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Hyporheic invertebrate assemblages at reach scale in a Neotropical stream in Brazil

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(With 4 figures)

Abstract

In the Neotropical Region, information concerning hyporheic communities is virtually non-existent. We carried out a sampling survey in the hyporheic zone of the Tijuca River, in the Tijuca National Park, located in the urban area of the city of Rio de Janeiro. Biological samples from the hyporheic zone were collected in three different stream reaches, in June 2012. The main objectives were: 1) to describe the structure of invertebrate assemblages in the hyporheic zone of a neotropical stream; 2) to apply a reach-scale approach in order to investigate spatial patterns of the hyporheic assemblages in relation to hydrology, depth and microhabitat typology. A total of 1460 individuals were collected and identified in 31 taxa belonging to Nematoda, Annelida, Crustacea, Hydrachnidia and Insecta. The class Insecta dominated the upper layer of the hyporheic zone. Copepods were the most abundant taxon among crustaceans and occurred mostly in the upwelling areas of the reaches. The results of this study represent one of the few contributions so far about hyporheic invertebrate assemblages of the Neotropical Region.

Keywords: hyporheic zone, Neotropical Region, stygobites, stygophiles, stygoxenes.

Assembleia de invertebrados em grande escala em um riacho Neotropical no Brasil

Resumo

Na Região Neotropical, informações sobre comunidades de invertebrados de zona hiporréica são praticamente inexistentes. Foi realizado um levantamento da zona hyporréica do Rio Tijuca, no Parque Nacional da Tijuca, localizado na área urbana da cidade do Rio de Janeiro. As amostras biológicas da zona hiporréica foram coletadas em três diferentes trechos do riacho, em junho de 2012. Os principais objetivos foram: 1) descrever a estrutura das assembléias de invertebrados na zona hiporréica de um riacho neotropical, 2) aplicar uma abordagem de grande escala de trecho de riacho com finalidade de investigar os padrões espaciais das assembléias hiporréicas em relação à hidrologia, profundidade e tipologia de microhabitat. Um total de 31 táxons foram identificados pertencentes à Nematoda, Annelida, Crustacea, Hydrachnidia e Insecta. A classe Insecta dominou a camada superior da zona hiporréica. Copépoda foi o taxon mais abundante entre os crustáceos e ocorreu principalmente nas áreas de resurgência de água. Os resultados deste estudo representam uma das poucas contribuições até o momento sobre assembléias de invertebrados de zona hiporréica em região Neotropical.

Palavras-chave: zona hiporréica, Região Neotropical, estigóbios, estigófilos, estigóxenos.

