Effects of small changes in riparian forest complexity on aquatic insect bioindicators in Brazilian subtropical streams

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Abstract. Riparian forests have positive effects on water quality and biodiversity. However, most studies have only tested the effects of distinct vegetation types or streams with and without forests, despite the fact that riparian forests differ in degrees of complexity. The aim of the present study was to test whether riparian forest complexity affected the composition and abundance of the Ephemeroptera, Plecoptera and Trichoptera (EPT) taxa across a small environmental gradient. We also measured whether EPT genera or feeding groups responded to changes in riparian forest complexity. The study was conducted in two protection areas (i.e. Lagoa do Peri Municipal Park, PERI; and the Permanent Protection Area of Ratones, RAT) of Santa Catarina Island, Brazil. Primary production increased in streams with lower canopy percentage, and EPT assemblages differed among streams with different riparian forest complexity. In RAT, the water quality and forest variables affected EPT composition; however, in PERI, only water quality variables were important. Indicator species analysis based on genera suggested *Kempnyia* (Plecoptera) and *Zelusia* (Ephemeroptera) to be indicative of streams with greater forest complexity, whereas *Farrodes* (Ephemeroptera) was significant in streams of intermediate riparian forest complexity; however, no one functional group dominated. The results of the present study show that small changes in riparian forest complexity influence the composition of EPT insects in subtropical streams.

Additional keywords: Atlantic Forest, Ephemeroptera, Plecoptera and Trichoptera insects, indicator species.

Introduction

Anthropogenic activity has changed landscapes primarily through conversion of native forest to urban occupation and agricultural land. At a watershed scale, these changes have resulted in the degradation of water resources due to sedimentation, changes in hydrology and physicochemical water characteristics, and biodiversity loss (Allan 2004; Nessimian *et al.* 2008). Deforestation of the riparian zone, which represents an important link between terrestrial and aquatic ecosystems, reduces the input of allochthonous organic matter, especially in forested headwater streams, where the main source of energy comes from the terrestrial ecosystem (França *et al.* 2009). The allochthonous organic matter (formed by coarse particulate organic matter) entering streams is composed of litterfall, primarily leaves, twigs and reproductive parts, regardless of vegetation type or geographic area (França *et al.* 2009). This material increases the supply of nutrients and organic matter to streams (Balian and Naiman 2005) and may also serve as a habitat to be colonised (leaves, branches and fruits), a refuge from predators or water flow and oviposition sites for fauna (Salles and Ferreira-Junior 2014).

Riparian vegetation has several other functions: it serves as a buffer zone that reduces the entry of sediment, fertilisers and pesticides entering streams through overland flow, assists in reducing run-off and bank erosion, provides substrate heterogeneity and helps maintain a constant stream water temperature by reducing light input (Vannote *et al.* 1980; Balian and Naiman 2005; Naiman *et al.* 2005). By removing the vegetation, the amount of light reaching the water increases, often leading to higher algal growth (primary production) and accelerated breakdown rates of organic matter, because these processes are temperature dependent (Richardson and Béraud 2014). At a regional scale, the riparian zone serves as a climate regulator, a carbon sink and a corridor for biota movement; the riparian zone also increases regional biodiversity and maintains water quality, among other environmental services (Naiman *et al.* 2005).

Given the positive effects of the riparian zone, the loss of riparian forest affects the nutrient concentrations of streams and physicochemical water parameters, alters the rates of organic matter transport and breakdown and reduces aquatic faunal diversity (Richardson and Béraud 2014). Aquatic insect diversity has been shown to decrease when natural riparian vegetation is replaced by agricultural land use, such as sugarcane crops and pasture (Corbi and Trivinho-Strixino 2008; Corbi and Froehlich 2010; Egler *et al.* 2012, Siegloch *et al.* 2014), banana (Kleine *et al.* 2011; Corbi *et al.* 2013) and eucalyptus or pine plantations (Stenert *et al.* 2012). Moreover, a reduction in the width of the riparian zone compromises aquatic assemblages in streams, a width greater than 15 m is necessary to maintain the composition and trophic conditions of fauna (Moraes *et al.* 2014). However, natural riparian forests are not homogeneous, and may vary in composition and structure because of differences in climatic conditions, soil type, topography, successional stage and the condition of adjacent areas (Naiman *et al.* 2005). Thus, the composition and structure of riparian vegetation may affect the physicochemical water properties in tropical streams (Souza *et al.* 2013) and consequently be an important predictor of aquatic insect assemblage composition, especially groups sensitive to environmental changes.

The structure of aquatic insect assemblages reflects the integrity of ecosystems. In general, high diversity and the presence of sensitive taxa are expected in streams with preserved riparian vegetation, whereas it is common to find a high abundance of generalist and tolerant taxa in degraded systems (Rosenberg and Resh 1993). Among aquatic insects, the orders Ephemeroptera, Plecoptera and Trichoptera (EPT; mayflies, stoneflies and caddisflies) are sensitive to the effect of human activities on streams, and their richness decreases with increasing disturbance (Rosenberg and Resh 1993). However, according to Ligeiro *et al.* (2013), in basins with a weaker disturbance gradient, natural habitat variability associated with stream size and microhabitats explained more variation in EPT richness than the disturbance index related to anthropogenic pressures on sites and catchment scale. Given the relationship between feeding behaviour and the availability of the food resources in streams, functional groupings in the analysis of aquatic insects is another tool used for stream assessment (Cummins *et al.* 2005). Streams with riparian forests are usually characterised by a high input of allochthonous material being consumed primarily by shredders, which use the coarse particulate component, as well as collectors, which use fine particulate organic matter. In well-illuminated streams, scrapers are abundant, feeding off the primary production of periphyton (Cummins *et al.* 2005; Brasil *et al.* 2014).

Although several studies have highlighted the negative effect of land use and marked degradation of riparian vegetation on water quality and biodiversity, it remains uncertain whether changes in riparian forest complexity, rather than just removal of riparian canopy, can also affect aquatic insect assemblages, primarily in tropical and subtropical regions where few studies have been conducted. Riparian buffer zones are even more important in headwater streams in mountainous areas of tropical and subtropical climates, where rainfall events are more intense and frequent, and so the riparian vegetation serves to cushion the input of organic and inorganic material into the system (Allan and Castillo 2007). Therefore, the aim of the present study was to test whether variables of riparian forest complexity and the physicochemical characteristics of streams affected the composition of EPT assemblages across a small gradient of riparian forest complexity in southern Brazil. In addition, we determined whether EPT genera or functional feeding groups responded to small changes in the complexity of the riparian forest in order to find potential bioindicators. We worked with the following hypotheses: (1) small changes in riparian forest complexity can affect the EPT assemblage; and (2) the expected changes in the availability of organic matter and primary production affect the feeding groups along a disturbance gradient of riparian forest complexity.

