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Ecological drivers of aquatic metacommunities in a riverine network

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Índice

1. PhD Overview

I divided my thesis in a general introduction, three chapters and a conclusion. In the general introduction, I present the theoretical framework that I based my research and the connection among chapters. The three chapters are in an article format and they are independent of each other regarding their aims. First and second chapters are connected by the same dataset. First chapter is published on *Ecological Indicators* (see footnote in the first page of this chapter). To evaluate the different hypothesis, I used different statistical methods. The figures and tables are included along the text to facilitate visualization and complementary tables are provided as supporting information after the references of each chapter. The last section includes a synthesis of my findings from these three chapters and some comments and perspectives for future studies. In the remainder of this section, I aim to highlight activities completed during my PhD that are external to the chapters in this thesis and part of my story during these 4 years.

I saw my PhD as an opportunity for a change, after completing my master's degree in a lab where I had been based for 7 years. I expanded my research interest from aquatic ecology to metacommunity ecology. My graduate advisor, Fábio de Oliveira Roque, was also interested in metacommunity ecology. I contacted him about possibly being my PhD supervisor and he promptly responded with a yes. After that, my life changed. I moved to Campo Grande, State of Mato Grosso do Sul with my fiancée at that time (my wife since 2012) and we started a new life together. We settled in happily in our new house and university.

During these 4 years I have known different researchers, been exposed to new ways of thinking, and made new friends and new scientific contacts. I was involved in activities that greatly improved my scientific knowledge as well as activities that familiarized me with other academic responsibilities, including learning how to organize an event, editing a book and appreciating the functioning and organization of a Graduate Program. During the first year of my PhD, I was part of the team that develop the Brazilian National Monitoring Program for Biodiversity. From this amazing experience, I learned how to work with a group to sum up individual project aspects into a cohesive whole. During my second and third years in the PhD program, I was elected as the student representative for the Ecology and Conservation Graduate Program activities. In this position, I learned how to manage the many different issues among students and professors that arose in the department. My most educational experiences as student representative occurred during council meetings, where the chair, 4 professors and I discussed and voted on different issues relate to the Graduate Program. In addition, I also taught some classes to undergraduate students of Biology. I furthered my academic training outside of the university by being part of organization on field ecology course, editing a book and attending some congresses, including international ones (Annual Meeting of The Association for Tropical Biology and Conservation and Ecological Society of America Annual Meeting).

I published 5 papers (see Appendix) and collaborated on projects external to my main research. Some of these collaborative projects are still in the writing phase and one is under review. I believe that collaborative research is a rewarding activity that encourages open-mindedness, bringing together varied points of view that contribute to and improve the quality of a manuscript. The chapters of my thesis are also the results of collaborations which have expanded my scientific network and improved my manuscripts.

My PhD project changed during the first 2 years, but the focus on metacommunity ecology did not. In fact, at the start of my PhD, I was interested in how riverine networks affect metacommunity structure, but not as clearly as I am now. It is worth mentioning that, the aquatic insect datasets, from 46 streams/rivers, presented in this thesis were collected together with my friend and colleague Marciel Elio Rodrigues. Undoubtedly, the fieldwork was a great time during which we made friends and visited wonderful places in Serra da Bodoquena. Apart from odonates (identified by Marciel) and Chironomidae (identified by Suzana Escarpinati and Carolina Bellodi), I identified all aquatic insects in these datasets with the help of undergraduate students.

In the last year of my PhD, I wanted to complete part of my research abroad and Fábio gave me a lot of incentive. I really enjoyed reading Christopher Swan's papers and felt that his research on stream networks aligned with my interests. After some email exchanges, Chris accepted me, for 7 months, in his lab at the University of Maryland, Baltimore County and he became my co-advisor. Working in Chris' lab was not only a great academic experience but also helped improve my English. I made many friends and established a cooperative research program with Chris.

Putting all together and reflecting on it, I think that my PhD was a great time that resulted in experiences that have prepared me for a future career as a scientist.

2. Abstract

Metacommunity ecology recognizes that both environmental control and spatial processes affect community structure. Riverine network, a complex hierarchical system connected by water flow, can be used as a natural boundary where i) biomonitoring programs could be implemented and ii) dispersal-related processes occur and could be represented by different spatial configuration of sites. In this PhD thesis, I investigated ecological drivers affecting metacommunities in a riverine network in three complementary chapters. In the first chapter, I investigated the influence of environmental gradient related to cattle ranching on larvae and adults odonates, organisms that undergo ontogenetic niche shift, and if they are congruent in their distributional pattern. In the second chapter, I sought to learn if the same ecological process affect distributional patterns of larvae and adults of odonates, using both environmental and spatial components as predictors. The last chapter, I aimed to understand whether environment and/or spatial configuration of sites (overland, watercourse and a centrality measure) were determinants of dissimilarity between sites of aquatic insects with different dispersal abilities and if centrality compared to other distance measures improve the detection of spatial process. Taken together, organisms that undergo ontogenetic niche shifts, in this case odonates, are partially structured by environmental control (species sorting) and both larvae and adults can be used as bioindicators of land use change by cattle ranching. By considering ontogenetic niche shifts in biomonitoring and metacommunity contexts, this thesis sheds light on the connection between terrestrial and aquatic ecosystems, opening new avenues for future studies. Also, species sorting was an important mechanism shaping dissimilarities between sites of aquatic insects with different dispersal abilities. Ontogenetic niche

shifts and dendritic network structure help us to understand ecological process affecting metacommunity structure.

3. Resumo

A ecologia de metacomunidades reconhece que tanto fatores ambientais quanto espaciais afetam a estrutura de comunidades. Bacias hidrográficas, sistemas hierárquicos complexos conectados por fluxo de água, podem ser usadas em estudos ecológicos como áreas naturais onde: i) sistemas de biomonitoramento podem ser implementados e ii) processos relacionados a dispersão ocorrem e podem ser representados pela configuração espacial entre pontos amostrais. Nessa tese, investiguei os processos ecológicos responsáveis por afetar a estrutura de metacomunidades em uma bacia hidrográfica em três capítulos complementares. No primeiro capítulo, procurei entender a resposta de larvas e adultos de libélulas, organismos que sofrem mudança ontogenética de nicho, a um gradiente ambiental relacionado ao uso do solo por pecuária e se diferentes estágios de vida são congruentes. No segundo capítulo, eu busquei compreender se os mesmos processos ecológicos afetam larvas e adultos, usando tanto preditores ambientais e espaciais. O último capítulo, investiguei se componentes ambientais e/ou espaciais (distâncias por terra, água e centralidade) foram determinantes da variação entre sítios de comunidades de insetos aquáticos com diferentes capacidades dispersoras e se a medida de centralidade melhora a detecção de processos relacionados a dispersão em relação aos outros componentes espaciais. Em geral, organismos que sofrem mudança ontogenética de nicho, nessa caso libélulas, são afetados por variáveis ambientais (paradigma escolha de espécies), e larvas e adultos podem ser utilizados como bioindicadores de impactos relacionados a pecuária. Por considerar mudanças ontogenéticas de nicho no contexto de biomonitoramento e metacomunidades, essa tese avança no sentido de conectar os sistemas terrestres e aquáticos, abrindo um caminho para futuros estudos. O paradigma escolha de espécies também foi parcialmente responsável pela variação de comunidades de insetos

aquáticos com diferentes habilidades de dispersão entre sítios. Mudanças ontogenéticas de nicho e redes dendríticas auxiliam no entendimento dos processos ecológicos estruturadores de metacomunidades.

4. Introduction

One of the main questions in ecology is how species are distributed in space and time and what processes affect them at multiple scales (Sutherland *et al*. 2013). In the classic view of community ecology, communities were described as localized and isolated entities driven by local factors, such as species interactions and environmental variables (Ricklefs 1987). Parallel to that, ecologists more interested in large spatial scale argued that dispersal was the ecological force explaining variation among communities (MacArthur & Wilson 1967). The acknowledgment that regional species pool is connected to local communities via dispersal changed these classic perspectives and the concept of metacommunity brings the importance of different scales (local to regional) to explaining spatial variation in community structure.

Metacommunity studies have been attracting attention since their syntheses published by Leibold *et al*. (2004). Metacommunity ecology recognizes that local communities are linked by dispersal of one or more of their constituent species (Leibold *et al*. 2004). The weights given to dispersal, species characteristics and local forces (both abiotic and biotic processes) structuring metacommunities were originally organized in four paradigms: species sorting, patch dynamics, mass effects and neutral model (Leibold *et al*. 2004). While metacommunity theory was being developed, a debate took place about pros and cons of niche versus neutral theories. Niche proponents suggested that deterministic processes, such as environmental variables and biotic interactions shape community distributions (e.g. Chase & Leibold 2003; Chase 2005), while proponents of neutral theory pointed out that stochastic factors, including some events related to colonization/extinction and ecological drift generated variation between communities (e.g. Hubbel 2001; Hubbel 2005). After that debate, both processes are recognized to affect simultaneously any community and ecologists focused on understanding the relative importance of niche and neutral processes structuring communities (Tilman 2004; Gravel *et al*. 2006; Leibold & McPeek 2006; Vellend *et al*. 2014). Metacommunity ecologists followed the same way and started thinking of metacommunity structure as a gradient from neutral to species sorting, in which the strength of dispersal varies, i.e. limiting, moderate and high dispersal (Ng *et al* 2009; Winegardner *et al*. 2012). Thus, metacommunity ecology has the general aim to tease apart the relative roles of dispersal processes and environmental control on the structuring of ecological communities (Leibold *et al*. 2004; Cottenie 2005; Logue *et al*. 2011).

Riverine networks are complex hierarchical systems connected by water flow as headwaters coalesce into mainstems. They constitute a natural boundary that are subject to anthropogenic impacts, such as deforestation and dam construction (Dudgeon *et al*. 2006). In this sense, they are valuable areas for conservation and biomonitoring planning. Also, they contain a high variety of habitat types and disproportionately large number of species (Dudgeon *et al*. 2006; Clarke *et al*. 2008; Vorosmarty *et al*. 2010), with great fraction of them comprising organisms that undergo metamorphosis, a process connecting two life stages of the same organism that has important ecological consequences. For example, aquatic insects inhabit aquatic systems as larvae and change their niche to terrestrial systems after metamorphosis (adult life stage). These changes are so abrupt that they are recognized as ontogenetic niche shifts (Wilbur 1980; Nakzawa 2014). However, metamorphosis is not necessarily a new beginning, since some environmental effects could be carried over to the next life stage. For example, damselfly larvae living in habitats with low food supply and high larvae density delay the time of emergence and decrease the adult mass, affecting adult dispersal (Anholt, 1990, 1991). The use of different life stages for biomonitoring purposes and to understand the ecological drivers affecting metacommunity structure has rarely been assessed, being more common the use of only one life stage (larvae or adult) and, consequently, in only one ecosystem (terrestrial or aquatic) (Soininen *et al*. 2015). In this way, considering ontogenetic niche shift in ecological studies is worth because organisms with complex life cycle have dispersal ability variable according to life stage, so assessing only one stage would generate an incomplete view about the processes structuring metacommunities. Further, including different life stages allow us to better understand how terrestrial and aquatic ecosystems are connected by organisms flux (Soininen *et al*. 2015), which was historically recognized in stream ecology (Vannote *et al*. 1980).

Riverine networks were first thought as longitudinal systems where changes in geomorphic, physical and biotic characteristic along the riverine habitat, including terrestrial ecosystem, lead to a predictable response on biological communities – i.e. the river continuum concept (Vannote *et al*. 1980). The river continuum concept recognized connectedness in river and terrestrial systems, assuming an aquatic energy shift along the longitudinal network that has consequences for biological communities. Despite its importance, it was criticized regarding its low generality (see review Tank *et al*. 2010) and some stream ecologists shifted the focus from energy to network properties (the network dynamic hypothesis - Benda *et al*. 2004) and longitudinal plus lateral dimensions (the riverine ecosystem synthesis - Thorp, Thoms & Delong 2006). Metacommunity ecologists followed this trend by assessing riverine network structure, such as differences in ecological processes affecting upstream versus downstream communities (Brown & Swan 2010; Göthe, Angeler & Sandin 2013) as well as using graph theory as a tool to investigate if the dendritic structure of riverine networks could explain dispersal-related processes (Erős, Schmera & Schick 2011; Carrara *et al*. 2012;

Altermatt, Seymour & Martinez 2013). In this way, the study of riverine networks moved from a longitudinal to a network view.

Following this view, riverine networks constrain different dispersal routes for aquatic insects, such as overland used by flying adults and watercourse used by both larvae and flying adults. Dispersal rates between communities is not easy to measure, so metacommunity studies usually use proxies that correlate to or affect dispersal, such as spatial configurations of sites and dispersal ability of organisms (Jacobson & Peres-Neto 2010). Spatial configuration of sites could be assessed by overland and watercourse distances as well as their use to generate spatial predictors through eigenvector techniques (e.g. Moran's eigenvector techniques see Landeiro *et al*. 2011; Dray *et al*. 2012; 1). Besides overland and watercourse distances, different spatial components could improve the explanatory power of spatial processes driving communities. Recent studies have tested landscape resistance based on topography, with mixed results regarding its improvement in detecting dispersal-related process (Cañedo-Argüelles *et al*. 2015; Kärnä *et al*. 2015; Morán-Ordóñez *et al*. 2015). Graph-based measures could also provide a valuable information for understanding spatial processes, because some connectivity measures reflect the degree of connectedness and isolation of sites in a network landscape, which are important drivers of richness and community structure (Carrara *et al*. 2012; Altermatt, Seymour & Martinez 2013; Carrara *et al*. 2014).

In this PhD thesis, I investigated ecological driver affecting aquatic metacommunities in dendritic networks in three complementary chapters (Fig. 4.1). They are connected by the use of a riverine network, which was assessed differently, since a target area for biomonitoring (first chapter), to the use of different spatial configurations to understanding ecological drivers of metacommunity structure (second and third chapters), including one derived from graph-based approach (third chapter). More specifically, first and second chapters are linked by ontogenetic niche shifts, a process that offers interesting applied and ecological questions, such as: i) Do larvae and adults respond in the same way to environmental gradients and carry the same information about anthropogenic impacts? (first chapter); ii) What is the relative role of environmental and spatial factors shaping metacommunities formed by organisms that undergo metamorphosis? (second chapter). Metacommunity ecology has accumulated evidence for the role of species sorting structuring communities (Cottenie 2005; Logue *et al*. 2011). The detection of spatial processes structuring metacommunities is not so common and could be related to the use of simple spatial configurations (e.g. Euclidean distance). Thus, in chapter three, I investigated the role of environmental and spatial process structuring community dissimilarities between sites using different spatial dimensions (overland, watercourse and centrality). Also, I sought to understand the role of dispersal ability changing the outcome of dispersal-related process and environmental control on community dissimilarities. To test these questions I used different datasets (odonate larvae/adults and aquatic insects) sampled in a nested manner in a river network (headwaters to mainstems) to capture the whole structure of the riverine network.

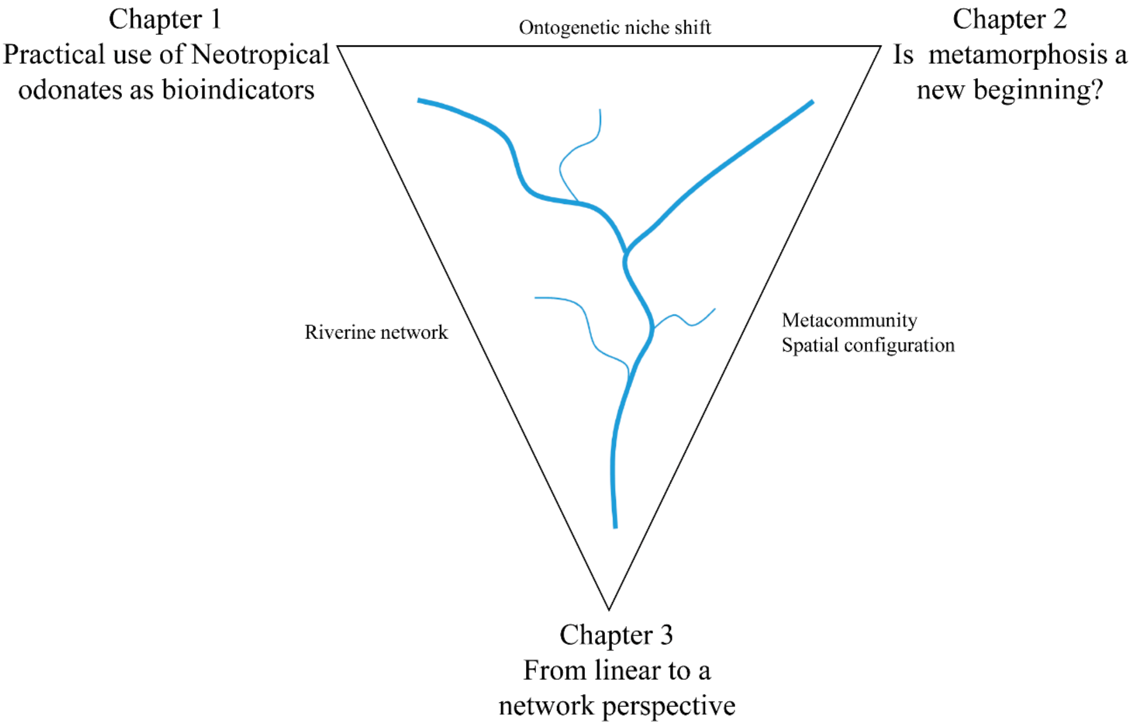

Fig. 4.1. Connection among three chapters with the general aim to understand ecological process driving community structure. Specifically, chapters are linked by ontogenetic niche shift, metacommunity ecology and riverine network.