1. Introduction

The hyporheic zone (HZ) is the interstitial active ecotone between the surface stream and ground water, where exchanges of water, biota, nutrients and organic matter occur (Orghidan, 1959; Schwoerbel, 1967; Boulton et al., 1998; Bencala, 2000, 2005). At reach scale, in opposition to surface water, the hyporheic water flow is laminar but not unidirectional. A maze of flow paths of different lengths, directions and velocities occurs, generated by reach-scale geomorphological features such as discontinuities in slope and depth of riffle-pool sequences, channel shape and streambed structure (Boulton et al., 1998). Along a single riffle-pool alternation, surface water enters the HZ in a downwelling area at the riffle head (or at the end of a pool), and hyporheic water returns to stream surface in an upwelling area at the riffle tail (or at the head of a pool) after travelling in the HZ (Thibodeaux and Boyle, 1987; Boulton et al., 1998 and references herein), in a process known as hyporheic exchange (Harvey and Wagner, 2000). Laterally, water flow enters and leaves the HZ proceeding from stream banks and vice versa, according to geomorphological features (Jones Junior and Holmes, 1996). Hyporheic flow patterns regulate physical and chemical conditions in the HZ, determining microbial activity and occurrence of hyporheic fauna too (Franken et al., 2001). According to the degree of adaptation to subsurface habitats, hyporheic species can be attributed to one of three diverse ecological categories: stygoxenes, that have no affinities with the underground environment and occur in it only accidentally; stygophiles, that actively explore the resources of the HZ, at least in part of their life cycle; stygobites, that are specialized to live in underground and complete their lifecycle in subsurface waters only (Gibert et al., 1994). The stygophiles can be further subdivided into three categories: the occasional hyporheic fauna, predominantly consisting of benthic insects, mainly larvae, that may spend part of their lifetime in this environment under specific circumstances like simulids; amphibites, namely species with lifecycle related to both surface and subsurface environments, mostly represented by insects like chironomids and some species of ephemeropterans; permanent hyporheos, namely species that may be present during all life stages either in benthic habitats and in ground water, like nematodes, oligochaetes, copepods, ostracods, cladocerans, tartigrades and mites (Gibert et al., 1994). Hyporheic invertebrate communities typically exhibit high spatial and temporal heterogeneity due to several biotic and abiotic factors, at all scales (Boulton et al., 1998; Brunke and Gonser, 1997). At reach scale, the structure of hyporheic assemblages may be strongly influenced by the nature of substratum which affects the ability of organisms to move, settle, gather food, and find shelter (Olsen et al., 2001; Omesová and Helešic, 2007; Omesová et al., 2008). The occurrence of riffle-pool sequences has been proved to be a major determining factor of the patterns of hyporheic assemblages. In particular, more oxygenated downwelling zones are often dominated by stygoxenes and stygophiles, whereas stygobites may prevail in the upwelling areas which are less rich in oxygen (Marmonier et al., 1992; Brooks and Boulton, 1991). The insect larvae are generally confined to the upper layers of the HZ; however, some species penetrate deeper and can be an important component of the hyphoreic fauna, called hyporheos (Stanford and Ward, 1988; Brooks and Boulton, 1991; Lencioni, 2004; Fenoglio et al., 2006; Lencioni et al., 2008). The benthic invertebrates may use the HZ in order to escape competition or predation (Schwoerbel, 1967; Williams and Hynes, 1974). Moreover, they actively exploit it as a nursery and shelter from the disturbance induced by the movement of the substrate and the variations of the environmental conditions due to seasonal flooding and droughts (Schwoerbel, 1967; Williams and Hynes, 1974; Dole-Olivier et al., 1997; Lencioni, 2004; Lencioni et al., 2008; James and Suren, 2009; Stubbington, 2012). Other groups perform vertical migrations: Tricladida, Nematoda, Annelida, Crustacea, Acari (Hynes, 1975; Godbout and Hynes, 1982; Olsen and Townsend, 2003).

In Brazil, as elsewhere in the Neotropical Region, information concerning hyporheic communities are scarce. This study was aimed at enhancing the bulk of information on this matter. The main objectives were: 1) to describe the structure of invertebrate assemblages in the HZ of a neotropical stream in the Rio de Janeiro State, Brazil; 2) to apply a reach-scale approach in order to investigate the spatial patterns of the hyporheic assemblages in relation to hydrology, depth and microhabitat typology.

2. Methods

2.1. Study area

The Tijuca National Park is entirely located in the urban area of the city of Rio de Janeiro (Figure 1), between S 22° 55'– S 23° 00' and W 43° 11' – W 43° 19', with a forested area of approximately 32 km^2. The vegetation is typical of the Atlantic forest. From a historical point of view, this area was extensively deforested for coffee cultivation in the XVII and XIX centuries and it was subsequently reforested using native and some exotic species (Mattos et al., 1976). The secondary forest is the typical tropical rainforest that occurs along the Brazilian coast. The climate is humid tropical with average annual temperature between 20 °C and 25 °C. The annual rainfall rate is higher than 1,500 mm. The rainy season occurs between November and February (rainfalls > 250 mm/month), and the dry season from June to September (rainfalls < 100 mm/month). The geological substratum predominantly consists in granite (Brasil, 1987).

Biological samples from the HZ were collected in three stream reaches (PI, PII and PIII), in June 2012, respectively located in the first, second and third Strahler order (*sensu* Strahler, 1957) in the Tijuca River (Figure 1). The three reaches are quite different. PI is the steepest; boulders represent 80% of the riverbed with a 20% of sand. The PII is the least steep and sand represents 60% of the riverbed, with regular riffle–pool alternations. In the PIII reach, boulders represent 60% of the riverbed; riffle-pool sequences are less regular and separated by long areas of

