PUBLIC HEALTH

Spatial and Temporal Distribution of Blackflies (Diptera: Simuliidae) in the Itatiaia National Park, Brazil

Ronaldo Figueiró[1,2,3], Carlos J.P.C. Araújo-Coutinho[4], Leonardo H. Gil Azevedo[1], Érika S. Nascimento[1] e Ricardo F. Monteiro[3]

[1]Depto. Entomologia, Instituto Oswaldo Cruz - Fiocruz, Av. Brasil, 4365, Manguinhos. Pavilhão Carlos Chagas 4º andar, C. postal 926, 21045-900, Rio de Janeiro, RJ
[2]Student of the Programa de Pós-Graduação em Ecologia of the Univ. Federal do Rio de Janeiro, RJ
[3]Depto. Ecologia, Instituto de Biologia, Univ. Federal do Rio de Janeiro, Av. Brigadeiro Trompowski, s/n. CCS, IB, Ilha do Fundão, Cidade Universitária, C. postal, 68020, 21941-590, Rio de Janeiro, RJ
[4]Visitor researcher SUCEN / FIOCRUZ

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Distribuição Espacial e Temporal de Simulídeos (Diptera: Simuliidae) no Parque Nacional de Itatiaia

RESUMO - A distribuição das taxocenoses de larvas de simulídeos (Diptera: Simuliidae) de diferentes altitudes no Parque Nacional de Itatiaia (localizado no sudeste do estado do Rio de Janeiro, Brasil 44º34‘ – 44º42‘ W , 22º16‘ – 22º28‘ S) foi investigada ao longo de quatro campanhas, cada uma em uma diferente estação do ano de 2003. Cada campanha foi composta de 60 amostras, obtidas a partir de “quadrats” de madeira de 30 x 30 cm, distribuídos através de quatro áreas: Taquaral, Véu da Noiva, Três Picos e Brejo da Lapa. Nas campanhas do inverno e da primavera, dois outros sítios, Alsene e Agulhas Negras, foram adicionados. Foram coletadas 3578 larvas e 292 pupas, sendo identificadas seis espécies: *Simulium clavibranchium* Lutz, *S. subnigrum* Lutz, *S. rappae* Py-Daniel, *S. incrustatum* Lutz, *S. stellatum* Gil-Azevedo, Figueiró & Maia-Herzog e *Lutzsimulium pernigrum* Lutz. As maiores abundância e diversidade foram encontradas no sítio de altura intermediária, o Brejo da Lapa. A análise de componentes principais (PCA) revelou que *L. pernigrum*, *S. stellatum* e *S. incrustatum* se mostraram associadas a rios de menor porte, com as duas primeiras espécies associadas a sítios com maior insolação e *S. incrustatum* com pouca insolação.

PALAVRAS-CHAVE: Inseto aquático, simulídeo, distribuição altitudinal

ABSTRACT - The distribution of the blackfly larval taxocenoses of different altitudes in the Itatiaia National Park (located in the southeast of Rio de Janeiro state, Brazil 44º34‘ – 44º42‘ W , 22º16‘ – 22º28‘ S) was investigated on four campaigns, one in each season of 2003. On each campaign 60 samples were collected, using 30 x 30 cm wooden quadrats, distributed at four sites: Taquaral, Véu da Noiva, Três Picos and Brejo da Lapa. The winter and spring campaigns included two additional sampling sites: Alsene and Agulhas Negras. A total of 3578 larvae and 292 pupae were sampled, representative of six species: *Simulium clavibranchium* Lutz, *S. subnigrum* Lutz, *S. rappae* Py-Daniel, *S. incrustatum* Lutz, *S. stellatum* Gil-Azevedo, Figueiró & Maia-Herzog and *Lutzsimulium pernigrum* Lutz 1910. The highest larvae abundance and diversity were reported at the intermediate altitude site, Brejo da Lapa. Principal Components Analysis (PCA) revealed that *L. pernigrum*, *S. stellatum* and *S. incrustatum* were associated with the smaller breeding sites, the first two species being associated with sunny sites, while *S. incrustatum* occurred in sites with little sunlight.

KEY WORDS: Aquatic insect, blackfly, altitudinal distribution

Simuliidae (Diptera) has a wide geographical range, which extends from the tropics to the Artic and Antarctic circles. The family comprises more than 1750 known species, most of which are hematophagous (Crosskey & Howard 1997). These insects have public health and economical relevance, since their bites can lead to allergic reactions, and because some of them are vectors of *Onchocerca volvulus* Bickel (Spirulida: Filariidae), aethiological agent of the Onchocerciasis in the Americas and on the African continent (Rey 1991). Futher, there are indications of a relation between blackfly

bites and the genesis of the self-immune disease Penfigus Foliaceus (Eaton *et al.* 1998).

