# Preliminary observations on the patterns of co-occurrence of Black fly (Diptera: Simuliidae) larvae and some of their potential macroinvertebrate predators

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## ARTICLE INFO

## ABSTRACT

Biotic factors such as predation, although important drivers of the black fly community, are rarely investigated in the literature. This study aimed to test the hypothesis that the patterns of co-occurrence of black fly larvae and its potential predators is not random and that there is a correlation between its frequencies and Simuliidae larvae abundances. Larvae were sampled from two localities in the Pedra Branca State Park, Rio de Janeiro, Brazil, during the dry season in june 2018. We collected *Simulium pertinax* Kollar, 1832, *Simulium subpallidum* Lutz, 1910, *Simulium* (*Inaequalium*) sp., *Simulium* (*Psaroniocompsa*) sp. and *Simulium* (*Trichodagmia*) sp. The predators families present were Perlidae, Hydropsychidae, Leptoceridae, Libellulidae and Chironomidae. The null models showed that species co-occurred significantly more than expected by chance. The canonical correlation analyses for the Vargem Grande and Pau da Fome areas area showed a significant relationship between black fly abundances and predator abundances. In Vargem Grande the abundance of Chironomidae showed a highly significant positive correlation to *S.* (*Psaroniocompsa*) sp., while Hydropsychidae was significantly correlated to *Simulium* sp. On the other hand, in Pau da Fome Libellulidae was significantly correlated to *S.* (*Psaroniocompsa*) sp. and *Simulium* sp. and Chironomidae correlated significantly to *Simulium* sp. The result is consistent with what would be expected of organisms that exercise mutual population regulation, although other factors than biotic interactions may be causing these patterns. However the lack of mechanistic evidences on the effect of biotic interactions on black fly populations pose a challenge on the understanding of these patterns.

Aquatic insects play an important role in the trophic relationships of freshwater systems (Garcia-Domingo and Saldaña, 2008), as they participate in various processes, such as the cycling of matter and energy flow (Cummins, 1992; Allan, 1995). Among the aquatic insects, a very abundant group in the streams are the black flies (Diptera, Simuliidae). This family contains 2,310 described species (Adler, 2019). Black flies are pests of humans and domestic animals and vectors of bird and mammal parasites (Adler et al., 2004), some species act as vectors of human diseases such as Onchocerciasis (Rey, 2001; Luz et al., 2014).

In the southern and southeastern regions of Brazil, there is no report of disease transmission by these insects, but the high rates of stinging by hematophagous species in areas of occurrence cause great discomfort in the population (Figueiró and Gil-Azevedo, 2010). Their larvae, however, play a key functional role in flowing waters (Malmqvist et al., 2004). Use their labral fans to filter particulate matter from the water column (Crosskey, 1990). Larvae also scrape material from the substrate, prey on aquatic organisms (Currie and Craig, 1988), and ingest dissolved organic matter (Hershey et al., 1996).

Black flies are ubiquitous organisms, found in most environments with running waters that may harbor their immatures (Currie and Adler, 2008), even though their local occurrence is influenced by a series of local abiotic factors such as substrate type (Hamada et al., 2002; Pepinelli et al., 2005; Figueiró et al., 2012), water flow velocities (Figueiró et al., 2008, 2015), water pollution (Docile et al., 2015, 2016)

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and altitude (Figueiró et al., 2006; Kuvangkadilok et al., 1999; Buitrago-Guacaneme et al., 2018; Cuadrado et al., 2019). Seasonality also plays an important role in the distribution of black flies, with wet and dry seasons influencing local (Srisuka et al., 2015; Pavitra et al., 2020) and regional diversity patterns (Figueiró et al., 2014).