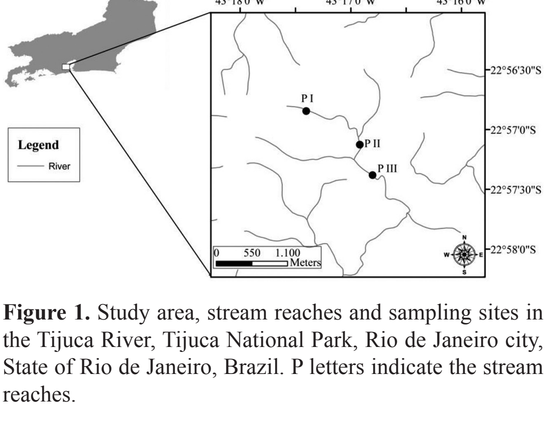

Figure 1. Study area, stream reaches and sampling sites in the Tijuca River, Tijuca National Park, Rio de Janeiro city, State of Rio de Janeiro, Brazil. P letters indicate the stream reaches.

deposition of fine sediments. Habitat assessment was done with visual-based habitat assessment (Barbour et al., 1999).

2.2. Sampling methodology

At each reach, PVC mini-piezometers, 2.5 cm in diameter and 150 cm in length, were used to collect samples from the HZ at three different depths: 10, 25 and 45 cm. The design of traditional piezometers (see Lee and Cherry, 1978; Valett, 1993;) was modified reducing the height of perforated bands to 5 cm, in order to allow a stratified sampling (Figure 2). The diameter of the slots was 5 mm. For each depth, five mini-piezometers were positioned in five different habitats: riffle heads, riffle tails, lateral side of pools, areas of sand accumulation, namely bars and curves. Riffle heads represented downwelling habitats. Riffle tails and lateral sides of curves were considered zones of longitudinal and lateral upwelling, respectively. Sand accumulation areas were taken into account as zones of equilibrium between up- and downwelling. In the PIII reach, at 45 cm in depth, no water was found. Consequently, 40 mini-piezometers were located, overall. In order to guarantee the independence of samples, the mini-piezometers were positioned at least 1.5 meters from each other (Boulton et al., 2007). Samples were collected using a modified Bou-Rouch pump (Vigna Taglianti et al., 1969). For each mini-piezometer, a sample of five liters of water and sediments was taken (Boulton et al., 2004; EA, 2009; Kibichii et al., 2009). In order to separate fine sediments, POM and invertebrates, the samples were elutriated in the field and filtered by a net with a mesh of 68 µm and preserved in a 75% alcohol solution. In laboratory, the samples were treated with rose bengal for 48 hours (Brown, 1975), before sorting under a stereomicroscope. The specimens were counted and removed using tweezers and glass pipettes. Individuals were identified at the lowest taxonomic level allowed by a magnification of 40 x: genus or family for insect´s larvae, order for crustaceans with exception of family Parastenocaridae and phylum for anelidians and nematans (Appendix 1).

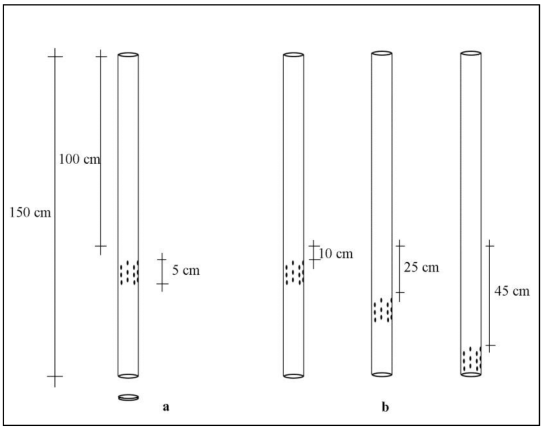

Figure 2. Mini-piezometers and position of perforated bands at different sampling depths.

2.3. Statistical analyses

We analyzed the biological data of the HZ of Tijuca River at each sampling site for abundances (individuals per site) and taxa diversity considering Strahler order of stream reaches, depth and microhabitat typology as factors influencing biological patterns. Taxonomic richness and Shannon's and Simpons' indices were used to asses taxa diversity. Shapiro-Wilk test was used to test for normal distribution of biological data Since the data were not normally distributed, a non-parametric ANOVA was applied. Kruskal-Wallis tests, followed by post-hoc t-tests corrected through Bonferroni method, was carried out in order to detect differences in abundances and taxa composition, separately. The test was applied only to those variables with homogeneous variances, after applying a non-parametric Levene test. Multifactorial analysis was additionally used to identify the taxa that mostly contributed to site discrimination. Biological patterns were explored by a Principal Coordinate Analysis (PCoA) on log-transformed abundances of all taxa. All statistical analyses were performed by the R software, version 2.15.0 (R Development Core Team, 2013).