In Brazil, the Simuliidae are of importance in the public health sphere, as vectors of Onchocerciasis in northern Roraima (Moraes & Chaves 1974), Goiás (Gerais & Ribeiro 1986) and northern Amazonas (Shelley *et al.* 1997) and socioeconomically, affecting specially tourism (Araújo-Coutinho & Lacey 1990). Eaton *et al.*(1998) have related the occurrence of the Penfigus Foliaceus to the presence of *Simulium nigrimanum* Macquart.

Even though the Simuliidae have a worldwide geographical range, these organisms are in general restricted to areas where lothic conditions create a suitable habitat for the development of their immature stages (Lake & Burger 1983).

Most of the studies on blackfly population dynamics and community structure concentrate on the temperate region (E.g.: Corkum & Currie 1987, Ciborowski & Adler 1990, McCreadie *et al.* 1995), whereas in the tropical region they are limited to few studies undertaken in Brazil (Hamada *et al.* 2002, Hamada & McCreadie 1999, Araújo-Coutinho *et al.* 1999), Guatemala (Okazawa & Takahashi 1981) and Venezuela (Grillet & Barrera 1997, McCreadie *et al.* 2005).

The studies undertaken in Brazil generally focus on a single species (Hamada 1993, Hamada & McCreadie 1999), or deal with species occurrences, such as Hamada *et al.* (2002). Of these studies, only that study of Araújo-Coutinho *et al.* (1999) was carried out outside Amazonia (in the state of Rio de Janeiro). The other studies on the Simuliidae of the southeastern Brazil consist of species inventories and do not deal with ecological aspects (Araújo-Coutinho *et al.* 1988, Pepinelli *et al.* 2003, Gil Azevedo & Maia-Herzog 2004).

The present study investigated the spatial and temporal distribution of blackfly larvae at different altitudes in the Itatiaia National Park.

Material and Methods

The present study was carried out during four campaigns in the Itatiaia Natonal Park, one in each season of the year 2003, in February (summer), April (autumn), August (winter) and November (spring).

The Park is located in the southeastern part of the state of Rio de Janeiro (44º34‘ – 44º42‘W and 22º16‘ – 22º28‘ S).

Sixty samples were collected on each campaign, using 30 X 30 cm wooden quadrat frames, distributed homogeneously throughout the sampling areas Taquaral, Véu da Noiva, Três Picos and Brejo da Lapa. During the winter and spring campaigns two additional areas were sampled, in order to allow further qualitative analyses of the altitudinal gradient: Alsene and Agulhas Negras, resulting in 30 additional samples on each of these two campaigns.

Each sampling area was subdivided into three sites, about 10 m far from each other, for the purpose of discovering possible heterogeneity within each area. Five samples were collected at each site, each sample consisting of riffle litter contained within a 30 x 30 cm quadrat, with a total of 15 samples per area. The biological material collected was stored in plastic bags and kept refrigerated, to conserve the organisms until their transference to 70% ethanol and posterior identification and quantification in the laboratory.

Sampling areas. Taquaral is located near to the headquarters of the Itatiaia National Park, at an altitude of 874 m a.s.l. This area is situated near a road, but due to difficult access, presents little evidence of visitation. The canopy is very dense, in spite of the proximity of the road. It is possible to observe arboreal and undergrowth layers with lianas. The river has a mean width of 4.5 m and many exposed rocks, which have resulted in many riffle litter patches throughout the sampled stretch of the river.

Véu da Noiva is part of the Alambari river, but due to the proximity to the waterfall of the same name, this denomination has been adopted to provide a clearer reference. This area is located at 1100 m a.s.l., and is one of the main touristic attractions of the Park; it is thus exposed to constant anthropic activity. The canopy provides less shadow than that of Taquaral. At some points on the river, the riparian vegetation is further from it, resulting in less exposed rocks and riffle litter. The riparian vegetation is very similar to that at Taquaral, with arboreal and undergrowth layers.

Três Picos is located at 1576 m a.s.l., and is of difficult access, requiring a 2h hike, which means that it is little visited. This area alternates parts with dense canopy with others where the vegetation is sparser, and thus, provides less shadow. There are few exposed rocks, which results in a smaller number of riffle litter patches. However, the streamside vegetation is thick, composed of arboreal and undergrowth layers, with many epiphytes and lianas, especially bromeliads, resulting in much aloctonous material falling in the river.