While the literature on black fly spatial distribution often focus on the influence of seasonality and/or abiotic factors (Rohmah et al., 2018, Hadi et al., 2019), the effect of biotic factors tend to be overlooked, with most of the studies in this perspective concentrating on the influence of parasitism (Ginarte et al., 2003, Araújo-Coutinho et al., 2004, Nascimento et al., 2007) due to its potential use in biological control (Figueiró and Gil-Azevedo, 2010). Predation can have a strong influence on community structure, affecting reproduction, feeding, adaptation to abiotic factors and defense, which are the four basic requirements of animals for survival and perpetuation (Peckarsky, 1984). In Brazil, few works have studied predators of black flies, and much of the existing information has been published in abstracts of papers presented at meetings or theses that are not widely circulated (Alencar et al., 1999).

Biotic factors such as predation (Alencar et al., 1999) and competition (Harding and Colbo, 1981; Hemphill, 1988), although important drivers of the black fly community structure, are rarely investigated in the literature. Thus, the present study held as objective to test the hypothesis that the patterns of co-occurrence of black fly larvae and its potential predators listed in the literature is not random and that there is a correlation between Simuliidae larvae abundances and the abundance of its potential predators.

The study was carried in the Pedra Branca State Park, which is the largest urban park in Brazil (Silva et al., 2019). This park had its creation in 1974 in the Rio de Janeiro municipality and harbors 8 hydrological basins (Vallejo et al., 2009). Due to the existence of these basins within this area its conservation is vital to the water supply of the Rio de Janeiro municipality.

The larvae was sampled from the Divisa/Moinho river, located in the Vargem Grande locality (Site 1: S22°50.224', W043°30.157'; Site 2: S22°57.067', W043°29.155'; Site 3: S22°57.164', W043°29.270'; Site 4: S22°57.166', W043°29.268') and rio Grande river, located in the Pau da Fome locality (Site 5: S22°52.922', W043°26.651', Site 6: S22°55.987', W043°26.667', Site 7: S22°56.009', W043°26.735') during the dry season in june 2018. While the Divisa/Moinho stream is adjacent to the Quilombola community Cafundá Astrogilda, which implanted an agroforest model of sustainable agriculture, the Rio Grande stream feeds a water catchment station and is located in an area more prone to visitation.

Macroinvertebrates were sampled manually: at each sampling site, which consisted of a 15 m stream/river section, a random sample of riffle litter from 10 quadrats (30 x 30 cm) was taken. This type of sampling was selected in detriment of a surber in order to focus the sampling on black fly distributions and its associated entomofauna that co-occurred in the same substrates.

In order to test if the abundances of black flies was just reflecting the overall macroinvertebrate abundances a spearman correlation coefficient was calculated using black fly larvae counts and all other macroinvertebrate immature counts finding no significant correlation pattern (p=0.3154, rs=0.175). After this procedure macroinvertebrate specimens from insect orders that have families described as black fly predators in the literature were separated and then identified down to family level. Individuals from insect families of black fly predators were then identified and quantified using the pertinent literature (Angrisano, 1995; Wiggins, 1996; Carvalho et al., 2002; Olifiers et al., 2004; Pes et al., 2005).

Black fly larvae were sorted into different morphotypes in the laboratory and then identified in species level when possible and in

subgenus level when species-level identification was not possible using the taxonomical literature (Hamada and Adler, 2001; Coscarón and Coscarón-Arias, 2007).

The null hypothesis that the co-occurrence pattern of black fly larvae and their potential predators was random was tested using the null modelling software ECOSIM (Gotelli and Entsminger, 2009) to create null models for co-occurrence, in which the C-score index (Stone and Roberts, 1990) with fixed sums for row and fixed column constraints was employed and all taxa with less than 50 individuals were removed according to Gotelli and Ulrich (2012).

Blanchet et al. (2020) recently concluded in their critical review of the use of co-occurrence models in ecology that interactions cannot be directly inferred from the output of these models, and suggest that although presence-absence data are still important to understand species distributions, abundance data could provide a more powerful framework to understand the patterns of occurrence of species. Thus, the relationship between the abundances of black fly larvae and the abundances of their predators was investigated using a canonical correlation analysis (CCorrA), which is a multivariate technique that allows measuring the strength of the relationships between two sets of variables. The first set of variables used in the CCorrA was composed by the abundances of the predator families used in this study (Perlidae, Leptoceridae, Hidropsychidae, Libellulidae, Chironomidae) whereas the second set of variables was comprised of the abundances of the black fly species/subgenera identified in this study. A distinct CCorrA was performed to each of the two areas.

