Morphometric Comparison of *Simulium perflavum* Larvae (Diptera: Simuliidae) in Relation to Season and Gender in Central Amazônia, Brazil

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Number of larval instars, age structure and environmental effects on these parameters represent basic information in the study of insect population biology. When species have economic importance, this information is essential in order to choose the best period to apply different control methods and to determine the stages of the life cycle of the insect that are most susceptible to each treatment. The family Simuliidae has many species of medical/veterinary importance in the world, and some studies in the temperate region have suggested that the number of larval instars and the larval size can vary according to the season, gender and some environmental factors, such as temperature and diet. This study, with the zoophilic species Simulium perflavum *Roubaud, is the first in the Neotropics observing some of these factors and will serve as a template for other species of medical importance in the region.* S. perflavum *larvae were collected in five streams in Central Amazônia (Manaus and Presidente Figueiredo counties, State of Amazonas), in Sept./Oct. 1996 (dry season) and Feb./Mar. 1997 (rainy season). These larvae were measured (lateral length of head capsule and width of cephalic apodema) to determine the number of larval instars (n=3985), to compare the larval size between seasons and genders (last and penultimate larval instars, n=200). Seven larval instars were determined for this species using frequency distributions, t-tests and Crosby´s growth rule. Significant differences were not detected (t-test, p>0.05) in larval size between seasons and genders. Our results differ from some found in temperate regions suggesting that in the Neotropical region the larval size in different seasons and different genders remains constant, although some environmental parameters, such as diet, change depending on the season.*

Key words: aquatic insects - black fly - biometry - larval instars - Amazônia

Several studies have been done to determine the number of larval instars and the time of development of Simuliidae larvae (e.g. Craig 1975, Ross & Merritt 1978, Elouard 1978, Ross & Craig 1979, De Moor 1982, Colbo 1989). This information is important for the understanding of the population dynamics and to determine the community structure of these insects, known worldwide for their medical and veterinary importance.

The number of larval instars in black fly species is variable with reports of 4, 6, 7, 8 and 9 instars; 6 and 7 being the most common numbers and 4, 8 and 9 the most rare (Ross & Merritt 1978, Ross & Craig 1979, De Moor 1982). In South America, the number of black fly larval instars reported are: 6 (Coscarón-Arias & Bramardi 1996), 7 (Hamada 1989, Cunha et al. 1998) and 8 (Gorayeb 1981, Coscarón-Arias & Bramardi 1996). Many studies, worldwide, have shown that larval instars can vary among seasons and genders within a given species (Craig 1975, Reisen 1975, Ruhm & Sander 1975, Ross & Merritt 1978, Brenner et al. 1981, De Moor 1982, Colbo 1982). Environmental factors such as discharge, water temperature and food availability can reduce the time of development, larval instar number and the size of black flies at maturity (Ross & Merritt 1978, Colbo & Porter 1979).

As studies reporting intraspecific and gender differences in size were done in the temperate region, we carried out the present study to determine the number of larval instars of a black fly species (*S. perflavum*) in tropical Central Amazônia. In temperate regions seasonal variations in rainfall and especially temperature greatly affect the larval biology of Simuliidae, whereas in Amazônia the major physical factor is rainfall and its effect on breed-

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ing grounds. We tested the following hypotheses: (a) larval instar numbers differ between dry and rainy seasons; (b) larval size differs between dry and rainy seasons; and (c) size differences exist between male and female larvae.

MATERIAL AND METHODS

The study was carried out in five streams in Central Amazonia, three of which were located in the area of the BR-174 highway, Manaus county and two in Presidente Figueiredo county (2°39'S, 60°2'W; 2°23'S, 59°59'W; 2°29'S, 60°1'W; 2°2'S, 59°59'W; 2°2'S, 59°52'W, respectively). These streams have many characteristics in common: all flow through yellow latosol soil and are located in areas that have been disturbed by anthropogenic action such as the opening of highways. In Manaus one can divide the year in two seasons: a rainy season from December to May (March being the rainiest month with an average of 281 mm), and a dry season from June to November (August being the month with the lowest precipitation, with an average of 39 mm) (Araújo 1970). In each stream, measurements were made of water conductivity (Cole-Parmer), temperature (mercury thermometer) and pH (Cole-Parmer pH Testr 2).

Immatures of *S. perflavum* were collected in a 10-30 m stretch in each stream. They were collected on all available substrates, placed in plastic bags with 80% ethanol or in glass vials containing Carnoy's solution (one part glacial acetic acid to three parts absolute ethanol). Larvae preserved in Carnoy's solution were used for gender determination. Larval head capsules were measured for determination of larval instar and comparison of larval size by season and gender. Larvae collected in three of the five sampled streams were used to determine the number of larval instars. To determine differences between gender, when a large number of last-instar larvae were needed, we used larvae from all five streams. Biometric measurements were made on larvae of different sizes, ranging from larvae with "egg bursters" (first instar) to those with dark histoblasts (last instar) (Ross & Craig 1979). Larvae of *S. perflavum* were measured for lateral length and width of the head capsule using the methods detailed by Gorayeb (1981) and Hamada (1989).