Brejo da Lapa is located in Itamonte county, Minas Gerais state, at 2145 m a.s.l. The region is at the headwater of a river, and is characterized by herbaceous riparian vegetation, mostly composed by grasses, and sparse bushes, resulting in considerable exposure to sunlight and less riffle litter, but unlike the other areas, there is a large amount of grass submerged in the river.

Alsene is located at 2400 m a.s.l. and received its name due to the proximity of a hotel of the same name. The riparian vegetation is mostly of bushes, which in some parts of the stream located at the bottom of a small steep-sided valley, provides a dense shade. The river contains many partially or totally submerged rocks, but riffle litter is very scarce in this area, in spite of the conditions favorable to this type of substrate.

Agulhas Negras is located at 2422 m a.s.l., and like the Brejo da Lapa, is the source of a small river of one meter’s average width. The riparian vegetation is composed of bushes and herbaceous plants, that only partially overhang the river surface. The river has few exposed rocks, but, like the Brejo da Lapa, presents submerged grasses that serve as substrate for the larvae. This area was only visited during the winter and spring campaigns.

Abiotic factors. In each campaign the following factors were measured in each area: pH, luminosity (Lux), water dischage (m^3/s), water temperature (ºC) and substrate dominance.

The luminosity was determined with a luximeter, always

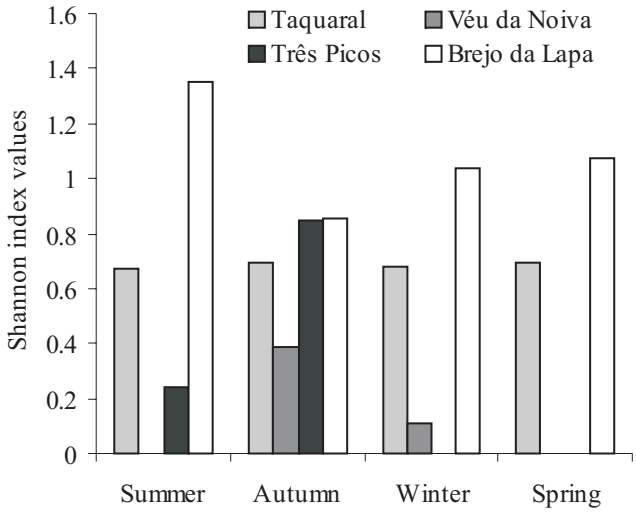

Figure 1. Shannon diversity index values for blackflies at each of the sites visited in all four campaigns.

around noon, under a clear sky. The water discharge was calculated using a flow meter (rotameter) and the substrate dominance was estimated in terms of the surface area occupied by each substrate type per quadrat.

Larvae identification. The blackfly larvae were separated into morphotypes, based on their cephalic spot patterns. The last instar larvae (those with mature respiratory histoblasts) of these morphotypes were separated for dissection and mounting on slides in accordance to Calvão-Brito & Maia-Herzog (2003). These slides were examined under an optical microscope and the morphotypes identified by comparison with specimens of the blackfly collection of the "Laboratório de Referência Nacional em Simulídeos e Oncocercose, Departamento de Entomologia - IOC / Fiocruz".

The larvae of the subgenus *Simulium* (*Inaequalium*) are difficult to differentiate in species, and therefore were treated as a group, since earlier instars were used in the analysis.

Data analysis. As the abiotic factors were intercorrelated, a Principal Components Analysis was performed in order to summarize these variables in a smaller number of statistically independent principal components. To compare the influence of the abiotic factors in the different seasons, the data of each season were analysed separately.

The abiotic variables that were not normally distributed were submitted to a Log 10 transformation (width, discharge and luminosity) or negative inverse (temperature). The areas were compared as regards similarity and their diversities estimated using the Shannon-Weaver index.

To describe the altitudinal distribution of blackfly larvae, another ordination technique, the Correspondence Analysis was applied. To reduce the effect of different sampling efforts and thus allow the inclusion of Alsene and Agulhas Negras in the analysis, the mean larvae abundances for each site were used.

Results

A total of 3578 blackfly larvae belonging to the following six species were sampled: *Lutzsimulium pernigrum* Lutz, *Simulium incrustatum* Lutz, *S. stellatum* Gil-Azevedo, Figueiró & Maia-Herzog, *S. clavibranchium* Lutz, *S. subnigrum* Lutz, *Simulium rappae* Py-Daniel, 1982, The last three belong to the subgenus *Simulium* (*Inaequalium*).

