.1"S and 52°19'28"W), near the city of Altamira, Pará, during the flood period, April 2015 (Figure 1). The Xingu River belongs to the Amazon River



**Figure 1.** Sampling points of aquatic insects on the Xingu River in the period of April 2015. The sample units are symbolized by the P code.

Basin, located on the right side of the river. With an extension of 1500 km from its source in the Brazilian Central Plateau until its mouth in the Amazon River, it drains an area of 540 km<sup>2</sup>. The pH ranges from 5.5 to 7.0 with a mean conductivity of 30  $\mu\text{S}/\text{cm}^{-1}$ , as well as high concentrations of oxygen resulting from the large volume of water (Sioli 1957, Salomão et al. 2007).

The average flow rate during the flood period varies from 8,000 to 10,000 m<sup>3</sup>/s and in the dry period the average is 2000 m<sup>3</sup>/s (Norte-Energia 2016). The flood period occurs between December and April and the dry season occurs between July and November. Because it is located near the equator, the Xingu River basin presents a warm climate and according to Köppen classification the climate is tropical and predominantly humid (Am, Sheffield et al. 2006). The mean annual temperature in the Altamira-PA region is 27°C, the rainy season starts in November and the dry season in July.

## 2. Sampling

The sampling occurred in seven locations on the Xingu River during the night period. We sampled two times in the central region of the river in each location. However, for our study we jointed the two samples. At each sampling point we measured abiotic variables (pH, OD, conductivity, temperature and current velocity). We used a plankton net with a 50 cm diameter ring and 1.5 m in length, with a mesh opening of 300  $\mu\text{m}$  (Bialecki et al. 1999). We attached a weight to the net, aiming at its balance in the water column. A flowmeter was attached to the net to determine the amount of filtered water and at the end of the net a collecting cup was added. We performed the samplings with the aid of a motorized canoe, which during the collection was kept on with low acceleration, with the bow upstream of the river. The net was positioned against the current, at an average depth of 2 meters, for a period of 10 minutes, adapting methodologies previously used in works with aquatic insects (Waters 1972, Castro et al. 2013a, b). The sampled individuals were preserved in 70% ethanol and identified to the genus level (Wiggins 1977, Domingues & Fernandez 2001, Pes et al. 2005, Oliveira 2006).

## 3. Data analysis

To verify the relationship between the number of genera and the abundance of individuals with the upstream-downstream gradient of the points sampled, a linear regression was performed, using the ordering of the samples in this gradient as a predictor variable. We

used NODF (Nestedness metric based on Overlapping and Decreasing Fill) (Almeida-Neto et al. 2008), to observe a nestedness pattern in the upstream-downstream gradient for the aquatic insect community within the river course. This metric works in a range between 0-100, with 100 representing a perfectly nestedness set. The data are organized in an array of rows and columns, where the columns are the genera and in the rows are the points sampled, the NODF is calculated in pairs of subsequent rows and columns, if the previous row has lesser or equal number of genera than the after, the value of the NODF will be zero. However, if the previous line has higher number of genera than the posterior one, the index uses the common occurrence in both lines to generated the value of the NODF. This calculation is performed for both rows and columns and at the end the overall mean will be achieved resulting in the general NODF (Milesi & Melo 2014, Pinha et al. 2016). In order to calculate the NODF in our study, we maintained the order of the sampling sites by fixing the points in the upstream-downstream direction, generating the real distribution of the genera of aquatic insects in the studied gradient. We performed the T test to compare the observed NODF values with the estimated distribution in a null model of 1000 iterations for the NODF values.

The dispersion among the communities of the points sampled was determined by the dispersion coefficient of the biogeographic direction, using the DD3 index (Legendre & Legendre 1984). This index determines in what direction the movement of species is occurring between communities of locations that are geographically connected (Borcard et al. 1995, Legendre & Legendre 1984). For the analysis, the Vegan package was used (Oksanen et al. 2013) available in the R program (R Development Core Team 2020).