4. Results

A list of hyporheic taxa, identified during the survey, and their relative abundances is given in the Appendix 1. A total of 31 taxa were identified belonging to Nematoda, Annelida, Crustacea, Hydrachnidia and Insecta. Insects were dominant with 23 taxa. A total of 1460 specimens were collected overall, of which 549 belonging to Insecta, 458 to Crustacea, and the remnants evenly distributed among Nematoda, Annelida and Hydrachnidia. The absolute abundances ranged from 4 to 170 individuals/site. Insects were not equally distributed among the various orders and families, being Chironomidae (Diptera) dominant with 369 individuals. The other 14 identified families of taxon Insecta accounted 180 individuals, overall. Copepods abundances accounted for 307 individuals; the rest of crustaceans, 151 individuals, were distributed in Cladocera, Ostracoda and Bathynellacea.

Both Shannon (H') and Simpson (D) indices showed higher values at 45 cm in depth (H' = 2.5; D = 0.9), while the lowest value was observed in the microhabitat represented by bars of sand accumulation (H' = 2.1; D = 0.8). However, the ranges of the value of the indices were very narrow, indicating no marked differences in diversity indices among the orders of the reaches, depths and microhabitats, when considering all taxa. Conversely, the hyporheic assemblage showed clear spatial distribution in terms of abundances. Overall insect abundances significantly differed with depth ($p<0.001$), being higher at 10 cm than at the other two sampling depths (Figure 3). This pattern is mainly due to Diptera abundances ($p<0.001$), and above all to Chironomidae ($p<0.001$), whose abundances were significantly different at all depths, and to a lesser extent to Ephemeroptera that had higher abundances at 10 cm, being almost absent at the other depths. Insect abundances did not show a significant pattern considering stream orders and microhabitat typology according to the Kruskal_Wallis test. Water mite abundances were significantly higher at 10 cm (p-value<0.001), compared to the other two depths. The abundances of the taxon Crustacea showed a pattern influenced by microhabitat typology (p-value = 0.049), being higher in riffle tails and in lateral sides of pools. The remnant taxa abundances were not explained by the examined factors. Taxa richness, both total and measured for each higher taxa (Insecta, Crustacea, etc.), did not differ significantly either according to stream orders, or to habitats, or to depth. The first two axes of PCoA plot accounted for 46% of the total cumulative variation (30.3% axis 1 and 15.8% axis 2, respectively). The major part of taxa, with the predominance of Chironomidae and Hydracnidia, showed higher abundances along the negative axis 1 where most of the sites at the lowest depth (10 cm), were located (Figure 4).

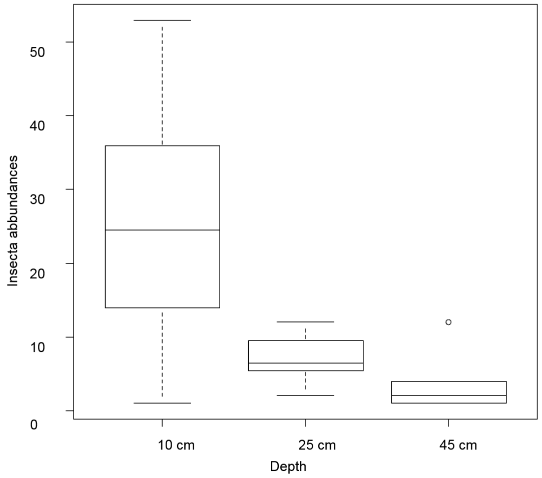

Figure 3. Boxplot indicating differences in insect abundances at the three depths. Number of individuals on the Y axis.

5. Discussion

Studies on HZ in the Neotropical Region are virtually absent and the ecology of interstitial habitats along the rivers remains uninvestigated. The study of hyporheic assemblages is of particular interest due to the ability of hyporheic communities to provide signals of declining water quality (Hancock et al., 2005; Di Marzio et al., 2009; Iepure et al., 2013 and references herein). In the Neotropical Region, the first study of interstitial fauna was accomplished by Fernández and Palacios (1989) using the collecting method Chamaran-Chapuis (Chappuis, 1942). Fernández (1993, 1994, 2001) published a sequence of papers on interstitial water mites using the Bou-Rouch method (Bou, 1974). Both studies occurred in streams in Argentina. In other countries, studies mainly dealt with water chemistry and water flow exchanges in the HZ only (e.g. Chestnut and McDowell, 2000; Romero-González et al., 2001; Medina et al., 2003; De Smedt et al., 2006; De Smedt, 2007; Fabian et al., 2011; Cuajinicuilapa et al., 2012; Marchetti and Carrillo-Rivera, 2013).