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5. Chapter 1

Toward a practical use of Neotropical odonates as bioindicators: testing congruence across taxonomic resolution and life stages*

Abstract

Odonates are suggested as bioindicators of human impact. However, their complex life cycles add additional challenges in the practical use as bioindicators, because the level of taxonomic identification could be dependent on life-history stage and, during their ontogeny, dramatic changes occur in their niche (ontogenetic niche shifts). Considering that larvae and adults have different biological characteristics, which could interfere in their performance as bioindicators, we first sought to understand how similar or different environmental factors affect larval and adult life stages in the Odonata. Second, we assessed the level of congruence between (larvae and adult) and within (adult genera and species) life-history stages, considering the taxonomic and numerical resolution. We sampled larvae and adults in 44 streams distributed along a riverine network in southwest Brazil. Larvae samples constituted 20 sampling units of 1m length each, using the kick sampling method; adults were collected for one hour at each site with a hand net along a 100-m transect parallel to the stream/river banks. The influence of environmental factors on larvae and adult was assessed by redundancy analysis coupled with forward selection. The congruence level between response matrices were determined by Procrustes analysis. Our results revealed that a set environmental variables explained a portion of larvae and adults distribution and some environmental factors affect both between (larvae and

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adults) and within (adult genera and species) life-history stages. Larvae and adult were about 54% congruent, regardless of taxonomic level of adults. Abundance of adult genera and species were 94% congruent, but numerical resolution (abundance vs. incidence) decreased the congruency by 10%. Environmental variables could influence larvae and adults individually or via carry-over effects, i.e., larval environmental conditions that could affect adult fitness components or vice-versa. In addition, some odonate behaviors, such as female selection of more appropriate habitats for laying their eggs, could also help us to explain our results, because it could determine larvae distribution. In a biomonitoring perspective, considering the cost-benefit of taxonomic level and sampling of larvae and adults, our results suggest that abundance of adult genera could be used in biomonitoring programs since they capture, respectively, 94% and 54% of the information carried by adult species and larvae.

Key words: biomonitoring; complex life cycles; congruence; ontogenetic niche shifts; surrogacy

Introduction

The establishment of rigorous and cost-effective environmental indicators that can be widely used and easily understood is crucial, considering the current rate of biodiversity loss (Balmford et al., 2005). This subject has been suggested as a priority for decision-making regarding the conservation and management of biodiversity in tropical regions (Barlow et al., 2010). Particularly, the selection of indicators include multiple factors (Bonada et al., 2006). Rationale and performance of a bioindicator are related to its sensitivity to environmental change, surrogacy, habitat choice and appropriate life-stages history (Balmford et al., 2005; Bonada et al., 2006; Gardner et al., 2008; Lindenmayer et al., 2012). The appropriate level of taxonomic identification and numerical resolution are practical issues and key aspects for the establishment of cost-benefit monitoring programs (Lovell et al., 2007; Landeiro et al., 2012). All these factors are important, due to paucity of funds, experts, time of processing and identification of biomonitoring samples (Gardner et al., 2008). Multi-taxa studies are particularly relevant as they have the ability to optimize resources of biological inventories and enable information integration (Lawton et al., 1998; Basset et al., 2004; Gardner et al., 2008; Landeiro et al., 2012). However, most of them consider the organism as a box, i.e., all individuals inside the box are functionally equivalent and no differences between life stages, age or size are generally recognized (Miller and Rudolf, 2011). For example, different life-history stages could differentially be affected by environmental variables (Stoks and Córdoba-Aguilar, 2012), revealing implications for biomonitoring purposes. In this context, it is worth investigating if different life-history stages of an organism are congruent, i.e., if only one or different life-history stages are required for its use as a bioindicator.

The use of organisms with complex life cycles, such as parasitoids, amphibians and insects, as bioindicators has an additional challenge because the level of taxonomic resolution could be dependent on life-history stage and, during ontogeny, dramatic changes occur in their niche (ontogenetic niche shifts) (Wilbur, 1980). Some examples of ontogenetic niche shifts include changes in habitat (aquatic vs. terrestrial ecosystem), which allow the same organisms to exploit different habitats and to interact with different communities, affecting important behaviors, including activity, dispersal and foraging (Miller & Rudolf, 2011; Stoks and Córdoba-Aguilar, 2012). For example, during aquatic insect development, larvae use the aquatic system mainly for growth and emergence, while adults shift their niches to terrestrial ecosystems and invest their energy in dispersal and reproduction (Wilbur, 1980). Thus, larvae and adult could be differently affected by environment, a fundamental issue for biomonitoring purposes.

Aquatic insects are interesting models to compare responses to environmental gradients and their congruency between life-history stages. For example, the order Odonata lives in different types of habitats, both lotic and lentic, and exhibit widespread distributions, are easy to collect, the identification is possible at the species (adult) or generic (larvae) level, and have different responses to environment changes (e.g. Kutcher and Bried, 2014; Dutra and De Marco, 2015; Monteiro-Junior et al., 2015). Both larvae and adults are potential bioindicators of environmental quality in aquatic ecosystems, although they differ in some characteristics. Exuviae may be the best option for a bioindicator, because they reflect the presence of both adult and larvae (Raebel et al., 2010), however this issue is still controversial because of problems related to repeated sampling, low detectability, protocol standardization and identification (Bried et al., 2012). Compared to larvae, adults have great dispersal ability, enabling them to easily evade environmental constraints and colonize patches with more amenable environmental conditions (Corbet, 1999). Because of that, adults may be captured in suboptimal sites, creating problems for its use as a bioindicator (Heino, 2013). For example, adults respond to removal of vegetation, conversion of natural areas to invasive alien trees and urbanization (Magoba & Samways, 2009; Simaika and Samways, 2011; Kutcher & Bried, 2014), but the distribution of some adults with great dispersal capacity (Anisoptera) is negatively affected or not related with human activities, such as urbanization (e.g. Dutra and De Marco, 2015; Monteiro-Junior et al., 2015).

Larvae exhibit relatively limited dispersal ability, depending more upon drift to disperse, and spend a long time (months to years) in aquatic systems (Corbet, 1999). This supports the notion that they are more susceptible to local environmental changes and could exhibit rapid responses to anthropogenic modification. As such, many studies report that they are bioindicators of pollution by heavy metals from herbicides and pesticides, vegetation removal, sedimentation, nutrient enrichment and cattle grazing (Foote & Hornung, 2005; Couceiro et al. 2007; Corbi et al., 2011). Despite the general agreement that odonates could be used as bioindicators for multiple impacts, few studies have evaluated different life-history stage performance to environmental gradients and their congruence (e.g. D'Amico et al. 2004; Raebel et al., 2010).

Based on the theoretical and practical importance of understanding the implications of ontogenetic niche shifts and the effect of taxonomic and numerical resolution on the use of Odonata as potential bioindicators, we sought to learn: i) how similar or different environmental factors affect larval and adult (genus and species) life-history stages distribution; and ii) if distributional patterns between larval and adult stages are congruent, considering taxonomic (abundance vs. abundance and incidence vs. incidence) and numerical resolution (abundance vs. incidence). We predicted that different types of environmental variables would affect larvae and adults. We also expected that the same set of variables would influence genera and species of adult, due the fact that species within the same genus exhibit morphological and ecological similarities. Based on the better dispersal ability of adults compared to larvae, we expected a low level of congruence between larvae and adults but high congruence between adult genera and species. We used 44 streams and rivers from a neotropical drainage basin, which have some advantages, such as the same regional species pool, environmental gradients ranging from headwaters to rivers and a natural boundary where biomonitoring programs could be implemented.

Material and Methods

Study area and sampling

The study was carried out in Bodoquena Plateau, located in the southwest of Mato Grosso do Sul, Brazil (Figure 1). The riverine network used here (Betione watershed) belong to Miranda River basin, a subunit of the Upper Paraguay River basin, which includes part of the Pantanal wetlands. The region was composed of a transition between Cerrado and Atlantic Rainforest forests, two biomes considered hotspots of biodiversity in Brazil (Myers et al., 2000). Currently, it is fragmented, mainly due the land use by cattle ranching. In general, the Betione watershed has karstic waters, no sewage release, and a gradient of forest cover ranging from dense riparian vegetation to different degrees of deforestation and degradation.

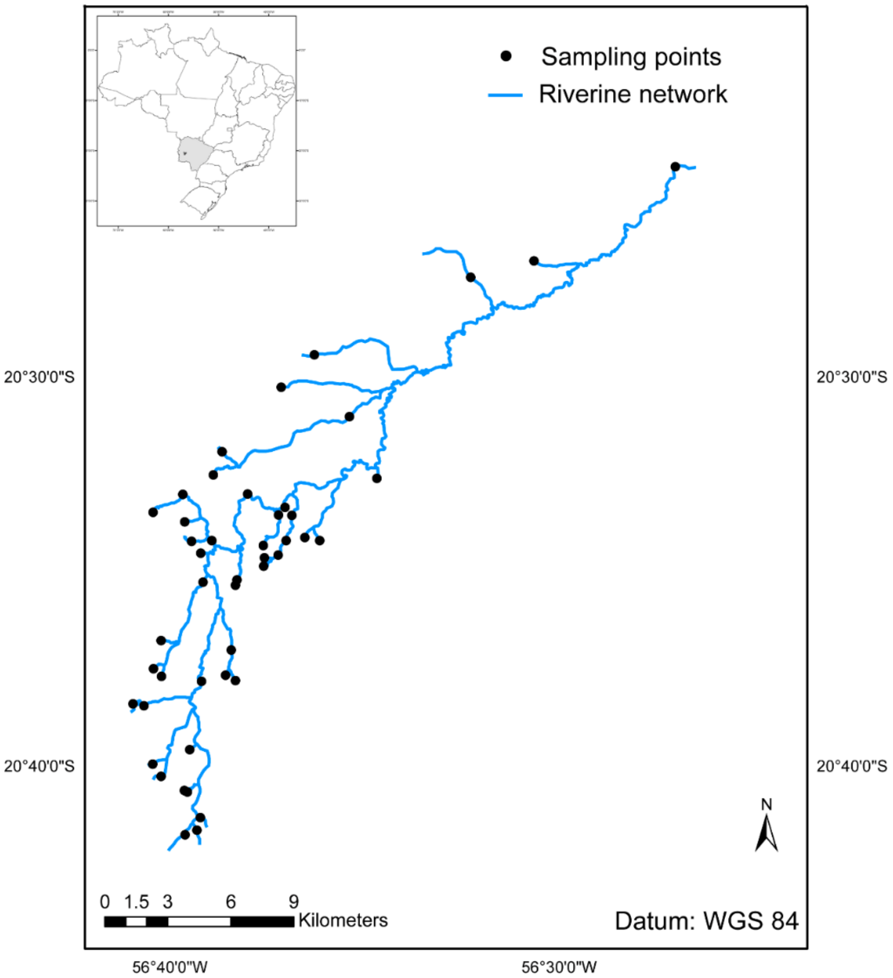

Fig. 5.1. Location of the 44 sampling sites along the Betione riverine network in Bodoquena, state of Mato Grosso do Sul, Brazil.

We selected 44 segments of the Betione watershed, including small streams and larger rivers with width varying from 0.5 to 7 m and depth varying from 0.1 to 1.6 m. We sampled both odonate larvae and adults in the same sites. For larvae, we used the multi-habitat approach (Barbour et al., 1996), consisting of 20 sampling units per stream/river proportionally distributed between different habitats. Each sampling unit consisted of 1m length and 0.3m width using a kick-sampling (0.5mm mesh size), totaling a sample of 6 m^2 of stream/river bottom. We used three reaches of 10m to estimate the proportion of the following habitats: rock outcrops, rock cobble, gravel, sand, mud silt, organic matter, wood, aquatic vegetation, leaf litter and roots. We then calculated the mean for the 30m reach and proportionally distributed the 20 sampling units among habitats. Larvae were identified to genus level using taxonomic keys, considering the limited knowledge for species identifications, including few descriptions of larvae species (Costa et al., 2004; von Ellenrieder, 2009). Some individuals lost morphological structures during sampling and sampling processing, such as gills and the pre-mentum, or some were too young, which affected the visualization of such structures. These structures are fundamental for a precise identification to genus level. We therefore morphotyped these individuals to family level.

The adults were actively collected during 1 hour using an entomological net along a 100-m transect parallel to the stream banks. Samplings were conducted once at each site on sunny days between 10:00 and 15:00h, due to known thermal restrictions in odonates. This method has been effectively used in other studies (e.g. Juen and De Marco, 2011). The identification of specimens to species or morphotype followed taxonomic keys and original descriptions (Garrison 2006, 2010; Lencioni, 2005, 2006). Voucher specimens are deposited in UFMS’s Zoological Collection (ZUFMS).

Environmental variables

Stream width, depth and flow were measured once every 6 meters along the same 30m reach that the samples were collected, totally 5 measurements. Altitude was measured once; physicochemical variables (temperature, conductivity, salinity, velocity, pH, dissolved oxygen and turbidity) and percentage of substrates were measured three times (once every 10m). Luminosity was estimated using 3 digital photographs of the canopy that was taken from the middle of the stream/river. The images were analyzed with ImageJ (version 1.47, Wayne Rasband, National Institute of Health, USA). We first transformed the image to black and white scale, and afterward measured the white pixels area of each photograph, i.e., the area that light pass through the canopy. The percentage of forest was calculated using ArcGis (version 10.1 ESRI, Redlands, California, USA) and a digital map of forest coverage with 30m resolution provided by Environmental Institute of the state of Mato Grosso do Sul (IMASUL, 2014). A buffer of 250m was used from the middle of each sampling point to estimate the percentage of forest cover. This distance was chosen considering that some adult damselflies use short distances to disperse (≤ 500m) (Keller and Holderegger, 2013). In case of environmental variables that were measured twice or more, we used its mean as the environmental predictor.

Data analysis

Each stream/river segment was defined as our sample, which was constituted by the sum of 20 subsamples for larvae and by 1 hour of active collection for adults. The abundance response matrices (larvae, adult genera and adult species) were transformed using Hellinger transformation prior to statistical analysis, because this method provide a more suitable dataset to linear-based analyses (Legendre & Gallagher 2001).

We used redundancy analysis (RDA) coupled with forward selection to verify if larval and adult stages (genera and species) were affected by environmental variables and if the same environmental variables would be selected considering the response matrices (larvae, adult genera and adult species). Prior to analysis, we proceeded with a Spearman correlation among environmental variables, because some of them could be redundant. The cutoff level for statistical significance was set at $p<0.05$ and $r_s >0.8$. The variables correlated were: width and depth; conductivity and turbidity; conductivity and salinity; turbidity and salinity. We selected conductivity and width, because of its importance for odonates distribution (Corbet, 1999; Juen and De Marco, 2011; Remsburg and Turner, 2009). Predictors included in the analysis were: altitude, width, percentage of forest, luminosity, physicochemical variables (temperature, conductivity, pH and dissolved oxygen) and percentage of substrates (rock outcrops, rock cobble, gravel, mud silt, sand, organic matter, wood, aquatic vegetation, leaf litter and roots) (see Supplementary material 1 for the variation of each environmental variable). First, we ran a global model using redundancy analysis with all explanatory variables, and verified the significance and adjusted R^2. If a global model was significant, we selected the most important variables using the forward.sel function implemented by Blanchet et al. (2008). In this approach, variable selection was stopped if a candidate variable was non-significant ($p > 0.05$) or if the adjusted R^2 of the reduced model was over the value of the adjusted R^2 of the global model (see details in Blanchet et al., 2008). The forward selection procedure reduces the probability of Type I error and overestimation of the explained variance (Blanchet et al., 2008).

To test the congruency between response matrices we used a Procrustes analysis (Figure 2). First, we carried out Principal Coordinates Analysis (PCoA) for each of the response matrices, to ordinate the samples based in community similarity. We used

Jaccard similarity index for incidence (presence/absence) response matrices and Euclidean distance for the Hellinger transformed abundance data. Then we used the scores of sites for all vectors generated by PCoA in a Procrustes analysis to test the degree of congruence between pairs of response matrices (larvae, adult genera and adult species) (Figure 2). Procrustes analysis minimizes the sum of squared distances between corresponding points of two matrices through a rotational-fit algorithm, resulting in the m^2 statistic, from which is derived the correlation statistic r (square-root of 1- m^2) (Peres-Neto & Jackson, 2001). The correlation statistic indicates the degree of association between pairs of response matrices, i.e, 0 and 1 indicate complete difference and overlap, respectively. The significance of Procrustes statistic was tested by permutation with the Procrustean randomization test – PROTEST (with 5000 permutations) (Jackson, 1995; Peres-Neto and Jackson, 2001). All analysis were performed in R software, using vegan (Oksanen et al., 2014) and packfor packages (Dray et al., 2011).