## Results

We collected 1760 individuals in total, divided into 13 families and 34 genera. The order Ephemeroptera consisted of 1614 individuals divided into 6 families and 21 genera. The order Trichoptera presented 146 individuals distributed in 7 families and 13 genera. The most abundant genera were *Lachlania*, *Camelobaetidius*, *Hydrosmilodon* and *Cloeodes* (Table 1). The families that obtained the highest representation were Baetidae (31.2%), Oligoneuriidae (29.01%) and Leptophlebiidae (22.26%). The values of physical and chemical variables showed low variability between the locations (Table 2).

**Table 1.** Aquatic insects' genera, abundance and occurrence in samples collected in the channel of Xingu River, during the flood period (April/2015).

Family	Genera	Individuals	%	Sampling Points
Oligoneuriidae	<i>Lachlania</i>	448	25.45	1, 2, 3, 4, 5, 6, 7
Baetidae	<i>Camelobaetidius</i>	374	21.25	1, 2, 3, 4, 5, 6, 7
Leptophlebiidae	<i>Hydrosmilodon</i>	261	14.82	1, 2, 3, 4, 5, 6, 7
Baetidae	<i>Cloeodes</i>	145	8.23	1, 2, 3, 4, 5, 6, 7
Leptophlebiidae	<i>Needhamella</i>	116	6.59	1, 2, 3, 4, 5, 6, 7
Polymitarcyidae	<i>Campsurus</i>	79	4.48	1, 2, 3, 4, 5, 6, 7
Hydropsychidae	<i>Leptonema</i>	70	3.97	1, 2, 4, 5
Oligoneuriidae	<i>Oligoneuria</i>	63	3.57	1, 2, 4, 5, 6
Leptohephidae	<i>Tricorythopsis</i>	49	2.78	1, 2, 3, 4, 5, 6, 7
Leptohephidae	<i>Leptohephes</i>	25	1.42	2, 4, 5, 6

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Family	Genera	Individuals	%	Sampling Points
Baetidae	<i>Spiritiops</i>	20	1.13	1, 2, 4, 5
Hydropsychidae	<i>Centromacronema</i>	19	1.07	1, 3, 6
Hydropsychidae	<i>Macrostemum</i>	19	1.07	1, 3, 5
Hydropsychidae	<i>Smicridea</i>	13	0.73	1, 6
Leptophlebiidae	<i>Farrodes</i>	9	0.51	1, 3
Baetidae	<i>Baetodes</i>	6	0.34	1
Helicopsychidae	<i>Helicopsyche</i>	6	0.34	3, 6
Hydropsychidae	<i>Synoestropsis</i>	5	0.28	2, 5
Leptohyphidae	<i>Tricorythodes</i>	5	0.28	1
Leptoceridae	<i>Oecetis</i>	4	0.22	1, 4, 5
Polycentropodidae	<i>Cyrnellus</i>	4	0.22	3, 4, 5
Baetidae	<i>Aturbina</i>	3	0.17	2, 6
Baetidae	<i>Cryptonympha</i>	2	0.11	1, 5
Leptophlebiidae	<i>Askola</i>	2	0.11	1, 6
Leptophlebiidae	<i>Ulmeritoides</i>	2	0.11	1
Philopotamidae	<i>Chimarra</i>	2	0.11	1, 6
Polymitarcyidae	<i>Asthenopus</i>	2	0.11	1, 3
Caenidae	<i>Caenis</i>	1	0.05	5
Ecnomidae	<i>Austrotinodes</i>	1	0.05	6
Hydroptilidae	<i>Hydroptila</i>	1	0.05	4
Hydroptilidae	<i>Neotrichia</i>	1	0.05	4
Leptoceridae	<i>Nectopsyche</i>	1	0.05	5
Leptophlebiidae	<i>Hagenulopsis</i>	1	0.05	6
Leptophlebiidae	<i>Tikuna</i>	1	0.05	3

