

# Stomach Content Analyses of *Simulium perflavum* Roubaud 1906 (Diptera: Simuliidae) Larvae from Streams in Central Amazônia, Brazil

Yamile B Alencar/<sup>+</sup>, Thelma A Veiga Ludwig\*, Climéia C Soares\*\*, Neusa Hamada

Coordenação de Pesquisas em Entomologia \*\*Coordenação de Pesquisas em Biologia de Água Doce, Instituto Nacional de Pesquisas da Amazônia, Caixa Postal 478, 69011-970 Manaus, AM, Brasil \*Laboratório de Ficologia, Departamento de Botânica, Universidade Federal do Paraná, Curitiba, PR, Brasil

*Stomach contents of Simulium perflavum Roubaud larvae were analyzed and compared with plankton and periphyton collected in five streams, in Central Amazonia (Manaus and Presidente Figueiredo counties), in Sep./Oct. 1996 (dry season) and Feb./Mar. 1997 (rainy season). A total of 1,400 last-instar larvae were dissected; the stomach contents were analyzed using different methods: fresh and after oxidation. A total of 87 taxa (algae, diatoms and rotifers) were found in the stomach contents. In each stream, qualitative samples of plankton and periphyton were collected; these were mounted between slides and cover slips. A total of 94 taxa of plankton and 54 taxa of periphyton were collected. One species of Rotifera was present in the stomach contents, plankton and periphyton. Cluster analysis based on species composition of the organisms present in the stomach contents grouped the streams into two major groups, each belonging to a different drainage area. Correlations based on presence/absence of species of microalgae in the stomach contents, plankton and periphyton indicated significant associations ( $p < 0.05$ ) between stomach contents and plankton and between plankton and periphyton ( $z$  test); the Sorenson coefficient and cluster analysis corroborate the same associations.*

Key words: aquatic insects - black fly - diatoms - microalgae - stomach contents - Amazônia - Brazil

Black fly larvae usually are abundant in running-water ecosystems and very important in the food chain because they process fine particulate organic matter making it available for other insects and fishes (predators). The size of particles ingested by black fly larvae is a factor influencing the effects of particulate insecticides on these larvae and on other organisms that live in the same habitat (Lacey & Lacey 1983). Knowing the minimum dimensions of particles ingested by the larvae can help in choosing the minimum dimensions of insecticides and microorganisms to be maintained in suspension in the water (Lozovei 1989).

Few studies of black fly larval feeding have been done in Brazil. Lozovei (1989) observed 118

species of diatoms (Bacillariophyta) in the larval stomach contents of three species of black fly, Dellome Filho (1989) reported 50 genera of Bacillariophyta, Chlorophyta, Cyanophyta and Euglenophyta in the stomach contents of *Simulium incrassatum* Lutz, 1910 larvae. Lacey and Lacey (1983) observed that food takes 29.2 min to pass through the intestine of *Simulium fulvinotum* Cerqueira & Nunes de Mello (= *Simulium rorotaense* Floch & Abonnenc) in an Amazonian stream with a velocity of 1 m/s and 0.37 mg/l particles in suspension.

Some authors (e.g. Craig 1977, Kurtak 1979) studying the feeding behavior and the functional morphology of black fly larvae have suggested that they are grazing and filter feeding. Wotton (1980) and Fredeen (1964) observed large amounts of bacteria in the stomach contents of black fly larvae.

This is the first study on the stomach contents of black fly larvae in the northern region of Brazil. The study identifies the organisms in the stomach contents of *S. perflavum* larvae and compares them with the plankton and the periphyton present in the streams. The five streams are compared with each other based on stomach contents of black fly larvae.

This study received partial financial support from PPG-BTRN/INPA, PPI-2-3230 (INPA/MCT) and CNPq.

<sup>+</sup>Fellowship from CNPq (139761/96-9) and corresponding author. Fax: +55-92-643.3195

E-mail: yamile@inpa.gov.br

Received 16 June 2000

Accepted 10 October 2000

## MATERIALS AND METHODS

This study was carried out in five streams in Central Amazônia, three of which were located in the area of the BR-174 highway, Manaus county and two in Presidente Figueiredo county ( $2^{\circ}39'S$ ,  $60^{\circ}2'W$ ;  $2^{\circ}23'S$ ,  $59^{\circ}59'W$ ;  $2^{\circ}29'S$ ,  $60^{\circ}1'W$ ;  $2^{\circ}2'S$ ,  $59^{\circ}59'W$ ;  $2^{\circ}2'S$ ,  $59^{\circ}52'W$ , respectively) (Fig. 1). These streams have many characteristics in common: all flow through yellow latosol soil and are located in areas that have been disturbed due to the opening of highways.

In the Manaus area the year can be divided into two seasons: a rainy season from Dec. to May (Mar. being the雨iest month with an average of 281 mm), and a dry season from Jun. to Nov. (Aug. being the month with the lowest precipitation, with an average of 39 mm) (Araújo 1970). The samples were taken during two months of the dry season (Sep./Oct. 1996) and two months of the rainy season (Feb./Mar. 1997). In each stream, measurements were made of water conductivity (Cole-Parmer conductivimeter), temperature (mercury thermometer) and pH (Cole-Parmer pH Testr 2).

Black fly stomach contents were analyzed using two different techniques. Seventy larvae from each stream were dissected monthly during the study period; from this total 20 larvae were maintained alive on ice, transported to the laboratory

and dissected on the same day, two slides were mounted with the larval black fly stomach contents using glycerine jelly (Lozovei & Luz 1976), each slide contained the stomach contents of 10 larvae. The remaining 50 larvae were preserved in Carnoy (3 parts absolute ethanol: 1 part glacial acetic acid) or 80% ethanol for later dissection and treatment using the slow-oxidation technique (Moreira Filho & Valente Moreira 1981); permanent slides were mounted using Hyrax (I.R = 1.7) as the mounting medium.

Permanent and semi-permanent slides were examined under a Zeiss compound microscope. The organisms present on these slides were counted over the whole field of the coverslip at 40x and 100x.

Plankton were collected using a net with 20 µm mesh during 30 min. The samples were placed in 40 ml vials and preserved in Transeau solution (1:1 proportion). Periphyton were collected in the streams from submersed leaves used as substrate by larvae. The material was removed with a brush, placed in 120 ml plastic vials and preserved in Transeau solution.

Identifications of Cyanophyta, Euglenophyta, Dinophyta and Chlorophyta were based on Bourrely (1968, 1970, 1972). The identifications of Bacillariophyta (diatoms) were based on Husted (1930), Krammer and Lange-Bertalot (1985, 1986,

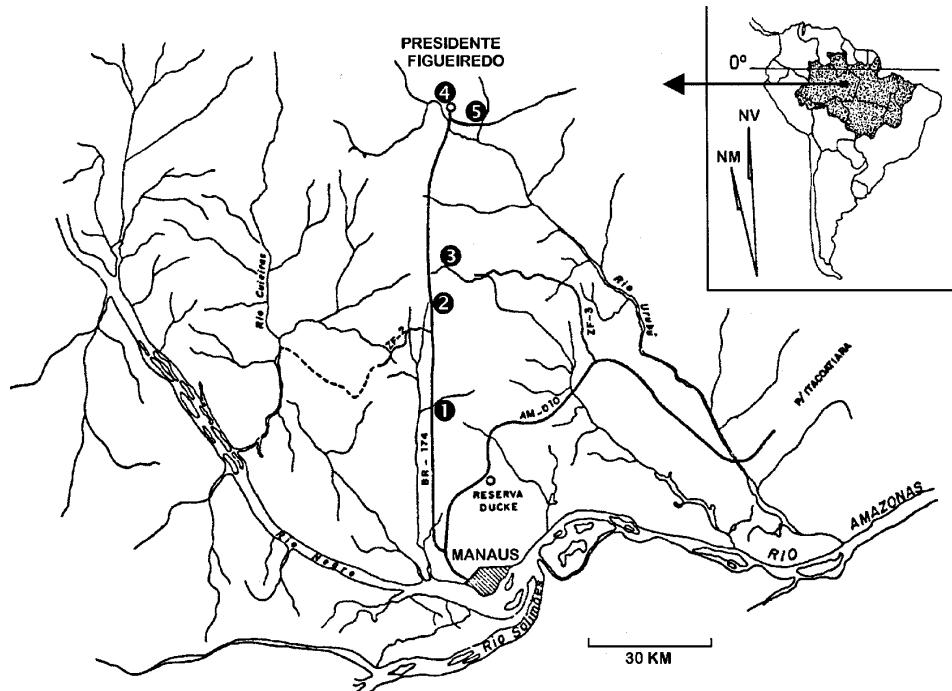


Fig. 1: collection places in Manaus and Presidente Figueiredo counties, Amazonas, Brazil. 1: Ibama stream; 2: Bueiro stream; 3: ZF3 stream; 4: Cemitério stream; 5: Escada stream

1988, 1991a,b), Kobayasi and Nagumo (1988), and Cox (1996).

Vouchers of *S. perflavum* (larval carcasses) were deposited in the invertebrate collection of the Instituto Nacional de Pesquisas da Amazônia (INPA). Samples of plankton and periphyton and the permanent and semi-permanent slides with stomach contents were deposited in the INPA and the Universidade Federal do Paraná (UFPR).