The highest diversity values were recorded at the Brejo da Lapa (2200 m) and Taquaral (874 m) sites. The first was

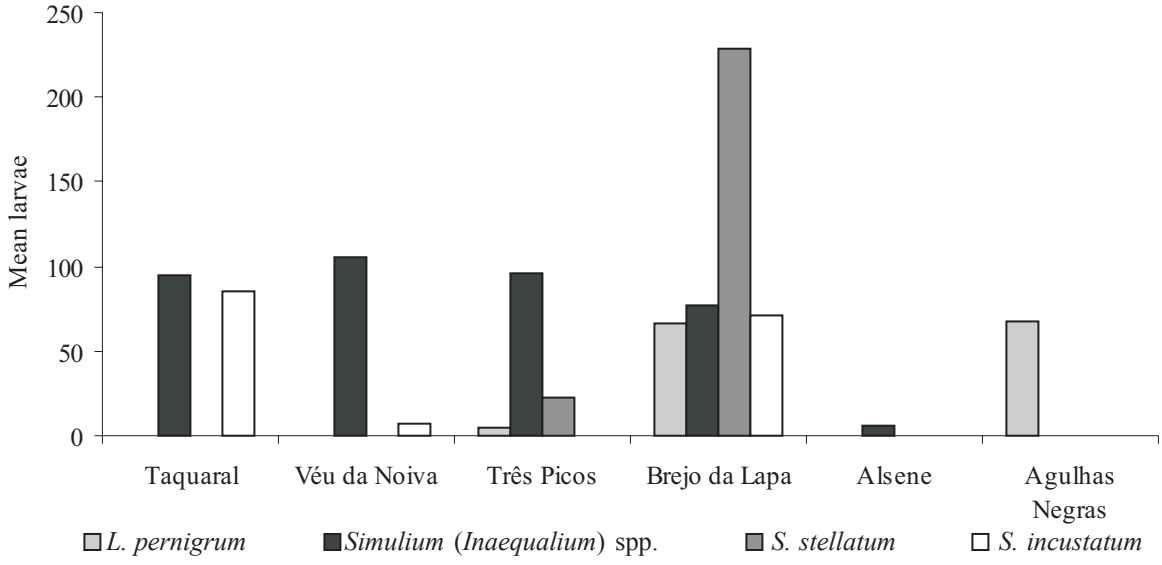

Figure 2. Mean larvae abundances for each sampling site, including the sites visited only during Winter and Spring campaigns (Alsene and Agulhas Negras).