**Table 2.** Coordinates and physical and chemical variables in the sampled locations.

Points	Latitude	Longitude	Water velocity (m.s <sup>-1</sup> )	Water temperature (°C)	Dissolved oxygen (ppm)	Electrical conductivity (µS.cm <sup>-2</sup> )	pH
1	03°34.807'	52°23.683'	6.34	28.2	7.3	13	7.3
2	03°12.826'	52°11.248'	10.11	28.1	7.2	14	7.4
3	03°19.260'	52°02.154'	9.78	27.5	7.8	15	7.5
4	03°35.753'	51°50.262'	8.33	27.2	8.2	14	6.4
5	03°23.312'	52°43.967'	19.15	27.7	6.5	15	6.5
6	03°07.745'	51°41.479'	6.96	28.3	7.4	15	7.6
7	03°53.014'	51°57.535'	13.91	28.1	7.8	16	7.2

The number of genera between the sampling points did not differ significantly, except for the last sampling point located downstream, where there was a reduction in the number of genera ( $F_{1,5} = 1.99$ ,  $p = 0.28$ ). However, this reduction should not be interpreted as an indication of decay, since the pattern is not clear, and more collections are needed before we can even visualize this distribution. The same pattern was observed for the abundance of individuals following the downstream gradient ( $F_{1,5} = 0.01$ ,  $p = 0.90$ ).

The distribution of the genera showed a turnover pattern (NODF observed: 28.88; NODF estimated: 58.51;  $T = 7.97$ ,  $p < 0.01$ ), wherein a genera substitution and each sampling point there is a differentiated community. The genera *Needhamella*, *Lachlania*, *Hydrosmilodon*, *Cloeodes*, *Campsurus*, *Camelobaetis*, *Tricorythopsis* were the

most expressive in the abundance being present at all sampling points, whereas the genera *Anacroneuria*, *Austrotinodes*, *Chimarra*, *Hagenulopsis*, *Caenis*, *Cryptonympha*, *Nectopsyche*, *Hydroptila*, *Neotrichia*, *Tikuna*, *Ulmeritoides* and *Tricorythodes* occurred at only one sampling point.

The result of the dispersion coefficient (DD3 index) indicated a possible existence of a dispersion pattern in the downstream direction of the river, where the organisms move following the current flow. Individuals were found to carry out a dispersion movement directed towards the last sampling point located further downstream (Figure 2). The positive values in the table indicate that the displacement is occurring in a downstream direction while negative values indicate that a reverse movement occurs (Table 3).

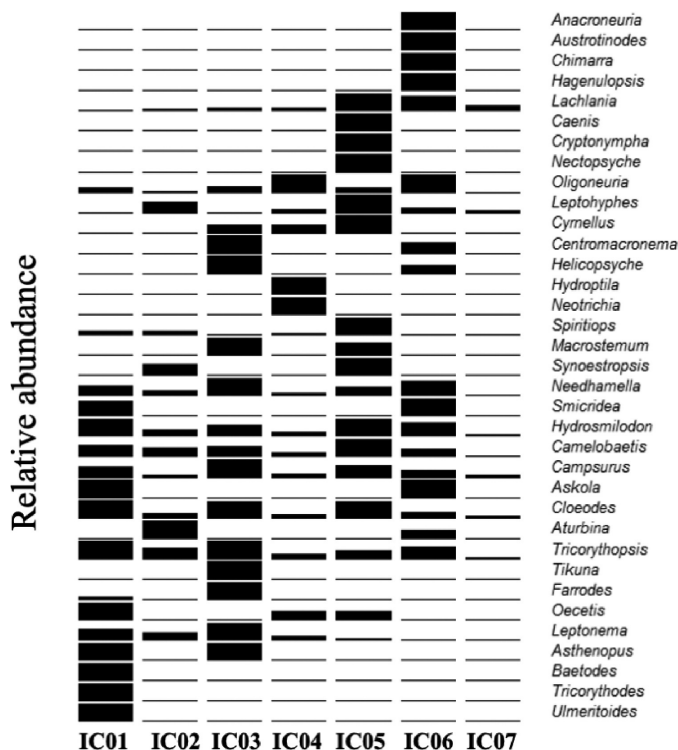


Figure 2. Relative abundance of the aquatic insects at the sampling points into the Xingu River.