Similarity patterns based on species present in the three levels (i.e. plankton, periphyton and stomach contents of *S. perflavum* larvae) were verified using the Sorenson coefficient based on presence/absence data. Sorenson coefficient =  $2A/2A + B + C$ , where A = number of species common to the two levels, B = number of species exclusive to level 1, and C = number of species exclusive to level 2. The significance of correlation among the three levels was tested using the z statistic, based on nominal data (Zar 1996). Similarity dendograms from the similarity matrix with the Sorenson coefficient (binary data) were derived using UPGMA methods (non-balanced means) based on Pielou (1984).

## RESULTS AND DISCUSSION

The streams in the study area had pH values of 4.4 to 5.3, conductivities of 9.4 to 12.6S/cm<sup>2</sup> and temperatures of 25° to 29°C, these being representative values of streams in the study, which are normally acidic and poor in mineral salts (Sioli 1965).

*S. perflavum* larvae feed on diatoms, algae and other organisms present in the plankton and periphyton (Figs 2-72). In the stomach-content analyses (permanent slides), during the dry and rainy seasons 40 species of diatoms were observed; the frequencies of occurrence are presented in Table I.

The Bueiro, ZF3 and Cemitério streams had *Peronia* sp. as the most frequent diatom species in the dry season (35%, 22% and 23%, respectively). In the rainy season, the most frequent diatom species were different in these three streams; in the Bueiro stream the most frequent species was *Frustulia crassinervia* (24%), in the ZF3 stream it was *Eunotia gibbosa* (66%) and in the Cemitério stream it was *Actinella brasiliensis* and *Eunotia conversa* (18% and 17.9%, respectively). The Escada stream had *E. gibbosa* as the most frequent diatom species in the dry and rainy seasons (21% and 14%, respectively). The Ibama stream had *F. crassinervia* as the most frequent species in the dry season (24%) and *E. bilunaris* in the rainy season (44%).

Species of *Actinella* and *Eunotia* are indicators of acidic waters (Patrick & Reimer 1966). By the frequent presence of these two genera in the stom-

ach contents of *S. perflavum* larvae we can characterize the breeding place of this black fly species as being tropical, oligotrophic, acidic waters.

The semi-permanent slides with the stomach contents of *S. perflavum* larvae had a predominance of non-silicose algae. A total of 42 species of non-silicose microalgae were identified, six species of diatoms and one species of Rotifera, *Lecane (Monostyla) quadridentata* Ehrenberg (Table II). *Oedogonium* sp. was the most frequent species in the Bueiro stream in the dry and rainy seasons (70.8% and 74.3%, respectively). In the ZF3 stream, the most frequent species in the dry and rainy seasons was *Microspora* sp. (44.6% and 63.1%, respectively). In the Cemitério stream, *Pleurotaenium minutum* Ralfs Delp. was the most frequent species in the dry season (34.6%) and *Pleurotaenium* sp. in the rainy season (27.8%). In the Escada stream, *Microspora* sp. was the most frequent species (47%) in the dry season and in the rainy season *Closterium* sp. was the most frequent (28.6%). In the Ibama stream, *Hyalotheeca* sp. was the most frequent species in the dry and rainy season (53.5% and 37.6%, respectively).

The dimensions of diatoms observed in the stomach contents of *S. perflavum* in the five studied streams were between 67.8 and 133.57 µm for *E. serra* Ehrenberg and *Stenopterobia curvula* (W. Smith) Krammer, respectively. For the other divisions of microalgae, the sizes range from 150 to 608 µm (*Closterium* sp., *Mougeotia* sp.) and from 65 to 1000 µm (*Oedogonium* sp.) (Burton 1973).

Depending on the classification of the phytoplankton, the size can vary from 2 to 500 µm. Kurtak (1978) conducted detailed studies on the size and proportion of the ingested particles of black fly larvae and observed a predominance of large particles (150 µm) compared to the smaller ones (5-10 µm).

A great variety of food items is reported in black fly larval diets: fungal spores and mycelia, silt, detritus, Rotifera, several species of algae and diatoms (Peterson 1956, Kuznetsov 1981). The diatoms may be important food items because they are reported to be the main components of autochthonous periphyton and are constantly removed from the substrate by the water current, becoming abundant in the plankton (Dudley et al. 1986, Thompson 1987).

In this study, the qualitative analysis of the plankton found 94 infrageneric taxa: 53 Chlorophyta, 29 Bacillariophyta, 7 Cyanophyta, 2 Euglenophyta, 2 Rhodophyta and 1 Dinophyta (Table III). In the qualitative periphyton samples taken during the dry and rainy seasons we found 33 species of microalgae, 21 species of diatoms

and one species of Rotifera, *L. quadridentata* (Table III).

The similarity patterns based on qualitative data on items in the larval black fly stomach contents, plankton and periphyton were verified with the Sorenson coefficient. The results indicated

58% similarity between organisms present in the stomach contents and plankton in the streams, with 41.4% of the species in common. The similarity between the periphyton and the larval stomach contents was 54%, with 36.8% of the species in common. The Sorenson coefficient also indi-

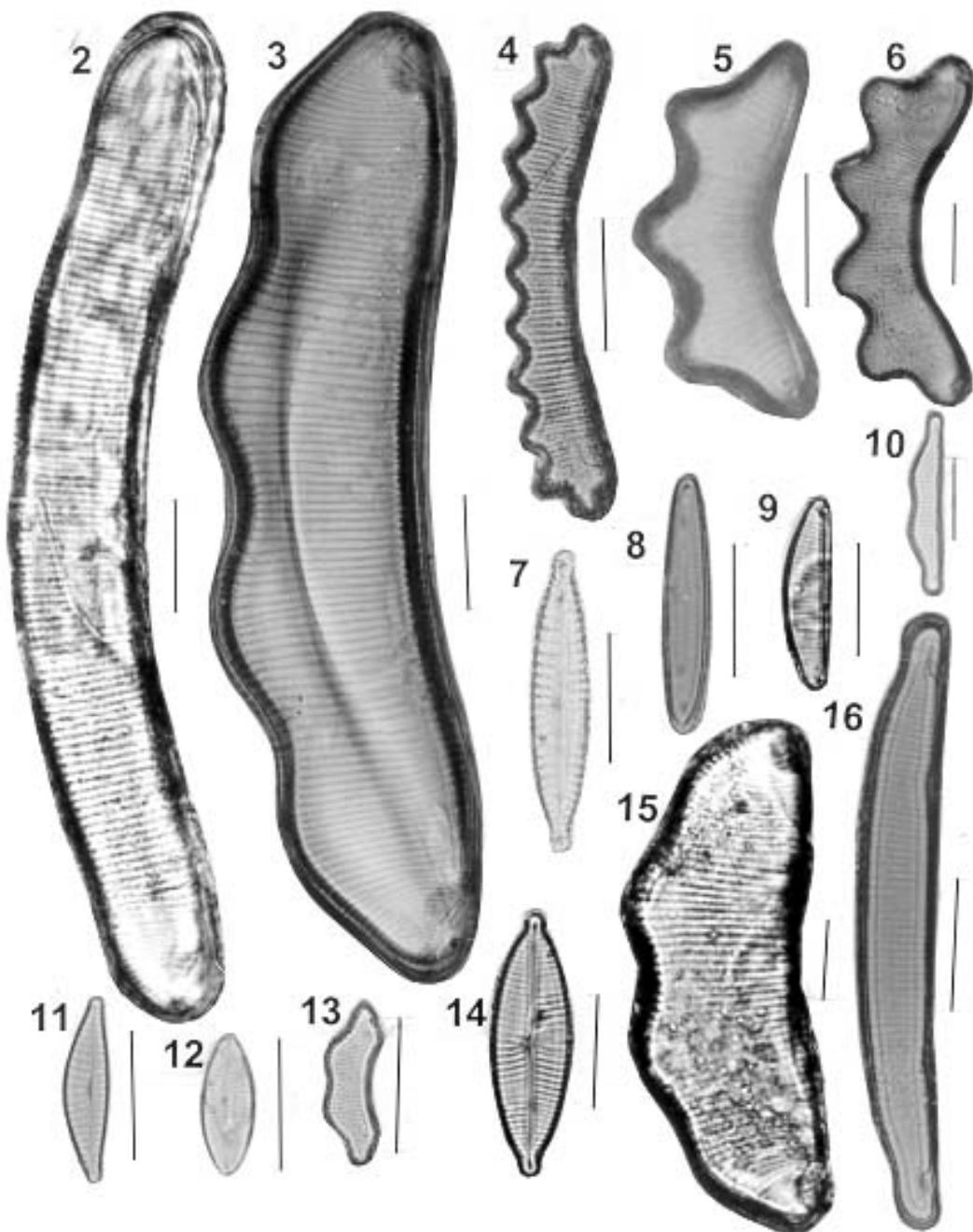


Fig. 2: *Eunotia maior*. Fig. 3: *E. zygodon*. Fig. 4: *E. serra*. Fig. 5: *E. triggiba*. Fig. 6: *E. subrobusta*. Fig. 7: *Cymbella difficilis*. Fig. 8: *Peronia brasiliensis*. Fig. 9: *E. minor*. Fig. 10: *E. bidentula*. Fig. 11: *C. minuta*. Fig. 12: *Achnanthes* sp. Fig. 13: *E. gibbosa*. Fig. 14: *Cymbella* sp. Fig. 15: *E. zygodon*. Fig. 16: *E. sudetica*. Bar = 10 µm

cated a 63% similarity among organisms in the plankton and periphyton with 45.6% of the species in common.