Materials and methods

The study was conducted in two areas of Atlantic Forest on Santa Catarina Island, southern Brazil. The forests on the island have been used since the arrival of the first European settlers in the second half of the 17th century (Caruso 1983. The main causes of deforestation on the island were vegetation clearing for building, energy production and the development of agriculture (Caruso 1983). In 1940, 83% of the native forest had disappeared, leaving only hilltop vegetation. Currently, the natural forests are fragmented, found mainly within conservation units or on hilltops and composed of secondary vegetation in different stages of succession and regeneration (Bisheimer *et al.* 2013).

The study areas were located in the last two larger forest fragments on the island: (1) the Lagoa do Peri Municipal Park (PERI; 27°43′30″S, 48°32′18″W) and the Permanent Protection Area of Ratones (RAT; 27°31′52″S, 48°30′45″W). PERI is located in the southern part of the island, with an area of 2030 ha. PERI is the first protected area of Santa Catarina Island declared since 1952, established as a park in 1981; it is one of the last remnants with pristine forest and various levels of dense rainforest vegetation succession on the island. RAT is located in the central north part of the island, covers an area of ~492 ha and has native vegetation ranging between 6 and 30 years of secondary forest development (Bonnet and Queiroz 2006). These areas are approximately 22 km apart, separated by several districts of the Florianópolis city with different histories of urban occupation. Currently, these areas represent sites of great relevance for conservation, because the Atlantic Forest Biome is considered a global biodiversity hot spot and has a high number of endemic species (Myers *et al.* 2000). The climate is classified as 'Cfa' according to the Köppen climate classification, with rainfall uniformly distributed throughout the year and no dry season. However, rainfall records since 1992 have shown higher rates of rainfall in the warmer months between December and March (Kleba Lisboa *et al.* 2015).

Sampling design

Within each area, samples were taken at five independent low-order streams (first and second order following Strahler 1957) along a gradient of riparian forest complexity: one stream was in the middle of a highly complex riparian forest, three streams were intermediates with regard to riparian forest complexity and one stream was inside the forest, but close to the fragment edge

and near urban residences. In total, across the two areas, 10 streams were sampled, each with at least a 50-m width of riparian forest (two streams had highly complex riparian forests, six streams were intermediates and two streams had low riparian forest complexity). In each stream, three sampling points were selected within 50 m of one another. At each sampling point, two samples of substrate were collected in riffles, one in gravel and another sample in organic material as leaves trapped in riffles. These subsamples were combined to give one sample per sampling point, totalling 15 samples in each area. Samples of aquatic insects were collected between January and March 2012 with a Surber sampler (area 0.0361 m^2, mesh 0.25 mm). In the laboratory, the material was fixed and preserved in 80% ethanol, passed through a 0.25-mm mesh and then sorted and identified under a stereomicroscope. The EPT were identified to the genus level using taxonomic keys (Wiggins 1996; Salles *et al.* 2004, Domínguez *et al.* 2006; Domínguez and Fernández 2009).

Environmental variables

Variables related to water, riparian forest structural complexity and soil cover were measured at each sampling point in order to test their effect on EPT assemblages. The following variables were measured within the streams: water velocity (m s^{-1}) using the float method (Bain and Stevenson 1999); width and depth of streams (cm), determined using a measuring tape; electrical conductivity (mS cm^{-1}); dissolved oxygen concentration; pH and water temperature, measured using a YSI Model Multiprobe; primary production, quantified through analysis of chlorophyll-*a*; and phaeopigment, scraped from the bedrock surface at each sample point and placed in a container with 200 mL of water. These samples were filtered (0.7 μm; Millipore AP40 glass fibre) and 90% acetone was used for pigment extraction from filters (Lorenzen 1967). The organic matter retained in each Surber sample was dried in an oven at 60°C for 5 days and then weighed. The last variable determined was predominant inorganic substrates, which were estimated visually according to Ward (1992).

The environmental variables of riparian forest and soil cover were measured using the adapted point-centred quarter method at each sampling location (Brower *et al.* 1998). Tree, shrub and soil variables were measured in each quadrant as follows: circumference at breast height for trees (when diameter at breast height >5 cm); size (basal area and height multiplied); top diameter of trees; distance away from the nearest tree to the centre of the cross, circumference at ankle height for shrubs (when <5 cm and with a minimum height of 1 m); canopy cover; percentage of litter cover; percentage of green cover; percentage of uncovered soil; height; and leaf litter biomass.

The height of trees and shrubs was estimated with a 4-m ruler. Top diameter, circumference and distance were determined using a measuring tape. The percentage of litter, soil covered by vegetation and uncovered soil (without vegetation and litter) was estimated visually following percentage classes (0–5, 6–25, 26–50, 51–75, 76–95 and 96–100%), using a square of 1 m^2, placed about 20 cm away from the cross. The biomass of the leaf litter was determined using a portion of litter removed from the square. Leaf litter was later dried (60°C, 72 h) and weighed to obtain dry biomass. The percentage of canopy cover was

estimated visually using the same classes described above. The basal area of trees and shrubs was calculated from the trunk circumference (based on the area of the circle). For each variable, a measure of central tendency was calculated based on the Shapiro–Wilk normality test. Thus, each environmental variable represented a central value (mean or median, as appropriate) of the four measurements at each point.

Data analysis

A principal components analysis (PCA) was performed to reduce the dimensionality of independent variables for physicochemical water and riparian forest characteristics for each area. The variables indicated by the PCA for the riparian forest were used to determine the degree of riparian forest complexity. The streams were labelled from A to E, beginning with streams of higher complexity descending to streams with lower complexity. Multiple linear regressions were used to determine the relationship between riparian forest variables and physicochemical water descriptors. The first two axes of each PCA (site scores, weighted sums of species scores) of riparian forest variables were used as independent variables in a linear regression model to evaluate the effects of riparian forest complexity on stream habitat and water quality variables. When necessary, the environmental variables were standardised and the analysis was run using the stats and vegan packages for *R* ver. 3.0.1 software (R Foundation for Statistical Computing, Vienna, Austria, see http://www.R-project.org, accessed 2015 January 2005). Permutational multivariate analysis of variance (PERMANOVA) was used to test for significant differences in insect assemblage composition between the two sampling areas. In this analysis, area was considered a fixed factor and crossover and lower hierarchical levels (streams) were considered fixed and nested (Anderson *et al.* 2008). This analysis was performed using PRIMER 6 version 6.1.16 with the PERMANOVA add-on version 1.0.6 (PRIMER-E Ltd, all rights reserved, Anderson *et al.* 2008). Based on the results obtained, each area was treated independently.