A frequency distribution of head-capsule widths was used to allow visualization of the number of larval instars, the limit of each instar being defined by the frequencies of the measurements, presence of the "egg burster" and by the presence of a dark histoblast (Elouard 1978, Ross & Merritt 1978, De Moor 1982). The Crosby growth rule (Craig 1975) was used to verify the precision in the grouping of larval instars. The means for each instar were also compared using the t-test (Ross & Craig 1979, De Moor 1982, Hamada 1989).

The genders of penultimate and last-instar larvae (n=200) were determined after staining the gonads with Feulgen (Rothfels & Dunbar 1953); females have elongated gonads and males round ones. Measurements of the width and lateral length of the head capsule were taken before the staining process (larvae were stored individually). The mean sizes of both genders were compared by means of the t-test (Zar 1996). All statistical tests were done at 5% probability.

The mean monthly air temperature varied little in the study area. In the dry season (Sept. and Oct. 1996) mean air temperature was 26.1°C and 25.7°C and, in the rainy season (Feb. and Mar. 1997) it was 24.3°C and 24.5°C. The monthly precipitation in the dry season varied from 74.4 to 178 mm and in the rainy season from 277.4 to 457.8 mm. The pH varied little: 4.7 to 5.3 in the dry season and 4.4 to 4.8 in the rainy season. Water temperature oscillated between 29°C (dry season) to 25°C (rainy season), and conductivity varied from 9.4 to 12.6 $\mu S/cm^{-2}$.

RESULTS AND DISCUSSION

In 3,985 larvae examined measurements of the head capsule were highly correlated (r=0.995), and the distribution frequency of the measurements of the width and lateral length of the head capsule, made in the three streams during the dry and rainy seasons, resulted in seven groups. The lateral length of the head capsule was selected for testing because it is less susceptible to errors (McCreadie 1991). The t-test indicated that the means of each instar were not significantly different ($p \geq 0.05$) between seasons. The Brook ratio between the first and second instars was greater than 10% in both seasons, a result that does not agree with this rule and suggests some problems. However, the t-test comparing instars did not indicate significant differences ($p \geq 0.05$) among the means (Table I). The mean values and standard errors of the measurements were plotted, and no overlapping of the data was observed, indicating that no instar was lost (Figure).

The t-test was used to compare the means of the measurements of the lateral lengths of the head capsules of 101 female and 99 male larvae in the sixth and seventh instars. We found no significant differences ($p \geq 0.05$) among these means (Table II).

Colbo and Porter (1979, 1981) demonstrated that the size of larvae of *S. vittatum* can be affected by feeding and temperature under laboratory conditions. Ross and Merritt (1978) and Westwood and Brust (1981) found seven instars in *S. vittatum*, while Colbo and Porter (1979) reported eight instars. Since this nominal species is composed of several cytospecies, the different results could be a reflection of the inadequacy of species identification based on gross morphology. Local weather conditions might have induced a reduction in the number of *Prosimulium mixtum/fuscum* Syme & Davies instars (Ross & Merritt 1978).

The streams in the study area have characteristics common to most streams in the Central Amazon region, being acidic and poor in dissolved salts (Sioli 1965). The mean air and water temperatures in the study area vary little throughout the year, but vary more widely over the course of the day (Salati 1985). Differences in precipitation between the dry and rainy seasons can affect water quality, especially in the type of habitat used by *S. perflavum*, usually associated to water impoundments (Hamada & McCreadie 1999). Water quality can directly affect microalgae and diatoms (Lozovei & Hohmann 1977), the main organisms used as food by black flies (Lozovei 1989, Dellome Filho 1989, Alencar 1998). These organisms are good food resources for black fly larvae in terms of growth and survival (Ladle & Hansford 1981). Physical perturbation, such as sudden floods, can affect the invertebrate and periphyton community in streams (Robinson & Minshall 1986) due to substrate washing (Fisher et al. 1982).

Alencar (1998) reported differences in the composition of microalgae among the five streams sampled in this study and between the two seasons, reporting higher species richness of microalgae in the stream located near the forest,

TABLE I

Mean of the measurements of the lateral length of the head capsule (µm) of the larvae of *Simulium perflavum* (Diptera: Simuliidae) in each instar in three streams (Manaus) in the dry (a) and rainy (b) seasons and the results of the Student's t test (± S.D.) limits of the classes (µm) and Crosby's growth rule

(a)

Instars	N	Limits of classes (µm)	Mean ± S.D.	Student's t	Brook ratio	Crosby's ratio (%)
I	368	71.4 - 119	104.19± 17.85			
II	267	142.8 - 166.6	159.20 ± 11.03	-44.54[a]	1.52	-10.52
III	335	190.4 - 238.0	217.61 ± 16.21	-50.28[a]	1.36	-3.67
IV	221	261.8 - 309.4	286.13± 15.95	-49.06[a]	1.31	-6.87
V	116	333.2 - 357.0	350.43 ± 10.68	-39.03[a]	1.22	-4.91
VI	487	380.8 - 428.4	408.99 ± 20.40	-29.93[a]	1.16	-5.17
VII	88	452.2 - 476	453.28 ± 4.98	-20.23[a]	1.10	