The hierarchical grouping analyses based on presence and absence with the Sorenson coefficient

resulted in 72% similarity between food items in the larval stomach contents and plankton in the streams, and 67% similarity between the larval stomach contents and the periphyton (Fig. 73). Therefore, the stomach content items were more

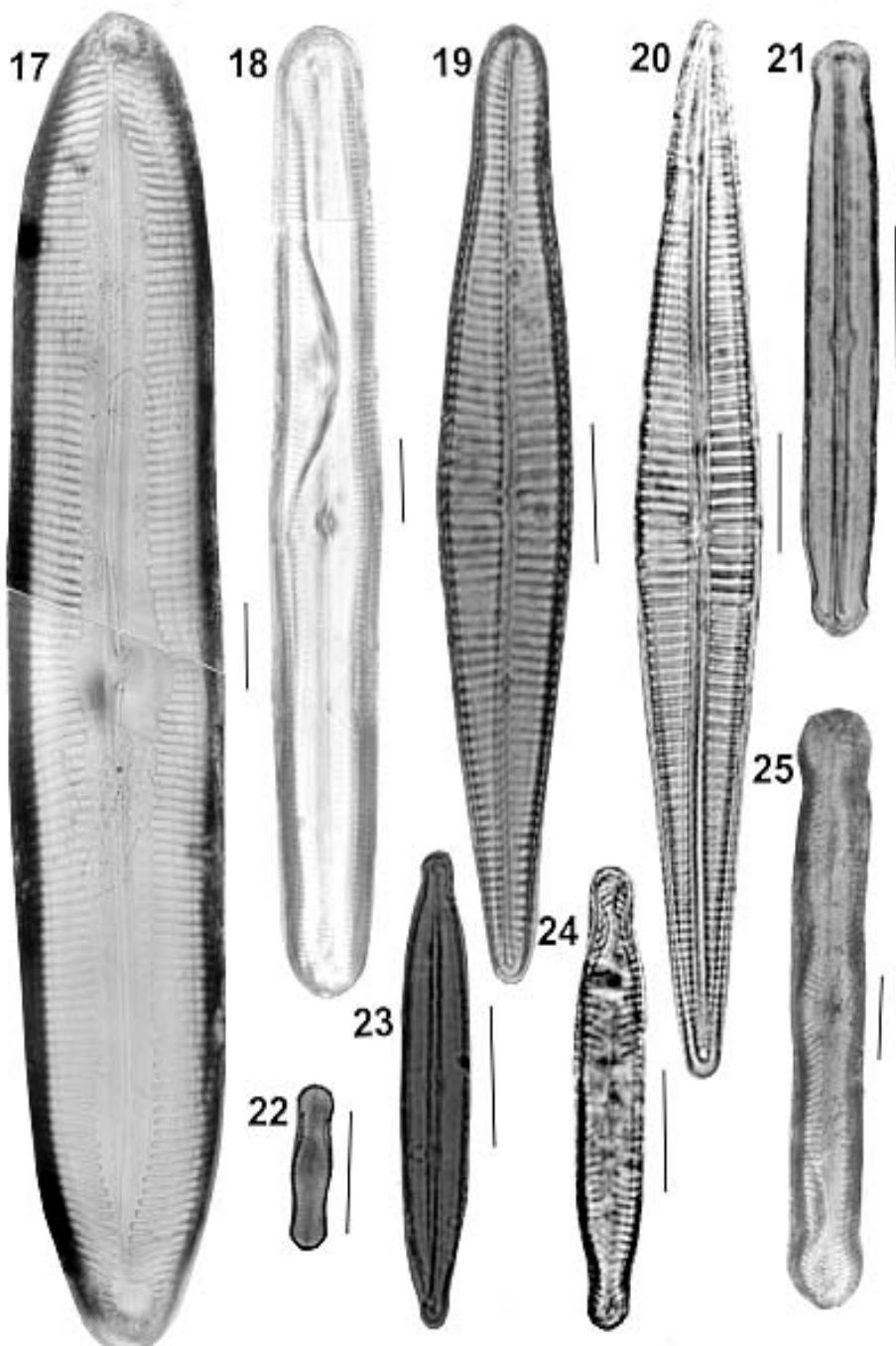


Fig. 17: *Pinnularia streptoraphe*. Fig. 18: *P. maior*. Fig. 19: *Gomphonema* sp. Fig. 20: *G. gracile*. Fig. 21: *Navicula subtilissima*. Fig. 22: *Navicula* sp. Fig. 23: *N. parasubtilissima*. Fig. 24: *P. subcapitata*. Fig. 25: *Pinnularia* sp. Bar = 10 µm

TABLE I

Frequency of diatoms (permanent slides) in the stomach contents of *Simulium perflavum* (Diptera: Simuliidae) larvae in five streams in Central Amazônia, during the dry (Sep./Oct. 1996) and rainy seasons (Feb./Mar. 1997)

Diatoms	Bueiro		ZF3		Cemitério		Escada		Ibama	
	Dry	Rainy	Dry	Rainy	Dry	Rainy	Dry	Rainy	Dry	Rainy
<i>Achnanthes</i> sp.	51	40	15	24	40	4	14	37	54	163
<i>Actinella brasiliensis</i> Grun.	127	145	122	151	180	296	113	309	287	83
<i>Actinella peronioides</i> Hustedt	168	190	77	152	141	2	12	1	226	104
<i>Actinella punctata</i> Lewis	16	57	5	—	8	—	—	—	15	—
<i>Brachysira kuntzei</i> (Reichelt)	28	—	21	150	90	—	16	—	19	6
Metzeltin & Lange-Bertalot										
<i>Brachysira serians</i> (Bréb.)	122	178	55	4	185	1	54	71	202	115
Round & Mann var.										
<i>brachysira</i> (Hus.) Cox.										
<i>Brachysira serians</i> (Brébisson)	156	177	54	209	232	198	405	1030	309	192
Round & Mann										
<i>Brachysira vitrea</i> (Grun.) Ross	285	231	182	2	398	5	802	142	336	27
<i>Cyclotella</i> sp.	—	3	—	2	—	—	—	—	—	1
<i>Cymbella difficilis</i> Krasske	15	3	5	261	43	3	3,085	1,589	38	38
<i>Cymbella minuta</i> Hilse	42	56	38	136	130	9	402	601	74	35
<i>Cymbella</i> sp.	—	1	1	—	—	—	1	3	1	1
<i>Eunotia bidentula</i> W. Smith	30	41	28	76	32	3	17	40	61	26
<i>Eunotia bilunaris</i> (Ehrenberg) Mills	175	574	127	1148	392	48	648	203	2,121	9,410
<i>Eunotia conversa</i> Hustedt	301	350	388	230	525	313	376	768	1,254	251
<i>Eunotia flexuosa</i> (Brébisson) Kützing	1,548	1,870	1,200	1,584	1,769	144	1,401	2,167	1,016	300
<i>Eunotia gibbosa</i> Grunow	220	251	294	19,614	1315	2	3,889	2,332	823	274
<i>Eunotia maior</i> (W. Sm.) Rabh.	—	—	1	—	—	—	—	—	—	—
<i>Eunotia minor</i> (Kützing) Grunow	1	—	—	—	—	—	—	—	—	—
<i>Eunotia serra</i> Ehrenberg	2	—	4	14	7	—	18	33	4	2
<i>Eunotia sudetica</i> O. Müller	106	57	125	224	209	38	390	37	70	21
<i>Eunotia trigibba</i> Hustedt	2	—	5	32	5	2	9	6	7	4
<i>Eunotia zygodon</i> Ehrenberg	1	3	2	38	35	—	266	345	3	4
<i>Fragilaria javanica</i> Hustedt	7	—	8	—	5	1	30	70	—	—
<i>Frustulia crassinervia</i> (Bréb.) Lange-Bertalot & Krammer	1,555	2,280	939	750	1,632	188	718	1,242	3,116	3,077
<i>Frustulia rhomboidea</i> (Ehrenberg) De Toni	53	81	63	574	383	87	1,102	1,679	471	161
<i>Gomphonema gracile</i> Ehrenberg	104	62	325	1920	580	1	482	316	253	154
<i>Gomphonema</i> sp. Ehrenberg	36	8	48	47	15	—	—	25	—	29
<i>Navicula cryptocephala</i> Kützing	497	505	321	127	1,312	113	420	941	678	311
<i>Navicula parasubtilissima</i> Kobayasi & Nagumo	1	—	—	—	—	—	—	—	—	—
<i>Navicula subtilissima</i> Cleve	112	59	67	21	126	56	140	153	131	48
<i>Peronia brasiliensis</i> Hustedt	3,156	2,124	1,552	302	2,865	62	920	1,203	1,095	170
<i>Pinnularia maior</i> (Kützing)	10	12	21	63	41	21	56	12	50	2
Rabenhorst										
<i>Pinnularia</i> sp.	15	32	17	20	27	55	28	74	7	36
<i>Pinnularia streptoraphe</i> Cleve	85	16	47	76	152	1	13	18	90	7
<i>Pinnularia subcapitata</i> Gregory	—	—	1	—	—	—	—	—	—	—
<i>Stenopterobia curvula</i> (W. Smith) Krammer	1	18	—	132	37	4	27	141	81	53
<i>Stenopterobia delicatissima</i> (Lewis) Van Heurck	30	11	44	48	28	—	10	8	6	5
<i>Surirella robusta</i> Ehrenberg	6	9	—	33	5	—	39	15	5	5
<i>Tabellaria</i> sp.	—	9	1	—	14	—	—	1	2	—
Total	9,064	9,453	6,203	28,164	12,958	1,657	15,903	15,612	12,905	15,115