Differences in observed richness of EPT taxa among streams with different levels of riparian forest complexity were explored using one-way analysis of variance (ANOVA). The structure of EPT assemblages was analysed using the Bray–Curtis similarity coefficient and was represented using non-metric multidimensional scaling (NMDS). An analysis of similarity (ANOSIM; one way) was used to test for differences in assemblage composition between the five streams in each area, using the permutation method on the Bray–Curtis resemblance matrix.

The most important water and riparian forest structure descriptors selected by the PCA were correlated with the EPT data matrix through a routine BioEnv (Clarke and Gorley 2006). The Bray–Curtis dissimilarity biotic matrix was correlated with the environmental matrix (Euclidean distance) through a Spearman correlation. The following environmental variables were used in the BIOENV analysis for PERI: electrical conductivity, depth, dissolved oxygen, percentage of boulders, amount of organic matter, chlorophyll-*a* and phaeopigments (physicochemical stream parameters), tree size and top diameter, distance from the nearest tree to the centre of the cross, canopy cover percentage, percentage of green cover and uncovered soil,
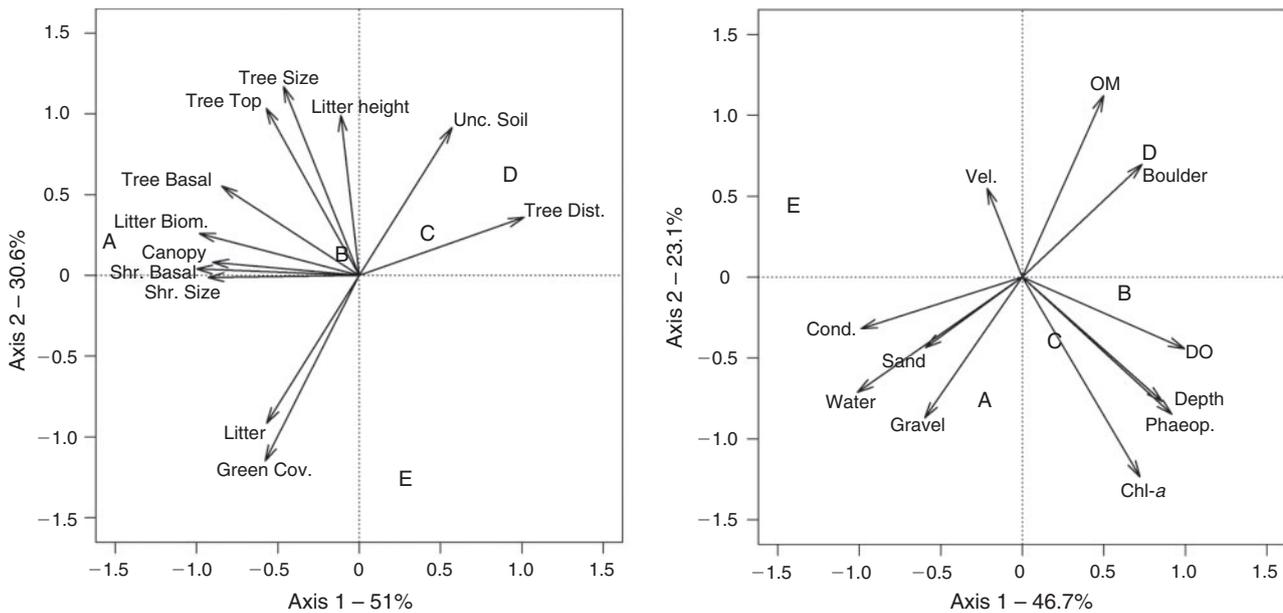
Fig. 1. Results of principal components analysis for riparian forest variables (left) and physicochemical stream parameters (right) in Streams A–E in Lagoa do Peri Municipal Park (PERI). Tree basal, basal area of trees; Tree Dist., tree distance; Shr. Basal, basal area of shrub; Shr. Size, size of shrub; Unc. Soil, percentage of uncovered soil; Green Cov., percentage of green cover; Litter Biom., litter biomass; Cond, electrical conductivity; DO, dissolved oxygen; Vel., water velocity; Water, water temperature; OM, amount of organic matter; Phaeop., phaeopigments; Chl-*a*, chlorophyll-*a*.

and litter biomass (riparian forest variables). For RAT, the following variables were used: water velocity, electrical conductivity, dissolved oxygen, substrate percentage, amount of organic matter, chlorophyll-*a* and phaeopigments (physicochemical stream parameters), tree size and top diameter, size of shrubs, percentage of green cover and uncovered soil, height and leaf litter biomass (riparian forest variables). EPT data were Hellinger transformed, and the environmental variables were square root transformed and standardised (Clarke and Gorley 2006).

From the PCA results, the streams were grouped into three groups of environmental quality (high, medium and low complexity). To test whether EPT genera and functional groups could be used to detect changes in riparian forest complexity, an indicator species analysis (Dufrêne and Legendre 1997) was undertaken with a Monte Carlo test used to assess the significance of the indicator value, using 999 permutations. The classification of the EPT genera in functional feeding groups was based on Cummins and Klug (1979), who define the following groups: brushers, filtering collectors, gathering collectors, shredders, scrapers, piercing and predators.

Results

The riparian forest complexity variables and physicochemical characteristics of water differed between the five streams in the two areas. In PERI, the first axis of the PCA (51% of total variation) for riparian variables represented the forest complexity gradient, where streams with high riparian forest complexity (A and B) had a higher basal area, tree and shrub size and top diameter, percentage canopy cover, biomass and litter height, whereas the second axis (30.6% of total variation)

separated the stream in worse condition (E; Fig. 1, Table S1). For physicochemical water variables, Stream A was associated with greater substrate heterogeneity, Streams B and C had higher primary production (chlorophyll-*a* and phaeopigments) and Stream D was associated with higher amounts of organic matter (Fig. 1). Multiple linear regressions showed that the amount of organic matter and chlorophyll-*a* were significantly related to the first axis of the PCA, performed with riparian forest data ($R = 0.4217$, $P = 0.04$). Chlorophyll-*a* values increased in streams with a lower percentage of canopy cover, and greater amounts of organic matter were found in streams with uncovered soil and greater distance among the trees.