(b)

Instars	N	Limits of classes (µm)	Mean ± S.D.	Student's t	Brook ratio	Crosby's ratio (%)
I	113	71.4 - 119	98.99 ± 18.42			
II	100	142.8 - 166.6	157.79 ± 11.54	-27.48[a]	1.59	-14.46
III	209	190.4 - 238.0	216.02± 16.56	-31.64[a]	1.36	-2.94
IV	308	261.8 - 309.4	286.99± 16.68	-47.59[a]	1.32	-7.57
V	263	333.2 - 357.0	351.57± 10.0	-54.88[a]	1.22	-4.91
VI	819	380.8 - 428.4	408.32 ± 20.28	-43.69[a]	1.16	-3.44
VII	291	452.2 - 476	459.97 ± 11.17	-41.26[a]	1.12	

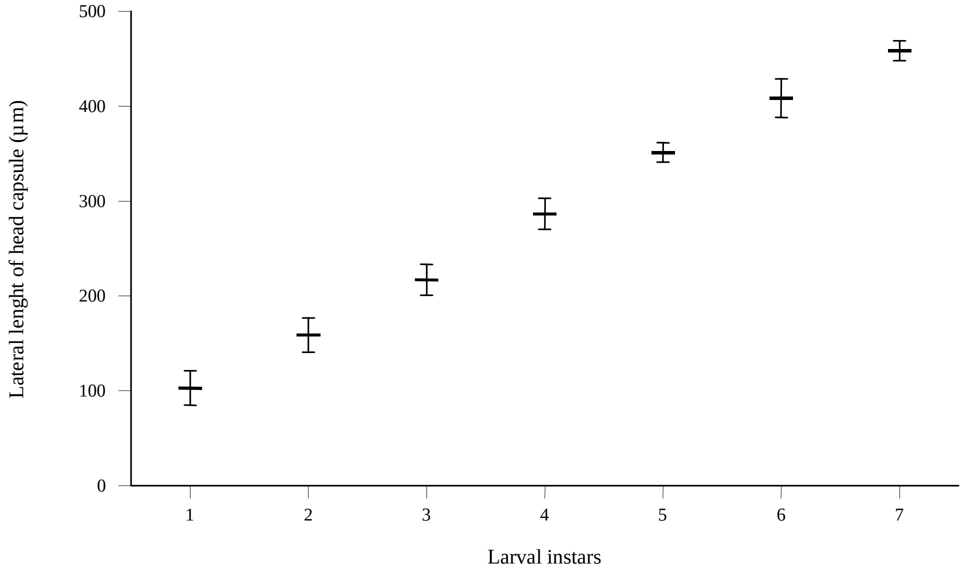
N: number of specimens; S.D.: standard deviation; t: Student's t test applied to the means of the two consecutive instars; Crosby's ratio (%): percent difference between two consecutive Brook ratios; *a*: p≥0.05

TABLE II

Mean of the measurements of the lateral length of the head capsule (µm) of male and female larvae in the sixth and seventh instar of *Simulium perflavum* (Diptera: Simuliidae) in five streams (Manaus and Presidente Figueiredo) and the results of the Student's t test (± S.D.)

Instars	Male		Female		Student's t
	N	Mean ± S.D.	N	Mean ± S.D.	
VI	41	422.01 ± 10.67	68	423.5 ± 10.53	0.709[a]
VII	58	456.71 ± 9.41	33	456.52 ± 9.32	-0.091[a]

N: number of specimens; S.D.: standard deviation; t: Student's t test applied to the means of the two consecutive instars; *a*: p≥0.05



Mean (±S.D.) size of the seven larval instars of *Simulium perflavum* (Diptera: Simuliidae) in Central Amazônia, Brazil

which is less degraded than the other ones. Even though differences in food type between season and streams were observed, mean measurements of lateral length of the larval head capsule of *S. perflavum* were not significantly different, suggesting that food type is not affecting larval size or number of larval instars in the study region, as happens in some temperate regions (Colbo & Porter 1979, 1981).

The fact that the Brook ratios between the first and second instars were not in agreement with Crosby's rule of growth could be the result of measurement error (Coscarón-Arias & Bramardi 1996). Craig (1975) reported a high Brook ratio (14%) between the first and second instars of *S. oviceps* Edwards, attributing this to apparently anomalous growth of antennal articles. However, we did not observe any anomalous growth in *S. perflavum* larvae that could explain this difference.

The mean measurements of the lateral length of the head capsule were also not significantly different between male and female larvae. Sexual dimorphism in the grouping of last larval instars has been reported in some studies (Craig 1975, Reisen 1975, Brenner et al. 1981). De Moor (1982) mentions that the sizes of the larval instars of *S. chutteri* Lewis can vary throughout the year and between genders. He observed that in pupae the females were significantly larger than the males in this species and that the decrease in size could also be seen in the sixth and seventh larval instars. The variation in size between males and females could be explained by the fact that the females would need larger reserves of nutrients for egg production. However, we did not find differences in size between males and females in the study area, thereby not supporting this hypothesis.

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