In the Vargem Grande area *Simulium pertinax* Kollar, 1832, *Simulium* (*Psaroniocompsa*) sp. and *Simulium* (*Trichodagmia*) sp. were the black flies identified and Perlidae, Hydropsychidae, Leptoceridae, Libellulidae and Chironomidae the potential predator families present, while in Rio Grande *S.pertinax*, *Simulium subpallidum* Lutz, 1910, *Simulium* (*Inaequalium*) sp., *Simulium* (*Trichodagmia*) sp. and *Simulium* (*Psaroniocompsa*) sp. were the black flies identified and Perlidae, Hydropsychidae, Libellulidae and Chironomidae were the potential predador families present (Table 1).

The null model showed that species co-occurred significantly more than expected by chance (observed index=116.40000, mean of simulated indices=110.30540, p-value=0.00280), thus indicating that co-occurrence patterns were not random.

The canonical correlation analysis for the Vargem Grande area showed a highly significant relationship between black fly abundances and predator abundances (p-value<0.001, canonical R=0.9098, R2=0.8277). The CCorrA for the Pau da Fome area also showed a significant relationship between black fly abundances and predator abundances (p-value=0.0021, canonical R= 0.9398, R2=0.8832). In both cases only the first canonical variable was significant. These correlations may also reflect that both black flies and their potential predators share the same microhabitat preferences.

The highest canonical loadings in module for the potential predators set in Vargem Grande were Chironomidae (0.8513) and Hydropsychidae (0.4764), while in the set composed by black fly larvae *S. (Psaroniocompsa)* sp. (0.9149) and *Simulium* sp. (0.2522) had the highest loadings in module (Table 2). The highest canonical loadings in module for the potential predators set in Pau da Fome were Libellulidae (1.1749) and Chironomidae (-0.6996) while in the set of black fly larvae *Simulium* (*Psaroniocompsa*) sp. (0.8781) *S. subpallidum* (-0.5666) and *S.* (*Trichodagmia*) sp. (0.5077) were the highest canonical loadings in module (Table 2).

In Vargem Grande the abundance of Chironomidae showed a highly significant positive correlation (p<0.01, r=0.838) to *S.* (*Psaroniocompsa*) sp., while Hydropsychidae was significantly correlated to *Simulium* sp. (p<0.01, r=0.531) (Fig. 1a). On the other hand, in Pau da Fome Libellulidae

**Table 1** Total abundances of Simuliidae larvae and potential predators for the Vargem Grande and Pau da Fome areas.

| | Vargem Grande | Pau da Fome |
|---|---|---|
| **Black fly species** | | |
| *S. pertinax* | 22 | 48 |
| *Simulium* sp. | 14 | 4 |
| *S.* (*Psaroniocompsa*) sp. | 9 | 27 |
| *S.* (*Trichodagmia*) sp. | 1 | 174 |
| *S.* (*Inaequalium*) sp. | 0 | 58 |
| *S. subpallidum* | 0 | 14 |
| **Potential predators** | | |
| Perlidae | 39 | 14 |
| Hydropsychidae | 18 | 15 |
| Leptoceridae | 4 | 0 |
| Libellulidae | 2 | 2 |
| Chironomidae | 14 | 55 |

**Table 2** Values of the CCorrA canonical loadings for the Vargem Grande and Pau da Fome areas.

| Vargem Grande | |
|---|---|
| Variables | Canonical loadings |
| *S. pertinax* | -0.2936 |
| *Simulium* sp. | 0.9502 |
| *S.* (*Psaroniocompsa*) sp. | -0.0613 |
| *S.* (Trichodagmia) sp. | 0.1512 |
| Perlidae | -0.2508 |
| Hydropsychidae | 0.4764 |
| Leptoceridae | -0.0052 |
| Libellulidae | 0.0082 |
| Chironomidae | 0.8513 |
| **Pau da Fome** | |
| Variables | Canonical loadings |
| *S. pertinax* | 0.0696 |
| *Simulium* sp. | 0.2019 |
| *S.* (*Inaequalium*) sp. | -0.0058 |
| *S.* (*Trichodagmia*) sp. | 0.5077 |
| *S.* (*Psaroniocompsa*) sp. | 0.8781 |
| *S. subpallidum* | -0.5666 |
| Perlidae | -0.1205 |
| Hydropsychidae | 0.1895 |
| Chironomidae | -0.6996 |
| Libellulidae | 1.1749 |