In RAT, the first principal component (PC1; 47.2% of variation) separated Streams A and B from streams with intermediate riparian forest complexity (Streams C and D). Higher values of basal area, tree and shrub size, top diameter, percentage of canopy cover and litter characterised Streams A and B. Streams C and D had higher percentages of green cover and litter layer (Fig. 2, Table S1). The second principal component (PC2; 28.9% of variation) separated Stream E, a site near the edge of the forest fragment, and was characterised by a greater distance from the nearest tree and uncovered soil. For physicochemical water variables, PC1 separated the streams by substrate composition and primary production. Stream A had homogeneous substrate and Stream C had higher primary production. Streams D and E had higher amounts of organic matter, water velocity and dissolved oxygen (Fig. 2). Regression analysis showed that the first axis of riparian forest variables was significantly related to phaeopigments and chlorophyll-*a* ($R = 0.455, P = 0.026$). Primary production increased in streams with a lower canopy cover percentage.
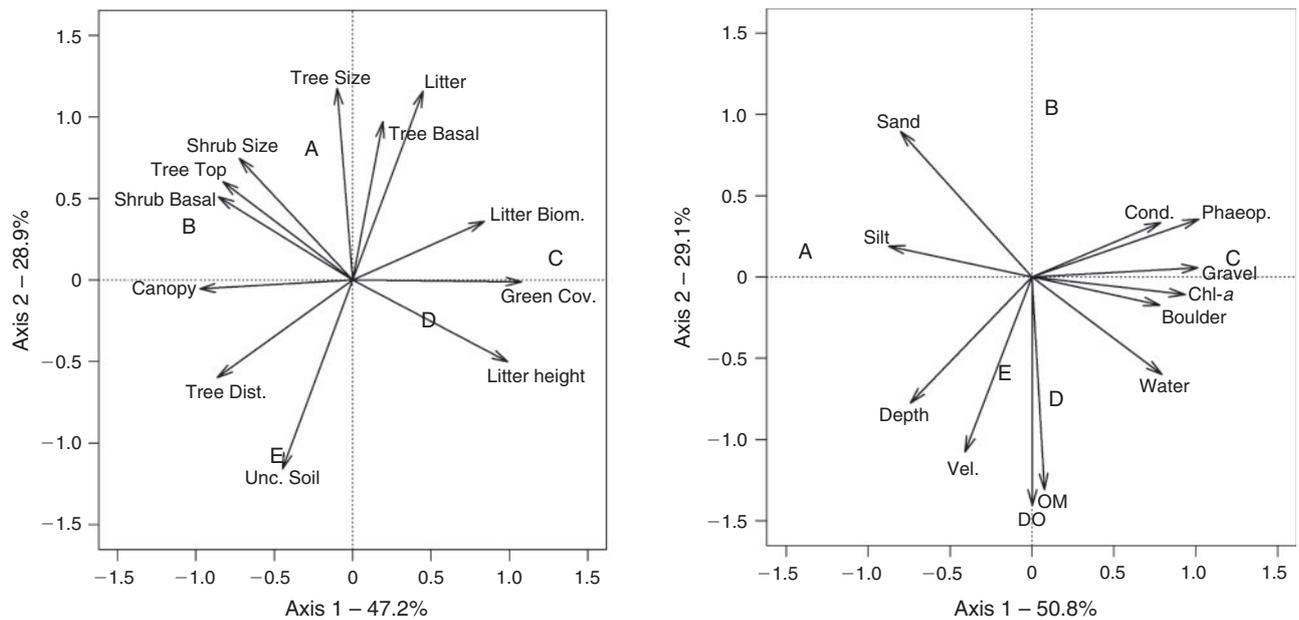
Fig. 2. Results of principal components analysis for riparian forest structural variables (left) and physicochemical stream parameters (right) in Streams A–E in the Permanent Protection Area of Ratones (RAT). Tree basal, basal area of trees; Tree Dist., tree distance; Shrub Basal, basal area of shrub; Unc. Soil, percentage of uncovered soil; Green Cov., percentage of green cover; Shrub Size, size of shrub; Litter Biom., litter biomass; Litter, litter percentage; Cond, electrical conductivity; DO, dissolved oxygen; Vel., water velocity; Water, water temperature; OM, amount of organic matter; Phaeop., phaeopigments; Chl-*a*, chlorophyll-*a*.

In all, 1493 individual EPT insects were collected during the study, distributed across 20 families and 37 genera (Table 1). The Trichoptera presented the highest abundance (53.5% of total) and genera richness (59% of total), whereas Plecoptera had the lowest abundance (17.3% of total) and only four genera. Despite the similar richness between the two areas (28 genera in PERI and 29 in RAT), PERMANOVA showed significant differences in the taxonomic composition of the EPT assemblages between the two areas ($F = 2.93$, $P = 0.003$), indicating that there are two distinct assemblages. Many genera occurred only in PERI (*Tricorythopsis*, *Massartela*, *Austrotinodes*, *Amphoropsiche*, *Chimarra*, *Polycentropus*), whereas others were found only in RAT (*Baetodes*, *Cotulma*, *Anchitrichia*, *Nectopsyche*, *Oecetis*, *Cyrnellus*, *Polyplectropus*, *Xiphocentron*). This analysis showed that each area has a different assemblage, with little genera turnover between sites, probably reflecting distance and geographical isolation of the areas. Similarity percentage analysis (SIMPER) showed an average dissimilarity of 53% between PERI and RAT. Many genera contributed to the dissimilarity between areas, with the most representative being *Helicopsyche* (6.67%), *Farrodes* (6.04%), *Smicridea* (5.85%), *Americabaetis* (5.46%), *Neotrichia* (5.36%) and *Anacroneuria* (5.07%). Thus, each area was treated independently.

The observed richness of EPT was similar among RAT streams (ANOVA, $F = 0.46$, d.f. $= 4$, $P = 0.76$), but differed among PERI streams, with higher richness in Stream B compared with the others (ANOVA, $F = 16.94$, d.f. $= 4$, $P = 0.0001$). Regarding taxonomic composition, *Phylloicus* (Trichoptera) and *Farrodes* (Ephemeroptera) genera were dominant in both areas, whereas *Kempynia* and *Tupiperla*

(Plecoptera) occurred only in streams with higher riparian forest complexity. The percentage of functional feeding groups (FFG) in streams is given in Table 2. Most FFG groups occurred independently of stream complexity; however, a higher percentage of scraper insects was recorded in streams with low riparian forest complexity.

