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LABORATORY STUDIES ON THE BIOLOGY OF *SIMULIUM NIGRITARSE* COQUILLETT AND *SIMULIUM ADERSI* POMEROY (DIPTERA: SIMULIIDAE)*

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ABSTRACT

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The eggs of both *Simulium nigritarse* and *S. adersi* took up to 13 days to hatch in water at a temperature of 25 °C. The larvae of *S. nigritarse* required a minimum of 20 days and those of *S. adersi* a minimum of 17 days to pupate when reared in water at 20±1 °C. No difference between the sexes was observed in the time taken by the larvae of either species to complete their life cycle. The duration of the pupal stage of *S. nigritarse* ranged from a minimum of 47 hours at 25 °C to a maximum of 569 hours (23,7 days) at 6 °C. An ambient temperature of 30±1 °C was lethal for both the larvae and the pupae of *S. nigritarse*. Eclosion of *S. nigritarse* reaches a peak after sunrise, then the rate declines towards sunset. A mean of 76% of the flies were found to hatch during the day. The time of eclosion of both males and females was similar. Pupation of *S. nigritarse* could take place at a water-depth of 2 m and was common at a depth of 1,1 m. In still water no negative geotropism could be detected in the behaviour of *S. nigritarse* larvae and they were positively phototropic. In agitated water larvae did not respond to a light gradient ranging from 5 to 1100 lux. Adult larvae became negatively phototropic before the onset of pupation, which took place in dark, fast-flowing water. *S. nigritarse* can overwinter in both the larval and the pupal stages.

Résumé

ÉTUDES DE LABORATOIRE SUR LA BIOLOGIE DU SIMULIUM NIGRITARSE *COQUILLETT ET DU* SIMULIUM ADERSI *POMEROY (DIPTERA: SIMULIIDAE)*

Les oeufs de Simulium nigritarse *et de* S. adersi *prirent une période allant jusqu'à 13 jours pour éclore dans l'eau à une température de 25 °C. Les larves de* S. nigritarse *nécessitèrent un minimum de 20 jours et celles de* S. adersi *un minimum de 17 jours pour accomplir leur transformation en nymphes quand elles furent élevées à une température de 20±1 °C. Aucune différence entre les sexes ne fut observée pendant la période prise par les larves des deux espèces pour accomplir leur cycle d'évolution. La durée du stade de nymphose de* S. nigritarse *se situa entre un minimum de 47 heures à une température de 25 °C jusqu'à un maximum de 569 heures (23,7 jours) à une température de 6 °C. Une température ambiante de 30±1 °C fut mortelle, à la fois pour les larves et pour les nymphes de* S. nigritarse. *L'éclosion des* S. nigritarse *atteignit une apogée après le lever du soleil; le degré déclinant ensuite vers le coucher du soleil. Une moyenne de 76% des mouches eurent leur éclosion pendant le jour. Le temps d'éclosion des mâles et des femelles fut similaire. La transformation du* S. nigritarse *pourrait prendre place à une profondeur de 2 mètres d'eau mais plus frequemment à une profondeur de 1,1 m. En eau calme, aucun géotropisme négatif ne fut observé dans le comportement des larves de* S. nigritarse *et elles furent positivement phototropiques. En eau courante les larves ne réagirent pas à une légère inclinaison qui variait entre 5 et 1100 lux. Les larves adultes devinrent négativement phototropiques avant le déclanchement de la nymphose qui prit place dans l'eau sombre coulant rapidement.* S. nigritarse *peut hiverner soit dans le stade larvaire ou encore au stade de nymphe.*

INTRODUCTION

The black flies, *Simulium nigritarse* and *S. adersi*, are widely distributed throughout Southern Africa and also in other parts of the African continent, usually in practically unpolluted streams. *S. nigritarse* is apparently ornithophilic (Freeman & De Meillon, 1953), while *S. adersi* in Africa will parasitize both man and chickens (Crisp, 1956, cited by Service, 1977). Huchzermeyer & Sutherland (1978) found evidence that *S. nigritarse* can transmit the sporozoan blood parasite, *Leucocytozoon smithi*, to turkeys in South Africa.

A laboratory study was undertaken to determine the duration of the egg and larval stages of *S. nigritarse* and *S. adersi*. The influence of temperature on the duration of the pupal stage, the occurrence of eclosion rhythms in adults and the position of pupal as well as larval attachment in the aquatic environment were also examined.

THE EGG INCUBATION PERIODS AND THE DURATION OF THE LARVAL STAGES OF *S. nigritarse* AND *S. adersi*

Materials and Methods

Simulium eggs, larvae and pupae were collected from the Modder River at the Agricultural College, Glen (28° 55′ S, 26° 18′ E), 25 km north of Bloemfontein in the Orange Free State. The specimens were transported to the University of the Orange Free State, Bloemfontein, where the research was conducted.