In our survey, the sampling sites located in the reach PIII at the depth of 45 cm were unsaturated. This fact may

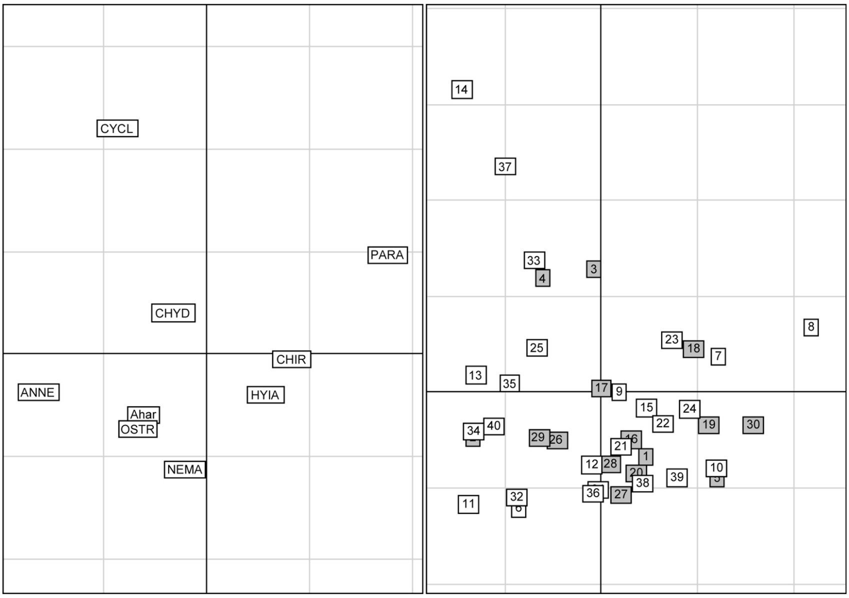

Figure 4. Principal Coordinates Analysis on log-transformed species abundance data of the 40 sampling sites in the HZ of the Tijuca River. Left) Plot showing both the site locations of at different depth (black triangles: –10 cm; dark gray x: –25 cm; light gray quadrats: –45 cm) and species ordination overlain. Right) Plot showing the abundances of taxon Chironomidae at each sampling site. Diameter of circles are proportional to number of chironomids collected in each site.

be due to the presence of an upstream cascade followed by a small dam of 1.5 m in height. Dams along small-order streams are known to alter the linkage between headwater and downstream reaches, potentially increasing at short distance hyporheic connectivity in the riparian zone (Duke et al., 2007). In particular, dams simplify alluvial system structure, reducing surface water peak flows and hyporheic flow and thus preventing refilling of alluvial aquifers (Berman and Pole, 2000).

The upper layers of HZ of Tijuca River were dominated by insects, particularly Chironomidae. This pattern copes with data from other studies, since insects are known to use HZ to seek for refuge during periods of disturbance (Sedell et al., 1990; Robertson et al., 1995; Dole-Olivier et al., 1997) or to find more stable habitats (Matthaei et al., 2000; Fowler and Death, 2001). Dole-Olivier (2011) reviewed the importance of the HZ as a protected "nursery" for eggs, early instar larvae, and quiescent stages of benthic invertebrates as Plecoptera, Ephemeroptera and Chironomidae. The Chironomidae were observed to be the most common insect in interstitial zone (Resh and Rosenberg, 1984; Ferrington Junior, 2008). They play a particularly important role in stream dynamics (Coffman and Ferrington Junior, 1996) and may be an important component of hyporheos (Lencioni et al., 2008). They typically are confined to surface sediment, but some species can penetrate deeper (Pinder, 1986) and larvae have been found invading the HZ at depths of up to 2 m, following high discharge events (Dole-Olivier et al., 1997). This strategy may in part account for the high resistance of these taxa to flood event (Anderson and Ferrington, 2012).

