

Potential phenotypic plasticity within *Simulium nigrimanum* Macquart, 1838 (Diptera: Simuliidae) larvae

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Abstract

Black fly larvae (Diptera: Simuliidae) are suspension filter-feeders which strongly depend on water velocity for proper feeding. Black fly species feature different microhabitat preferences. Studies of Holarctic black fly larvae revealed their phenotypic plasticity in response to water current velocity variation, but such studies have been rarely undertaken with Neotropical black flies. The current work presents results on the phenotypic plasticity of the black fly species *Simulium nigrimanum* Macquart. Twelve last instar larvae, sampled from the Brazilian Cerrado, were photographed under a stereoscopic microscope and measured using the CMEIAS Image tool software. Linear regressions with water velocity as the independent variable were performed, indicating that while body size and anal disk diameter correlated positively with water velocity, labral fan length correlated negatively. The observed relationships between water velocity and labral fan length and anal disk diameter were consistent with the literature, while the pattern of body size variation partially corroborated previous studies. The present work results suggest that potential phenotypic plasticity can be observed in black fly larvae within one population distributed in different microhabitats of the same stream section, as opposed to previous reports implying that such variation is only found among population from different streams.

Keywords: Black fly; labral fans; anal disk; water current.

1. Introduction

Although black flies (Diptera: Simuliidae) are a cosmopolitan insect family with world wide geographic range, their local occurrence is limited by suitable microhabitat characteristics allowing immature filter feeding black fly larvae to breed develop [1]. Black fly populations are regulated by biotic and abiotic factors, the former being competition, predation, and disease [2, 3]; and the latter involving to microhabitat features such as substrate and water velocity [4, 5, 6, 7]. Black flies are key organisms in lotic systems, regarded as ecosystem engineers. This is due to the silk their larvae produce essentially altering the microhabitat enabling other organisms to fixate more easily in the substrate. The water velocity influences their ability to capture organic matter in suspension in the water column [7], and due to their ability to convert smaller particles of organic matter into larger nutritive fecal pellets of organic matter because of their low digestive efficiency they are considered key organisms in the boreal biome [7, 8], and also the adults of some species are important disease vectors [9, 10, 11].

Black fly larvae usually reach their pupal stage on the same microhabitat they spent their last instar larval stage [7].





Ecological theory suggests that niche partitioning through differential use of resources is a key factor to diversity, as it promotes the local coexistence of ecologically similar black fly species [12]. This coexistence can be reached due to differential adaptations to microhabitat aspects; Nearctic and Palearctic black fly larvae are known to show different labral fan morphologies in relation to species level preferences [13, 14]. Likewise, body size is strongly related to water velocity and food availability [15, 16]. Even though these morphological relations are often studied in Holarctic black fly larvae, in their Neotropical relatives, these phenomena have rarely been approached [17].

In addition to its role as potential vector of the onchocerciasis, literature has suggested that the black fly species *Simulium nigrimanum* Macquart 1838 is potentially related to the skin condition known as Pemphigus Foliaceus [18, 19]. This makes the investigation of the bionomic and ecological traits of this species extremely relevant to public health. *Simulium nigrimanum* is widely distributed in South America [18, 19] and their larvae live associated to rocky substrates on small streams with dense canopy cover [11]. In this paper we investigate the relationships among *S. nigrimanum* morphological traits and water velocity; evaluating if these traits are correlated to this abiotic factor and among themselves.

2. Materials and Methods

2.1. Study area

The larva used in this study were originally sampled in from the Córrego do Mato (S12°39'33.0", W48°18'27.3"), which is a small stream shaded by a dense canopy cover located in the Brazilian Cerrado from the state of Tocantins in northern Brazil [11]. The Cerrado is a savanna biome which presents a typical vegetation consisting of grasses and trees that do not provide a close canopy cover, due to the small size and low density of its trees, with the exception of the gallery forests, which are the riparian vegetation of this biome.

2.2. Larvae sampling and identification

Black fly larvae of later instars were sampled by hand during six campaigns on alternate months from October 2004 to August 2005. A 15 m stream section had 15 quadrats (30 cm × 30 cm) sampled during each campaign.

Larvae were fixed in 70 % ethanol and later sorted according to morphotype in the laboratory. Last instar specimens were dissected and identified using the taxonomic literature [20, 21] and direct comparison with pupae collected in the sites and with the material deposited at the Laboratório de Simulídeos e Oncocercose/Instituto Oswaldo Cruz (LSO-IOC).

Twelve last instar individuals of *S. nigrimanum* were then separated for use in the present study. Larvae were then photographed in a stereoscopic microscope equipped with a digital camera and later measured with the use of CMEIAS software [22]. The decision to analyze only last instar larvae was made, compromising total sample size, to avoid any potential bias from measuring different stage larvae, which may still be prone to drift and morphological structure growth.