was significantly correlated to *S.* (*Psaroniocompsa*) sp. (p<0.01, r=0.683) and *Simulium* sp. (p<0.05, r=0.450) and Chironomidae correlated significantly to *Simulium* sp. (p<0.01, r=0.544) (Fig. 1b).

The results of the null models for co-occurence corroborated the hypothesis that the patterns of co-occurrence of Simuliidae and its predators is not random, while the results of the CCorrA corroborate the hypothesis that black fly larvae abundances are correlated to their potential predators.

The CCorrA also suggests that in Vargem Grande Chironomidae are the potential predators with stronger relation to black fly larvae abundances together with Hydropsychidae, while in Pau da Fome Libellulidae were the potential predators with stronger relation to black flies, although Chironomidae also showed strong association to black flies.

This analysis also indicates that in Vargem Grande Chironomidae is more associated to *S.* (*Psaroniocompsa*) sp., but it is important to remark that these organisms are reported in literature as predators of black fly eggs, not larvae (Crosskey, 1990; Werner and Pont, 2003), and that this insect family has extremely diverse feeding habits. In this same area Hydropsychidae was correlated basically to *Simulium* sp., which may suggest that it feeds primarily on this subgenus. On the other hand, the results of the CCorrA performed for the Pau da Fome data indicates that potential predation by Libellulidae sould be probably more generalistic, possibly occurring on *Simulium* sp. and *S.* (*Psaroniocompsa*) sp., which showed significant correlations to libellulidae.

Perlidae nymphs are known in the literature to be generalist predators, feeding on several macroinvertebrate groups such as Simuliidae, Chironomidae, Ephemeroptera and Trichoptera (Siegfried and Knight, 1976; Alencar et al., 1999), probably due to their great energy demands during their rapid growth immature stages (Cummins and Klug, 1979). However, Gamboa et al. (2009) observed that black fly larvae accounted for aproximately 20% of the diet of four *Anacroneuria* (Plecoptera: Perlidae) in the Andes, so Simuliidae actually may represent a small fraction of its feeding, so the absence of significant correlations in this study can be a reflection of this.

Trichopterans are usually regarded in literature as the main invertebrate predators of black fly larve, specially the families Hydropsychidae, Limnophilidae and Ryacophilidae (Kuralova and Olejnicek, 1985; Schorscher, 1991; Alencar, et al., 1999), so the correlation between Hydropsychidae and Simuliidae abundances is consistent with the literature, although the weight of its effects as predators on black fly larve, inferring from the correlations and canonical loadings observed,
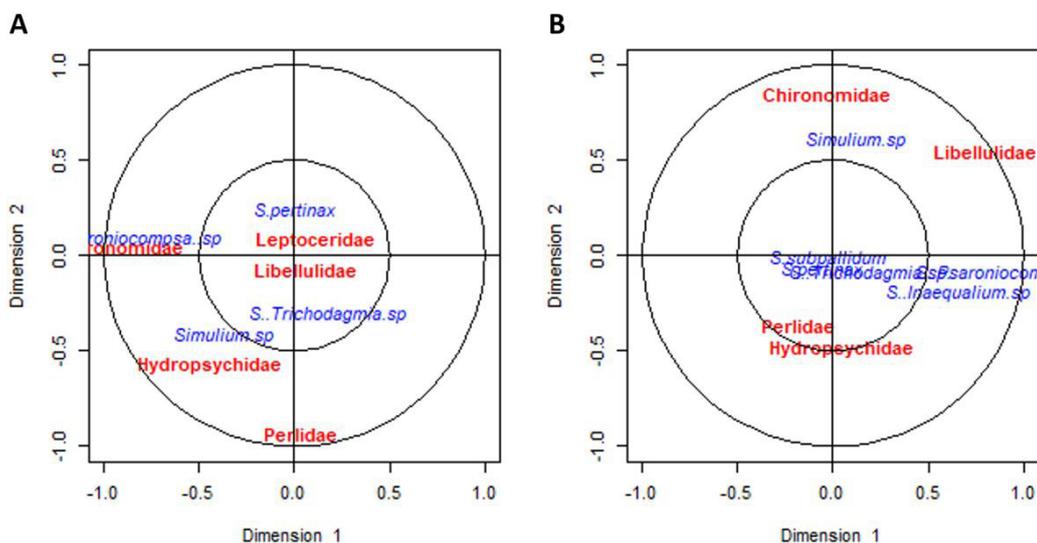