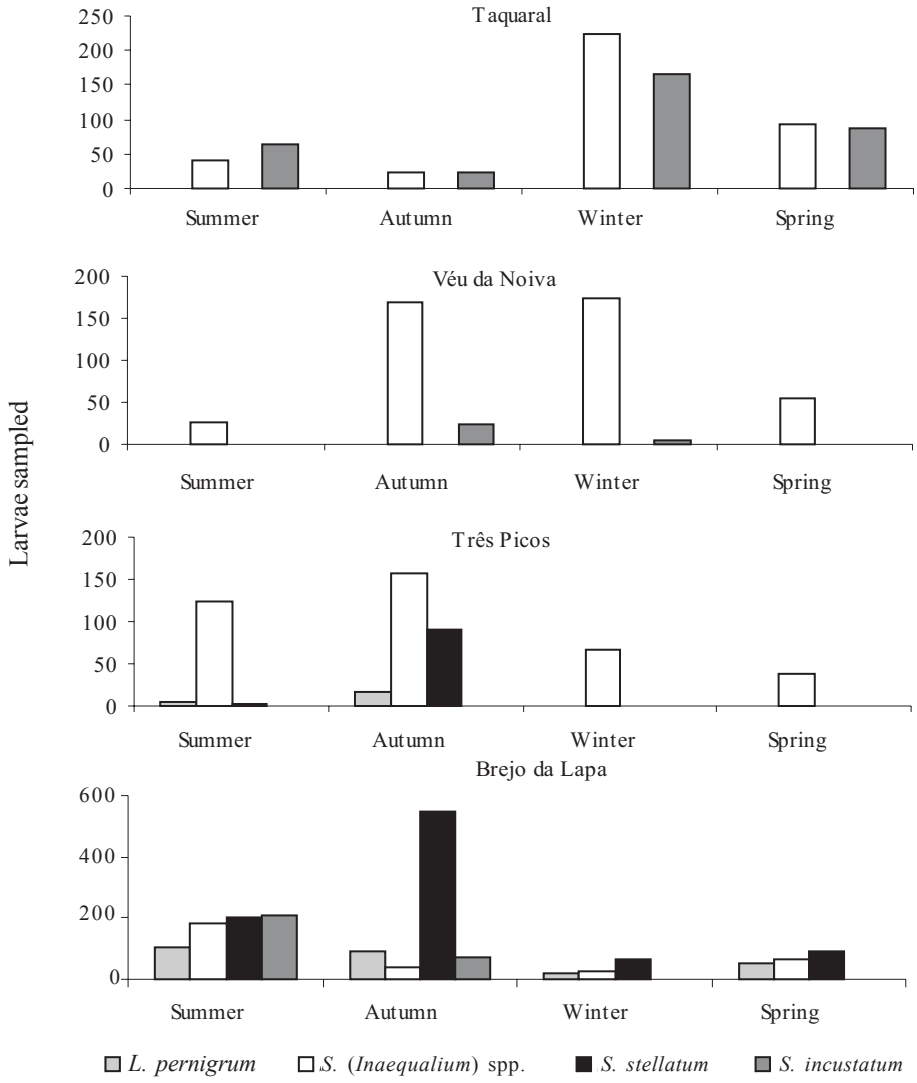

Figure 3. Seasonal larval abundance per sampling área.

greater in the summer, while the second had little fluctuation. Véu da Noiva and Três Picos had their highest values in the autumn (Fig. 1).

The morphotaxon *Simulium* (*Inaequalium*) spp. present in all the areas sampled, except Agulhas Negras (Fig. 2). This group was the most abundant in the summer, winter and spring, and the second most abundant in the autumn (Fig. 3). Its dominance was expected because this group consists of at least three species.

S. incrustatum occurred at Taquaral, Véu da Noiva and Brejo da Lapa, and its highest abundance was recorded in the summer, when it was dominant in Taquaral and Brejo da Lapa (Fig. 2, 3). The highest abundances of this species occurred in areas of lower discharge (Fig. 4).

S. stellatum occurred at Três Picos and Brejo da Lapa (Fig. 3). At the Três Picos, it was found during two campaigns (Summer and Autumn), whereas in Brejo da Lapa it was present in all seasons (Fig. 3). It was the dominant species at Brejo da Lapa in all seasons, except for the summer (Fig. 2).

L. pernigrum occurred at Três Picos, Brejo da Lapa and Agulhas Negras (Fig. 2). This species, as occurred with *S. stellatum*, was not recorded at Taquaral or Véu da Noiva, and was found at Três Picos on summer and autumn (Fig. 3). At Brejo da Lapa its occurrence was observed during all seasons, with particularly high values in the summer and autumn campaigns (Fig. 3). Brejo da Lapa and Agulhas Negras are physiognomically similar rivers, both being small and surrounded by herbaceous riparian vegetation.

Comparing the Principal Components Analyses of the four seasons, it is clear that the principal components assumed different meanings in each analysis, and the river size was the only common factor (Tables 1, 2). Due to this variation among the prominent factors acting on the species abundances in each campaign, it could have been predicted

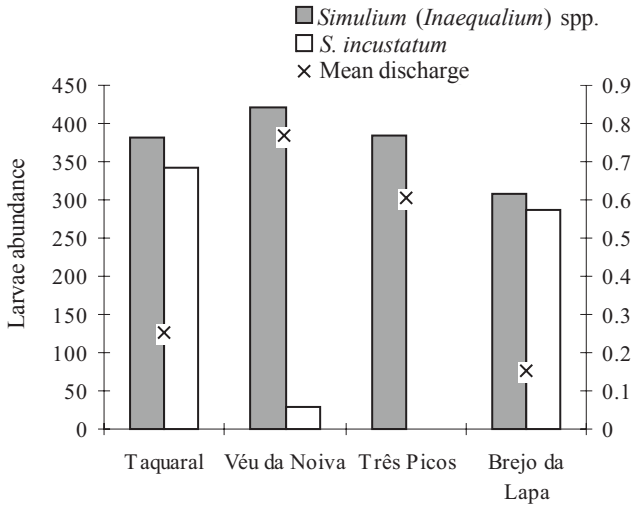

Figure 4. Comparison of the mean discharges and the abundances of *S. incrustatum* and *Simulium* (*Inaequalium*) spp., showing the association of the former with larger streams, and the association of the latter with smaller streams.

that pooling all the data in one single analysis would result in overshadowing of the abiotic factors, a fact that reinforces the need for the separate interpretation of each campaign PCA.

The Principal Components Analyses only had their first two axes interpreted, since they represented most of the variance of the data. This indicated the river size and intensity of sunlight as the major factors acting on the distribution of blackfly larvae (Fig. 5, Tables 1, 2).