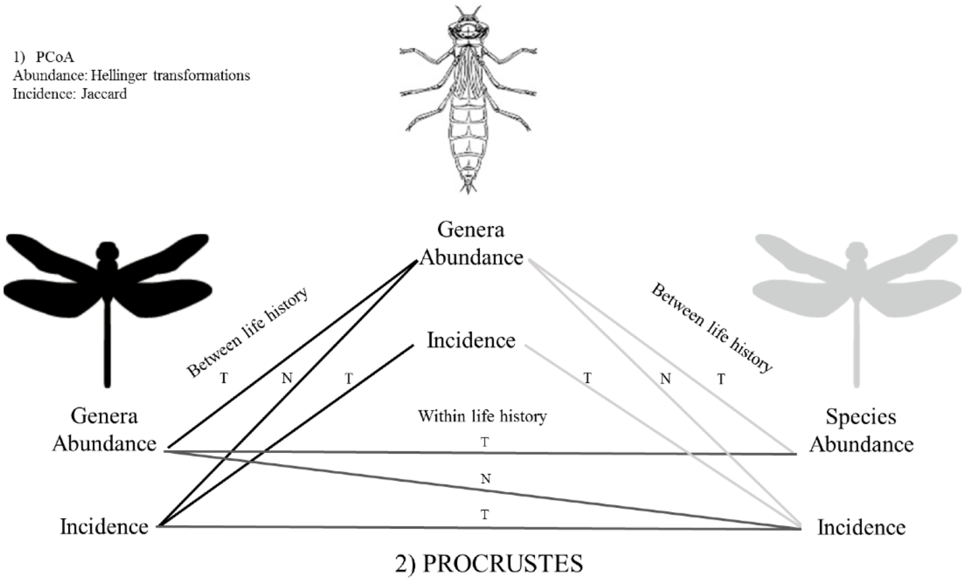

Fig. 5.2. General description of comparisons performed between responses matrices. For each matrix (abundance and incidence), we first ran a Principal Coordinates

Analysis (PCoA) to ordinate samples. After that, we performed pair-wise comparison using PROCRUSTES analysis between and within life history. Abbreviations: T=taxonomic resolution; N=numerical resolution.

Results

We sampled 1227 adult individuals, representing 21 genera and 39 species. The most abundant genera was *Argia* (310 individuals), *Hetaerina* (170 individuals), *Oxyagrion* (168 individuals), *Acanthagrion* (159 individuals) and *Neoneura* (158 individuals). Some genera were low in abundance (<5 individuals), including *Progomphus*, *Staurophlebia*, *Elga*, *Enallagma*, *Aeolagrion*, *Metaleptobasis*, *Tigriagrion* and *Micrathyria*. The most abundant species were *Argia chapadae* Calvert, 1909 (175 individuals), *Hetaerina rosea* Selys, 1853 (166 individuals), *Oxyagrion sulmatogrossense* Costa, Souza & Santos, 2000 (165 individuals) and the least abundant included: *Aeolagrion dorsale* (Burmeister, 1839), *Argia mollis* Hagen in Selys, 1965, *Macrothemis flavencens* (Kirby, 1897), *Perithemis mooma* Kirby, 1889, *Elga newtonsantosi* Machado, 1992, *Progomphus* sp, *Staurophlebia reticulate* (Burmeister, 1839) and *Micrathyria stawiarskii* Santos, 1953 (Supporting information, Tables S5.2 and S5.3).

We collected 780 larval individuals comprised of 32 genera. *Coenagrionidae* sp, *Argia* and Libellulidae sp were the most abundant taxa with, respectively, 195, 87 and 87 individuals. It is important to pointing out that the morphotypes used here were resulted from difficulties in identification, due to loss of morphological structure or too young stage. Some genera exhibiting low abundance (< 5 individuals) included *Castoraeschna*, *Anax*, *Rhionaeshna*, *Planiplax* and *Oligoclada* (Supporting information, Table S5.2).

Environmental variables

Global models using all set of predictors were significant for all response matrices (larvae: Adjusted R^2=0.14, p=0.004; adult genera: Adjusted R^2=0.25, p=0.003; adult species: R^2=0.24, p=0.002). Individually, each environmental variable selected had a weak explanatory power. The distribution of larvae and adults, both genera and species, were associated with dissolved oxygen (larvae: adjusted R^2=0.037, p=0.001; adult genera: adjusted R^2=0.03, p=0.013; adult species: adjusted R^2=0.033, p=0.003). Vegetation, temperature and conductivity also affected larvae distribution. Width was retained in forward selection in both adult matrices (adult genera: adjusted R^2=0.041, p=0.006; adult species: adjusted R^2=0.04, p=0.002). Adult genera and species was also affected, respectively, by water velocity (adjusted R^2=0.096, p<0.001) and altitude (adjusted R^2=0.033, p=0.003) (Table 5.1).

Table 5.1. Variables retained by the forward selection procedure in the redundancy analysis for larvae, adult genera and adult species matrices, with respective adjusted R^2, F and p values. Explanatory variables used in the global test were: altitude, width, percentage of forest, luminosity, physicochemical variables (temperature, conductivity, pH and dissolved oxygen) and percentage of substrates (rock outcrops, rock cobble, gravel, mud silt, sand, organic matter, wood, aquatic vegetation, leaf litter and roots).

Response matrix	Variables selected	R^2	R^2 Cum	Adj R^2	F	p
Larvae	DO	0.060	0.060	0.037	2.690	0.001
	Vegetation	0.045	0.103	0.023	1.959	0.021
	Temperature	0.038	0.141	0.017	1.785	0.042
	Conductivity	0.037	0.179	0.018	1.803	0.041
Adult genera	Velocity	0.117	0.117	0.096	5.582	<0.001
	Width	0.060	0.177	0.041	3.008	0.006
	DO	0.050	0.228	0.033	2.616	0.013
Adult species	DO	0.102	0.102	0.081	4.810	<0.001
	Width	0.059	0.162	0.04	2.916	0.002
	Altitude	0.051	0.213	0.033	2.617	0.003

Taxonomic resolution between and within life history

Larvae and adult abundance matrices were ~54% congruent, using either genera or species of adult (larvae vs. adult genus: r=0.522, p=0.005; larvae vs adult species: 0.541, p=0.005), i.e., the ordination of sampling points using larvae was about 54% similar to that using adults (Figure 5.3; Supporting information S5.4). Adult genera and species were highly congruent (r=0.939, p<0.001). Comparisons using incidence vs. incidence showed similar results for comparison between life history stages (larvae vs. adult genus: r=0.559, p<0.001; larvae vs adult species: 0.585, p<0.001). The congruency of larvae vs. adult using only incidence decreased 7%, compared to the abundance-abundance result.

Numerical resolution between and within life history stages

The ordination patterns of the abundance-incidence (numerical resolution) data exhibited different results. Numerical resolution comparisons of larvae and adult showed a similar level of 54% compared to abundance-abundance comparisons (Figure 5.3; Supporting informantion S5.4). However, the congruency within life stages (adult genera and adult species) decreased ~10%, compared to abundance-abundance comparison.

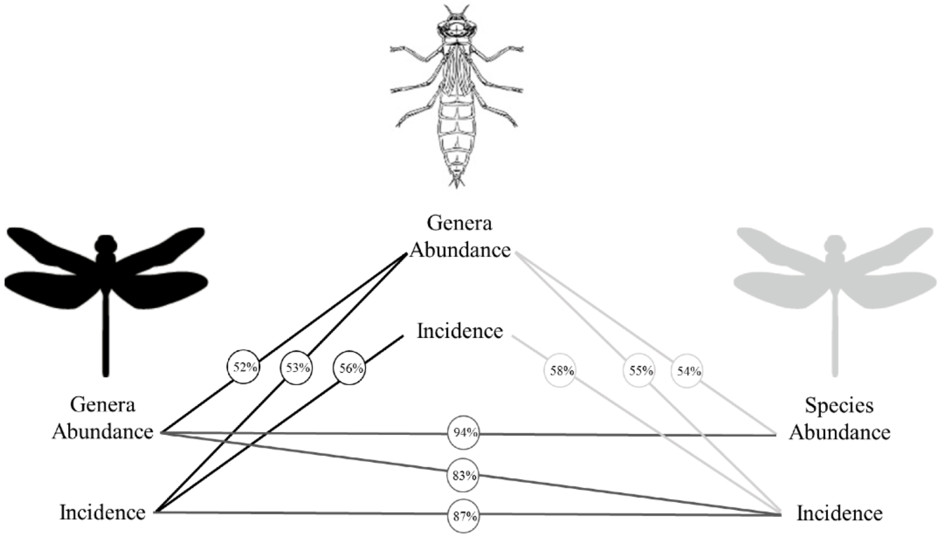

Fig. 5.3. Percentage of congruence between response matrices derived from correlation statistic r (Procrustes analysis). All results showed were significant ($p<0.05$).

Discussion

Potential governing processes behind congruent patterns

Our results revealed that environmental variables partially influenced the patterns of congruence between and within life-history stages. This influence on complex life cycle organisms, such as odonates, could be explained by how they affect each stage individually or via carry-over effects. Different behaviors could also help us to explain the congruence pattern.

The influence of physicochemical variables on aquatic communities from landscapes with agricultural/cattle ranching land use has been variable, because the environmental gradient could not be so strong (Allan, 2004), such as in this study. Considering all environmental predictors together, they explain a reasonable portion of variance in larvae and adult distribution, but individually, each variable had a weak explanatory power. However, it is worthy discussing these results because they agree with a bulk of literature. It is well known that physicochemical variables affect aquatic insect survivorship. Dissolved oxygen acts as a filter for larvae, because different species have distinct sensitivity to levels of dissolved oxygen (Steiner et al., 2000). Larvae were also related to vegetation cover, temperature and conductivity; the last two could also be related to suitable habitats for larval development and survivorship (Stoks and Córdoba-Aguilar, 2012), since high temperatures could decrease larval development time (Corbet, 1999). Forest cover influences in-stream habitat availability (Remsburg and Turner, 2009), as it inputs different organic substrates fundamental to larvae distribution, which have variable habitat preferences, including burrowing, climbing, grabbing and free living (Corbet, 1999). Forested streams provide different organic habitats used by fauna as habitat and foraging, such as leaves and wood; otherwise, open streams or streams with narrow riparian vegetation could be appropriate habitats for the development of aquatic vegetation, due to high input of sunlight.

The influence of oxygen dissolved and stream/river width on adult distribution (both genera and species) could be related to oviposition behavior, because females may select more appropriate habitats for laying eggs, such as in more oxygenated fast flowing water (Siva-Jothy et al., 1995). Further, width affects the availability of sunlit sites for reproduction and perching structures for thermoregulation (Corbet, 1999; Juen and De Marco, 2011). For example, large ectothermic adult species are favored in large sunlit streams and rivers while small ectothermic odonate species prefer shaded and small stream (De Marco Jr. et al 2015). Adult genera distribution was also related to water velocity, which is related to oviposition site selection by females (Siva-Jothy et al., 1995) and species affected by altitude, indicating that species relative abundances are differentially distributed along headwater-mainstem gradients (Corbet, 1999).

Carry-over effects, i.e., larval environmental conditions that may affect adult fitness components and vice-versa (see reviews of Benard and McCauley, 2008; Stoks and Córdoba-Aguilar, 2012), are also important for explaining our results. Aquatic habitat differences (both biotic and abiotic) experienced by egg and larval stages could alter individual development, resulting in adult dispersal asymmetries, which have consequences for metacommunity dynamics (Benard and McCauley, 2008). This mechanism could affect positive or negatively the next stage. For example, damselfly larvae living in habitats with low food supply and high larvae density delay the time of emergence and decrease the adult mass, affecting adult dispersal (Anholt, 1990, 1991). Adult examples of this mechanism are scarce, including the choice of optimal habitat for oviposition by *Calopteryx splendens*, which increases egg development significantly and decreases mortality, compared to suboptimal habitats (Siva-Jothy et al., 1995). In this way, the larvae and adult are interconnected and the conditions experienced by one stage could affect the next, depending on selection pressure on that trait across metamorphosis (Stoks and Córdoba-Aguilar, 2012). However, these effects could be decoupled by compensatory mechanisms in the other stage. For example, even if larvae experience suboptimal conditions, adults could increase their body mass during maturation period and no effect on fecundity is detected (Richardson and Baker, 1997).

Our congruence results could be explained by other factors. Adult genera and species congruency could be a result of the small number of species belonging to each genus (genus/species ratio=0.53) (Bevilacqua et al., 2012), and that the most abundant genera were also the most species rich, a pattern that was also observed in Nepomorpha (Hemitptera) (Giehl et al., 2014). Further, some adult Odonate behavior provide complementary explanations for our between life-history stage congruence results. Despite the greater flight ability of adults (Keller and Holderegger, 2013), this does not correspond directly to effective dispersal, because it depends on a complex trade-off among dispersal vs. fitness that also involves habitat conditions of larvae (Benard and McCauley, 2008). Several Odonate taxa have male territoriality and site fidelity (Corbet, 1999), factors that decrease dispersal among patches and allow larvae and adults to co-occur in the same area. Also, the strong competitive interaction experienced by adults (both inter- and intraspecific) could cause adult permanence in the same habitat after emergence instead of dispersing to other habitats (Corbet, 1999; Benard and McCauley, 2008).

Oviposition behavior is also important to determine the distribution of both larvae and adults, as we introduced above. Larvae have low dispersal ability and sparsely occur in the drift, depending on disturbance related to flood conditions (Brittain & Eikeland, 1988). Thus, larvae are limited dispersal organisms and adult females may compensate that through dispersal and oviposition, taking their offspring to appropriate sites. For example, suppose that a female lays its eggs in an inappropriate environment, consequently, eggs would not develop (Siva-Jothy et al., 1995) or, after larval emergence, they could die because of suboptimal conditions (Buskirk and Sherman, 1985). In this context, adult dispersal and oviposition may optimize the fitness of their offspring by selecting appropriate oviposition habitats (Buskirk & Sherman, 1985). Adults of *Calopteryx splendens* choose optimal habitat for oviposition (high velocity waters), which decreases mortality, compared to suboptimal habitats (low velocity) (Siva-Jothy et al., 1995). Oviposition behavior is also important for larvae distribution of many other aquatic insects, including mayflies (Lancaster et al., 2011) and caddisflies (Bovill et al., 2013). In this way, oviposition behavior may be key to understanding congruence between organisms with complex life cycles and the environmental controls on them, opening a good avenue for future studies.

Implications for using Odonate as bioindicators

Aquatic biomonitoring is a science-based process in which biodiversity metrics are used to assess environmental conditions or pre-established protection goals (Bonada et al., 2006; Lindenmayer et al., 2012). As a practical activity, biomonitoring programs should be efficient and established based on rigorous data collected at low effort and cost, and easily communicated to general public (Gardner et al., 2008). For selecting groups with complex life cycles, like odonates, it is important to consider a stepwise approach. It should start with the problem formulation (e.g. if the group is related to environmental variables recognized by applying a clearly stated set of statistical rules) towards a more operational criteria (e.g. if we need to use low taxonomic resolution or if it is more cost-effective to collect in aquatic or terrestrial systems).

Our study offers evidence that both larvae and adults of odonates were partially affected by environmental gradients (Stoks and Córdoba-Aguilar, 2012; Kutcher and Bried, 2014; Dutra and De Marco, 2015; Monteiro-Junior et al., 2015). More strikingly, larvae and adults were congruent and the niche changes experienced by larval and adult life-history stages (ontogenetic niche shift) did not imply in incongruent or idiosyncratic response. The congruence level was not as high as recommended in other studies for surrogacy (Lovell et al., 2007; Heino, 2010), despite this issue does not have a clear threshold (e.g. Landeiro et al., 2012). In a practical context, where the paucity of funds, time and knowledge is a reality, leading to choose which organisms would be surveyed, the pattern of congruence gives support for using larvae or adults for assessing environmental gradients from headwater to rivers dominated by cattle ranching impact. It could be hypothesized that areas with stronger environmental gradients, such as urban ones, the distribution of larvae and adults would be better explained by environmental variables and this could lead to a higher congruence among them.

For operational reasons, larval and adult sampling have advantages and disadvantages. Adults are collected using hand net (e.g. Juen and De Marco, 2011) and they do not require estimation of habitats proportions as larvae need, because the latter has a broader range of habitat use (Cobert, 1999), which demand more time in the field and laboratory for screening. Adults can also be sampled using close-focus binoculars, commonly used in Africa (see Magoba & Samways 2010; Samways & Sharrat 2010; Kietzka et al. 2015), which avoids community disturbance, killing dragonflies, is less time consuming and more efficient, since it includes species that are difficult to collect using net sampling. This approach has great potential in the Neotropical region. However, it is worth mentioning that there is no field identification guide available and some genera (e.g. *Argia*, *Epipleura*, *Neoneura*) have species with similar size and colors or even differ by the shape of small morphological structures, such as females superior and inferior appendage or males genitalia (Lencioni 2005). To implement such sampling approach it would be required the development of field identification guide and specialists training. Adults are active during sunlight periods, mainly the hottest ones, which could have some problems, such as sampling during cloudy days or cold periods of the day (Kutcher and Bried, 2014). On the other hand, there is no weather restriction to larvae sampling, but it demands a larger time commitment for processing samples in the laboratory, i.e., sorting species from sediment. Furthermore, larvae identification could be more time consuming, because some of them could miss important parts for identification in sampling processing or are too young, which have certain structures absent (Costa el al., 1999). Also, taxonomic studies have been done with adults, including a good knowledge about odonates in Neotropical region while larvae are poorly known to species level (Lencioni, 2005, 2006; von Ellenrieder 2009; Garrison, 2006, 2010). Putting this together, one could find a trade-off between field requirements and laboratory processing of larvae and adult sampling. In our case, we suggest that adult sampling is more advantageous, mainly because they do not need laboratory processing and have all structures developed and are easily observable for identification.