Table 3. Dispersion between communities of the Xingu River in a downstream gradient. At the top of the table are the values for index DD3, while at the bottom are the associated p values. Bold values indicate  $p < 0.05$

Sampling Points	Destination (Upstream – Downstream)						
	1	2	3	4	5	6	7
1		0.18	-0.07	0.12	-0.16	-0.06	0.06
2	0.13		-0.20	0.09	-0.19	-0.18	0.11
3	0.57	0.37		0.17	-0.11	-0.03	0.12
Origin 4	0.37	0.43	0.77		-0.14	-0.19	0.14
5	1.00	0.05	0.57	0.27		0.13	0.13
6	1.00	0.13	0.60	0.44	1.00		0.16
7	<b>&lt;0.01</b>	<b>&lt;0.01</b>	<b>&lt;0.01</b>	<b>&lt;0.01</b>	<b>&lt;0.01</b>	<b>&lt;0.01</b>	

### Discussion

The dispersion movement within aquatic insect communities is responsible for the interaction between communities from different localities, resulting in the colonization of environments and increasing local diversity. The physical and chemical characteristics of each locality will generate different organizations of aquatic insects, where the most adapted to a certain condition will be able to establish themselves (Rodrigues-Filho et al. 2015). The aquatic insect communities that perform their locomotion through the water column in the Xingu River did not demonstrate a nestedness distribution, refuting our first hypothesis. In order to occur a nestedness distribution, the community of aquatic insects found downstream should be a subset of the community found downstream, however we observed a species substitution for each

sampling point, a pattern normally observed when the environment is relevant to the community structure (Heino 2009).

The results obtained in this study showed no variation in the number of insect genera along the course of the river, resulting in a uniformity in the community of aquatic insects. The environmental variables don't showed a relevant variation between the sample points, and the marginal vegetation surround the river is well preserved. The marginal vegetation is a great driver in the chemical conditions of the water (Salomão et al. 2007, Sawakuchi et al. 2015), and the lack of major alterations in the course of sampling points and due to this uniformity in the environment, may be results in the stability of aquatic insect communities. As water level rises, there will be a greater interconnection between the environments facilitating the movement of dispersion between the environments (Wilson & McTammany 2016, Barbosa et al. 2015).

However, the variability of environmental conditions in the Xingu River is related to the seasonality (Salomão et al. 2007, Sawakuchi et al. 2015). During the rainy season the river showed an increase of environmental heterogeneity, because large portions of the bottom of the river (included big stones and mud stretch) were exposed out of the water. In other hand, during the period of flood occurs a uniformity of the environments, because the water covers great portion of the margins, and in the period of drought the environment becomes more heterogeneous with the emergence of rocky outcrops that will make the flow of water be varied (Sawakuchi et al. 2015). It was found in this study that during the flood period the structure of the aquatic insect community does not show large changes between the sampling points, and studies are needed to compare how insect community structuring occurs during the dry season.

Our results indicated the last downstream point as the destination of the genera. All genera observed at the most upstream points are scattering to this location, generating a drift pattern of genera that tend to follow the flow of the river. The dispersion movement is necessary for the colonization of new areas, in view of the fact that communities are interlinked to the dispersion movement and aim to establish the equilibrium of populations in the environment, keeping population sizes in line with environmental support capacity (Waters 1972, Mazzucco et al. 2015). However, that statement is need to be tested using other methods to verify the locomotion of individuals, like mark-recapture methods. In addition, we need caution to interpret the result of our study, because the reduced number of sample locations (seven) may be representing a relevant, but limited, source of information about this aquatic community.

The presence of aquatic insects in the water column has ecological relevance, such as food for larger organisms such as fish, processing of organic matter and provides energy within the trophic web. The location of the occurrence of aquatic insects in the regions of the river column may be related to the life strategies of the animals, where they can counterbalance the food encounter with the dispersion and leakage capacity provided by the water flow, in the water column due to poor fixation on the substrate. Understanding how the dispersion movement occurs is necessary so that we can understand patterns of distribution, richness and interaction between species from different localities (Junior & Suarez 2015).