The Correspondence Analysis showed that whereas most of the sites were very similar in their species compositions, Brejo da Lapa and Agulhas Negras stood out from the others (Fig. 6). Agulhas Negras behaved as an outlier. *Simulium* (*Inaequalium*) spp. was strongly associated with Taquaral (880 m) and Três Picos (1576 m), while *S. incrustatum* was equidistant in the multidimensional space of Taquaral (880 m), Três Picos (1576 m) and Brejo da Lapa (2200 m), and *L. pernigrum* behaved as an outilier, closer to Agulhas Negras (2500 m) (Fig. 6).

Discussion

Brejo da Lapa represented 52% of the total larvae sampled, as well as being the area with highest diversity and species richness on all the campaigns. The great exposure to sunlight in this area may be one of the factors determining the apropriate conditions for the colonization and estabilishment of different immature blackfly populations, the result of greater primary production. Microalgae are also an important part of the blackfly larvae diet (Alencar *et al.* 2001).

Comparing the four sampling areas according to their physiognomic characteristics, the Brejo da Lapa clearly stands apart from the others because of its herbaceous riparian vegetation that does not overshadow on the river. This greater exposure to sunlight, together with the small size of the river, allows us to infer that in this area the daily temperature variation is greater than in the other areas.

Vannote *et al.* (1980) postulated in their river continuum concept that systems with highly stable physical structure tend to have low biological diversity, while systems with lower stability tend to have higher diversities. According to

Table 1. Variable contributions to the formation of the axes in the principal components analyses.

	PC 1	PC 2
Summer		
Vegetal substrate	0.246156	0.069427
Luminosity	0.031275	0.484451
Width	0.274133	0.110939
Discharge	0.339953	0.000217
Water temperature	0.108483	0.334965
Autumm		
Vegetal substrate	0.288492	0.021612
Luminosity	0.294843	0.054124
Width	0.15965	0.26939
Discharge	0.000304	0.538549
Water temperature	0.256711	0.116325
Winter		
Vegetal substrate	0.242512	0.009912
Luminosity	0.246153	0.041067
Width	0.185976	0.267762
Discharge	0.243568	0.015625
Water temperature	0.081791	0.665634
Spring		
Vegetal substrate	0.224058	0.000297
Luminosity	0.229362	0.001737
Width	0.141757	0.614637
Discharge	0.229949	0.000296
Water temperature	0.174874	0.383033

PC: principal components

Table 2. Correlations between larvae abundance and the principal components.

	Summer	
	PC 1 (river size)	PC 2 (sunlight)
Lutzsimulium pernigrum	-0.519574	0.602178
Simulium (*Inaequalium*) spp.	-0.141092	0.44281
S. stellatum	-0.589951	0.697698
S. incrustatum	-0.661699	0.465142
Total larvae	-0.496707	0.567815
	Autumn	
	PC 1 (canopy cover)	PC 2 (river size)
L. pernigrum	-0.619784	0.008639
S. stellatum	-0.738404	-0.008418
S . incrustatum	-0.366747	0.209614
Simulium (*Inaequalium*) spp.	0.333463	-0.423557
Total larvae	-0.525943	0.010302
	Winter	
	PC 1 (river size)	PC 2 (water temperature)
L. pernigrum	-0.449059	-0.004177
S. stellatum	-0.580731	0.064793
S .incrustatum	0.290493	0.63594
S.(*Inaequalium*) spp.	0.405555	0.287796
Total larvae	0.256186	0.507163
	Spring	
	PC 1 (sunlight)	PC 2 (river size)
L. pernigrum	0.919446	0.02564
S. (*Inaequalium*) spp.	0.156357	0.000603
S. stellatum	0.939469	0.031776
S . incrustatum	-0.369759	-0.009003
Total larvae	0.717436	0.018554

PC: principal components

this concept, since Brejo da Lapa is a smaller and more exposed stream, it is reasonable to assume that it has a greater daily range of water temperature. Thus, this may be responsible for the higher diversity in this area. This conclusion is based on the fact that each organism prefers a particular optimal temperature, so a greater variation in temperature would allow more organisms to attain their optimal metabolic activity at some time during the day (Vannote *et al.* 1980).