2.3. Larval measurements and statistical analyses

Each larva had its anal disk diameter, body length, and labral fan length measured according to the methodology described in Figueiró *et al.* [17] (Fig. 1). Whereas in this aforementioned study three different populations of *Simulium subpallium* Lutz 1909 from different lotic systems had their morphological traits compared, the present study assessed phenotypic plasticity within the same *S. nigrimanum* population investigating the patterns of variation within a single lotic system. All statistics and graphics were performed using PAST 4.3. These measures and the water velocities had their normality investigated using the Lilliefors test, and as all distributions were normal correlations among them and the water velocity were determined using the Pearson correlation coefficient. These morphological measures then were used as dependent variables in linear regressions in which the independent variable was the water velocity, which was measured in the field through the head rod method [23, 11].

3. Results and Discussion

A total of 430 *S. nigrimanum* larvae were collected, from which 15 were last instar larvae. Three of these 15 specimens were dissected in order to confirm species identity and the remaining larvae were measured (Table 1). All variables were significantly correlated (Table 2). Linear regressions indicated that water velocity correlated positively with anal disk diameter ($p < 0.01$, $r^2 = 0.73$) and body length ($p < 0.05$, $r^2 = 0.42$) and negatively with labral fan length ($p < 0.01$, $r^2 = 0.69$) (Fig. 2).



Figure 1. Measurements of black fly larvae morphology taken using CMEIAS: Anal disk diameter (AD), Body length (BL), and Labral Fan Length (LF).

Table 1. Larval measurements and water velocity from which each specimen was collected.

Specimens	velocity	Anal disc diameter	body length	labral fan length
1	0.828 674	0.04	0.58	0.05
2	0.828 674	0.04	0.57	0.05
3	0.828 674	0.04	0.57	0.04
4	0.828 674	0.03	0.59	0.06
5	0.828 674	0.03	0.62	0.05
6	0.885 889	0.05	0.71	0.04
7	0.885 889	0.05	0.65	0.04
8	0.990 454	0.05	0.65	0.04
9	0.990 454	0.05	0.67	0.04
10	1.252 837	0.06	0.68	0.03
11	0.990 454	0.04	0.67	0.03
12	1.252 837	0.07	0.68	0.02

The labral fan patterns were consistent with previous studies in the literature on Holarctic species, with smaller labral fans selected in faster water flow velocities due to the decrease in the cost of drag [15, 16, 24], however the observed body size variation pattern differed from that in these studies in which small larvae were observed in streams with the highest water flow velocities [15].

This divergence of the pattern of body size correlation to water velocity can be attributed to the range of water velocities sampled (0.82 m s^{-1} to 1.25 m s^{-1}). Zhang & Malmqvist [15] found that maximum body sizes were observed at moderate velocities with size decaying at faster velocities or, as suggested by Lucas & Hunter [24], due to food availability. The abundance of food can also influence morphological traits in the case of optimal conditions for this species being found on faster flows.

The anal disk diameter was consistent with the pattern observed for *S. subpallidum* Lutz, 1910 larvae [17], with larger diameters observed at higher water flow velocities, probably due to these diameters accommodating more hooks or more robust hook structures that allow larvae to resist drag.

Table 2. Correlations between the morphological characteristics of *Simulium nigrimanum* larvae (Pearson's correlation coefficient). All correlations were significant.

	Water flow velocity	Anal disk diameter	Body length	Labral fan length
Water flow velocity	-	0.853 88	0.648 91	-0.832 42
Anal disk diameter	0.853 88	-	0.660 33	-0.834 49
Body length	0.064 891	0.660 33	-	-0.681
Labral fan length	-0.832 42	-0.834 49	-0.681	-

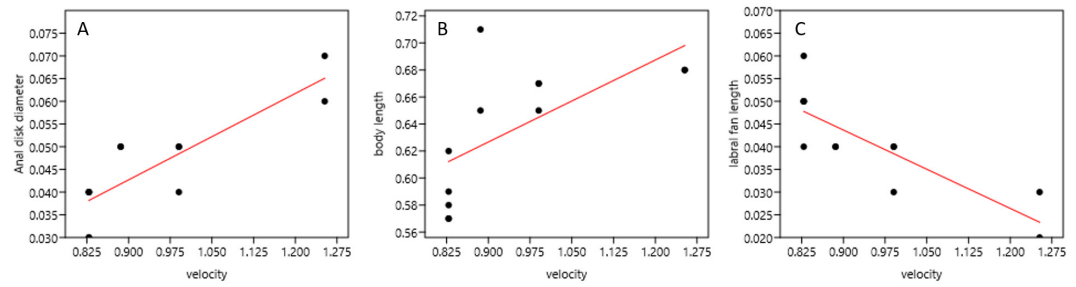


Figure 2. Linear regression of (A) water velocity versus anal disk diameter, showing a positive correlation ($p < 0.01$, $r^2 = 0.73$); (B) water velocity versus body length, showing that larger larvae were associated to faster velocities ($p < 0.05$, $r^2 = 0.42$); and (C) water velocity versus labral fan length being negatively correlated ($p < 0.01$, $r^2 = 0.69$).