In our study, water mites were found with the highest abundances in the upper layers of the HZ of the Tijuca River. In surface water habitats, Hydrachnidia can be very abundant and diverse (Smith and Cook, 1991; Di Sabatino et al., 2000). In general, in the HZ they are less abundant, although in some case they can show a diversity compared with other meiofaunal groups (Di Sabatino et al., 2000). In the Neotropical Region many species are able to exploit both benthic layers and the HZ of lotic systems with frequent vertical migrations (Fernández and Fossati-Gaschignard, 2011). Identification to species level and further investigations are required in order to better explain the pattern highlighted in this study. Oligochaetes and nematods were fairly represented in the HZ of the Tijuca River, although they were not dominant as previously observed in other studies (Mary and Marmonier, 2000; Lafont and Vivier, 2006). The high abundances in the HZ may be favoured by the worm-shaped body, that facilitates the movement in the interstices of HZ sediments (Omesová and Helešic, 2007; Omesová et al., 2008). Crustaceans, in particular copepods, ostracods and cladocerans, can be both abundant and species-rich in running waters and HZ (Dole-Olivier et al., 2000; Galassi, 2001). Only seven known species of cladocerans may be regarded as true inhabitants of subterranean environments, although they actively colonize ecotonal habitats (Forró et al., 2008). Van Damme et al. (2009) found specimens of Chidoridae up to a depth of 60 cm beneath the riverbed. In our samples, cladocerans were distributed at all depths, although they were mainly found in the upper layers (68% at 10 cm). In our study, copepods were the most abundant crustaceans. A similar condition was observed by other authors (Boulton et al., 1992; Hunt and Stanley, 2003; Di Lorenzo et al., 2013 and references herein). Harpacticoids are mainly burrowers and often dominant in HZ due to their holobenthonic lifecycle (Dole-Olivier et al., 2000; Galassi, 2001). Cyclopods are also common in benthic and HZ habitats (Dole-Olivier et al., 2000; Galassi, 2001; Brancelj and Dumont, 2007; Galassi et al., 2009), although they prefer coarse sediments. Copepod abundances, mainly related to cyclopoids, were higher in the riffle-tail and pool habitats of the HZ of the Tijuca River, where upwelling of ground water was supposed to be consistent (Dole-Olivier, 1998). A similar result was found by Mori and Brancelj (2011). According to this, the high abundances observed in this survey might be due to the massive presence of stygobiotic species. However, identifications to species level are required in order to shed light on this hypothesis. The occurrence of specimens from Parastenocarididae (Copepoda, Harpacticoida) in the HZ of the Tijuca River requires special mention. At present, the family includes five genera, four of which are exclusively found in the Neotropical Region (Galassi, 2001). In the HZ of the Tijuca River, specimens of Parastenocarididae were mainly collected in the sandier reach PII (60% of sand in the streambed). Bathynellacea (Syncarida) are stygobionts (Camacho and Valdecasas, 2008). They were only found in the deeper layers of the HZ in our survey. Remarkably, Amphipoda and Isopoda were not found in this first investigation. However, their presence cannot be excluded and further surveys are necessary.

6. Conclusions

The research in the HZ in Neotropical Region is still a challenge due to the paucity of basic information. The ecology of many invertebrate taxa found in the HZ of neotropical streams is almost unknown, especially for amphibite species. Only a few stygobiont species were described up to now. The results of this study represent new data about hyporheic invertebrate assemblages in this region. Seasonal surveys in the HZ of the Tijuca River are required in order to confirm the distribution patterns highlighted in this study. The analysis of other abiotic variables, such as granulometry, organic matter content and chemical-physical conditions, might highlight new patterns and better explain those analyzed in this first survey. Identification at species level is required for hyporheic specimens, in order to allow the attribution of the species either to the stygobiotic or non-stygobiotic categories, in this way obtaining a better assessment and understanding of the HZ biodiversity.

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Appendix 1. Sampling sites and taxa abundances (number of individuals). Site codes include information about: Strahler order (I: first Strahler order; II: second Strahler order; III: third Strahler order); sampling depth: numbers indicates cm of depth beneath the streambed (10, 25 and 45); habitats type (A: riffle head; B: riffle tail; C: lateral side of pools; D1: bars: D2: curves). CHIR: Chiromidae; DIXI: Dixidae; oCERA: other Ceratopogonidae; CULI: Culicoides; LEPT: Leptoconops; MARU: Maruina; TIPU: Tipulidae; SIMU: Simulidae; PSYC: Psychodidae; dLARV: Elmidae Larva d; NEOE: Neoelmis; DYTI: Dytiscidae; PERL: Perlidae; niEPHE: non identified Ephemeroptera; BAET: Baetidae; LEPT: Leptophlebiidae; OECE: Oecetis; NECT: Nectopsiche; HELI: Helicopsiche; GRUM: Grumicha; MARI: Marlia; CYRN: Cyrnellus; HYDR: Hydroptilidae; COLL: Collembola; CYCL: Cyclopoida; PARA: Parastenocarididae; Ahar: Harpacticoida morphotype "a"; OSTR: Ostracoda; CHYD: Chidoridae; BATH: Bathynellacea; HYIA: Hydrachnidia; NEMA: Nematoda; ANN: Annelida.