The EPT orders have a wide global distribution and there are many studies with this group about its ecology and bionomics characteristics (Marques et al. 1999, Baumgartner et al. 2004, Galdean et al. 2001,

Gualdoni & Oberto 2012, Godoy, Valente-Neto, et al. 2022, Godoy, Queiroz, et al. 2022). We can better understand the dynamics of dispersion and drift when we observe the characteristics of each order separately. The order Ephemeroptera is strongly influenced by the organic matter present in association with this type of material (Hamada et al. 2019). The samplings were carried out during the flood period when there is a large quantity of organic matter. The high level of the river transports the organic matter present in the marginal region to the channel resulting in an increase in the passive drift of these organisms, besides this factor, we have the high-water flow that exerts strong pressure for the detachment of these organisms from the substrate. Due to these conditions, the order Ephemeroptera was the most abundant and diverse in our samples (Bauernfeind & Moog 2000, Barbosa et al. 2015). Ephemeroptera presents a high diversity in lotic environments, where some genera have preference for places with higher current velocity (Hamada et al. 2019, Sawakuchi et al. 2015). This ability to adapt to this type of environmental condition is related to its body structure that has as characteristic the flattened and elongated body, besides the presence of abdominal gills that aid in the displacement, most of the collected individuals are filterers and adapt easily to this type of environment, due to these characteristics occurred an expressive number of individuals obtained (Castro et al. 2013a).

During the dry season, the Xingu River provides an environment with high heterogeneity, with the presence of riffles in some localities, which will be used for fixation by organisms of the order Trichoptera (Spies et al. 2006, Braun et al. 2014). This study was carried out during the flood period characterized by high water level and uniformity in the landscape, however, even when submerged the riffles serve as shelter for the genera of the order Trichoptera, which has the capacity to build shelters with sediment and suspended material. These shelters are normally fixed to the substrate and are not easily carried by the stream, reducing the probability to found individuals of these group present in the water column (de Moor & Ivanov 2007). The beginning of the dispersion movement may be related to the population density that the increase of density forcing individuals to disperse in the water column, like other organisms, aquatic or not (Munday et al. 2001, Munday 2004, Yu et al. 2001).

Since this relationship exists between the flow of a river and the processes of dispersion and drift, changes in the water dynamics of a river can drastically alter the distribution of aquatic insects, since these spatial patterns are directly related to the drift movement. In order to generate energy, Hydroelectric Power Plants are being built all over Brazil, however such developments cause great changes in the natural course of the river. As there is a relationship between aquatic insects and water flow, these modifications will alter the structure of communities, generating a new equilibrium in the environment (Fearnside 2016). Understanding how the environment is in its pristine state is essential to create conservation strategies for ecosystem processes, allowing the maintenance and use of natural resources (Oldmeadow et al. 2010). In addition, it is necessary to establish parameters to be used in monitoring human-modified environments (Gray et al. 2011, Hauer et al. 2012).

Our study demonstrates an organization of these organisms in an environment without major changes. However, the Belo Monte hydroelectric plant is being built on the Xingu River, which is modifying the original landscape of the river, in which some parts of the river were closed for the construction of a reservoir. These changes directly

influence the community of aquatic insects that depend on the flow of the river. The barriers that have been created will make some communities isolated and over time this may result in changes in population genetics in these communities, as well as loss of diversity due to loss followed by non-replenishment of individuals of the species. This study will serve as a reference on how the community of aquatic insects was structured before the changes occurred in the river, being necessary the monitoring to observe how the community will be structured by the changes in the environment.

## Acknowledgments

We thank the CNPq for the scholarship of Leal TB; to Eletronorte for the field support; to Federal University of Pará for the support in the production of the manuscript (06/2022 – PAPQ/PROPEP); Kostek L and Santos W for help in identifying the material; and we thank the two anonymous reviewers who evaluated the manuscript.

## Associate Editor

José Mermudes

## Author Contributions

Leal TB and Godoy BS conceived the ideas, designed methodology; Leal TB collected and analyzed the data; Leal TB, Oliveira RRS, Giarrizzo T and Godoy BS wrote the original manuscript. All authors contributed critically to the drafts and gave final approval for publication.

## Conflict of Interest

The authors declare that they have no conflict of interest.

## Data Availability

Supporting data are available at <<https://doi.org/10.5281/zenodo.7186623>>

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Received: 08/04/2022

Accepted: 24/01/2023

Published online: 31/03/2023