Bray–Curtis similarity analysis showed distinct EPT assemblages among streams in the two areas (Fig. 3). In PERI, the NMDS graph revealed four groups, with a clear distinction among streams, except for Streams B and C. In RAT, two groups were formed, one with Streams A and B of higher riparian forest complexity, and the second group with Streams C, D and E. One-way ANOSIM indicated significant differences in the EPT assemblages among the five streams in PERI (R $= 0.899$, $P = 0.001$) and RAT (R $= 0.567$, $P = 0.001$).

The Spearman correlation using BIOENV demonstrated that, in PERI, only environmental water variables ($r = 0.95$, $P = 0.044$) affected EPT assemblage composition. However, in RAT, water variables ($r = 0.94$, $P = 0.011$) and riparian forest variables ($r = 0.90$, $P = 0.039$) were important. In PERI, the analysis indicated that the following water variables determined the variation in EPT assemblages: electrical conductivity, depth, boulder percentage and phaeopigments. However, in RAT, the variables of riparian forest (size and top diameter of trees and size shrubs) and physicochemical water characteristics (water velocity, sand and boulder percentage, amount of organic matter, chlorophyll-*a*) affected the EPT assemblage.

From the PCA results, the streams were grouped into three groups of environmental quality (high, medium and low complexity) to run the indicator species analysis. This analysis indicated that there were two genera in streams with greater

Table 1.   Total abundance and taxonomic composition of Ephemeroptera, Plecoptera and Trichoptera recorded in five streams (A–E, from highest to lowest riparian forest complexity) within two sampled areas (Lagoa do Peri Municipal Park (PERI) and Permanent Protection Area of Ratones (RAT)) on Santa Catarina Island, southern Brazil

The functional feeding groups (FFG) of insects collected were as follows: brushers (Bs), filtering collectors (Cf), gathering collectors (Cg), shredders (Sh), scrapers (Sc), piercing (Pi) and predators (Pr)

Order and Family	Genera	FFG	PERI					RAT				
			A	B	C	D	E	A	B	C	D	E
Ephemeroptera												
Baetidae	*Americabaetis*	Cg	3	15	5	0	0	4	12	15	18	8
	Baetodes	Sc	0	0	0	0	0	0	0	4	9	1
	Cloeodes	Sc	0	2	5	0	0	0	0	0	0	0
	Zelusia	Cg	0	6	0	2	0	9	8	5	0	4
Caenidae	*Caenis*	Sc	1	0	0	0	0	0	0	0	0	0
Euthyplociidae	*Campylocia*	Cf	0	4	1	0	0	0	1	2	1	0
Leptohyphidae	*Tricorythopsis*	Sc	0	1	27	0	0	0	0	0	0	0
Leptophlebiidae	*Askola*	Bs	0	2	0	1	15	8	0	0	0	0
	Farrodes	Bs	5	20	62	1	0	2	3	42	37	13
	Thraulodes	Bs	1	11	0	0	0	0	0	4	11	7
	Massartela	Bs	0	2	0	0	0	0	0	0	0	0
Plecoptera												
Perlidae	*Anacroneuria*	Pr	2	35	10	5	0	2	6	2	32	43
	Kempnyia	Pr	0	11	0	1	0	20	8	0	0	1
Gripopterygidae	*Paragripopteryx*	Sh	1	7	5	20	1	8	6	0	2	2
	Tupiperla	Sh	0	0	5	0	0	18	3	1	0	0
Trichoptera												
Anomalopsychidae	*Contulma*	Sc	0	0	0	0	0	0	0	0	0	3
Calamoceratidae	*Phylloicus*	Sh	20	23	11	8	27	9	29	157	37	32
Ecnomidae	*Austrotinodes*	Cf	0	2	2	0	1	0	0	0	0	0
Glossossomatidae	*Itauara*	Sc	0	12	1	0	0	0	0	1	41	5
Helicopsychidae	*Helicopsyche*	Sc	5	12	3	15	37	0	1	9	0	1
Hydrobiosidae	*Atopsyche*	Pr	1	9	13	10	4	2	2	2	9	7
Hydropsychidae	*Leptonema*	Cf	0	0	1	0	1	1	2	0	0	0
	Smicridea	Cf	0	5	3	0	0	22	7	3	12	1
Hydroptilidae	*Anchitrichia*		0	0	0	0	0	0	0	0	7	0
	Alisotrichia	Sc	4	0	0	1	0	0	0	4	2	11
	Metrichia	Pi	0	0	0	0	0	0	0	1	0	0
	Neotrichia	Sc	0	8	2	0	0	22	0	4	3	10
Leptoceridae	*Amphoropsyche*		0	0	0	1	0	0	0	0	0	0
	Nectopsyche	Sh	0	0	0	0	0	0	0	0	0	1
	Oecetis	Pr	0	0	0	0	0	2	1	0	0	0
	Triplectides	Sh	1	1	0	0	1	1	2	1	1	0
Odontoceridae	*Marilia*	Sh	1	0	0	0	0	0	1	0	0	0
Philopotamidae	*Chimarra*	Cf	0	3	2	0	2	0	0	0	0	0
Polycentropodidade	*Cyrnellus*	Cf	0	0	0	0	0	0	0	0	0	1
	Polycentropus	Pr	0	1	0	0	0	0	0	0	0	0
	Polyplectropus	Cf	0	0	0	0	0	0	2	0	0	0
Xyphocentronidae	*Xiphocentron*	Sc	0	0	0	0	0	0	1	0	0	0

riparian forest complexity (*Kempnyia* – Plecoptera (indication value, IV = 0.787; P = 0.003) and *Zelusia* – Ephemeroptera (IV = 0.635; P = 0.047)), and one genus in streams with intermediate complexity (*Farrodes* – Ephemeroptera; IV = 0.773; P = 0.02). However, the analysis did not indicate any functional groups as bioindicators of riparian forest complexity.