—: absence

TABLE II

Frequency of organisms (semi-permanent slides) in the stomach contents of *Simulium perflavum* (Diptera: Simuliidae) larvae in five streams in Central Amazônia, in the dry (Sept./Oct. 1996) and rainy seasons (Feb./ Mar. 1997)

Organisms observed	Bueiro		ZF3		Cemitério		Escada		Ibama	
	Dry	Rainy	Dry	Rainy	Dry	Rainy	Dry	Rainy	Dry	Rainy
<i>Actinotaenium curcubita</i> (Bréb.) Teiling	—	1	—	—	—	—	—	—	—	—
<i>Actinotaenium globosum</i> (Bulnheim) Teiling	—	—	—	—	1	1	1	—	1	3
<i>Actinotaenium</i> sp.	—	—	—	—	—	—	2	—	—	—
<i>Actinotaenium wollei</i> Kütz	—	—	—	—	—	—	1	—	1	—
<i>Anabaena</i> sp.	—	—	—	—	1	—	—	—	—	—
<i>Bambusina brebissonii</i> Kütz	1	1	91	125	—	—	—	—	5	12
<i>Cosmarium</i> sp.	—	—	—	—	—	—	—	—	1	1
<i>Closterium</i> sp.	2	3	44	41	6	4	112	22	74	26
<i>Closterium dianai</i> Ehrenberg	—	—	3	1	—	—	32	9	2	—
<i>Closterium regulare</i> Brébisson	—	—	—	1	—	1	1	—	—	—
<i>Closterium pronum</i> Brébisson	—	—	—	—	—	—	1	1	2	—
<i>Closterium navicula</i> (Bréb.) Lütkemüller	—	—	—	—	—	—	2	1	1	—
<i>Closterium subulatum</i> (Kütz) Bréb.	—	—	—	—	—	—	—	7	4	—
<i>Desmidium elegans</i> (Racib.) Grönblad.	—	—	1	3	—	—	—	—	—	—
<i>Desmidium</i> sp.	—	—	—	—	—	—	—	—	—	2
<i>Desmidium cylindricum</i> Greville	—	—	—	—	—	—	—	—	1	—
<i>Euastrum sinuosum</i> Lenorm	—	1	—	—	—	—	—	1	—	1
<i>Euastrum</i> sp.	1	—	—	—	—	—	—	—	—	—
<i>Euglena acus</i> Ehrenberg	1	—	1	—	—	—	—	—	1	—
<i>Eunotia flexuosa</i> (Brébisson) Kütz	—	—	1	—	—	—	—	—	—	—
<i>Eunotia</i> sp.	—	—	1	—	3	—	1	—	—	—
<i>Frustulia rhomboidea</i> (Ehrenberg) De Toni	1	—	—	—	—	—	1	5	1	—
<i>Hyalotheca dissiliens</i> (Smith) Bréb.	1	—	—	—	—	—	—	—	787	190
<i>Hyalotheca</i> sp.	2	7	—	1	1	—	1	2	1,440	402
<i>Microspora</i> sp.	3	7	202	492	1	3	193	4	258	277
<i>Micrasterias</i> sp.	—	—	—	1	—	—	2	—	1	—
<i>Micrasterias torreyi</i> Bailey	—	—	2	—	—	—	—	—	—	—
<i>Micrasterias radiosa</i> Ralfs	—	—	—	—	—	—	1	—	—	—
<i>Micrasterias rotata</i> (Greville) Ralfs	—	—	—	—	—	—	1	4	3	2
<i>Mougeotia</i> sp.	—	1	—	20	—	—	—	—	—	—
<i>Navicula</i> sp.	1	—	—	—	—	—	2	—	—	—
<i>Netrium</i> sp.	—	—	—	—	—	—	—	—	3	—
<i>Oedogonium</i> sp.	68	81	4	48	4	1	7	6	79	115
<i>Oscillatoria</i> sp.	1	—	—	1	—	—	24	2	—	—
<i>Peridinium</i> sp.	1	—	1	3	—	—	—	—	—	—
<i>Pleurotaenium minutum</i> (Ralfs) Delp.	1	2	—	—	28	—	4	7	—	2
<i>Pleurotaenium</i> sp.	4	3	7	8	16	5	4	1	5	2
<i>Pleurotaenium trabecula</i> (Ehrenberg) Nägeli	1	—	1	—	20	—	3	—	—	—
<i>Pseudoanabaena</i> sp.	—	—	—	2	—	—	—	—	—	—
<i>Spondylosium</i> sp.	—	—	—	—	—	—	—	1	—	—
<i>Spyrogyra</i> sp.	1	1	87	8	—	—	13	4	17	35
<i>Surirella linearis</i> W. Smith	1	—	—	—	—	—	—	—	—	—
<i>Surirella</i> sp.	3	—	—	—	—	—	1	—	—	—
<i>Tetmemorus laevis</i> (Kützing) Ralfs	1	1	4	4	—	3	1	—	—	—
<i>Tetmemorus granulatus</i> (Bréb.) Ralfs	—	—	—	1	—	—	—	—	—	—
<i>Xanthidium canadense</i> (Joshua) Förster	—	—	—	5	—	—	—	—	—	—
<i>Xanthidium siolii</i> Grönblad & Croasdale	—	—	3	5	—	—	—	—	2	—
<i>Xanthidium</i> sp.	—	—	—	3	—	—	—	—	1	—
Rotifera	—	—	—	—	—	—	—	—	—	—
<i>Lecane (Monostyla) quadridentata</i> Ehrenberg	1	—	—	1	—	—	—	—	—	—
Total	96	109	453	774	81	18	411	77	2,690	1,070

—: absence

similar to the plankton than to the periphyton. However, organisms found in the periphyton also were similar to the plankton (45.6%), possibly because the periphyton detached from the substrate and entered the water column.

Correlations based on qualitative data on items in the stomach contents, plankton and periphyton agree with the Sorenson coefficient. The correlations between the stomach content organisms and the plankton and between the plankton and the pe-

riphyton were significant ( $z$  test,  $p < 0.05$ ), while the correlation between stomach contents and periphyton was not significant ( $z$  test,  $p > 0.05$ ) (Table IV).

Based on the organisms found in the stomach contents of black fly larvae, the sampled streams can be divided into two groups with 77% similarity. The first group was composed of the Bueiro and ZF3 streams, and the second of the Cemitério, Escada and Ibama streams, being the latter group less similar to the other streams (Fig. 74).