Taxonomic and numerical resolution are key for the establishment of cost-benefit monitoring programs (Lovell et al., 2007; Landeiro et al., 2012). Our study showed that abundance of adult genera is sufficient to capture 94% species information, but the numerical resolution decrease 10% of this relationship. These results are similar to Giehl et al. (2014) that showed for Nepomorpha (Hemiptera), the genus level may be used instead of species to detect impacted areas and that the congruency is higher using abundance data. Also, between life-history stages, the numerical resolution showed similar results of abundance vs. abundance comparisons.

We highlight that our aim was to give support for the practical use of odonates in biomonitoring programs. In this context, we suggest that biomonitoring programs can use genera of either larvae or adults to detect environmental changes caused by cattle ranching in aquatic systems from Neotropical region. Knowledge about adult genera identification is reasonable in this region (Lencioni, 2005, 2006; Garrison, 2006, 2010) and could be done by non-specialists, after some training. Larval identification has several problems, some of them related to few described larvae and keys for most genera were not yet developed (Corbet, 1999; Lencioni, 2005, 2006; von Ellenrieder 2009; Garrison, 2006, 2010), highlighting the need for more taxonomical studies with larvae, a fundamental issue for their knowledge and its use in biomonitoring. In general, larval generic identification is more time consuming, because i) it requires larvae dissection to have access to specific structures (e.g. labrum and gill) and ii) they need to be in the final stage of development or near it, because there is great morphological variation between the various stages of larval development (Corbet, 1999). In this context, considering the land use in the riverine network assessed here, our study suggests the use of abundance of adult genera for biomonitoring purposes. We recognize that this would not be the case in other regions, such as Africa, where assessment at the species level is critical (Magoba & Samways 2010; Samways & Sharrat 2010; Kietzka et al. 2015). This is because some genera could be more species rich (e.g. *Pseudagrion*, *Trithemis* and *Orthetrum*) than that present in our dataset and using just the genus level may not reflect all the environmental ranges needed by different species (e.g. Magoba & Samways 2010). Fortunately and as discussed in a previous paragraph, this extra refinement can be easily achieved for African adult odonates using the close-focus binocular sampling methodology and would be still more advantageous than sampling larvae, agreeing with our results.

Our study is a step forward because, besides documenting the relationship between odonates and environmental factors, we showed that their use in biomonitoring programs could be viable in terms of life-history stages, either larvae or adult. Also, we suggest that abundance of adult genera could be used in South America instead of species. Identification of adults to species is not always necessary, considering the time commitment and the experts requirements. These issues are fundamental to biomonitoring program development and strategy, because they require that both bioindicators be rigorous and cost-effective. Future studies should consider looking inside the box (Miller and Rudolf, 2011), because it could save time, money and make biomonitoring programs more efficient.

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Supporting information

Table S5.1. Variation for each eenvironmental variables measured in 44 streams/rivers along a riverine network in Bodoquena, Mato Grosso do Sul State, Brazil.

	Mean	Standard deviation	Maximum	Minimum
Altitude	258.8	66.05	358	118
Width	224.4	147.3	728	49.6
Water velocity	0.1	0.08	0.4	0.02
Temperature	24.8	1.9	31.2	21.7
Conductivity	462.0	170.3	717	32.3
pH	7.6	0.4	8.5	6.6
Dissolved oxygen	3.9	1.7	7.7	0.5
% vegetation	41.1	22.2	100	0
% rock outcrops	2.9	7.6	41.6	0
% rock cobble	5.0	11.1	40	0
% gravel	33.7	22.1	80	0
% mud silt	5.0	11.1	55	0
% sand	13.7	13.7	40	0
% organic matter	7.5	7.4	23.3	0
% wood	0.3	1.1	6.6	0
% aquatic vegetation	2.3	10.2	66.6	0
% leaf litter	24.4	13.0	55	0
% roots	4.2	4.7	23.3	0
luminosity	15.8	11.4	74.9	7.0

Table S5.2. Abundance of each genera of larvae and adults sampled in 44 streams/rivers along a riverine network in Bodoquena, Mato Grosso do Sul State, Brazil.

Genera	Larvae	Adults
Acanthagrion	71	159
Aeolagrion		2
Anax	2	
Archaeogomphus	4	
Argia	87	310
Brechmorhoga	36	
Castoraeschna	1	
Coenagrionidae sp	195	
Dythemis	22	41
Elasmothemis		6
Elga	4	1
Enallagma	4	2
Epigomphus	3	
Epipleoneura	14	8
Erythemis	4	
Erythrodiplax	6	14
Gomphoides	7	
Hetaerina	49	170
Idiataphe	8	
Libellulidae sp	87	
Macrothemis	56	12
Metaloptobasis		2
Micrathyria		4
Neoneura	19	158
Oligoclada	1	
Orthemis	10	7
Oxyagrion	3	168
Peristicta	5	117
Perithemis	14	41
Phyllocycla	21	
Phyllogomphoides	9	
Planiplax	1	
Progomphus	10	1
Rhionaeschna	2	
Staurophlebia		1
Telebasis	5	
Tibiagomphus	20	
Tigriagrion		3

Table S5.3. Abundance of each adult species sampled in 44 streams/rivers along a riverine network in Bodoquena, Mato Grosso do Sul State, Brazil.

Species	Adults
Acanthagrion aepiolum	138
Acanthagrion gracile	21
Aeolagrion dorsale	2
Argia chapadae	175
Argia croceipennis	10
Argia hasemani	75
Argia mollis	2
Argia reclusa	48
Enallagma novaehispaniae	2
Epipleoneura venezuelensis	8
Hetaerina mortua	4
Hetaerina rosea	166
Metaleptobasis lillianae	2
Neoneura bilinearis	3
Neoneura ethela	43
Neoneura rubriventris	3
Neoneura sylvatica	109
Oxyagrion sulmatogrossense	165
Oxyagrion terminale	3
Peristicta aeneoviridis	117
Tigriagrion aurantinigrum	3
Erythrodiplax fusca	9
Dythemis multipunctata	41
Orthemis discolor	3
Orthemis cultriformis	4
Elasmothemis cannacrioides	6
Macrothemis sp1	10
Macrothemis flavencens	1
Perithemis electra	18
Perithemis mooma	1
Perithemis lais	1
Perithemis thais	21
Macrothemis sp1	1
Erythrodiplax lativittata	5
Elga newtonsantosi	1
Progomphus sp1	1
Staurophlebia reticulata	1
Micrathyria pseudhypodidyma	3
Micrathyria stawiarskii	1

Table S5.4. Results of Procrustes analysis for each pair of comparison between response matrices. The comparisons were grouped into taxonomic (abundance vs. abundance or incidence vs. incidence) and numerical (abundance vs. incidence) resolutions between (larvae vs. adult) and within (adult genera and species) life history stages. It is also showed the r statistic and p value for each comparison. Abreviations: A=abundance; I=incidence

		Data matrices	r	p
Taxonomic resolution	Between life stages	A larvae vs. A. adult genera	0.522	0.005
		A larvae vs. A adult species	0.541	0.005
		I larvae vs. I adult genera	0.5592	<0.001
		I larvae vs. I adult species	0.5852	<0.001
	Within life stage	A adult genera vs. A adult species	0.939	<0.001
		I adult genus vs. I adult species	0.8721	<0.001
Numerical resolution	Within dataset	A larvae vs. I larvae	0.934	<0.001
		A adult genus vs. I adult genus	0.846	<0.001
		A adult species vs. I adult species	0.891	<0.001
	Between life stages	A larvae vs. I adult genera	0.528	<0.001
		A larvae vs. I adult species	0.548	0.003
	Within life stage	A adult genus vs. I adult species	0.833	<0.001

6. Chapter 2

Is metamorphosis a new beginning? Species sorting drives variation in both larval and adult odonates in a riverine network**

Abstract

Metamorphosis is a process that connects two life stages of the same organism. For some aquatic insects, it produces an abrupt change on life stages niche – known as ontogenetic habitat shifts – from aquatic to terrestrial ecosystem and vice-versa. During odonate life cycle, aquatic larvae use their energy for growth and emergence, while, after metamorphosis, adults shift their niches to terrestrial ecosystems and invest their energy in dispersal and reproduction. We aimed to investigate whether environment (in-stream and terrestrial components) and/or dispersal dimensions are determinants of larvae and adult odonates. We sampled larvae and adults in 44 streams distributed along a riverine network in southwest Brazil. We included environmental predictors in two components (in-stream and terrestrial habitat variables) and used different dispersal dimensions of riverine network (overland vs. watercourse) as spatial predictors. The influence of environmental factors and spatial predictors on larvae and adult were assessed by redundancy analysis coupled with forward selection. Global spatial models did not explain distribution of either larvae or adults. Both distribution of larvae and adults were explained by environmental global models, including both terrestrial and in-stream variables. Our results suggest that environmental factors were important for both larvae and adult distribution, indicating that metamorphosis might not necessarily be a new beginning. The absence of spatial signal on assemblage variation using different

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**I wrote this article in collaboration with Fabio de Oliveira Roque, Marciel Elio Rodrigues, Tadeu Siqueira and Christopher Swan.

spatial configurations (overland and in-water) suggests that odonates may track variation in environmental conditions in the spatial extent of our study area.

Introduction

Metamorphosis is an abrupt ontogenetic change experienced by some organisms that undergo complex life cycles (e.g. amphibians, insects), generating discrete larval and adult stages. This process can couple ecosystems functioning and dynamics through reciprocal flows of organisms, creating trophic cascades and regime shifts that transcend their boundaries (Baxter *et al*. 2005; Knight *et al*. 2005; Schreiber & Rudolf 2008). The changes that accompany metamorphosis are so abrupt that they are recognized as ontogenetic niche shifts (Wilbur 1980; Nakzawa 2014), which enable the same organism to partition resources and habitats, avoiding competition among age, size classes or life stages (Wilbur 1980). Metamorphosis also affects organism behavior, dispersal and foraging (Miller & Rudolf 2011; Wilson & Krause 2012; Stoks & Córdoba-Aguilar 2012). For example, it can change aquatic insects and amphibian dispersal ability, enabling adult species disperse outside the habitat experienced by larval stage (Wilbur 1980; Peckarsky, Taylor & Caudill 2000; Keller, Strien & Holderegger 2012). Following these ideas, one may think that different niche dimensions would differently affect larval and adult stages, i.e., metamorphosis would restart which ecological processes affect the different stages (Peckarsky, Taylor & Caudill 2000). However, environmental effects experienced by one life stage could change fitness-related traits in the next life stage (carry-over effects). Thus, metamorphosis might not necessarily be a new beginning (Pechenik 2006; Benard & McCauley 2008). For example, odonate larvae reared at high density or low food level emerged later and with a lower mass, affecting their dispersal (Anholt 1990).

The consequences of individual-based ontogenetic niche shifts on community structure and dynamics, mainly on aquatic food web plant–insect interactions are well understood (see review by Nakazawa 2014). Few studies have considered a dispersal mode that is dependent of ontogenetic niche shift, i.e., different life stages may differ their dispersal ability, which may have consequences for community structure (Nakazawa 2014). In this way, metacommunity ecology provides a general framework to understand the relative role of local control and dispersal-related process in structuring communities that undergo ontogenetic niche shifts (Leibold *et al*. 2004; Cottenie 2005; Logue *et al*. 2011). It recognizes that local communities are linked by species dispersal, a fundamental process that may change local community dynamics (Leibold *et al*. 2004). This framework could help to understand if metamorphosis is a new beginning at community level, following the reasoning of which process govern patterns of variation in community composition of different life stages.

Hierarchical and directional systems, such as riverine networks, are interesting models to understand metacommunity structure because they impose dispersal restriction to some organisms. For example, community structure of strict aquatic groups (e.g. aquatic earthworms, molluscs, crustaceans and fishes) are more affected by spatial process, because their dispersal limitation prevent them to reach at suitable habitats (De Bie *et al*. 2012; Heino 2013). In the same way, aquatic insects larvae are in general poor dispersal organisms, restricted to waterbodies boundaries and depending on drift to disperse between patches. This creates an expectation of dispersal limitation affecting larvae that did not use active drift behavior (Brittain & Eikeland 1988). However, their complex life cycles have a consequence: flying adults could track environment gradient, avoiding unsuitable habitats both for them and for their offspring (larval stage) by selecting more suitable habitats for oviposition (De Bie *et al*. 2012;

Heino 2013). Indeed, female dispersal and selection of suitable habitats to lay their eggs is fundamental for larvae distribution of different aquatic insects (Siva-Jothy, Gibbons & Pain 1995; Lancaster, Downes & Arnold 2011; Bovill, Downes & Lancaster 2013). In this way, organisms with complex life cycle have dispersal ability variable according to life stage, so assessing only one stage would generate an incomplete view about the processes structuring the metacommunity.

Odonates provide a good target organism to investigate the relative role of processes shaping community variation of different life stages in a riverine network. The larval stage develops in freshwater (both lotic and lentic) and, as a sit-and-wait predator with territorialism behavior, they are low mobile organism and drift do not influence the composition of local species communities (Corbet 1999). Adult has good dispersal ability (Keller, Strien & Holderegger 2012; Petersen *et al.* 2004) and females may select suitable habitats to lay their eggs (Buskirk & Sherman 1985; Wolf & Waltz 1988; Siva-Jothy, Gibbons & Pain 1995). These adult characteristics could compensate larval low mobility, which increases the expectation of local control in both life stages, as demonstrated separately for each one (Curry & Baird 2015; De Marco, Batista & Cabette 2015). Despite this expectation of local control in both life stages, the relative role of local factors governing variation in larvae and adult community composition could be differently. Larvae distribution is more affected by local water conditions (named here in-stream variables), such as substrate, pH, flow, dissolved oxygen and water velocity (Corbet 1999). Conversely, adults mainly depend on factors related to their eco-physiological requirements for thermoregulation (De Marco, Batista & Cabette 2015), such as luminosity, forest cover and channel width (named here terrestrial habitat variables). In this way, considering that aquatic and terrestrial gradients could not be spatially concordant, these different components should be appropriate for larvae and adults establishment and occurrence at the same site.

We investigate different ecological variables that could affect both larvae and adult life stages, in this case environment and spatial configuration of sites, using both overland and watercourse as distance sources. We expected that metamorphosis is not a new beginning, i.e., both larvae and adults odonates would be affected similar ecological process. In this case, we expect that both life stages would be affected by environmental variables (Fig. 6.1), and that i) larvae would be relatively more affected by in-stream (E_S) variables, whereas adults would be more influenced by terrestrial habitat variables (E_T), due to their different characteristics and physiological limitations to environment; ii) variation in community composition of larvae would be more explained by spatial variables than adults, due their restricted dispersal ability and iii) spatial variables derived from watercourse distances would explain a greater proportion of community variation than those derived from Euclidean distances, because adults disperse mainly by riverine network and larvae are restricted to aquatic system.

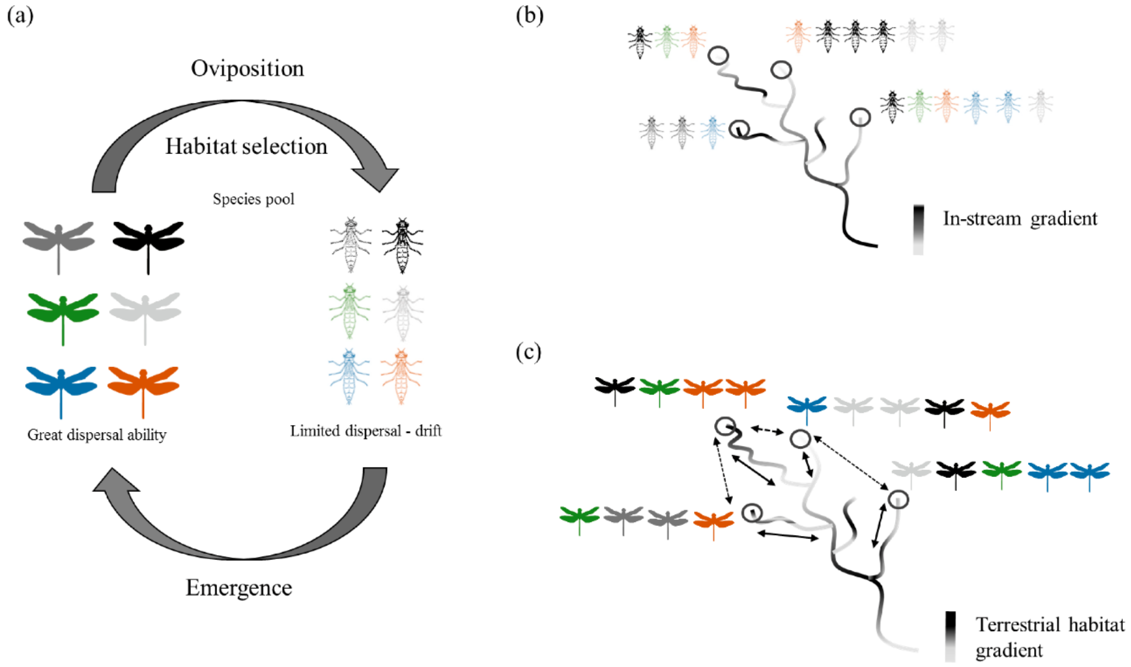

Fig. 6.1. Hypothetical explanation for larvae and adult of odonate distribution along a riverine network. (a) Biological cycle of odonates, including some life-stage characteristics: adults have great dispersal ability and females select better habitats for egg and larval development; larvae has restrict dispersal. (b) and (c) Both larvae and adults would be affected by environmental factors due to their biological characteristics and the connection between the two phases by oviposition behavior of females. However, as they have different niches, we predict that (b) larvae would be affected by in-stream environmental predictors and (c) adults would be influenced by terrestrial habitat predictors.