Discussion

Studies in tropical and subtropical streams have shown that the removal of the riparian zone or replacement of the native vegetation by agriculture or forestry land use can significantly reduce aquatic invertebrate richness (Nessimian *et al.* 2008; Corbi *et al.* 2013; Siegloch *et al.* 2014) and change physico-chemical properties of streams (Souza *et al.* 2013). Recently, it was reported that the quality of riparian vegetation could also affect richness, diversity and density of collector–gatherers of invertebrate assemblages (Mesa 2014). Moreover, the presence of a riparian forest buffer appeared to significantly reduce the effects of deforestation from adjacent areas on aquatic assemblages (Lorion and Kennedy 2009). According to Lorion and Kennedy (2009), the diversity and structure of invertebrate

Table 2. Total abundance (number of individuals), richness (number of genera) and percentage of functional feeding groups in five streams (A–E, from highest to lowest riparian forest complexity) within two sampled areas (Lagoa do Peri Municipal Park (PERI) and Permanent Protection Area of Ratones (RAT)) on Santa Catarina Island, southern Brazil

|	PERI					RAT				
	A	B	C	D	E	A	B	C	D	E
Abundance (*n*)	45	192	158	65	89	130	95	257	222	151
Richness	12	22	17	11	9	15	18	17	14	18
Functional feeding groups (%)										
Brushers	13.3	18.2	39.2	3.1	16.9	7.7	3.2	17.9	21.6	13.2
Filtering collectors	0	7.3	5.7	0	4.5	17.7	12.6	1.9	5.9	1.3
Gathering collectors	6.7	10.9	3.2	3.1	0	10.0	21.1	7.8	8.1	7.9
Predators	6.7	29.2	14.6	24.6	4.5	20	17.9	1.6	18.5	33.8
Shredders	51.1	16.1	13.3	43.1	32.6	27.7	43.2	61.9	18.0	23.2
Scrapers	22.2	18.2	24.1	24.6	41.6	8.6	2.1	8.6	24.8	20.5
Piercing	0	0	0	0	0	0	0	0.4	0	0
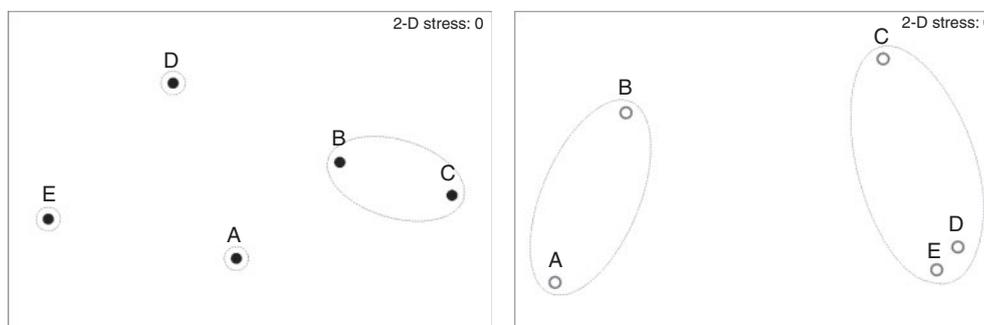
Fig. 3. Non-metric multidimensional scaling (NMDS) plots of Ephemeroptera, Plecoptera and Trichoptera assemblages of streams sampled in Streams A–E in Lagoa do Peri Municipal Park (PERI; filled symbols) and the Permanent Protection Area of Ratones (RAT; open symbols), where Streams A have greater riparian forest complexity and Streams E have lower complexity. Data were transformed using Hellinger and Bray–Curtis similarity measurements. Dashed circles represent 60% dissimilarity among groups in both areas.

assemblages in reaches with a forest buffer, with some exceptions, are very similar to those in forested reference reaches. A study conducted in the subtropical region of Brazil showed that a riparian zone with a width greater than 15 m is necessary to maintain the composition and trophic conditions of aquatic invertebrates similar to those in reference states of conservation (Moraes *et al.* 2014). The results of the present study showed that EPT richness was similar among streams with small differences in riparian forest complexity, but the new contribution is that small changes in riparian forest complexity can affect EPT assemblage composition and reduce the abundance and frequency of occurrence of the most sensitive genera, as well as cause changes in the availability of organic matter and primary production in streams.

Many studies have shown that EPT insects respond to marked changes in habitat and water quality, especially when there is a reduction in dissolved oxygen and the accumulation of fine sediments that can be deposited in the gills, reducing respiratory capacity (Rosenberg and Resh 1993; Compin and Céréghino 2003). In addition, it seems that this group does not respond homogeneously to environmental variations, because some genera are more sensitive, whereas others are more tolerant. For example, in the study of Corbi *et al.* (2013), the

Americabaetis (Ephemeroptera) and *Leptonema* (Trichoptera) genera were common in streams edged with banana plantations, whereas Plecoptera families and other Trichoptera genera were related to streams with natural riparian forest.

In the present study, the composition of EPT genera also responded to riparian forest complexity, considering a small-scale gradient. The greater occurrence and abundance of *Farrodes* genera (Ephemeroptera) in streams with medium riparian forest complexity seems to be associated with periphyton availability (chlorophyll-*a* and phaeopigments) and the amount of organic matter, a food source for brushers such as these insects (Baptista *et al.* 2006). In well-illuminated environments, scrapers are abundant, feeding on autochthonous periphyton; when these insects exploit other particles adhered to the substrate, they are classified as brushers (Brasil *et al.* 2014). The *Kempnyia* (Plecoptera) and *Zelusia* (Ephemeroptera) indicated streams with higher riparian forest complexity, corroborating the results of Corbi *et al.* (2013), who found that 40% of the Plecoptera genera recorded in their study were sensitive to environmental changes. Plecoptera taxa tend to decline at less intense levels of human disturbances compared with Ephemeroptera and Trichoptera, and they require a high proportion ($\geq$54%) of forest with limited anthropogenic

influence to maintain similar taxonomic richness to reference conditions (Törnblom *et al.* 2011).

On a local scale, it is possible that riparian forest complexity is an important moderator of physicochemical water variables and habitat, and consequently of aquatic insect assemblages. A study conducted in the same area as the present study showed that forest environmental heterogeneity was the main factor structuring dung beetle communities (Silva and Hernández 2014). In the present study, riparian forest variables seem to have affected the primary production and amount of organic matter in streams, and thus indirectly or directly the distribution pattern of EPT assemblages. In south-eastern Brazil, a study conducted in rural streams showed that composition (e.g. percentage of trees, grasses, vines, bamboo, canopy cover) and riparian forest structure (e.g. tree density and height, vertical canopy structure, mean basal area and diameter at breast height) affected water quality variables such as the amount of fine sediments, conductivity, dissolved oxygen and ammonia concentrations (Souza *et al.* 2013).