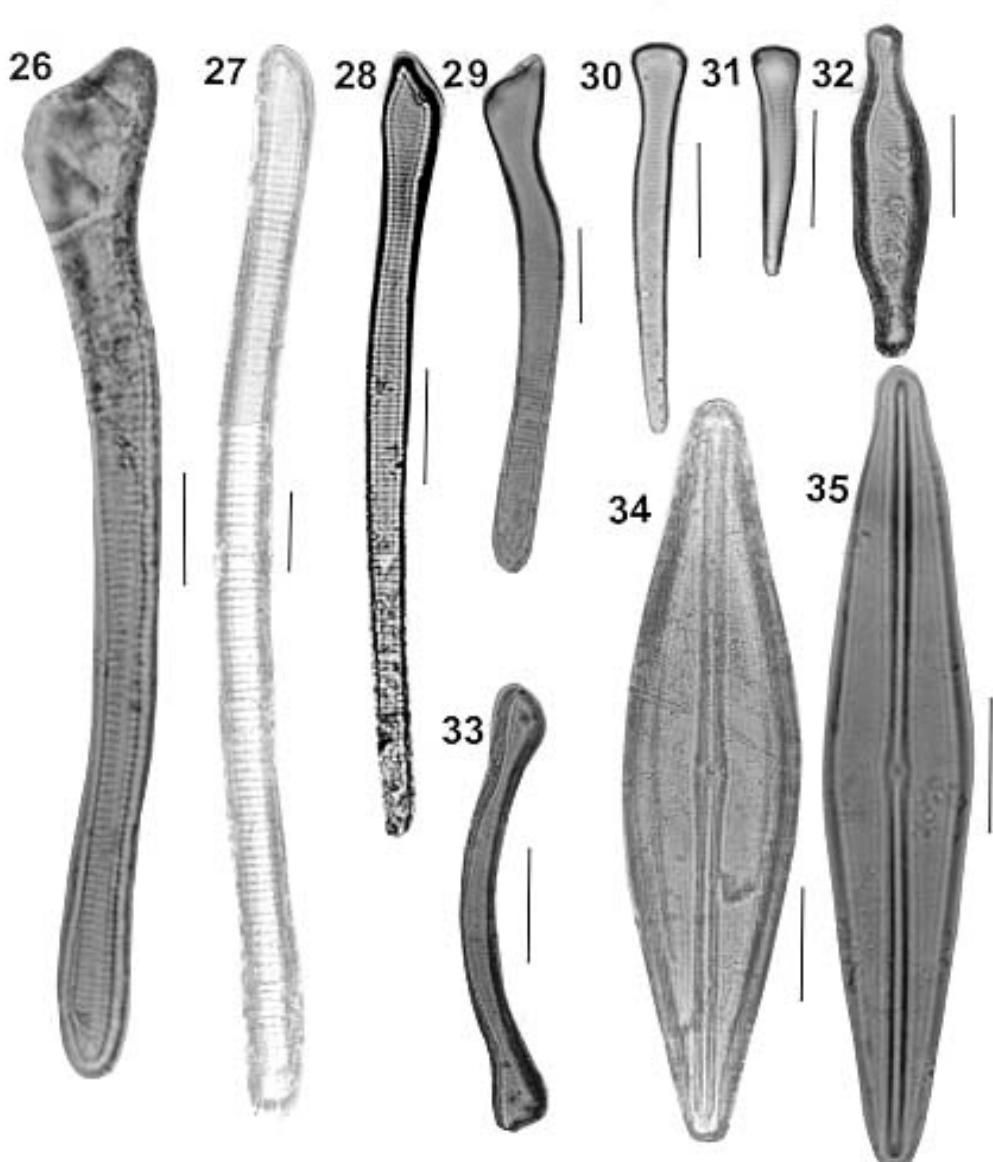
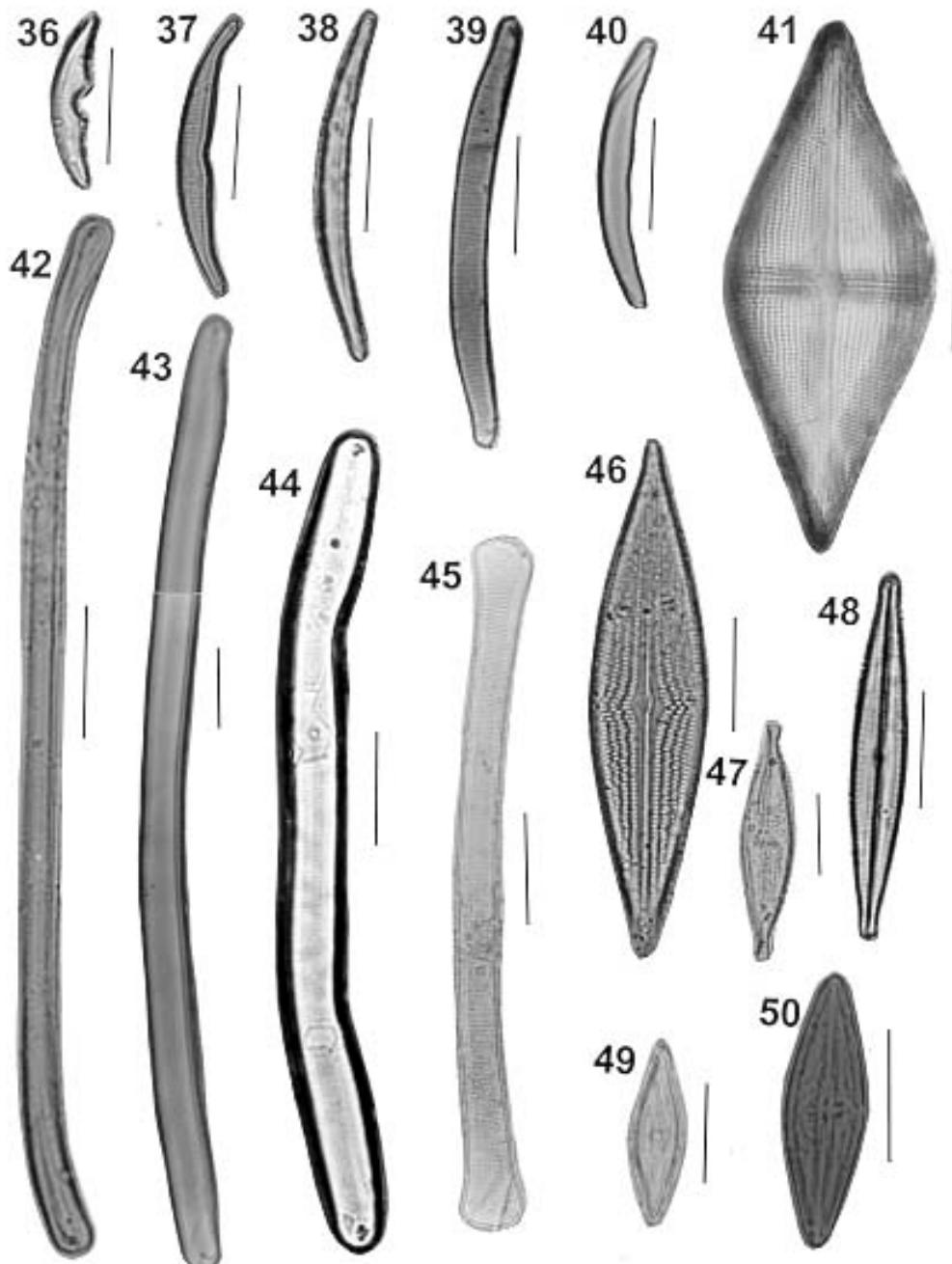


Fig. 26: *Actinella brasiliensis*. Fig. 27: *A. robusta*. Figs 28-29: *A. brasiliensis*. Figs 30-31: *A. peronioides*. Fig. 32: *Fragilaria javanica*. Fig. 33: *A. punctata*. Fig. 34: *F. rhomboides*. Fig. 35: *F. crassinervia*. Bar = 10 µm

Considering the geographical proximity of the studied streams we observed that they have geological, pedological and climatic characteristics in common. The Bueiro and Ibama streams belong to the Tarumã River Basin, ZF3 stream belong to the Rio Preto da Eva River Basin while Cemitério

and Escada streams belong to the Urubu River Basin. This grouping result may be an indication that, even though the studied streams were disturbed by anthropogenic influences, they maintained characteristics of the hydrographic basins to which they belong, reflected in the composition



Figs 36-40: *Eunotia bilunaris*. Fig. 41: *Brachysira kuntzei*. Fig. 42: *E. conversa*. Figs. 43-45: *E. flexuosa*. Fig. 46: *Brachysira serians*. Fig. 47: *B. vitrea*. Fig. 48: *N. cryptocephala*. Fig. 49: *B. serians* var. *brachysira*. Fig. 50: *B. serians*. Bar = 10 µm

of the phycoflora. Many workers have found that the classes of food items in the stomach contents of black fly larvae reflect, in general, the items available in the environment (e.g. Wotton 1977, Kurtak 1979).

Knowledge of black fly food items can provide important information on larval nutrition and

help to clarify differences observed in population productivity of larvae in different habitats (Colbo 1982). More studies will be necessary, not only on larval feeding, but also on the ecological associations between breeding places, black fly larvae, plankton and periphyton, in order to improve our understanding of black fly bioecology.