Fig. 1 Canonical Correlation Analysis ordination diagrams indicating the highly significant positive correlation of Chironomidae and *S.* (*Psaroniocompsa*) sp. (p<0.01, r=0.838), and the significant positive correlation between Hydropsychidae and *Simulium* sp. (p<0.01, r=0.531) in Vargem Grande (A) and the positive correlation between Libellulidae and *S.* (*Psaroniocompsa*) sp. (p<0.01, r=0.683) and *Simulium* sp. (p<0.05, r=0.450) and the correlation of Chironomidae to *Simulium* sp. (p<0.01, r=0.544) in Pau da Fome (B).

was coherent to what would be expected in Vargem Grande but unexpectedly low in Pau da Fome.

Libellulidae has been shown to be a black fly larvae predator in the literature (Gorayeb and Pinger, 1978; Crosskey, 1990), however its effect on black fly larvae populations has not been approached on previous studies, with the exception of Santos-Junior et al.(2006), in which the authors verified a small presence of black fly larvae in the stomach content of these organisms which contrasts to the high correlations observed in the present study, suggesting that different drivers other than predation may be influencing this pattern.

There are already reports in the literature predation on black fly larvae by larvae of Hydropsychidae (Trichoptera), Pyralidae (Lepidoptera), Libellulidae (Odonata) and Perlidae (Plecoptera) has been detected by means of stomach dissection and serological tests (precipitine and immuno difusion)(Gorayeb and Pinger, 1978; Gorayeb and Mok, 1982; Andrade, 1992; Ferreira, 1996).

The patterns of correlations between black fly larvae and its potential predators is consistent with what would be expected of organisms that exercise mutual population regulation, however the lack of literature (Hart, 1986; Malmqvist and Sackmann, 1996) on the effect of biotic interactions on black fly populations pose a challenge on the understanding of this mechanics, thus further studies like the present one are needed in order to confirm and unveil the extent of predator regulation on black fly distributions.

Due to the dynamic nature of density-dependent processes such as predation, negative and positive correlation may alternate in different moments, so further studies that address the seasonal dynamics could allow a better interpretation of the preliminary patterns that were observed. It is also important to state that these correlations don't necessarily imply causality nor interaction, so it is possible that abiotic factors such as habitat preferences may be influencing the patterns of association. However, in the light of the literature about potential black fly predators it would be reasonable to hypothesize that the results could suggest causality, although this should be further investigated possibly through manipulative experiments to be confirmed.

Studies on trophic relationships are key elements to determine the role of organisms in community structure, which has rarely been studied in black flies. In addition, studies on the potential black fly predators are needed to offer information for future integrated methods of controlling these insects, since some species in this family are of medical and veterinary importance.

## Acknowledgments

## Conflicts of interest

The authors declare no conflicts of interest.

## Author's contributions

RF contributed with the experimental design, data analysis and manuscript writing, SSS contributed in the field data sampling and organism identifications, TND contributed with data analysis and manuscript review, TRC and CAF contributed in the field data sampling and organism identification, LHGA supervised the organism identification and contributed to the manuscript writing.

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