In PERI and RAT, the higher values of primary production (chlorophyll-*a* and phaeopigments) found in streams was due to a low canopy cover percentage. This result was expected because the more open the canopy over a stream, the greater the light incidence and, consequently, the greater the growth of periphyton on the substrate (Vannote *et al.* 1980). The amount of allochthonous organic matter appeared to be more related to channel morphology, slope and the retention capacity of organic material. A higher amount of organic material was found in Stream D of PERI and seemed to be associated with a lower retention capacity of the surrounding terrestrial system. This stream had a steep slope, greater percentage of uncovered soil and greater distance between trees, which may have facilitated the input of organic matter into the stream by rain.

The EPT assemblages seem to be affected by multiple environmental predictors. Several predictors are driving EPT assemblages in the study area, which are related to riparian forest complexity (tree and shrub size and top diameter), as well as the composition of inorganic substrate, amount of organic matter, primary production and physicochemical characteristics (electrical conductivity, depth, water velocity). In conclusion, the results of the present study show that EPT insects can indicate small changes in riparian forest complexity, a reflection of processes generated by local disturbances.

Acknowledgements

References

Allan, J. D. (2004). Landscapes and riverscapes: the influence of land use on stream ecosystems. *Annual Review of Ecology Evolution and Systematics* **35**, 257–284. doi:10.1146/ANNUREV.ECOLSYS.35.120202.110122

Allan, J. D., and Castillo, M. M. (2007). 'Stream Ecology: Structure and Function of Running Waters', 2nd edn. (Springer)

Anderson, M. J., Gorley, R. N., and Clarke, K. R. 2008. PERMANOVA + for PRIMER: Guide to Sofware and Statistical Methods. PRIMER-R: Plymouth, UK.

Bain, M. B., and Stevenson, N. J. (1999). 'Aquatic Habitat Assessment: Common Methods.' (American Fisheries Society: Bethesda, MD, USA.)

Balian, E. V., and Naiman, R. J. (2005). Abundance and production of riparian trees in the lowland floodplain of the Queets River, Washington. *Ecosystems* **8**, 841–861. doi:10.1007/S10021-005-0043-4

Baptista, D. F., Buss, D. F., Nessimian, J. L., Da Silva, E. R., De Moraes Neto, A. H. A., Carvalho, S. N., De Oliveira, M. A., and Andrade, L. R. (2006). Functional feeding groups of Brazilian Ephemeroptera nymphs: ultrastructure of mouthparts. *Annales de Limnologie – International Journal of Limnology* **42**, 87–96. doi:10.1051/LIMN/2006013

Bisheimer, M. V., Santos, C., and Carlson, V. E. (2013). 'A mata atlântica da ilha de Santa Catarina', 2nd edn. (Lagoa Editora: Florianópolis, Brazil.)

Bonnet, A., and Queiroz, M. H. (2006). Estratificação vertical de bromélias epifíticas em diferentes estádios sucessionais da Floresta Ombrófila Densa, Ilha de Santa Catarina, Santa Catarina, Brasil. *Revista Brasileira de Botanica. Brazilian Journal of Botany* **29**, 217–228.

Brasil, L. S., Juen, L., Batista, J. D., Pavan, M. G., and Cabette, H. S. R. (2014). Longitudinal distribution of the functional feeding groups of aquatic insects in streams of Brazilian Cerrado Savanna. *Neotropical Entomology* **43**, 421–428. doi:10.1007/S13744-014-0234-9

Brower, J. E., Zar, J. H., and Von-Ende, C. N. (1998). 'Field and Laboratory Methods for General Ecology', 4th edn. (McGraw-Hill Science/Engineering/Math: Boston, MA, USA.)

Caruso, M. M. L. (1983). 'O desmatamento da Ilha de Santa Catarina de 1500 aos dias atuais.' (Universidade Federal de Santa Catarina: Florianópolis, Brazil.)

Clarke, K. R., and Gorley, R. N. (2006). 'Primer v.6: User Manual/Tutorial.' (PRIMER-E.)

Compin, A., and Céréghino, R. (2003). Sensitivity of aquatic insect richness to disturbance in the Adour-Garonne stream system (France). *Ecological Indicators* **3**, 135–142. doi:10.1016/S1470-160X(03)00016-5

Corbi, J. J., and Froehlich, C. G. (2010). Bioaccumulation of metals in aquatic insects of streams located in areas with sugar cane cultivation. *Quimica Nova* **33**, 644–648. doi:10.1590/S0100-40422010000300030

Corbi, J. J., and Trivinho-Strixino, S. (2008). Relationship between sugar cane cultivation and stream macroinvertebrate communities. *Brazilian Archives of Biology and Technology* **51**, 769–779. doi:10.1590/S1516-89132008000400015

Corbi, J. J., Kleine, P., and Trivinho-Strixino, S. (2013). Are aquatic insect species sensitive to banana plant cultivation? *Ecological Indicators* **25**, 156–161. doi:10.1016/J.ECOLIND.2012.09.020

Cummins, K. W., and Klug, M. J. (1979). Feeding ecology of stream invertebrates. *Annual Review of Ecology and Systematics* **10**, 147–172. doi:10.1146/ANNUREV.ES.10.110179.001051

Cummins, K. W., Merritt, R. W., and Andrade, P. C. N. (2005). The use of invertebrate functional groups to characterize ecosystem attributes in selected streams and rivers in south Brazil. *Studies on Neotropical Fauna and Environment* **40**, 69–89. doi:10.1080/01650520400025720

Domínguez, E., and Fernández, H. R. (2009). 'Macroinvertebrados bentónicos sudamericanos: sistemática y biología.' (Fundación Miguell Lillo: Tucumán, Argentina.)

Domínguez, E., Molineri, C., Pescador, M., Hubbard, M., and Nieto, C. (2006). 'Aquatic Biodiversity in Latin America: Ephemeroptera of South America', Volume 2. (Pensoft: Sofia, Bulgaria.)

Dufrêne, M., and Legendre, P. (1997). Species assemblages and indicator species: the need for a flexible asymmetrical approach. *Ecological Monographs* **67**, 345–366.