The diversity and abundance of blackflies found in this study do not correspond to the patterns regarded as typical of tropical regions. The pattern described by Hamada *et al.* (2002), establishes a positive relation between high species

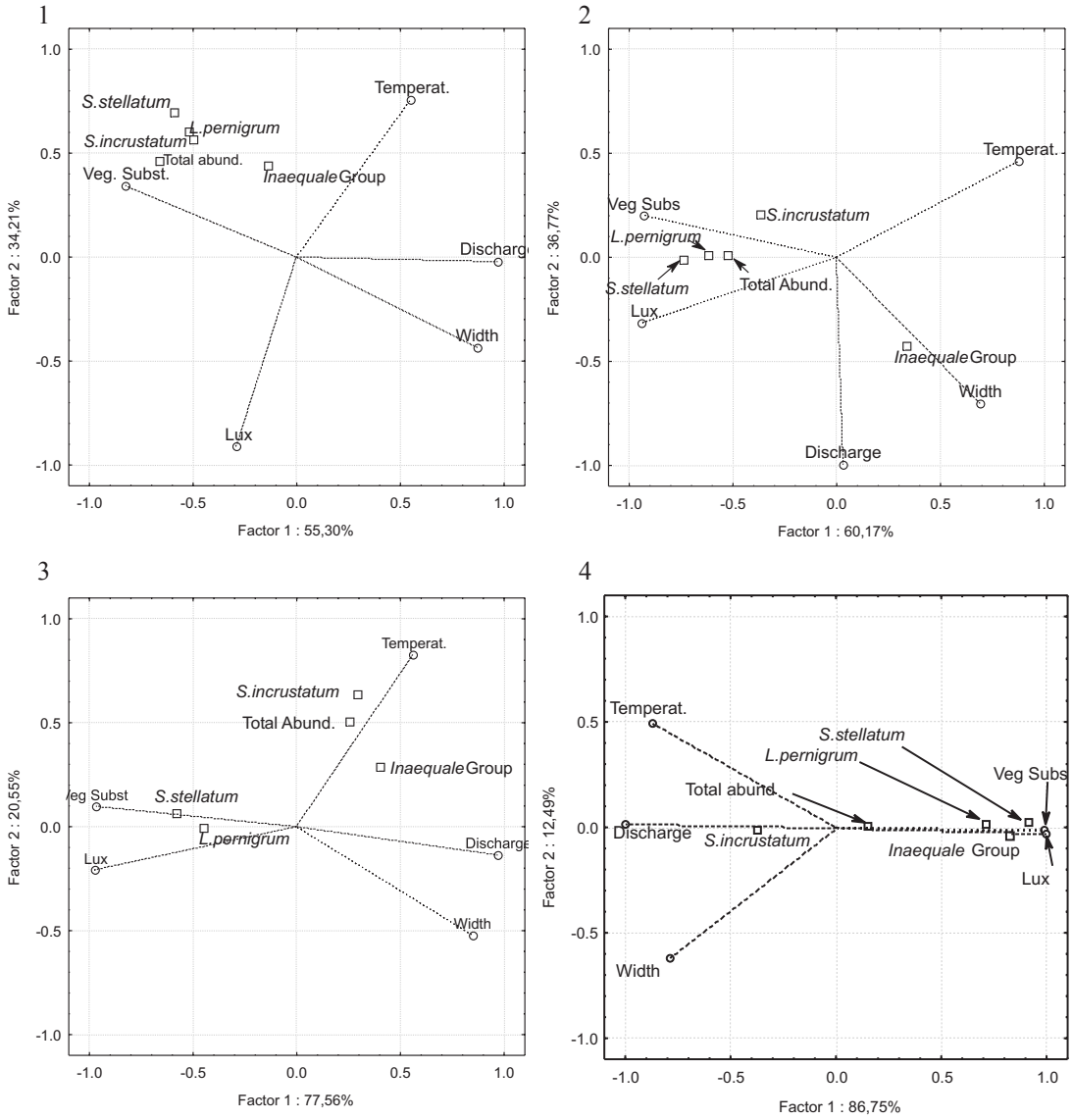

Figure 5. Principal components analyses for Summer(1), Autumn(2), Winter(3) and Spring(4).Width = river width, temperat = water temperature, lux = luminosity

richness and large fast flowing breeding sites, with dense canopy cover and cool temperature. This a pattern is also described by Grillet & Barrera (1997).

This difference between the pattern described by Hamada *et al.* (2002) and that observed in this project may be partially explained by the distinct climatic and physiognomical conditions of the breeding sites in the Amazonian region studied by those authors as compared to the sites investigated in the present study.

Of the six species found in the present study, five had already been previously recorded in the states of Rio de Janeiro and Minas Gerais. There *L. pernigrum*, *S. stellatum* and *S. incrustatum* were associated with smaller breeding sites. The first two occurred in sunnier breeding sites and the last in less sunny breeding sites. However, in order to determine habitat preference patterns, further studies at a larger number of sampling sites are necessary.