Material and Methods

Data collection

We carried out this study in a riverine network (Betione watershed, Bodoquena Plateau), located in the southwest of Mato Grosso do Sul State, Brazil (see Chpter 1, Fig. 5.2). The region was composed of a transition between Cerrado and Atlantic Rainforest biomes. Currently, the region is fragmented, mainly due to land conversion to cattle ranching. In general, the Betione drainage basin has karstic waters, no sewage release, and a gradient of forest cover ranging from dense riparian vegetation to different degrees of deforestation. All streams sampled had running water, but 9 streams did not maintain water surface connection with the network, due to the karstic soil. We prefer to use this term (no water surface connection) instead of intermittency, since we did not know if this is a repeated temporal pattern or even if streams may be connected by subterranean flow.

We used a nested sampling strategy by stream order to capture the structure of the network, i.e., each segment of the riverine network was selected and sampled, totaling 44 streams and rivers of the Betione riverine network. We sampled both larvae and adult odonates in the same sites. For larvae, we used the multi-habitat approach (Barbour *et al*. 1996), consisting of the sum of 20 sampling units per stream/river proportionally distributed between different habitats. We used three reaches of 10 m to estimate the proportion of the following habitats: rock outcrops, rock cobble, gravel, sand, mud silt, organic matter, wood, aquatic vegetation, leaf litter and roots. We then calculated the mean for the 30m reach and distributed proportionally the 20 sampling units among habitats. Each sampling unit consisted of 1 m length using a kick-sampling (0.5 mm mesh size; covering 0.3 m^2), totaling a sampling effort of 6 m^2 of stream/river bottom. Larvae were identified to genus level using taxonomic keys (Costa, Souza & Oldrini 2004; Heckman 2006, 2008; Neiss & Hamada 2014).

We actively collected the adults during 1 hour using an entomological net along a 100 m transect parallel to the stream banks. Samplings were conducted once at each site on sunny days between 10:00 and 15:00h, due to known thermal restrictions in the odonates. This method has been effectively used in other studies (e.g. Juen & De Marco 2011). The identification of specimens to species or morphotype followed taxonomic keys and original descriptions (Heckman 2006, 2008; Lencioni 2005, 2006). Voucher specimens are deposited in UFMS’s Zoological Collection (ZUFMS).

Environmental matrix

We measured abiotic environmental variables, such as stream width, depth and flow once every 6 meters along the same 30 m reach that the larvae samples were collected, totaling 5 measures. Altitude was measured once; in-streams variables (temperature, conductivity, pH, dissolved oxygen and turbidity) were measured using a Hanna HI 9828 multiparamenter probe three times (once every 10 m), as well as percentage of substrates. We estimated shading using 3 digital photographs of the canopy that was taken from the middle of the stream/river. The images were analyzed with ImageJ (version 1.47, Wayne Rasband, National Institute of Health, USA). We first transformed the image to black and white scale, after we measure the white pixels area of each photograph, i.e., the area that light pass through the canopy. We estimated the percentage of forest using ArcGis (version 10.1 ESRI, Redlands, California, USA) and a digital map of forest coverage with 30 m resolution provided by Environmental Institute of state of Mato Grosso do Sul (IMASUL 2014). A buffer of 250 m was used from the middle of each sampling point to estimate the percentage of forest cover. This distance was chosen considering that some adult damselflies use short distances to disperse (≤ 500 m; Keller, Van Strien & Holderegger 2012; Keller & Holderegger 2013). Streams not connected by superficial water flow to riverine network were categorized (i.e. connected or unconnected) and used as dummy variables in data analysis, named here “connection”. We grouped the proportion of some substrates classes as follows: rocks (>256 mm; composed by rock outcrops and rock cobble), gravel (2-255mm), sand (0.125–2 mm), mud (<0.125; composed by mud silt and organic matter) and organic substrates (wood, aquatic vegetation, leaf litter and roots). These substrates classes were usesd as environmental predictors.

We used macroinvertebrate abundance as a measure of prey availability for odonate larvae. Macroinvertebrates were collected following the same sampling methodology applied for odonate larvae. We included the following orders, known as odonate prey: Diptera, Ephemeroptera, Plecoptera, Trichoptera, Oligochaeta, Odonata and Coleoptera (Corbet 1999).

We separated the environmental variables in two groups, one related to important terrestrial variables, such as habitat structure for adults, and another one related to in-stream variables, more related to larvae habitat. Mantel test using

Euclidean distance as measure showed no correlation between these two environmental components (r=-0.01; p=0.46), enabling us to interpret their influence on community composition independently. Altitude, width, percentage of forest, luminosity, abundance of adults were grouped into the component named terrestrial habitat variables (E_T), because these variables are related to sunlight availability, perching structures, reproduction and thermoregulation sites for adults (Corbet, 1999; De Marco, Batista & Cabette 2015). In-water variables were formed by the following variables: temperature, conductivity, pH, dissolved oxygen, water velocity, percentage of substrates (gravel, sand, organic matter, organic substrates), connection and prey abundance (component E_S) (see Supporting Information, Table S6.1 for the variation of each environmental variable).

Distances calculation and spatial matrices

We based our riverine network in three sources of information: geographic position of sites along stream/river branches obtained in the fieldwork, high resolution (1 m) Google Earth images and, as a baseline, we used the Betione network flowline vector provided by Brazilian Institute of Geography and Statistics (IBGE 2014)(scale 1:250,000). Based on our fieldwork and Google Earth images, we manually corrected distortions in Betione network from IBGE source using a small spatial scale (1:10,000). This ensured a more accurate position of each branch in the riverine network.

We calculated overland distance (the shortest distance between sites in two-dimensional space) using the Euclidean distance among geographic coordinates of all sampling sites. Watercourse distance (the distance between sites following the riverine dendritic network) among all sites was calculated using ArcGis (version 10.1 ESRI, Redlands, California, USA) (Network Analysis Toolbox⁄OD Cost Matrix Analysis Tool).

The spatial structure of sampling sites based on overland (S_O) and watercourse (S_W) distance was modelled using distance-based Moran’s eigenvector maps (dbMEM; Borcard & Legendre 2002; Dray, Legendre & Peres-Neto 2006), which decompose spatial distances into orthogonal variables (eigenvectors). First, we used the maximum distance from each distance matrix in the minimum spanning tree that maintains all sites connected (overland=7.8km; watercourse=14.17km) as the truncation distance threshold. The truncated matrices were submitted to a Principal Coordinate Analysis (PCoA), producing eigenvectors. We selected eigenvectors with significant patterns of spatial autocorrelation, i.e., with significate ($P < 0.05$) and positive Moran’s I (see Sokal & Oden 1978). These eigenvectors represent distinct spatial patterns, from broad to fine-scale patterns of relationships among sampling sites, that are both mutually orthogonal and linearly unrelated (Dray, Legendre & Peres-Neto 2006; Griffith & Peres-Neto 2006). The selected eigenvectors were used as spatial explanatory variables in our analyses (see Borcard & Legendre 2002).

Data analysis

The abundance of genera of both larvae and adults were the response matrices. They were standardized using Hellinger transformation prior to statistical analysis, because this method provide a more suitable dataset to linear-based analyses, such as redundancy analysis (Legendre & Gallagher 2001).

We used a set of redundancy analysis (RDA) coupled with forward selection to verify if larval and adult stages were affected by environmental variables and or spatial configuration of sites (spatial eigenvectors based on overland and watercourse distances). This is commonly tested using a variation partitioning applied to RDA. However, variation partitioning is only required when both biological and environmental matrices are spatially structured, which would be necessary to test environment component filtering out the spatial variation (Legendre *et al*. 2002; Peres-Neto & Legendre 2010). Thus, we adopt a stepwise approach. First, we tested for the presence of spatial signals on biological matrices (larvae and adults separately) by fitting spatial redundancy analysis (RDA) global models using eigenvectors based on Euclidean and water distances. The following analysis is dependent of the significance of spatial global models.

If the spatial global models were not significant (i.e., species are not spatially structured), we just carried out environmental RDA global models, because spatial autocorrelation in just environmental variables does not affect significance of regression coefficients (Legendre *et al*. 2002). Following this logic, we ran environmental RDA global models separately using terrestrial habitat and in-stream variables. If these global models were significant, we selected the most important variables using forward selection implemented by Blanchet, Legendre & Borcard (2008). Then, we refitted a RDA environmental model using only the selected variables.

If spatial global models were significant (i.e., species are spatially structured), we selected the most important spatial variables by forward selection (Blanchet, Legendre & Borcard 2008). Then, we proceeded with a variation partitioning applied to RDA to decompose the relative importance of selected environmental and spatial variables in explaining the variation in the composition of larvae and adults of odonates (Borcard, Legendre & Drapeau 1992).

In the forward selection used here, the selection of variables is stopped if a candidate variable had a non-significant p-value ($p>0.05$) or if the adjusted R^2 of the reduced model was over the value of the adjusted R^2 of the global model (see details in Blanchet, Legendre & Borcard 2008). This forward selection method reduces the probability of Type I error and overestimation of the explained variance (Blanchet, Legendre & Borcard 2008). All analyses were performed in R software, using vegan (Oksanen *et al*., 2014), packfor (Dray, Legendre & Blanchet 2011) and PCNM packages (Legendre *et al*. 2013).

Results

We sampled 1227 adult individuals, representing 21 genera (see Supporting information of Chapter 1, Table S5.2). Damselflies were the most abundant suborder, comprising 1099 individuals. The most abundant genera were *Argia* (310 individuals), *Hetaerina* (170 individuals), *Oxyagrion* (168 individuals) and some of the least abundant (<5 individuals) included: *Aeolagrion, Elga, Metaleptobasis* and *Tigriagrion*.

We sampled 780 larval individuals comprising 32 taxa (see Supporting information of Chapter 1, Table S5.2,). Dragonflies were more abundant (452 individuals) than damselflies (328 individuals). Coenagrionidae sp, *Argia* and Libellulidae sp were the most abundant larvae with, respectively, 195, 87 and 87 individuals. Some genera exhibiting low abundance (< 5 individuals) included *Castoraeschna*, *Anax*, *Rhionaeshna*, *Planiplax* and *Oligoclada*.

Both watercourse and overland distances used in distance-based Moran's eigenvector maps generated seven eigenvectors with positive and significant Moran's *I*. Spatial matrices based in this two distances did not explain the variation in composition of either larvae (S_L: p=0.82, Adjusted R^2=-0.02; S_W: p=0.60, Adjusted R^2=-0.008) or adults (S_L: p=0.28, Adjusted R^2=0.01; S_W: p=0.10, Adjusted R^2=0.04). In this way, larvae and adult distribution was not spatially structured and we proceed with environmental analysis. Terrestrial habitat and in-water components explained significantly the variation in community composition of larvae and adults (Table 6.1). For larvae community variation, the most important variable of terrestrial component was percentage of vegetation; dissolved oxygen, percentage of gravel, velocity, prey abundance and pH were selected in the in-stream component. Width and altitude (terrestrial habitat) and velocity (in-stream) were the most important variables for adult distribution. Reduced models based on terrestrial habitat and in-water selected variables explained significantly the distribution of both larvae and adults. Terrestrial habitat component explained more variation of adult community (adjusted R^2=0.10) than larvae (adjusted R^2=0.03). In-water component explained an equal amount of variance for larvae (adjusted R^2=0.11) and adult (adjusted R^2=0.10). Most of the variation of both larvae and adults community was unexplained by any of the measured variables (Table 6.2).

Table 1. Results of redundancy analysis global models using spatial and environmental components and further forward selection of the most important variables to explain distribution variation of larvae and adults odonates. Explanatory variables used in the terrestrial habitat variables (E_T) global test were: altitude, width, percentage of forest and luminosity; in-water variables (E_S) used in the environmental global test: temperature, velocity, conductivity, connection, pH, dissolved oxygen (DO), prey abundance and percentage of substrates (organic material, organic substrates, gravel, sand). The spatial components, resulted from distance-based Moran's eigenvector maps using watercourse (S_{Wc}) and overland distances (S_L), included seven eigenvectors (variables) each one.

Response matrix	Global model	Adj R^2	P	Variables selected
Larvae	S_{Wc}	0.0008	Ns	-
	S_L	-0.02	Ns	-
	E_T	0.05	0.004	Vegetation

	E_S	0.12	0.001	DO, gravel, velocity, prey abundance, pH
Adult	S_W	0.04	Ns	-
	S_L	0.003	Ns	-
	E_S	0.09	0.001	Width, altitude
	E_W	0.08	0.04	Velocity

Table 2. Reduced environmental RDA models using variables retained by the forward selection procedure independently for terrestrial habitat (E_T) and in-stream (E_S) components used for explaining variation on larvae and adults distribution.

Response matrix	Environmental component	Variable(s) used	Adj R^2	F	p	Residual
Larvae	E_T	Vegetation	0.03	2.279	0.01	0.97
	E_S	DO, gravel, velocity, prey abundance, pH	0.12	2.151	0.001	0.89
Adult	E_T	Width, Altitude	0.11	3.780	0.001	0.89
	E_S	Velocity	0.10	5.582	0.001	0.90

Discussion

Our results indicate that the variation in larvae and adult composition are related to environmental factors and not related to high or limited dispersal. Both in-water and terrestrial structure environmental components explained variation in both larvae and adults composition. In the metacommunity framework used here and following our predictions, these results suggested that the same process affect both life stages and could be interpreted as metamorphosis is not a new beginning (Pechenik 2006). The use of different spatial configurations (overland and watercourse) reinforce the absence of dispersal related process structuring odonates community. Previous studies assessing ecological factors affecting separately on larvae or adults suggested that local factors were responsible for structuring separately larvae and adults of odonates (Siepielski & McPeek 2012; Monteiro-Júnior, Juen & Hamada 2015; Dutra & De Marco, 2015; De

Marco, Batista & Cabette 2015; Mendes, Cabette & Juen 2015). In our case, assessing larvae and adults simultaneously and at the same sites allow us to identify common patterns and possible processes governing variation in community of larvae and adults.

Processes shaping metacommunity patterns in riverine networks are context dependent (Brown & Swan 2010; Carrara *et al*. 2012; Heino et al. 2012; Göthe, Angeler & Sandin 2013; Carrara *et al*. 2014). For example, macroinvertebrates in stream networks are structured by species sorting in upstream habitats (Brown & Swan 2010; Göthe, Angeler & Sandin 2013) and by mass effects and dispersal limitation in the whole catchment scale (Göthe, Angeler & Sandin 2013). Recent studies using different spatial configurations also indicated the role of environmental processes structuring metacomunities (Cañedo-Arguëlles *et al.* 2015; Karna *et al*. 2015). In general, our results agree with these recent studies and with the common established role of species sorting shaping metacommunities (Cottenie 2005; Logue *et al*. 2011; but see Heino *et al*. 2015), even using different types of spatial configuration (i.e., spatial variables based on overland and water distances). The riverine network used in this study exhibited a wide range of environmental conditions (e.g. altitude, percentage of forest and in-water variables), which together with sufficient dispersal of adults, allowed species to be sorted along the environment gradient. Further, this study area comprises a small to intermediate spatial extent (~1200 km^2) for odonates where species dispersal could be enough to achieve their preferred habitats. From an odonate perspective, our spatial extent is neither small enough for the effect of high dispersal, leading to community homogenization, nor large enough for spatial patterns caused by dispersal limitation (Heino *et al*. 2012; Heino *et al*. 2014).

In general, stream communities are controlled by multi-scale environmental filters (Poff 1997). Our results give support to this view, because both in-stream and terrestrial environmental components explained variation in community composition of both larvae and adults. In-water environmental component has a relatively stronger influence on larvae composition variation compared with terrestrial variables, as we predicted. In-water variables (dissolved oxygen, velocity, pH and percentage of gravel) are related to adequate environment for larval development and survivorship. For example, odonates differ in their tolerance and sensitivity to dissolved oxygen levels, such as the damselflies *Platycnemis pennipes* and *Enallagma cyathigerum*, which comparatively have high and low oxygen requirement, respectively (Steiner *et al*. 2000). Forest cover – the only terrestrial variable retained for larvae – affects the input of organic substrates, fundamental to larvae distribution, considering that they have variable habitat preferences, including burrowing, climbing, grabbing and free living (Corbet, 1999).