4. Conclusions

These results suggest that potential phenotypic plasticity can be observed in black fly larvae within one population distributed through different microhabitats of a stream section, and this also represents the first data regarding *S. nigrimanum* phenotypic plasticity in response to microhabitat features. This data allows for a better comprehension of the spatial distribution of *S. nigrimanum*, which is considered a potential vector of the Onchocerciasis. The patterns observed in this study also suggest that although some of the trends observed in Neartic and Palearctic black fly larvae may be similar to those of Neotropical black flies, possibly some traits show markedly different patterns in the Neotropics.

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6. Conflict of interest

The authors declare no conflict of interest.

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Plasticidad fenotípica potencial dentro de las larvas de *Simulium nigrimanum* Macquart, 1838 (Diptera: Simuliidae)

Resumen: Las larvas de la mosca negra (Diptera: Simuliidae) son filtradoras de material en suspensión que dependen en gran medida de la velocidad del agua para alimentarse adecuadamente. Las especies de mosca negra muestran diferentes preferencias de microhábitat. Los estudios de las larvas de mosca negra del Holártico revelan plasticidad fenotípica en respuesta a las variaciones en la velocidad de la corriente, pero este tipo de estudios raramente se han llevado a cabo con moscas negras del Neotrópico. El presente trabajo presenta resultados acerca de la plasticidad fenotípica de la especie de mosca negra *Simulium nigrimanum* Macquart. Se fotografiaron 12 larvas en el último instar, muestreadas en el Cerrado brasileño, con estereoscopio-microscopio y se midieron usando el software CMEIAS Image. Se llevaron a cabo regresiones lineales con la velocidad del agua como variable independiente, que indicaron que, mientras el tamaño de cuerpo y el diámetro del disco anal se correlacionaron positivamente con la velocidad del agua, la longitud del abanico labral se correlacionó negativamente. Las relaciones observadas entre la velocidad del agua y la longitud del abanico labral y el diámetro del disco anal fueron consistentes con la literatura, mientras el patrón de variación del tamaño corporal corroboró parcialmente estudios previos. Los resultados del presente trabajo sugieren que se puede observar plasticidad fenotípica potencial en la larva de la mosca negra dentro de una población distribuida en diferentes microhábitats de la misma sección de la corriente, en oposición a reportes previos que implican que tal variación solo se encuentra entre poblaciones de distintas corrientes.

Palabras Clave: mosca negra; abanicos labrales; disco anal; corriente de agua.

Plasticidade fenotípica potencial de larvas de *Simulium nigrimanum* Macquart, 1838 (Diptera: Simuliidae)

Resumo: As larvas de borrachudos (Diptera: Simuliidae) são animais filtradores que realizam alimentação em suspensão. Sua ótima alimentação depende da velocidade da água e diferentes espécies preferem diferentes microhabitats. Estudos em borrachudos holoárticos têm mostrado plasticidade fenotípica em relação a mudanças na velocidades da corrente de água. Porém, existem poucos estudos feitos com borrachudos neotropicais. Este trabalho apresenta resultados sobre a plasticidade fenotípica da espécie de borrachudo *Simulium nigrimanum* Macquart. Doze larvas de último ínstar, amostradas do cerrado brasileiro, foram fotografadas em um estereomicroscópio e medidas usando o software de imagens CEMEIAS. A regressão linear realizada usando velocidade da água como variável independente indicou que o tamanho do corpo e o diâmetro do disco anal tinham uma correlação positiva com a velocidade da água. Em contraste, o comprimento dos espiráculos labrais tinham uma correlação negativa com a velocidade da água. As correlações observadas entre velocidade da água e comprimento dos espiráculos labrais e diâmetro do disco anal foram consistentes com a literatura. O padrão de variação no tamanho do corpo foi corroborado parcialmente por estudos prévios. Este estudo sugere que uma plasticidade fenotípica potencial pode se observar em larvas de borrachudos dentro de uma população distribuída em diferentes microhabitats da mesma seção de riacho, o que contradiz relatórios prévios que sugerem que esta variação pode ser encontrada unicamente entre populações de diferentes riachos.

Palavras-chave: Borrachudos; espiráculos labrais; disco anal; corrente de água.

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