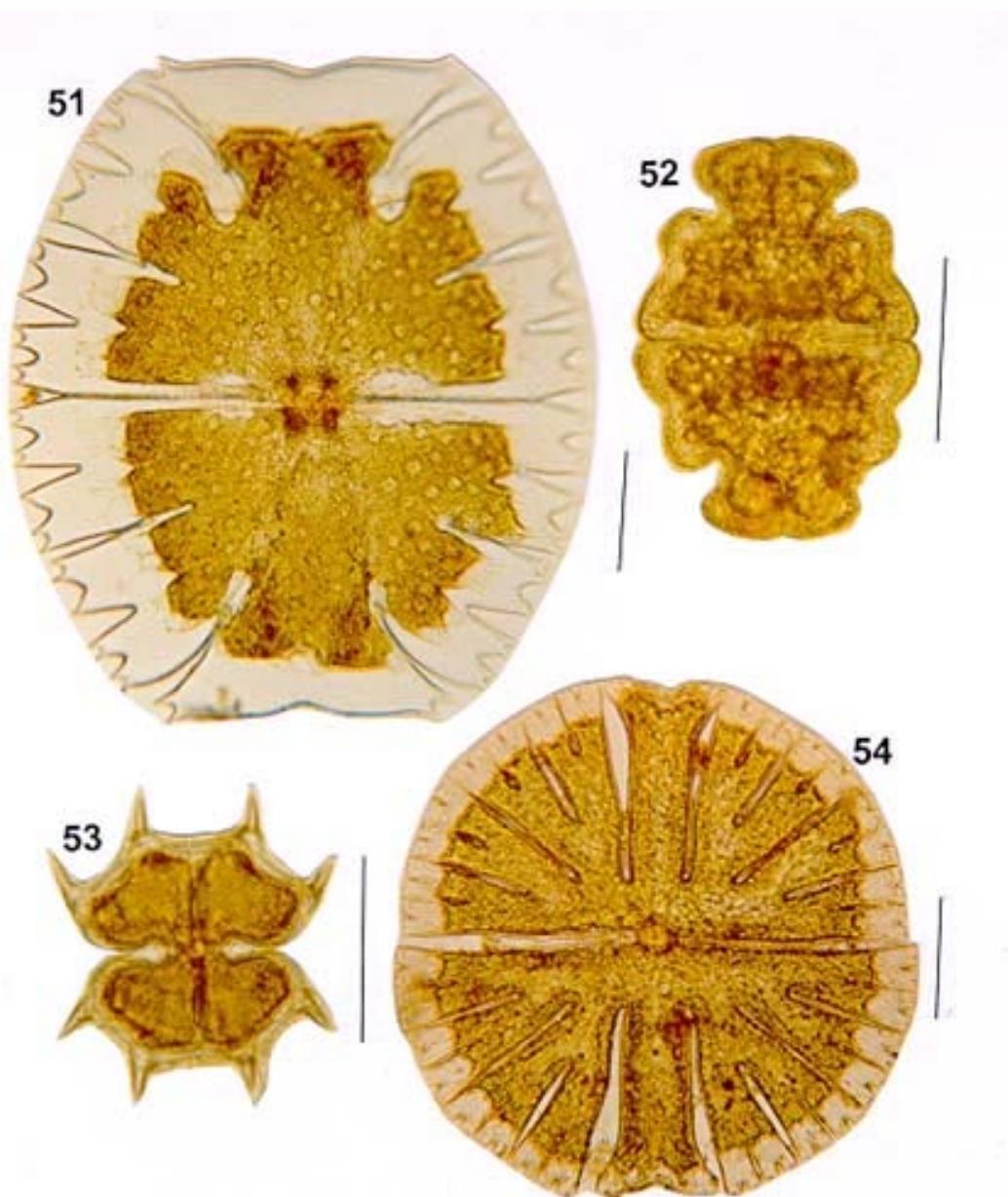


Fig. 51: *Micrasterias truncata*. Fig. 52: *Euastrum sinuosum*. Fig. 53: *Xanthidium siolii*. Fig. 54: *M. radiosoa*. Bar = 50 µm

TABLE III

Presence (X) or absence (—) in the plankton and periphyton samples and stomach contents of *Simulium perflavum* (Diptera: Simuliidae) larvae collected in five streams in Central Amazonia, observed in the study period (Sep./ Oct. 1996 and Feb./Mar. 1997)

	Plankton	Periphyton	Food items
<b>Cyanophyta</b>			
<i>Anabaena</i> sp.	X	—	X
<i>Aphanotece</i> sp.	X	—	—
<i>Chroococcus turgidus</i> (Kützing) Nügeli	X	—	—
<i>Hapalosiphon</i> sp.	X	—	—
<i>Merismopedia</i> sp.	X	—	—
<i>Oscillatoria sancta</i> (Kützing) Gomont	X	—	—
<i>Oscillatoria</i> sp.	X	—	X
<i>Pseudoanabaena</i> sp.	—	—	X
<b>Euglenophyta</b>			
<i>Euglena acus</i> Ehrenberg	X	—	X
<i>Trachelomonas armata</i> (Ehrenberg) Stein	X	—	—
<b>Bacillariophyta</b>			
<i>Achnanthes</i> sp.	—	—	X
<i>Actinella brasiliensis</i> Grun.	—	—	X
<i>Actinella mirabilis</i> Grun.	X	X	—
<i>Actinella punctata</i> Lewis	—	—	X
<i>Actinella peronioides</i> Hustedt	X	X	X
<i>Actinella guianensis</i> Grun.	X	X	—
<i>Brachysira serians</i> (Brebisson) Round & Mann var. <i>brachysira</i> (Hust.) Cox.	—	—	X
<i>Brachysira serians</i> (Brebisson) Round & Mann	X	X	X
<i>Brachysira vitrea</i> (Grun.) Ross	X	X	X
<i>Brachysira kuntzei</i> (Reichelt) Metzeltin & Lange-Bertalot	—	—	X
<i>Cyclotella</i> sp.	—	—	X
<i>Cymbella difficilis</i> Kraske	—	—	X
<i>Cymbella minuta</i> Hilse	—	—	X
<i>Cymbella</i> sp.	—	—	X
<i>Dinobryon sertularia</i> Ehrenberg	X	X	—
<i>Eunotia</i> sp.	—	—	X
<i>Eunotia bidentula</i> W. Smith	—	—	X
<i>Eunotia flexuosa</i> (Brébisson) Kütz.	X	X	X
<i>Eunotia gibbosa</i> Grunow	X	—	X
<i>Eunotia robusta</i> Ralfs	X	—	—
<i>Eunotia bilunaris</i> (Ehrenberg) Mills	X	X	X
<i>Eunotia sudetica</i> O. Müller	X	—	X
<i>Eunotia maior</i> (W. Smith) Rabh.	—	—	X
<i>Eunotia minor</i> (Kützing) Grunow	—	—	X
<i>Eunotia serra</i> Ehrenberg	—	—	X
<i>Eunotia triodon</i> Ehrenberg	X	X	—
<i>Eunotia trigibba</i> Hustedt	—	—	X
<i>Eunotia zygodon</i> Ehrenberg	X	—	X
<i>Eunotia conversa</i> Hustedt	X	X	X
<i>Fragilaria javanica</i> Hustedt	—	—	X
<i>Frustulia crassinervia</i> (Bréb.) Lange-Bertalot & Krammer	—	X	X
<i>Frustulia rhomboides</i> (Ehrenberg) De Toni	X	X	X
<i>Gomphonema gracile</i> Ehrenberg	X	X	X
<i>Gomphonema</i> sp. Ehrenberg.	X	X	X
<i>Aulacoseira</i> sp.	X	—	—
<i>Navicula</i> sp.	X	X	X
<i>Navicula cryptocephala</i> Kützing	—	—	X
<i>Navicula subtilissima</i> Cleve	—	—	X
<i>Navicula parasubtilissima</i> Kobayasi & Nagumo	—	—	X
<i>Pinnularia maior</i> (Kützing) Rabenhout	—	X	X
<i>Pinnularia subcapitata</i> Gregory	—	—	X
<i>Pinnularia streptoraphe</i> Cleve	—	—	X
<i>Pinnularia</i> sp.	X	—	X
<i>Peronia brasiliensis</i> Hustedt	X	X	X
<i>Stauroneis</i> sp.	X	—	—
<i>Stenopterobia curvula</i> (W. Smith) Krammer	X	X	X ↓

	Plankton	Periphyton	Food items
<i>Stenopterobia delicatissima</i> (Lewis) Van Heurck	—	—	X
<i>Stenopterobia intermedia</i> Lewis	X	X	—
<i>Surirella linearis</i> W. Smith	X	—	X
<i>Surirella robusta</i> Ehrenberg	X	—	X
<i>Surirella</i> sp.	—	—	X
<i>Synedra</i> sp.	X	—	—
<i>Fragilaria</i> sp.	X	X	—
<i>Tabellaria</i> sp.	X	X	X
Rhodophyta			
<i>Audouinella violaceae</i> (Kütz) Hamel	X	X	—
<i>Batrachospermum moniliferum</i> Roth	X	—	—
Dinophyta			
<i>Peridinium</i> sp.	X	—	X
Chlorophyta			
<i>Actinotaenium curcubita</i> (Bréb.) Teiling	X	X	X
<i>Actinotaenium globosum</i> (Bulnheim) Teiling	X	—	X
<i>Actinotaenium</i> sp.	X	X	X
<i>Actinotaenium tumidum</i> (Gronbl.) Teiling	X	—	—
<i>Actinotaenium wollei</i> Kütz.	X	X	X
<i>Bambusina brebissonii</i> Kütz.	X	X	X
<i>Closteriopsis</i> sp.	X	—	—
<i>Closterium</i> sp.	X	X	X
<i>Closterium dianai</i> Ehrenberg	X	X	X
<i>Closterium gracile</i> Brébisson	—	X	—
<i>Closterium kuetzingii</i> Brébisson	X	X	—
<i>Closterium libellula</i> Forcke	X	X	—
<i>Closterium macilentum</i> Brébisson	X	X	—
<i>Closterium navicula</i> (Bréb.) Lütkemüller	X	—	X
<i>Closterium primum</i> Brébisson	X	—	X
<i>Closterium regulare</i> Brébisson	X	—	X
<i>Closterium setaceum</i> Ehrenberg	X	X	—
<i>Closterium subulatum</i> (Kütz.) Bréb.	X	X	X
<i>Closterium tumidum</i> Johnson	—	X	—
<i>Cosmarium incrassatum</i> (Fritsch & Rich)	X	—	—
<i>Cosmarium pseudoconnatum</i> Nordstedt	X	—	—
<i>Cosmarium siolii</i> Forster	X	—	—
<i>Cosmarium</i> sp.	X	X	X
<i>Desmidium laticeps</i> Nordstedt	X	—	—
<i>Desmidium cylindricum</i> Greville	X	—	X
<i>Desmidium grevelli</i> (Kütz.) DeBary	X	—	—
<i>Desmidium quadratum</i> Nordstedt	X	—	—
<i>Desmidium elegans</i> (Racib.) Grönblad.	—	—	X
<i>Desmidium</i> sp.	X	X	X
<i>Euastrum</i> sp.	—	X	X
<i>Euastrum brasiliensis</i> Borge	—	X	—
<i>Euastrum sinuosum</i> Lenorm	X	—	X
<i>Hyalotheca</i> sp.	X	X	X
<i>Hyalotheca dissiliens</i> (Smith) Bréb.	—	—	X
<i>Micrasterias borgei</i> Krieger	X	—	—
<i>Micrasterias radiosa</i> Ralfs	X	X	X
<i>Micrasterias rotata</i> (Greville) Ralfs	X	X	X
<i>Micrasterias siolii</i> Scott & Croasdale	X	X	—
<i>Micrasterias truncata</i> (Corda) Brébisson	X	—	—
<i>Micrasterias torreyi</i> Bailey	—	—	X
<i>Micrasterias</i> sp.	—	—	X
<i>Microspora</i> sp.	X	X	X
<i>Mougeotia</i> sp.	X	X	X
<i>Netrium</i> sp.	—	—	X
<i>Oedogonium</i> sp <sub>1</sub>	X	X	X
<i>Oedogonium</i> sp <sub>2</sub>	X	—	—
<i>Plerotaenum tridentulum</i> Grönblad & Croasdale	X	—	—
<i>Pleurotaenium coronatum</i> (Bréb.) Rabenhorst	X	—	—
<i>Pleurotaenium minutum</i> (Ralfs) Delp.	X	X	X
<i>Pleurotaenium</i> sp.	X	X	X ↓