Unlike larvae, adults were equally explained by in-stream and terrestrial environmental components. Terrestrial environmental component has a direct relationship with sites for reproduction, feeding, and thermoregulation. Width and altitude were the two terrestrial variables selected for adults and they may be considered surrogates for upstream-downstream gradient, i.e., composition variation of adults change at some extent along the longitudinal profile of the riverine network used here. In fact, adults have different eco-physiological requirements and behaviors for thermoregulation along longitudinal gradient, creating a variation in community composition (De Marco, Batista & Cabette 2015). Narrow and shaded streams has relative more small perchers dependent on air temperature and able to thermoregulate in shaded areas, while large rivers with high incidence of sunlight is predominantly composed by larger perchers (De Marco, Batista & Cabette 2015). In-stream component do not affect other life-history processes of adults than female selection for suitable oviposition sites. Interestingly, water velocity was selected as important in-stream variables for both larvae and adults, meaning that adults may select suitable habitat based on water velocity for their offspring development. For example, *Calopteryx splendens* exhibits preference for laying eggs in fast-flowing waters, which increases egg development significantly and decreases mortality (Gibbons & Pain 1992; Siva-Jothy, Gibbons & Pain 1995). Thus, larvae and adults may have some similar niche requirements and ontogenetic niche shifts did not restart which ecological processes affect these different stages, i.e., metamorphosis might not necessarily be a new beginning (Pechenik 2006; Benard & McCauley 2008). In a more applied issue, such as biomonitoring, these results may indicate that the use of both life stages could be viable and one life stage could be used as surrogate of the other (see Valente-Neto *et al*. 2015).

Variation in community composition of larvae was not explained by any spatial predictors (explanation values were close to zero), despite their limited mobility. This reinforces that adults are the main dispersal life stage, since they compensate the low mobility of larval stage. Interestingly, adults composition were more explained by spatial predictors than larvae, although both spatial components were not significant. In general, pure spatial variables explained a little percentage of community variation in aquatic insects (Logue *et al*. 2011; Heino *et al*. 2014), but this depends on the type of habitats, its structure, the organisms group examined, their dispersal abilities and spatial extent (De Bie *et al*. 2012; Heino *et al*. 2014). Indeed, for odonates in lotic systems, some studies showed that spatial variables were not important in magnitude or they did not explain significantly variation in community composition (McCauley *et al*. 2008; Curry & Baird 2015), whereas other demonstrated strong dispersal limitation in a smaller spatial extent than that used here (McPeek 1989; McCauley 2006). The hierarchical nature of riverine network and the spatial extent used in this study could facilitate enough dispersal between habitats that allow species to track environmental variation.

Despite we detected that both larvae and adults were explained by in-stream and terrestrial environmental components, they have a small predictive power. This is not surprising considering that many others studies showed that environmental component when controlling for the effect of spatial correlation explained a low percentage of variation in aquatic insects composition (Beisner *et al*., 2006; Göthe *et al*., 2013; Grönroos *et al*., 2013; Heino *et al.*, 2015), including for odonates in lotic systems (Curry & Baird 2015). First, environmental gradients used here may not be strong enough to produce biological variation. However, we included different set of environmental variables recognized to affect variation in odonates community composition (e.g. Curry & Baird 2015; De Marco, Batista & Cabette 2015) distributed along a riverine network gradient, i.e., since headwater to mainstem. Second, variation in community composition in streams could be due to high variability in stream hydrology as well as in environmental heterogeneity, as hypothesized by Heino *et al*. (2015) using a large dataset of stream insects distributed around the world. This would create a large number of rare species in local communities in a certain point in time and single snapshot studies, such as this study, would not detect or would detect a low explanation of environmental predictors on biological community variation (Erős *et al*. 2012). Several samplings of the community composition of odonates may include important compositional information for this dynamic group and future studies should consider that (Erős *et al*. 2012).

In conclusion, we found that similar environmental factors are determinant for both larval and adult distribution, and suggest that metamorphosis might not necessarily be a new beginning. The absence of spatial signal on assemblage variation using different spatial configurations (overland and in-water) suggests that odonates may track variation in environmental conditions in the spatial extent of our study area. Larvae have low dispersal ability and drift propensity, thus their dispersal would be mainly via adult females dispersal and oviposition. Our study open new venues to understanding metacommunity drivers of organism that undergo ontogenetic niche shift.

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Supporting Information

Table S6.1. Mean, standard deviation (SD) and range of all environmental variable along 44 sites in Betione riverine network.

Variable	Mean (SD)	Range
Altitude (m)	258.8 (66.0)	118-358
Width (cm)	224.4 (147.3)	49.6-728
Velocity (m/s)	0.1 (0.08)	0.01-0.4
Temperature (ºC)	24.8 (1.9)	21.7-31.21
Conductivity (µS.cm^{-1})	462.0 (170.3)	32.3-717
pH	7.6 (0.4)	6.6-8.5
Dissolved oxygen (mg/l)	3.9 (1.7)	0.5-7.7
Vegetation (%)	41.1 (22.2)	0-100
Gravel (%)	33.7(22.1)	0-80
Sand (%)	13.7(13.7)	0-40
Organic matter (%)	12.7(15.1)	0-55
Organic substrates (%)	31.3(15.2)	5-76.6
Luminosity (%)	15.8 (11.4)	7-74.9
Prey abundance (abundance)	429.5(494.4)	15-2588
Connection (dummy)	0.8 (0.4)	0-1

7. Chapter 3

From linear to a network perspective: does landscape centrality improve our detection of spatial processes in empirical riverine metacommunities?***

Abstract

Dispersal is a key factor in metacommunity ecology. However, dispersal-related processes have not been detected so frequently as niche processes, which could be related to the use of simple dispersal proxies, such as Euclidean and watercourse distances. Riverine networks could be better described using graph network measures, because it captures how sampling sites connect to each other, from more central sites to more isolated ones. We investigated whether environment and/or spatial configuration of sites, calculated by overland, watercourse and centrality, are determinants of dissimilarity of aquatic insects in a dendritic network. We tested if centrality measure (closeness) improved the explicability of dispersal-related processes compared to overland and watercourse distances. Also, we tested if dispersal ability of species affected the outcome of spatial and niche processes on community dissimilarities between sites. We used a nested sampling strategy by stream order to capture the structure of the network, i.e., each segment of the riverine network was selected and sampled, totaling 46 streams and rivers. The influence of spatial predictors and environmental factors were assessed through distance decay relationship and Mantel and partial Mantel tests. In general, our results pointed for a environmental control of community dissimilarity between sites and that community dissimilarity were

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***I wrote this article in collaboration with Fabio de Oliveira Roque and Christopher Swan.

very high at small spatial scales. Spatial predictors have little effect on the community dissimilarity-environmental distance relationship and closeness did not improve the explicability of dispersal-related process compared to overland and watercourse distances. Dispersal ability of aquatic insects did not change the strength of environmental control on driving community dissimilarity. The intermediate spatial extent of our study together with the connection among streams by water may be enough for different dispersal ability groups track environmental variation between sites in this dendritic network.

Key words: aquatic insects, dendritic network, environmental control, graph theory

Introduction

Dispersal is a key factor for maintaining biodiversity (MacArthur & Wilson 1967; Cadotte 2006b) and is one of the central concept in the metacommunity framework (Leibold *et al*. 2004). This is related to its strength to modify local community dynamics through colonization and immigration, adding species to local communities (MacArthur & Wilson 1967; Cottenie *et al*. 2003; Mouquet & Loreau 2003; Leibold *et al*. 2004; Cadotte 2006a, 2006b). At the same spatial extent, species dispersal ability and metacommunity configuration, i.e. isolation and connectedness, interact and affect species dispersal rates (MacArthur & Wilson 1967; Cottenie *et al*. 2003; Mouquet & Loreau 2003; Heino *et al*. 2014). For example, a general pattern is that community dissimilarity increases with distance, a classical extension of the first geography law – all is related with everything, but near things are more related than distant ones (Tobler 1970). As distance increases, habitats becomeless connected and species could become dispersal limited (Nekola & White 1999; Soininen *et al*. 2007). More connected habitats could increase the strength of spatial process and environmental control driving metacommunity structure, since connectivity facilitates species moving around and could maintain source-sink dynamics or even species sorting (e.g. Göthe, Angeler & Sandin 2013). Despite these theoretical and empirical predictions about spatial process affecting metacommunity structure, dispersal continues to be a notoriously difficult process to measure for entire community, consequently, there is little information about dispersal rate between communities (Jacobson & Peres-Neto 2010). In this context, an alternative way is measuring proxies that correlate to or even affect dispersal rates, an approach commonly applied in metacommunity studies (see Jacobson & Peres-Neto 2010; Heino *et al*. 2014).

Dendritic riverine networks are naturally complex hierarchical systems connected by water, in which headwater coalesce to form large rivers. In these networks some organisms, such as aquatic insects, could use different routes for dispersal, being overland (flight by adults) and watercourse (larvae and adult drift and adults flying). So, different spatial configuration and structure of riverine networks could be assessed and revealed complex aquatic metacommunities patterns (Landeiro *et al*. 2011; Brown & Swan 2010; Göthe, Angeler & Sandin 2013). In this way, the detection of spatial process may be dependent to which spatial predictors are included in metacommunity studies. This issue have received a lot of attention recently (Cañedo-Argüelles *et al*. 2015; Kärnä *et al*. 2015; Morán-Ordóñez *et al*. 2015), because the lack of finding spatial processes affecting metacommunities structure (see reviews Cottenie 2005; Logue *et al*. 2011; Heino *et al*. 2014) or their unpredictability (Heino *et al*. 2015) could be related to the use of simple measures of spatial configuration (e.g. Euclidean distance). Besides Euclidean and watercourse distances, some empirical metacommunity studies include asymmetric eigenvector maps as spatial components, showing that these measures could capture better spatial patterns than overland distances (Göthe, Angeler & Sandin 2013; Liu *et al*. 2013). Further, the use of landscape resistance based in topography showed both a little (Cañedo-Argüelles *et al*. 2015; Kärnä *et al*. 2015) and great improvement (Morán-Ordóñez *et al*. 2015) in the explanation of spatial component compared to Euclidean and watercourse distances.

A more intuitive way to think spatial configuration of riverine networks is through graph theory, because a network can be treated as a hierarchical organized system composed of nodes connected by links along which individuals or resources flow (Urban & Keitt 2001; Grant, Lowe & Fagan 2007). For instance, connectivity measures, such as centrality ones, captures how sample sites connect to each other, from more central sites to more isolated ones (Carrara *et al*. 2014), is useful to detect spatial processes and relatively simple index that could be incorporated in metacommunity studies (Carrara *et al*. 2012; Carrara *et al*. 2014; Seymour, Fronhofer & Altermatt 2015). Most knowledge about centrality and graph measures as spatial predictors of community (dis)similarity have been done with experimental microcosm systems (Carrara *et al*. 2012; Carrara *et al*. 2014; Seymour, Fronhofer & Altermatt 2015; but see Altermatt, Seymour & Martinez 2013), that, despite of great importance to understanding complex systems, do not allow for direct extrapolation to natural systems. This opens a good avenue for testing graph theory related measures as another source of spatial configuration in empirical metacommunity studies.

Although spatial configuration may affect dispersal rates, species perceive a given spatial configuration differently and have different dispersal abilities, which could interfere in interpreting the relative roles of spatial processes and environmental constraints on community structure (Lowe & McPeek 2014). In general, organisms with high dispersal ability lead to more similar local communities, because of homogenizing effects (Cadotte *et al*. 2006b), while low dispersal ability may limit species to certain habitats, generating local communities comprised of different species. For example, strong dispersers can actively select habitats, while intermediate to weak aerial dispersers are relatively less affected by environmental control and the role of spatial processes increase due to dispersal limitation (De Bie *et al*. 2012; Heino 2013; Padial *et al*. 2014).

We aimed to understand whether environment and/or spatial configuration of sites, calculated by overland, watercourse and centrality, are determinants of dissimilarity of aquatic insects in a dendritic network. We hypothesized that:

i) Environmental difference between sites would be the most important factor explaining dissimilarity patterns, since: a) intermediate spatial scales, such as the riverine networks used here, increase the influence of species sorting (Heino *et al*. 2014); b) the longitudinal gradient of riverine network has a wide range of environmental conditions, where species can track different environmental conditions;

ii) Overland and watercourse distances would be highly correlated (Cañedo-Argüelles *et al*. 2015; Kärnä *et al*. 2015) and, consequently, they would explain similar amounts of variation in dissimilarity between sites. We expected that centrality (closeness) would be more important for explaining community variation than overland and watercourse distances after taking into account the effect of environmental distance, because it captures how sample sites are connected to each other, from more central sites to more isolated ones;

iii) Dispersal ability of aquatic insects could modify the outcome of first hypothesis (Brow & Swan 2010; Heino 2013). Insects with high dispersal capacity are able to find suitable sites along the environmental gradient and they would be relatively more affected by environmental differences between sites, being the least affected by spatial distances between sites, while those with low dispersal ability would be dispersal limited to certain habitats, reinforcing the effect of spatial configuration and decreasing the strength of environmental control. Medium dispersal ability group would have intermediate effects of both environmental and spatial distances between sites.

Material and Methods

STUDY AREA AND SAMPLING

The study was carried out in a riverine network (Betione watershed) in Bodoquena Plateau, located in the southwest of Mato Grosso do Sul, Brazil. The region was originally composed of a transition between Cerrado and Atlantic Rainforest forests and, currently, it is affected by land conversion to cattle ranching. In general, Betione drainage basin has karstic waters, no sewage release, and different degrees of deforestation. Due to the karstic soil, we detected 10 streams that did not maintain water surface connection with the network. We prefer to use this term (no water surface connection) instead of intermittency, since we did not know if this is a repeated temporal pattern.

We used a nested sampling strategy by stream order to capture the structure of the network, i.e., each segment of the riverine network was selected and sampled, totaling 46 streams and rivers of the Betione riverine network. We sampled aquatic insects using the multi-habitat approach (Barbour *et al*. 1996), consisting of the sum of 20 sampling units per stream/river proportionally distributed between different habitats. We used three reaches of 10 m to estimate the proportion of the following habitats: rock outcrops, rock cobble, gravel, sand, mud silt, organic matter, wood, aquatic vegetation, leaf litter and roots. We then calculated the mean for the 30 m reach and distributed proportionally the 20 sampling units among habitats. Each sampling unit consisted of 1 m length using a kick-sampling (0.5mm mesh size; covering 0.3m^2), totaling a sampling effort of 6 m^2 of stream/river bottom. Aquatic insects were identified to genus level using taxonomic keys (Domínguez and Fernández 2009; Segura, Valente-Neto &

Fonseca-Gessner 2011; Trivinho-Strixino2011; Hamada, Nessimian & Quirino 2014; Salles & Lima 2014).

ENVIRONMENTAL VARIABLES

We measured stream width, depth and flow once every 6 meters along the same 30 m reach that the samples were collected, totaling 5 measures. Altitude was measured once; in-streams variables (temperature, conductivity, pH, dissolved oxygen, salinity and turbidity) were measured using a Hanna HI 9828 multiparamenter three times (once every 10m), as well as percentage of substrate cover (visually estimated). Luminosity was estimated using 3 digital photographs of the canopy that was taken from the middle of the stream/river. The images were analyzed with ImageJ (version 1.47, Wayne Rasband, National Institute of Health, USA), by first transforming the image to black and white scale and then measuring the white pixels area of each photograph, i.e., the area that light pass through the canopy. The percentage of forest was calculated using ArcGis (version 10.1 ESRI, Redlands, California, USA) and a digital map of forest coverage with 30m resolution provided by Environmental Institute of state of Mato Grosso do Sul (IMASUL 2014). A buffer of 250m was used from the middle of each sampling point to estimate the percentage of forest cover. All streams sampled had running water, but some were not connected in the riverine network. We used dummy variable for characterizing streams without water surface connection with the network (i.e. connected or unconnected), named here “connection”. Note that, because the study area was located in a karstic region, streams may be connected by subterranean flow. In case of environmental variables that were measure twice or more, we used the mean as environmental predictor.

We grouped some of the substrates classes as follows: rocks (>256 mm; composed by rock outcrops and rock cobble), gravel (2-255mm), sand (0.125–2 mm), mud (<0.125; composed by mud silt and organic matter) and organic substrates (wood, aquatic vegetation, leaf litter and roots). These substrate classes were used as environmental variables.

DATA PREPARATION

Distance matrices

We characterized our riverine network on three sources of information: geographic position of sites along stream/river branches obtained in the fieldwork, high resolution (1m) Google Earth images and, as a baseline, we used the Betione network flowline vector provided by Brazilian Institute of Geography and Statistics (IBGE 2014)(scale 1:250,000). Based on our fieldwork and Google Earth images, we manually corrected distortions in Betione network from IBGE source using a small spatial scale (1:10,000). This ensured a more accurate position of each branch in the riverine network.