L. pernigrum is dominant, according to the data of Strieder *et al.* (2002), at the headwaters of the Rio dos Sinos basin (Rio Grande do Sul State, Brazil). Brejo da Lapa and Agulhas Negras (areas where this species was found), are both headwaters, like the area these authors studied.

No clear altitudinal distribution was found in this study, as the Correspondence Analysis showed (Fig. 6). The species occurrence seemed to be more related to site specific characteristics than to altitude related factors, which may be observed in the graphical distribution of the sites in the multidimensional space of the Correspondence Analysis: Taquaral (880 m) was more similar to Três Picos (1576 m) than to Véu da Noiva (1100 m), and Alsene (2400 m) was more closely similar to Três Picos (1576 m) than to Brejo da Lapa (2200 m) or Agulhas Negras (2500 m).

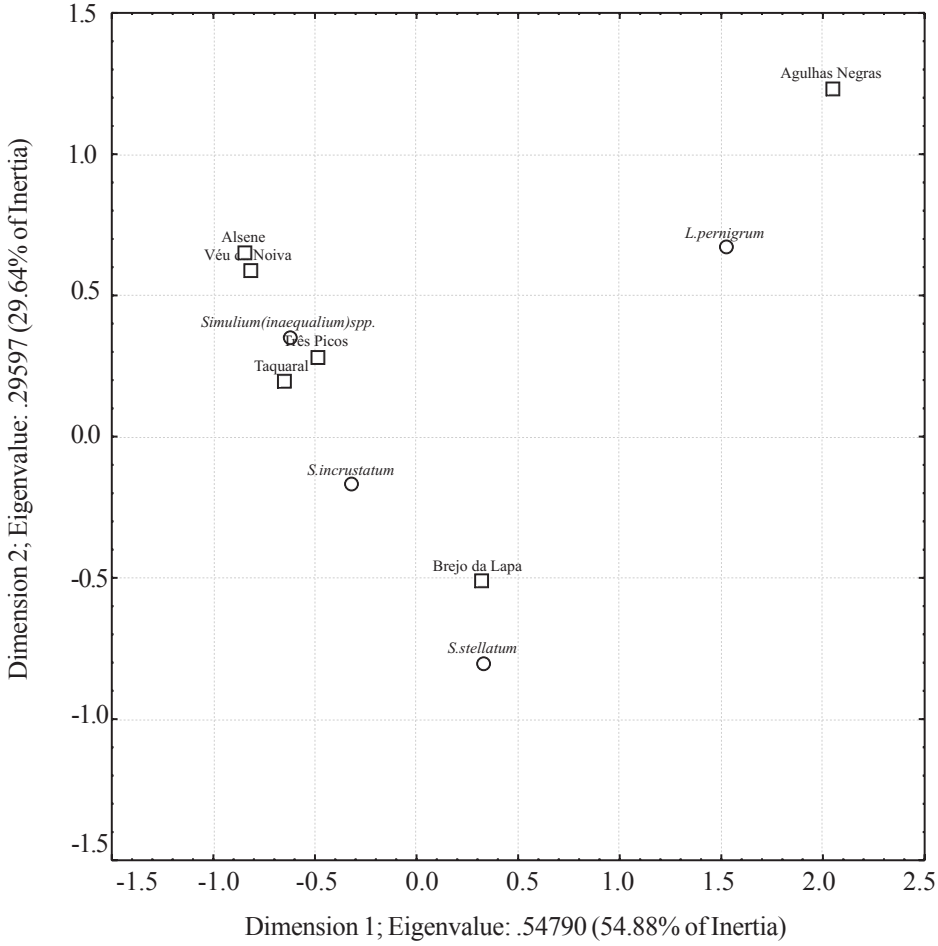

Figure 6. Correspondence analysis for larvae abundance per sampling site, indicating species associations with each site.

The position of a given species in the multidimensional space of the correspondence analysis may be intepreted as the place where its optimal conditions are to be found. This means that *Simulium* (*Inaequalium*) spp. found their optimal conditions at Taquaral (880 m) and Três Picos (1576 m), while *S.incrustatum* found its optimal conditions at Taquaral (880 m), Três Picos (1576 m) and Brejo da Lapa (2200 m). On the other hand, *S. stellatum* clearly found its optimal conditions only at Brejo da Lapa (2200 m), and *L. pernigrum*, even though it is not as clear as with *S. stellatum* seemed to find its optimal conditions at Agulhas Negras (2500 m).

The small number of studies in the literature regarding the community ecology of the Simuliidae in Brazil does not yet allow us to draw any consistent general patterns nor understanding the processes which determine them. This points to the need for further studies, embracing a larger variety of biomes, in order to establish consistent distributional patterns for Simuliidae communities.

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