ID	Site	CHIR	DIXI	oCERA	CULI	LEPT	MARU	TIPU	SIMU	PSYC	dLARV	NEOE	DYTI	PERL	niEPHE	BAET	LEPT	OECE	NECT	HELI	GRUM	MARI	CYRN	HYDR	COLL	CYCL	PARA	Ahar	OSTR	CHYD	BATH	HYIA	NEMA	ANNE
26	I10A	5	0	0	5	0	0	3	0	0	0	2	0	0	0	0	0	0	0	0	0	0	2	0	0	1	0	4	1	0	0	4	0	4
27	I10B	12	0	0	17	1	1	0	0	1	0	0	0	0	2	0	0	1	0	0	0	0	0	0	1	1	0	14	1	0	0	14	0	1
28	I10C	20	0	0	3	0	0	1	0	0	0	0	0	0	0	2	0	0	0	0	2	0	0	0	0	0	0	9	4	1	0	5	2	8
29	I10D1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	1	2	1
30	I10D2	7	0	0	2	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	3	0	0
31	125IA	3	0	0	2	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	1	0	0	4	1	1
32	125B	3	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	2	3	5
33	I25C	4	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	10	1	4	0	1	0	4	1	3
34	125D1	7	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	1	0	0	0	1	0	0	2	11
35	I25D2	2	0	0	1	0	0	2	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	2
36	I45A	5	0	0	2	0	0	1	3	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	2	4	7	0	0	7	9	2
37	I45B	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	10	1	1	1	0	0	3	0	2
38	I45C	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	“0	0	0	0	0	3	0	0	0	5	0	0
39	I45D1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	3	0	0
40	I45D2	1	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	3	0	0	0	0	1	1
1	II10A	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	1	0	2	0	0	9	4	2
2	II10B	15	0	0	14	0	0	3	0	0	0	0	0	1	2	0	0	0	0	0	0	0	0	0	0	6	9	9	12	14	0	7	17	61
3	II10C	21	0	0	0	0	0	1	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	25	7	5	3	13	0	9	4	4
4	II10D1	27	0	0	3	0	0	3	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	27	4	5	0	2	0	7	1	20
5	II10D2	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0
6	II25A	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	1	1
7	II25B	42	0	6	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	2	7	7	1	0	3	0	6	6	0
8	II25C	30	0	2	0	0	0	0	0	0	0	1	0	0	2	0	0	0	0	0	0	1	0	0	0	1	29	1	0	3	0	13	1	0
9	II25D1	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	1	1	0
10	II25D2	3	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	1	0
11	II45A	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	14	2	1	3
12	II45B	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	1	0
13	II45C	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6	0	8	3	5	1	1	3	5
14	II45D1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	9	0	1	0	0	1	2	0	1
15	II45D2	7	1	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	1	0	3	0	1	0	1	1	0
16	III10A	17	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	2	2	4	2	11	0	9	14	1
17	III10B	21	0	0	0	0	0	0	0	0	0	1	0	0	0	3	0	0	0	0	0	0	0	0	0	8	1	6	7	13	0	14	5	4
18	III10C	15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	2	1	0	0	0	0	0	0	2
19	III10D1	24	0	0	3	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	1	0	1	1	0	0	7	2	1
20	III10D2	36	0	0	2	2	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	2	3	4	2	0	12	18	10
21	III25A	7	0	0	4	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	2	2	0	0	0	5	5	4
22	III25B	7	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	2
23	III25C	5	0	0	1	0	0	0	0	0	0	3	0	0	0	0	0	0	0	1	0	0	0	0	0	1	4	0	2	7	0	2	1	0
24	III25D1	4	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	1	0	0	0	6	0	0
25	III25D2	3	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	4	0	1	1	4	0	2	3	2