We calculated distance matrices between sites based on Euclidean distance (overland) (the shortest distance between sites in two-dimensional space) and watercourse distance (the distance between sites following the riverine dendritic network). For the overland distance, we created a distance matrix (Euclidean distance) between sampling points using the geographic coordinates. Watercourse distances between sites were calculated using ArcGis (version 10.1 ESRI, Redlands, California, USA) (Network Analysis Toolbox⁄OD Cost Matrix Analysis Tool).

Closeness matrix

We constructed an adjacency matrix (sites by sites) connecting sampling points, i.e., a symmetric matrix connecting adjacent sampling points by the corresponding watercourse distance value between them. We considered each sampling site as a node and watercourse distances between sites as edges.

Centrality measures attempt to capture the degree of connectedness or isolation of a node in a network graph. Closeness is defined as the inverse of mean distance of one specific site to all other sites, thus, highest values are more central in the network – close to many other nodes (Fig. 7.1). As an inverse measure, closeness could assume low values, so we multiply them by 1000. Using these values, we then calculated a distance matrix between sampling points using Euclidean distance. We performed the analysis in R software, using the igraph package (Csardi & Nepusz 2006)

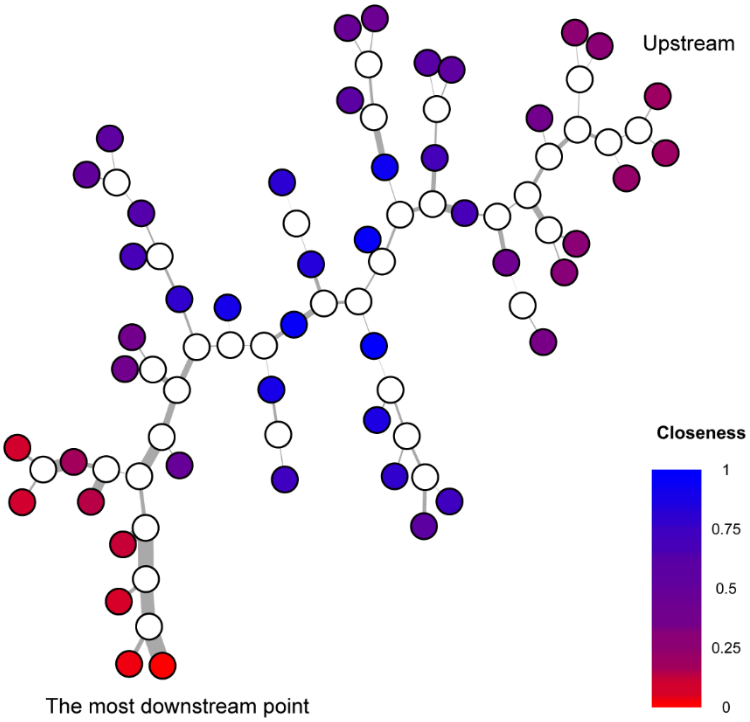

Fig. 7.1. Graph depicting closeness values for all sampling points. White circles are confluences (not sampled) and width of each edge is weighted by the respective watercourse distance (i.e. as wider the edge, more distant two points are to each other).

Species and environmental matrices

In addition to the total species composition and to know the influence of dispersal ability on the outcome of the relative role of environmental and spatial distances on community dissimilarity, we deconstructed aquatic insect species composition according to their flight dispersal ability based on literature and size (Poff *et al*. 2006; Heino 2013; Saito *et al*. 2015). We separated taxa into low (i.e. riffle beetles and midges, including chironomids), medium (mayflies, most of caddisflies, stoneflies and most Hemiptera) and high directional flight ability (odonates, some caddisflies, diving/scavenger beetles and Belostomatidae). For all species compositional matrices (total and low, medium and high flight dispersal abilities) we used Bray-Curtis index to calculated pairwise dissimilarity of abundance data between sampling sites.

Predictors included in environmental component were: altitude, width, percentage of forest, luminosity, in-water variables (temperature, water velocity, conductivity, pH and dissolved oxygen) and percentage of substrates (rocks, gravel, sand, mud, organic substrates). These variables were used to calculate pairwise Euclidean distances between sampling sites.

DATA ANALYSIS

We used the function bioenv from vegan package (Oksanen *et al.*, 2013) to select environmental variables with the maximum correlation with community dissimilarities matrices (total, low, medium and high). This function generates all possible subsets of environmental variables, scaling the variables and calculating Euclidean distances with each subset and then it finds the subset with the greatest correlation with community dissimilarities (Bray-Curtis).

We used Mantel tests between all spatial predictors matrices to understand their relationship and to understand the correlation between spatial distance matrices. To assess our first and third hypothesis, we used distance decay relationship (DDR) to relate species compositions dissimilarity to spatial predictors and environmental. This approach is useful because it estimates the rate of composition change through space (Nekola & White 1999; Soininen *et al.* 2007). The intercept or initial (dis)similarity reflects beta diversity or turnover at small spatial extents (Soininen *et al.* 2007). In case of dissimilarity measures, high values indicate high turnover at small scales, generally resulting from environmental gradient or dispersal limitation (Soininen *et al.* 2007). Pairwise data are inherently non-independent, so all regression statistics were tested using permutation tests (Manly 1991) with 1000 random permutations. We shuffled the y-values between the x-values and re-estimates the slope. Then, the significance was calculated by comparing the observed values with that generated by permutations.

Complementary to linear regression approach and to tease apart environmental from spatial effects on fauna dissimilarity, we used partial Mantel tests. First, each spatial distance matrix was used as explanatory variable of each dissimilarity matrix controlling for the effect of environmental distance matrix. Conversely, each dissimilarity matrix was correlated with environmental distance matrix accounting for the effect of each spatial distance matrix.

Results

We sampled 17,560 aquatic insects, representing 199 taxa. Chironomidae (Diptera) was the most abundant and rich taxa, comprising 7640 individuals belonging to 65 genera. Following chironomids, mayflies (4956 individuals, representing 21 genera), riffle beetles (1823 individuals from 12 different genera) and caddisflies (823 individuals belonging to 17 genera) were highly representative taxa. Some groups were low in abundance, such as the stoneflies.

The environmental variables vary considerably among sampling sites (Table 7.1), such as width, because our study area range from headwaters to mainstems. Some of in-stream variables also have a high range, such as conductivity and dissolved oxygen, while others were less variable (temperature, pH and velocity). In those cases, streams could be classified as having warm, neutral to alkaline and low velocity waters. Variables related to forest structure (% forest cover and % of luminosity) also have a high range as well as percentage of different substrates. Closeness (Fig. 7.1), overland and watercourse distances also varies between sites.

Table 7.1. Mean, standard deviation (SD) and range of all environmental variable along 46 sites in Betione riverine network.

Variable	Mean (SD)	Range
Altitude (m)	258.8 (66.0)	118-358
Width (cm)	224.4 (147.3)	49.6-728
Velocity (m/s)	0.1 (0.08)	0.01-0.4
Temperature (ºC)	24.8 (1.9)	21.7-31.21
Conductivity (µS.cm^{-1})	462.0 (170.3)	32.3-717
pH	7.6 (0.4)	6.6-8.5
Dissolved oxygen (mg/l)	3.9 (1.7)	0.5-7.7
Vegetation (%)	41.1 (22.2)	0-100
Rock (%)	8.9 (13.0)	0-41.6
Gravel (%)	34.4 (22.2)	0-80
Organic matter (%)	12.2 (15)	0-55
Organic substrates (%)	30.8 (15.1)	5-76.6
Sand (%)	13.0 (13.7)	0-40
Luminosity (%)	15.8 (11.4)	7-74.9
Connection (dummy)	0.8 (0.4)	0-1

In general, bioenv routine resulted in similar subset of variables (Table 7.2) for all dissimilarities matrices (total, low, medium and high). All dissimilarity matrices showed slightly different correlation values with environmental distance matrix subsets, being around 30 to 40% (Table 7.2). Overland and watercourse distance were highly correlated (Mantel r=0.88, p<0.001), while the correlations between closeness and watercourse/overland were weaker (overland: Mantel r=0.46, p<0.001; watercourse: Mantel r=0.50, p<0.001).

Table 7.2. Variables selected by bio-env routine for each response matrix (total, low, medium and high dispersal) and their respective correlation values

Response matrix	Variables selected	r
Total	Altitude, velocity, conductivity, dissolved oxygen, % forest, gravel, sand, connection	0.374
Low	Altitude, conductivity, pH, dissolved oxygen, organic substrates, sand, connection	0.399
Medium	Altitude, velocity, conductivity, dissolved oxygen, % forest, sand, connection	0.299
High	Conductivity, dissolved oxygen, % forest, gravel, luminosity, connection	0.352

Linear regressions analyses showed, in general, that differences in community dissimilarity increased with both spatial and environmental distances and that initial dissimilarity (i.e. intercept) were very high for all biological matrices (Fig 7.2; Supporting information, Table S7.1). Closeness distances between sites did not explain significantly dissimilarity between sites of low and high dispersal ability and it was the spatial predictor that less explained community dissimilarity between sites. However, in general, all spatial predictors were low related with community dissimilarity between sites (Fig. 7.3; Supporting information, Table S7.1). Environmental predictors varied in explanatory power among dispersal ability groups, being community dissimilarity of total and low flight dispersal ability better explained (Fig. 7.3; Supporting information, Table S7.1). In general, dispersal ability of aquatic insects did not change the general relationship between community dissimilarity with spatial and environmental distances (Fig. 7.2; Supporting information, Table S7.1).

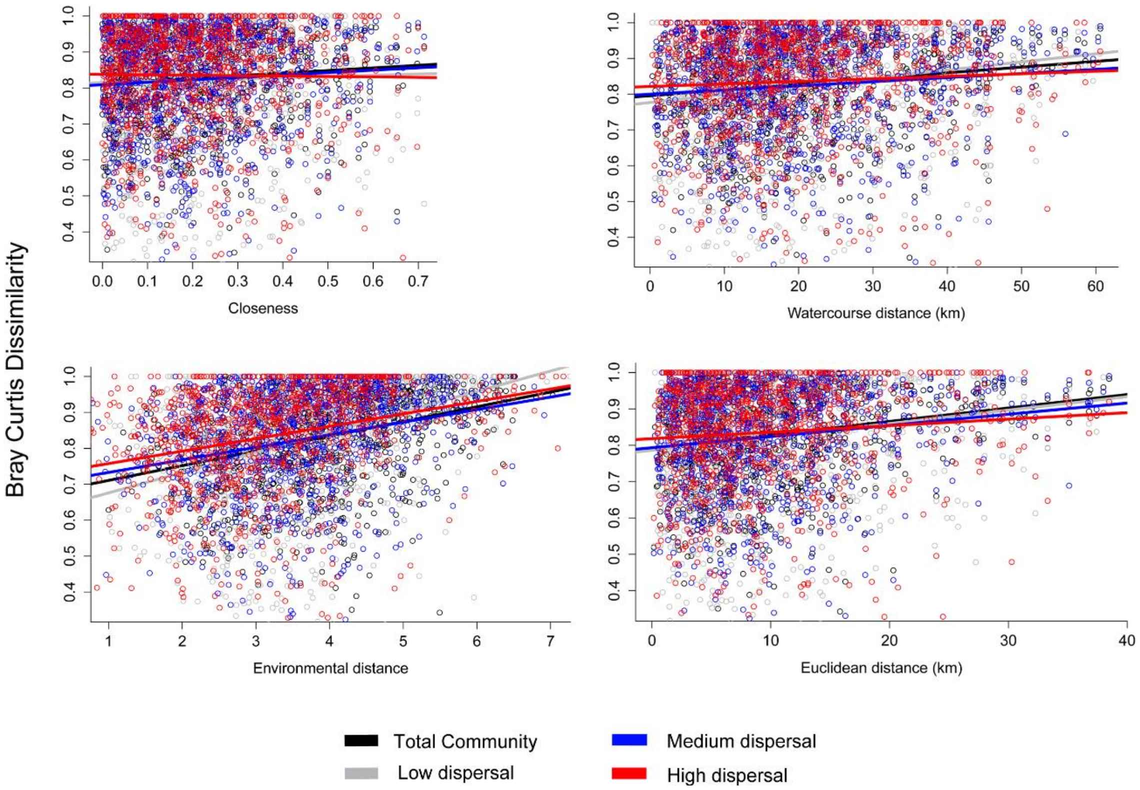

Fig. 7.2. Relationship between dissimilarity matrices (total, low, medium and high dispersal) and distance predictors (closeness, watercourse, Euclidean and environmental).

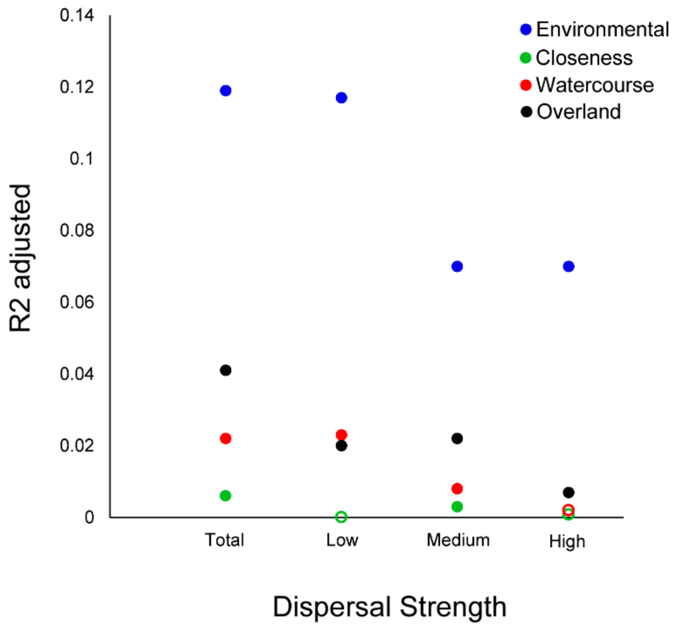

Fig. 7.3. Values of the adjusted R^2 of each linear regression between dissimilarity matrix (total, low, medium and high dispersal ability) and distance predictors (subsets of environmental variables, closeness, watercourse and overland). Filled and open circles indicated, respectively, significant and non significant relationship between dissimilarity matrices and distance predictors.

The correlations between environmental distance and dissimilarity after controlling for the effect of spatial distance matrices were stronger (Mantel and partial Mantel tests, Fig. 7.4; Supporting information, Table S7.2). Indeed, spatial distances had little effect on the community dissimilarity-environmental distance relationship. None of spatial distances controlled for the effect of environmental distances was significantly correlated with community dissimilarity. Also, closeness did not increase the explanation of community dissimilarity and, actually, Euclidean distance matrix showed higher correlation values with fauna dissimilarity than watercourse and closeness. Dispersal ability of species did not changed the outcome of relative importance of the explanatory matrices, despite the influence of spatial distances was weaker on high dispersal ability dissimilarity (Fig. 7.4, Supporting information, Table S7.2).

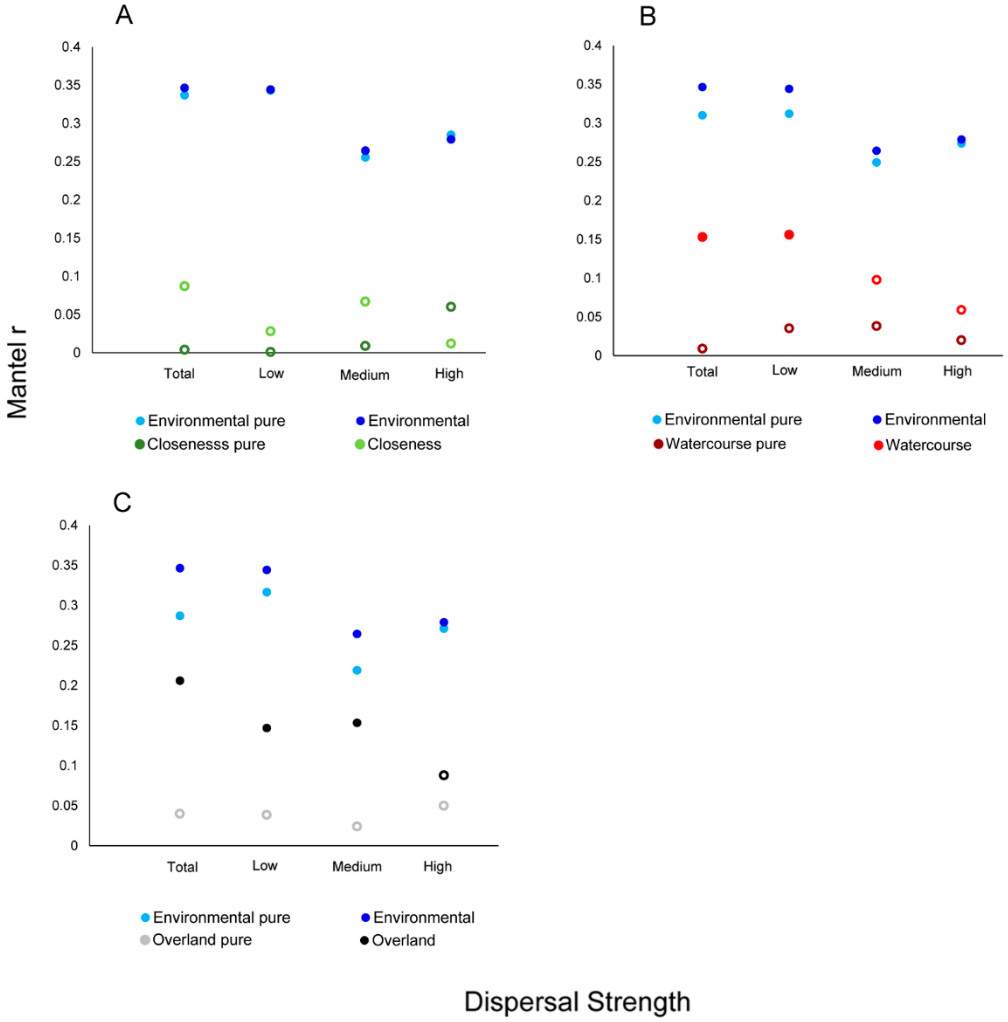

Fig. 7.4. Mantel and partial Mantel results between different dissimilarity matrix (total, low, medium and high dispersal ability) and distance predictors (A: closeness; B: watercourse; and C: overland). Environmental distance was included in all sets of partial Mantel tests. The terms pure refers to partial mantel that measure the effect of environmental taking into account for the effect of the of respective distance predictor, and vice-versa. Filled and open circles indicated, respectively, significant and non significant correlation between dissimilarity matrices and predictors.