	Plankton	Periphyton	Food items
<i>Pleurotaenium trabecula</i> (Ehrenberg) Nägeli	X	—	X
<i>Spirogyra</i> sp.	X	X	X
<i>Spondylosium</i> sp.	—	—	X
<i>Staurastrum quadrangulare</i> Brébisson	X	—	—
<i>Staurastrum setigerum</i> Cleve	X	—	—
<i>Staurastrum</i> sp.	X	X	—
<i>Staurodesmus</i> sp.	X	—	—
<i>Tetmemorus laevis</i> (Kützing) Ralfs	X	X	X
<i>Tetmemorus granulatus</i> (Bréb.) Ralfs	X	—	X
<i>Xanthidium amazonense</i> Scott & Croasdale	X	—	—
<i>Xanthidium siolii</i> Grönblad & Croasdale	X	X	X
<i>Xanthidium canadense</i> (Joshua) Forster	X	—	X
<i>Xanthidium</i> sp.	—	X	X
<i>Zygnuma</i> sp.	X	X	—
Rotifera			
<i>Lecane (monostyla) quadridentata</i> Ehrenberg	X	X	X

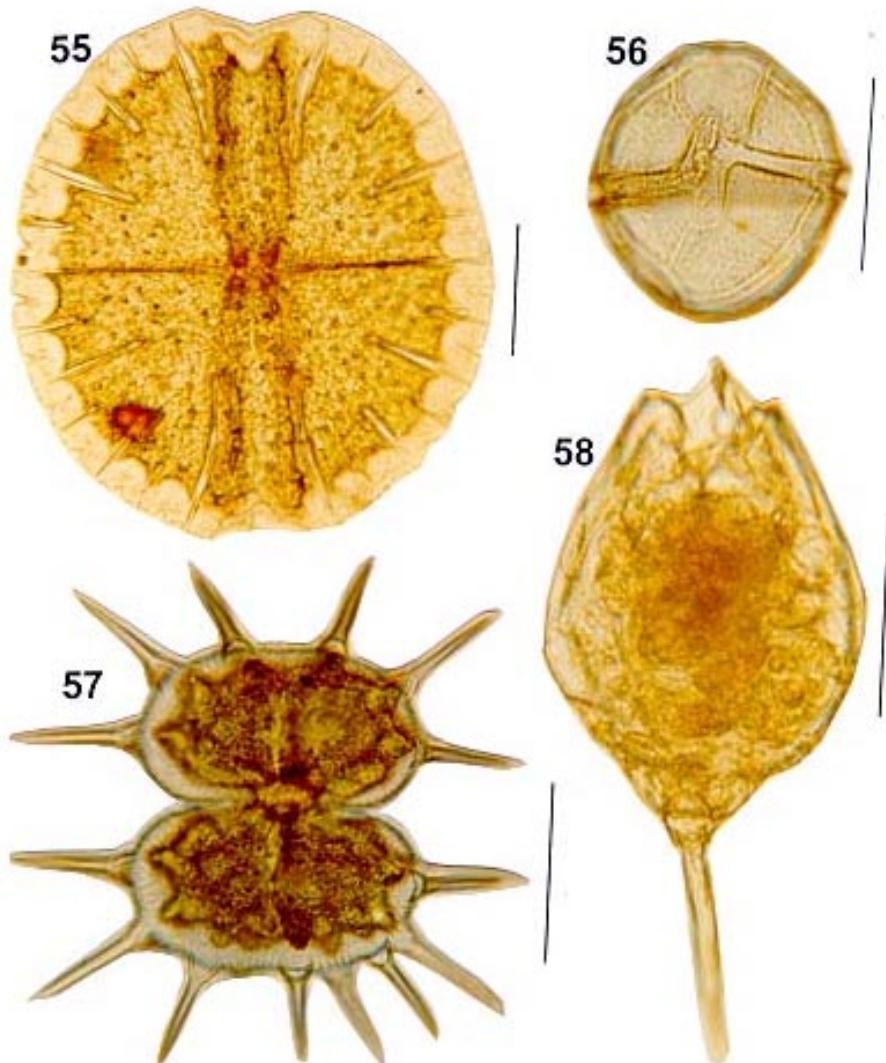


Fig. 55: *Micrasterias rotata*. Fig. 56: *Peridinium* sp. Fig. 57: *Xanthidium amazonense*. Fig. 58: *Lecane quadridentata*. Bar = 50 µm

TABLE IV

Correlation coefficient (*r*) and significance test (*z* value) between the organisms present in the stomach contents of *Simulium perflavum* (Diptera: Simuliidae) larvae, plankton and periphyton

	<i>r</i>	<i>z</i> value	<i>n</i>
Stomach content x plankton	- 0.34	- 3.85 <sup>a</sup>	131
Stomach content x periphyton	0.06	0.70	131
Plankton x periphyton	0.25	2.81 <sup>a</sup>	131

*a*:  $p < 0.05$



Fig. 59: *Closterium regulare*. Fig. 60: *Hyaloteca* sp. Fig. 61: *Spirogyra* sp. Fig. 62: *Microspora* sp. Fig. 63: *Audouinella violaceae*. Fig. 64: *Mougeotia* sp. Fig. 65: *Closterium* sp. Fig. 66: *Actinotaenium wollei*. Bar = 50  $\mu$ m

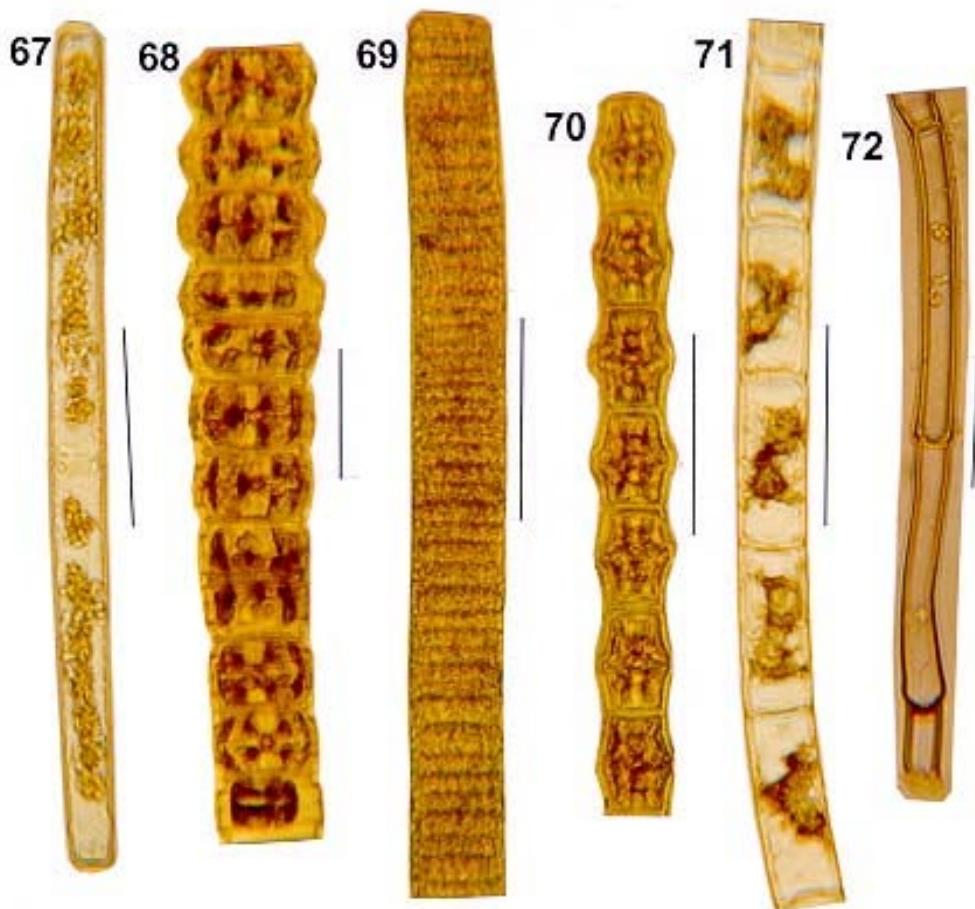


Fig. 67: *Pleurotaenium* sp. Fig. 68: *Desmidium cylindricum*. Fig. 69: *Oscillatoria* sp. Fig. 70: *Bambusina brebissonii*.  
Fig. 71: *Zygnema* sp. Fig. 72: *Oedogonium* sp. Bar = 50  $\mu$ m