Egler, M. A., Buss, D. F. A., Moreira, J. C. B., and Baptista, D. F. (2012). Influence of agricultural land-use and pesticides on benthic macro-invertebrate assemblages in an agricultural river basin in southeast Brazil. *Brazilian Journal of Biology* **72**, 437–443. doi:10.1590/S1519-69842012000300004

França, J. S., Gregório, R. S., Paula, J. D., Gonçalves, J. F., Ferreira, F. A., and Callisto, M. (2009). Composition and dynamics of allochthonous organic matter inputs and benthic stock in a Brazilian stream. *Marine and Freshwater Research* **60**, 990–998. doi:10.1071/MF08247

Kleba Lisboa, L. K., Silva, A. L., Siegloch, A., Gonçalves, J. J., and Petrucio, M. (2015). Temporal dynamics of allochthonous coarse particulate organic matter in a subtropical Atlantic Rainforest Brazilian stream. *Marine and Freshwater Research* **66**, 674–680. doi:10.1071/MF14068

Kleine, P., Trivinho-Strixino, S., and Corbi, J. J. (2011). Relationship between banana plant cultivation and stream macroinvertebrate communities. *Acta Limnologica Brasiliensia* **23**, 344–352. doi:10.1590/S2179-975X2012005000012

Ligeiro, R., Hughes, R. M., Kaufmann, P. R., Macedo, D. R., Firmiano, K. R., Ferreira, W. R., Oliveira, D., Melo, A. S., and Callisto, M. (2013). Defining quantitative stream disturbance gradients and the additive role of habitat variation to explain macroinvertebrate taxa richness. *Ecological Indicators* **25**, 45–57. doi:10.1016/J.ECOLIND.2012.09.004

Lorenzen, C. J. (1967). Determination of chlorophyll and pheopigments: spectrophotometric equations. *Limnology and Oceanography* **12**, 343–346. doi:10.4319/LO.1967.12.2.0343

Lorion, C. M., and Kennedy, B. P. (2009). Relationship between deforestation, riparian forest buffers and benthic macroinvertebrates in Neotropical headwater streams. *Freshwater Biology* **54**, 165–180. doi:10.1111/J.1365-2427.2008.02092.X

Mesa, L. M. (2014). Influence of riparian quality on macroinvertebrate assemblages in subtropical mountain streams. *Journal of Natural History* **48**, 1153–1167. doi:10.1080/00222933.2013.861937

Moraes, A. B., Wilhelm, A. E., Boelter, T., Sternet, C., Schultz, U. H., and Maltchik, L. (2014). Reduced riparian zone width compromises aquatic macroinvertebrate communities in streams of southern Brazil. *Environmental Monitoring and Assessment* **186**, 7063–7074. doi:10.1007/S10661-014-3911-6

Myers, N., Mittermeier, R. A., Mittermeier, C. G., Fonseca, G. A. B., and Kents, J. (2000). Biodiversity hotspots for conservation priorities. *Nature* **403**, 853–858. doi:10.1038/35002501

Naiman, R. J., Décamps, H., and Mcclain, M. E. (2005). 'Riparia: Ecology, Conservation, and Management of Streamside Communities.' (Elsevier Academic Press: Burlington, MA, USA.)

Nessimian, J. L., Venticinque, E. M., Zuanon, J., De Marco, P. J. R., Gordo, M., Fidelis, L., Batista, J. D., and Juen, L. (2008). Land use, habitat integrity, and aquatic insect assemblages in Central Amazonian streams. *Hydrobiologia* **614**, 117–131. doi:10.1007/S10750-008-9441-X

Richardson, J. S., and Béraud, S. (2014). Effects of riparian forest harvest on streams: a meta-analysis. *Journal of Applied Ecology* **51**, 1712–1721. doi:10.1111/1365-2664.12332

Rosenberg, D. M., and Resh, V. H. (1993). 'Freshwater Monitoring and Benthic Macroinvertebrates.' (Chapman & Hall: New York.)

Salles, F. F., and Ferreira-Junior, N. (2014). Hábitat e hábitos. In 'Insetos aquáticos na Amazônia brasileira: taxonomia, biologia e ecologia'. (Eds N. Hamada, J. Luiz Nessimian, and R. Barbosa Querino.) pp. 39–49. (Editora do INPA: Manaus, Brazil.)

Salles, F. F., Da Silva, E. R., Serrão, J. E., and Francischetti, C. N. (2004). Baetidae (*Ephemeroptera*) na Região Sudeste do Brasil: novos registros e chave para os gêneros no estágio ninfal. *Neotropical Entomology* **33**, 725–735. doi:10.1590/S1519-566X2004000600010

Siegloch, A. E., Suriano, M., Spies, M., and Fonseca-Gessner, A. (2014). Effect of land use on mayfly assemblages structure in Neotropical headwater streams. *Anais da Academia Brasileira de Ciencias* **86**, 1735–1747. doi:10.1590/0001-3765201420130516

Silva, P. G, and Hernández, M. I. M. (2014). Local and regional effects on community structure of dung beetles in a mainland–island scenario. *PLOS One* **9**(10), e111883. doi:10.1371/JOURNAL.PONE.0111883

Souza, A. L. T., Fonseca, D. G., Libório, R., and Tanaka, M. O. (2013). Influence of riparian vegetation and forest structure on the water quality of rural low-order streams in SE Brazil. *Forest Ecology and Management* **298**, 12–18. doi:10.1016/J.FORECO.2013.02.022

Stenert, C., Bacca, R. C., Moraes, A. B., Ávila, A. C., and Maltchik, L. (2012). Negative effects of exotic pine invasion on macroinvertebrate communities in southern Brazil coastal ponds. *Marine and Freshwater Research* **63**, 283–292. doi:10.1071/MF11169

Strahler, H. N. (1957). Quantitative analysis of watershed geomorphology. *American Geophysical Union Transactions* **38**, 913–920. doi:10.1029/TR038I006P00913

Törnblom, J., Degerman, E., and Angelstam, P. (2011). Forest proportion as indicator of ecological integrity in streams using Plecoptera as a proxy. *Ecological Indicators* **11**, 1366–1374. doi:10.1016/J.ECOLIND.2011.02.011

Vannote, R. L., Minshall, G. W., Cummins, K. W., Sedell, J. R., and Cushing, C. E. (1980). The river continuum concept. *Canadian Journal of Fisheries and Aquatic Sciences* **37**, 130–137. doi:10.1139/F80-017

Ward, J. V. (1992). 'Aquatic Insect Ecology: Biology and Habitat.' (Wiley: New York.)

Wiggins, G. B. (1996). 'Larvae of the North American Caddisfly Genera (Trichoptera)', 2nd edn. (University of Toronto Press: Toronto, ON, Canada.)