Discussion

Our results suggest that environmental difference were related to community dissimilarity between sites, as we hypothesized (hypothesis 1). Community dissimilarities were high at small spatial extent (i.e. initial similarity or intercept on DDR; Fig. 7.2) for all dispersal groups and environmental distances significantly affected community dissimilarities after controlling for the effect of spatial matrices. This agrees with previous studies that suggested species sorting as the main mechanism structuring metacommunities (see reviews Cottenie 2005; Logue *et al*. 2010; Heino *et al*. 2014; but see Heino *et al*. 2015). The use of different types of dispersal dimensions reinforces our results, since none of them was significantly correlated to aquatic insect dissimilarity between sites. Also, spatial distances had a little effect on community dissimilarities-environmental relationships, as indicated by the Mantel and partial Mantel test of environmental distance with community dissimilarities when controlling for the effect of spatial distances. Recent studies also showed that species sorting was the main mechanism driving metacommunities in dendritic networks, despite the use of different dispersal dimensions (Brown & Swan 2010; Cañedo-Argüelles *et al*. 2015; Kärnä *et al*. 2015).

Spatial scale is a key factor in understand dispersal-related processes driving metacommunity structure. This is because a large spatial extent could increase the strength of dispersal limitation whereas small extent could improve the role of homogenization between communities, if dispersal is high enough to generate source-sink dynamics (Ng, Carr & Cottenie 2009; Heino *et al*. 2014) or species sorting when dispersal is sufficient. Intermediate spatial extent increases the relative contribution of environmental filtering on community variation (Heino *et al*. 2014) as species could choose more suitable habitats. Our riverine network could be considered as a small to intermediate spatial extent (~1200km^2) with a longitudinal gradient, since headwaters to mainstem (Table 7.1).

Centrality measures did not increase the explicability of the spatial processes compared to other spatial distances, contrary to our second hypothesis. However, network closeness captures a different spatial configuration compared to overland and watercourse distances. Indeed, mesocosm and empirical studies have shown that network centrality was useful to detect spatial processes responsible to community dissimilarity structure (Carrara *et al.* 2012; Altermatt, Seymour & Martinez 2013; Carrara *et al.* 2014). The shape of dendritic network is one interesting aspect to be considered here and may help to explain the absence of spatial processes affecting communities and the low explicability of closeness. Rectangular and elongate basin shape (as assessed here) lacks the formation of larger tributaries downstream compared to heart-shaped networks. Rectangular basins decrease the effects related to downstream runoff of sediments, organic matter and aquatic insects drift compared to heart shaped networks (see Benda *et al.* 2004). Also, rectangular basins have branches farther apart from each other compared to heart shaped networks. These characteristics would lead to an expectation of increasing environmental control in rectangular networks, as we demonstrated here, whereas heart shaped networks would be expected to sustain mass effects dynamics or even homogenizing effects (e.g. Milesi & Melo 2013). The use of different dendritic network architecture seems to be a good avenue in future metacommunity studies.

Aquatic insects are known to have different dispersal abilities, from a few meters to kilometers (Bilton *et al.* 2001; Petersen *et al.* 2004) As such, they would be expected to perceive a given spatial configuration differently and would be structured by different ecological processes. However, environmental distances affected dissimilarity between sites of all dispersal groups. Dissimilarities between sites of high dispersal aquatic insects did not show any effect of spatial distances between sites (Fig. 7.4), suggesting that this group can track different environmental conditions between sites independently of their spatial structure (De Bie *et al*. 2012; Padial *et al*. 2014). This is reasonable considering that high dispersal groups are composed mainly by odonates, diving and scavenger beetles, known to have strong dispersal ability and to avoid unsuitable sites (Bilton *et al.* 2001). Dissimilarity between sites of low dispersal aquatic insects was affected by both overland and watercourse distances between sites, but not when controlled by the effect of environmental distances. In the same way, medium dispersal ability dissimilarity was also affected by watercourse distances. These results suggest that low and medium dispersal aquatic insects dissimilarity are shaped by environmental variables spatially structured and they cannot track environmental variation independently of space, i.e., they inhabit sites closer each other with similar environmental conditions. Low dispersal ability was the most affected by environmental distance between sites, followed by medium and high with similar correlation values. Low dispersal ability organisms could be highly dispersed by wind, even great distances (Kovats, Ciborowski & Corkum 1996), but because wind is not as directional as active dispersal, just organisms that arrive in suitable habitats would survive. This finding suggest that the riverine network works as a dispersal route for all dispersal ability groups, enable them directly or indirectly (via wind currents) find suitable habitats in this spatial extent.

Overland and watercourse distance showed similar correlations with community dissimilarity between sites, even after controlling for the effect of environmental distances (DDR, Fig. 7.3; Mantel and partial Mantel tests, Fig 7.4). Overland and watercourse distances between sites were highly correlated (Mantel r=0.88), providing, thus, similar community patterns, as demonstrated in other studies (Nabout *et al*. 2009; Grönroos *et al*., 2013; Cañedo-Arguëlles *et al*. 2015; Kärnä *et al*. 2015).

We recognize that our study is a snapshot of one season in a highly variable environment due to the disconnection of some streams from network. Connection, a dummy variable, representing stream linkage to the whole network, was correlated to all dispersal groups used here, being selected as important variable. We sampled in a dry season, which is expected to enhance dispersal-related processes in intermittent streams (Göthe, Angeler & Sandin 2013), because severe drought may generate clumped patterns of aquatic insects distribution through limited dispersal, increasing community dissimilarity with distance. Our results did not support limited dispersal since we did not find any influence of dispersal-related processes. Another study showed that environmental differences were more important than spatial distances for biological dissimilarities in intermittent streams (Cañedo-Argüelles *et al.* 2015). This issue deserves future investigation, as periodic environmental fluctuations could change the relative role of different process structuring metacommunities (see Göthe, Angeler & Sandin 2013).

Our results point to environmental control of aquatic insects dissimilarity between sites. All dispersal ability groups can find suitable habitats in the riverine network, suggesting that these different groups may track environmental variation. Despite the potential of graph-based measures to detect spatial processes (Carrara *et al*. 2014), our results did not showed a better performance of the centrality measure to detect spatial processes compared to overland and watercourse distances.

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Supporting Information

Table S7.1. Distance decay relationship results showing adjusted R^2, intercept, slope and significance of different models (biological matrices explained by environmental and spatial predictors individually). Abbreviations: Med=medium dispersal ability; SClos=closeness; SWater=watercourse distances; SOver= overland distances; Env=environmental distances

Model	R^2adj	Intercept	Slope	p (1000)
Total~SClos	0.006	0.812	0.072	0.005
Low~SClos	0.0001	0.814	0.034	0.34
Med~SClos	0.003	0.809	0.067	0.01
High~SClos	0.0008	0.837	-0.011	0.735
Total~SWater	0.022	0.795	0.001	<0.001
Low~SWater	0.023	0.776	0.0022	<0.001
Med~SWater	0.008	0.799	0.0011	0.002
High~SWater	0.002	0.821	0.0007	0.05
Total~SEucl	0.041	0.791	0.003	<0.001
Low~SOver	0.020	0.786	0.003	<0.001
Med~SOver	0.022	0.793	0.001	<0.001
High~SOver	0.0069	0.817	0.198	0.004
Total~Env	0.119	0.669	0.041	<0.001
Low~Env	0.117	0.619	0.056	<0.001
Med~Env	0.07	0.697	0.034	<0.001
High~Env	0.07	0.722	0.034	<0.001

Table S7.2. Mantel and partial mantel tests among predictors and biological matrices (total, low, medium and high dispersal). The terms Env, Sclos, SWater and SOver refer to results of Mantel test. Pure term depicts the effects of one predictor when controlling for the effect of the another one in the same set of analysis (partial Mantel). Abbreviations: SClos=closeness; SWater=watercourse distances; SOver= overland distances; Env=environmental distances

		Response (Bray-Curtis dissimilarity)			
Predictors		Total	Low	Medium	High
Env		0.3464***	0.3443***	0.2643***	0.279***
Env+SClos	Env pure	0.337***	0.3432***	0.2557***	0.285***
	SClos pure	0.004	0.001	0.009	-0.006
	SClos	0.087	0.028	0.067	-0.012
Env+Water	Env pure	0.314**	0.3121***	0.2493***	0.274***
	SWater pure	-0.009	0.0352	-0.038	-0.02
	SWater	0.153*	0.156*	0.098	0.059
Env+Overl	Env pure	0.287**	0.3165***	0.219**	0.271***
	SOver pure	0.04	0.0385	0.024	0.050
	SOver	0.206*	0.147*	0.1534*	0.088

*0.01<p<0.05

**0.001<p<0.01

***p<0.001

8. Conclusion, comments and perspectives

In this thesis I assessed issues of both theoretical and applied ecology using different theories/concepts – ontogenetic niche shifts and the metacommunity concept – in a riverine network. This special type of habitat could be used in two different ways: as a natural area where biomonitoring programs could be implemented or to extract different spatial predictors that could be used to explain biological community structure. I investigated both questions here.

In the first chapter, the results showed that larvae and adults were affected by common environmental variables and that they were congruent, with higher correlation values using both abundance data compared to abundance-incidence comparisons (numerical resolution). Also, adult genera and species were highly congruent. These results could be due to carry-over effects, i.e., larval environmental conditions that could affect adult fitness components or vice-versa, as well as female selection of more appropriate habitats for laying their eggs, which could determine larvae distribution. For practical reasons, including time and money for sampling and identifying larvae and adults, I recommended abundance of adults genera to be used as bioindicators in Neotropical region where the main human impact is cattle raising. These issues are fundamental to biomonitoring program planning and development, because they require that both bioindicators be rigorous and cost-effective.

In the second chapter, I found that terrestrial and in-stream environmental components affect both larvae and adults, suggesting that metamorphosis might not necessarily be a new beginning. The absence of spatial signal on assemblage variation using different spatial configurations (overland and in-water) suggests that odonates may track variation in environmental conditions in the spatial extent of our study area. Larvae have low dispersal ability and drift propensity, thus their dispersal would be mainly via adult females dispersal and oviposition. Our study open new venues to understanding metacommunity drivers of organism that undergo ontogenetic niche shift.

The results of later chapter also pointed to a environmental control of dissimilarities between sites and that centrality did not improve the detection of dispersal-related process. Different dispersal abilities did not change the relative role of environmental and spatial components, which together with the strong effect of environmental control, suggested that all dispersal ability groups perceive this riverine network in the same way and could find suitable habitats via dispersal.

Considering the three chapters together (Fig. 8.1), I conclude that: i) both larvae and adults of odonates could be used as bioindicators of anthropogenic impacts; ii) both larvae and adults of organisms that undergo ontogenetic niche shifts are structured by species sorting and are affected by environmental variables from different scales (in-water and terrestrial), iii) species sorting also drives community dissimilarities between sites of aquatic insects with different dispersal abilities, iv) centrality measure (closeness) did not improve the detection of dispersal-related process. These results agreed with the well established role of species sorting shaping metacommunities, suggesting that species with different dispersal abilities or in different life stages could track the environmental variation among sites in the extension of the study area used here. Using different riverine networks shape could be interesting to detect the relative role of spatial process and environmental control shaping metacommunity structure, opening a good avenue for future studies.

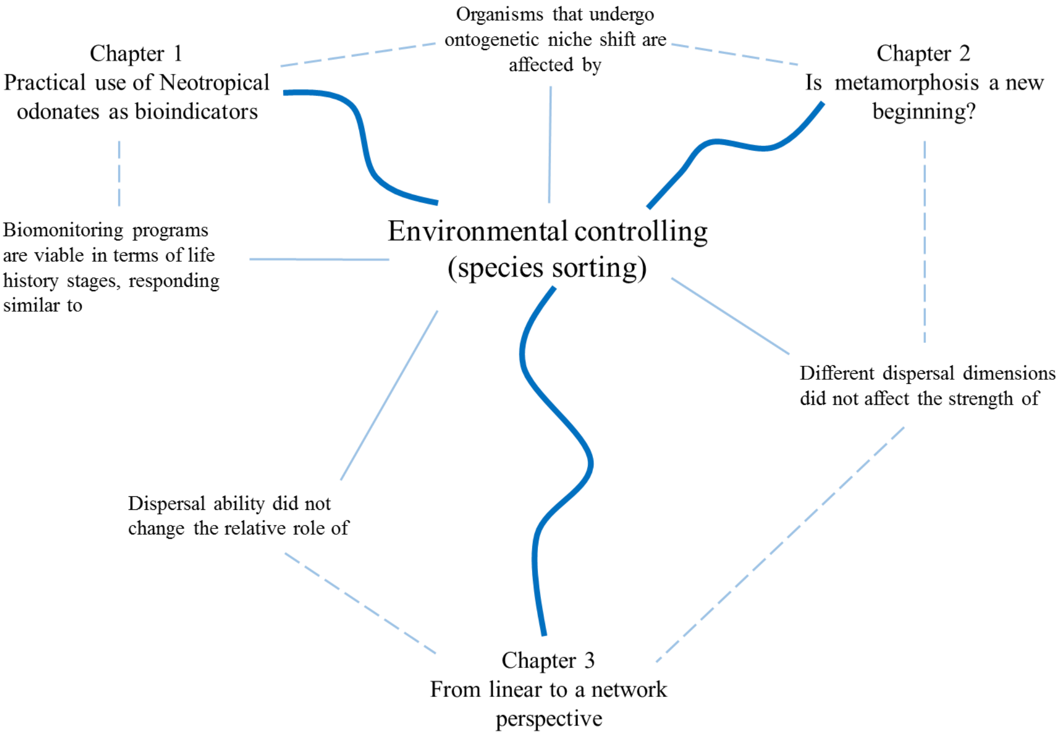

Fig. 8.1. Summary of common results found in the three chapters.

9. Appendix

List of articles published, manuscripts in revision and book resulted from different collaborative projects.

Articles

Published:

1) Francisco Valente-Neto; Ricardo Koroiva; Alaíde Aparecida Fonseca-Gessner & Fabio de Oliveira Roque (2015) The effect of riparian deforestation on macroinvertebrates associated with submerged woody debris. *Aquatic Ecology*, 49, 115-125.
2) Fabio de Oliveira Roque; Suzana Cunha Escarpinati; Francisco Valente-Neto & Neusa Hamada (2015) Responses of Aquatic Saproxylic Macroinvertebrates to Reduced-Impact Logging in Central Amazonia. *Neotropical Entomology*, doi: 10.1007/s13744-015-0295-4
3) Susana Trivinho-Strixino; Fabio Laurindo da Silva & Francisco Valente-Neto (2012). First record of larvae of Chironomidae (Insecta, Diptera) as prey of *Temnocephala* sp. (Platyhelminthes, Temnocephalidae), an ectosymbiont on larvae of Corydalidae (Megaloptera). *Revista Brasileira de Entomologia*, 56, 387-389.
4) Melissa Ottoboni Segura; Francisco Valente-Neto & Alaíde Aparecida Fonseca-Gessner (2012) Checklist of the Elmidae (Coleoptera: Byrrhoidea) of Brazil. *Zootaxa*, 3260, 1-18.

In review:

1) Fabio de O. Roque, Nayara Karla Zampiva, Francisco Valente-Neto, Jorge F. S. de Menezes, Neusa Hamada, Mateus Pepinelli, Tadeu Siqueira & Christopher Swan. Gradual deconstruction of black fly richness reveals that environmental and spatial signals in metacommunity organization depends on species commonness. *Freshwater Biology*.
2) Francisco Valente-Neto, Victor S. Saito, Tadeu Siqueira & Alaide Aparecida Fonseca-Gessner. Metacommunity drivers of aquatic beetles associated with woody debris in a transitional region between Cerrado and Atlantic Rainforest biomes. *Aquatic Ecology*.

Book

1) Fava, W. S.; Zucca, C. F.; Delatorre, M.; Valente-Neto, F.; Rodrigues, M. E.; Paula, G. A.; Araujo, A. C.; Fischer, E. ; Roque, F. O.; Laps, R. R. (Eds) (2014) *Ecologia do Pantanal curso de campo*. Editora UFMS, Campo Grande. 387pp.