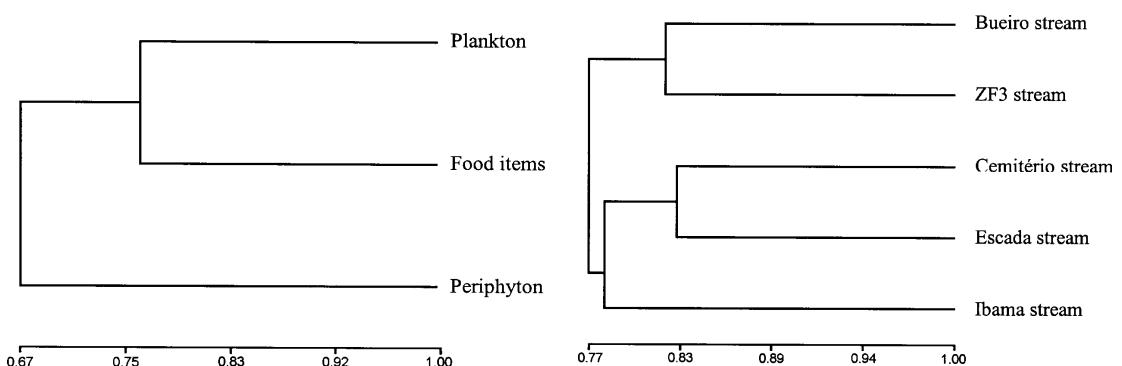


Fig. 73: similarity dendrogram between the organisms in the stomach contents of *Simulium perflavum* (Diptera: Simuliidae) larvae and the food availability in the habitat (plankton and periphyton) (Sorensean coefficient, UPGMA)

Fig. 74: similarity dendrogram between five streams in relation to the organisms in the stomach contents of *Simulium perflavum* (Diptera: Simuliidae) larvae (Sorensean coefficient, UPGMA)

## ACKNOWLEDGMENTS

To the master's students of Laboratório de Ficologia, UFPR and Dr PS Mera for helping to identify the microalgae; Dr Mera also helped with the photographs presented in this paper. To MSc. Marcelo Garcia who made helpful suggestions in many steps of this project and Dr PM Fearnside, who made helpful suggestions and revised the manuscript.

## REFERENCES

- Araújo VC 1970. Fenologias de essências florestais amazônicas. *Bol INPA, Pesquisas Florestais* 4: 1-25.
- Bourrelly P 1968. *Les Algues d'Eau Douce, Initiation à la Systematique, Tom. II, Les Algues Jaunes et Brunes*, N. Boubée & Cie, Paris, 438 pp.
- Bourrelly P 1970. *Les Algues d'Eau Douce, Initiation à la Systematique, Tom. III, Les Algues Blues et Rouges*, N. Boubée & Cie, Paris, 512 pp.
- Bourrelly P 1972. *Les Algues d'Eau Douce, Initiation à la Systematique, Tom. I, Les algues Vertes*, N. Boubée & Cie, Paris, 569 pp.
- Burton GJ 1973. Feeding of *Simulium hargreavesi* Gibbins larvae on *Oedogonium* algal filaments in Ghana. *J Med Ent* 10: 101-106.
- Colbo MH 1982. Size and fecundity of adult Simuliidae (Diptera) as a function of stream habitat, year and parasitism. *Can J Zool* 60: 2507-2513.
- Cox EJ 1996. *Identification of Freshwater Diatoms from live Material*, Chapman & Hall, London, 158 pp.
- Craig DA 1977. Mouthparts and feeding behavior of Tahitian larval Simuliidae (Diptera: Nematocera). *Quaest Entomol* 13: 195-218.
- Dellome Filho J 1989. Simuliofauna do rio Marumbi, Morretes, PR, Brasil. Microalgas como alimento de larvas de *Simulium incrassatum* Lutz, 1910 (Diptera, Simuliidae). *Mem Inst Oswaldo Cruz* 84(Suppl. IV): 157-163.
- Dudley TL, Cooper SD, Hemphill N 1986. Effects of macroalgae on a stream invertebrate community. *N Am Bentholol Soc* 5: 93-106.
- Fredeen FJH 1964. Bacteria as food for blackfly larvae (Diptera: Simuliidae) in laboratory cultures and in natural streams. *Can J Zool* 42: 527-548.
- Hustedt F 1930. Bacillariophyta (Diatomeae). In A Pascher, *Die Süsswasser-flora Mitteleuropas*, Iena, Gustav Fisher, Heft 10, 466 pp.
- Kobayasi H, Nagumo T 1988. Examination of the type materials of *Navicula subtilissima* Cleve (Bacillariophyceae). *Bot Mag Tokyo* 101: 239-253.
- Krammer K, Lange-Bertalot H 1985. Naviculaceae: neue und Wening bekannte Taxa, neue Kombination und Synonyme Sowie Berneukungen zu einigen Gattungen. *Bibl Diatomol Berlin* 9: 5-230.
- Krammer K, Lange-Bertalot H 1986. Bacillariophyceae: Naviculaceae. In H Ettl, J Gerloff, H Heyning, D Mollenhauer (eds), *Süßwasserflora von Mitteleuropa*, Vol. 2, part. 1, G. Fischer, Stuttgart, 876 pp.
- Krammer K, Lange-Bertalot H 1988. Bacillariophyceae: Bacillariaceae, Epithemiaceae, Suriellaceae. In H Ettl, J Gerloff, H Heyning, D Mollenhauer (eds), *Süßwasserflora von Mitteleuropa*, Vol. 2, part. 2, G. Fischer, Stuttgart, 595 pp.
- Krammer K, Lange-Bertalot H 1991a. Bacillariophyceae: Centrales, Fragilariae, Eunotiaceae. In H Ettl, J Gerloff, H Heyning, D Mollenhauer (eds), *Süßwasserflora von Mitteleuropa*, Vol. 2, part. 3, G. Fischer, Stuttgart, 576 pp.
- Krammer K, Lange-Bertalot H 1991b. Bacillariophyceae: Achnanthaceae. Kristische Ergänzungen zu *Navicula* (Lineolatae) und *Gomphonema*. In H Ettl, J Gerloff, H Heyning, D Mollenhauer (eds), *Süßwasserflora von Mitteleuropa*, Vol. 2, part. 4, G. Fischer, Stuttgart, 437 pp.
- Kurtak DC 1978. Efficiency of filter feeding of black fly larvae (Diptera: Simuliidae). *Can J Zool* 56: 1608-1623.
- Kurtak DC 1979. Food of black fly larvae (Diptera: Simuliidae): Seasonal changes in gut contents and suspended material at several sites in a single watershed. *Quaest Ent* 15: 357-374.
- Kuznetsov AV 1981. The diet of black fly larvae (Diptera: Simuliidae). *Rev Appl Entomol* 69: 134.
- Lacey LA, Lacey JM 1983. Filter feeding of *Simulium fulvinotum* (Diptera: Simuliidae) in the Central Amazon Basin. *Quaest Entomol* 19: 41-51.
- Lozovei AL 1989. Diatomáceas (Bacillariophyceae) como alimento das larvas de *Simulium* spp. (Diptera, Simuliidae) no rio Passaúna, Curitiba, Paraná, Brasil. *Arq Biol Tecnol* 32: 339-376.
- Lozovei AL, Luz E 1976. Diptera Culicidae em Curitiba e arredores. II. Alimentação. *Arq Biol Tecnol* 19: 43-84.
- Moreira Filho H, Valente Moreira IM 1981. Avaliação taxonômica e ecológica das diatomáceas (Bacillariophyceae) epífitas em algas pluricelulares obtidas nos litorais dos estados do Paraná, Santa Catarina e São Paulo. *Bol Museu Botânico Municipal* 47: 1-17.
- Peterson BV 1956. Observations on the biology of Utah black flies (Diptera: Simuliidae). *Can Entomol* 88: 496-507.
- Patrick R, Reimer W 1966. *The Diatoms of the United States*, Vol. 2, The Livingston Publishing Company, 213 pp.
- Pielou EC 1984. *The Interpretation of Ecological Data*, John Wiley & Sons, New York, 263 pp.
- Sioli H 1965. A limnologia e a sua importância em pesquisas da Amazônia. *Amazoniana* 1: 11-35.
- Thompson BH 1987. The use of algae as food by larval Simuliidae (Diptera) of Newfoundland streams. III. Growth of larvae reared on different algal and other foods. *Arc Hydrobiol Suppl.* 76: 459-466.
- Wotton RS 1977. The size of particles ingested by moorland stream blackfly larvae (Simuliidae). *Oikos* 29: 332-335.
- Wotton RS 1980. Bacteria as food for blackfly larvae (Diptera: Simuliidae) in a lake-outlet in Finland. *Ann Zool Fennici* 17: 127-130.
- Zar HJ 1996. *Biostatistical Analysis*, Prentice-Hall do Brasil, Rio de Janeiro, 662 pp.