On 4 June 1978 *Simulium* eggs were collected from reeds at depths of up to 500 mm in water at 15 °C. They were transported to the laboratory in 300 mℓ glass jars filled with river water, then transferred to Petri dishes filled to a depth of 10 mm with aged water and left at 25 °C till they hatched.

Water was aged by mixing 20 ℓ of detritus from the bottom of a local open reservoir with 300 ℓ of tap water in an open asbestos drum and then leaving it out of doors for 14 days prior to use. Throughout the study tap water was added to the drum to keep the water level fairly constant. During the experimental period the pH in this water, measured with a Radiometer 29* pH meter, fluctuated between 8,3 and 9,2, and its conductivity, measured with a Horizon model† conductivity meter, varied between 400 and 500 micro-ohms per centimetre.

Groups of approximately 2 000 1st instar larvae were transferred from the Petri dishes to 2 ℓ glass jars containing 1,8 ℓ of aged water. To ensure that this water contained adequate food supplies for the larvae it was thoroughly stirred before it was removed from the asbestos drum. It was then homogenized in a Waring blender to prevent contamination by unwanted macro-organisms such as *Daphnia*, mosquito larvae and filamentous algae.

The jars were aerated with ordinary aquarium pumps which forced air through porous aeration stones, thus creating turbulence in the water, a method essentially similar to that used by Tarshis (1968). The

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* Radiometer, Copenhagen

† Horizon Ecology Co., USA

water speed in the jars, which was determined by timing the distance moved by suspended particles varied between 120 mm and 240 mm per second. In the incubator room the breeding jars were exposed to an artificial day-night cycle with a maximum light intensity of 200 lux (Philips "daylight" illumination) measured with a Yew type 3281* light meter. An ambient temperature of 25 °C and humidity of ±55% maintained the water temperatures in the rearing jars at 20±1 °C. Once a week 300 mℓ of homogenized water was added to each jar to compensate for evaporation. In 2 jars, which had been in use for 69 days, the pH was 8,8, while the conductivity was 800 micro-ohms per centimetre in one and 1 600 in the other.

Newly-formed pupae were transferred twice daily to 25 mℓ glass vials containing damp absorbent paper and plugged with perforated plastic tops. The transfer of the pupae was necessary because the tubulence in the jars frequently prevented the newly-emerged flies from escaping from the water surface.

Results and Discussion

The eggs of both *S. adersi* and *S. nigritarse* took from 3–13 days to hatch (Table 1). Once hatching had started in an individual batch of eggs, the process was completed within 3 days.

The hatching of simuliid eggs, the duration of the larval stages and the time spent as pupae are largely influenced by the temperature of their environment (Freedeen, 1959). The time required for the eggs to hatch is usually not synchronized in a *Simulium* population and fluctuates even more in species which lay diapause eggs (Freedeen, 1959). Chutter (1972) found that the duration of the egg stage of *S. nigritarse* was less than a week in the Fish and Bloukrans Rivers in the Eastern Cape Province, but no records of the water temperature were given.

Second instar larvae appeared 4–5 days after the 1st instar larvae had been liberated in the breeding jars. The larvae were characterized by the numerous silken threads to which they were attached but these threads became less conspicuous within 2 days. When the 3rd instar larvae appeared, they and all the subsequent larval instars preferred the side of the jar and the aeration pipe as attachment sites. Chutter (1972) found that 1st instar *S. nigritarse* larvae were mainly present in drift samples taken from the Fish and Bloukrans Rivers in the Eastern Cape Province. The spontaneous spinning of silk threads by 2nd instar *S. adersi* and *S. nigritarse* larvae under laboratory conditions may support the finding by Rühm (1970) that *Boopthora erythrocephala* and members of the *Odagmia ornata* complex (Simuliidae) drift away from oviposition sites and thus become dispersed during the 2nd larval stage.

The first pupae were formed by *S. adersi* after 17 days and the last by *S. nigritarse* after 69 days had elapsed (Fig. 1 & 2). There appeared to be no direct relationship between the time taken by the eggs to hatch and the time that elapsed before the pupae developed. The shortest time taken by *S. nigritarse* larvae to develop into pupae was 20 days, although these larvae had initially taken 13 days to hatch.

Colbo & Porter (1979) showed clearly that, since both the time required by the larvae of *S. vittatum* and *S. verecundum* to reach the pupal stage and the synchronization of larval development are greatly influenced by the availability of food, the available nutrients may have been insufficient under the rearing conditions used in this study. This could have prevented the synchronization of larval development and thus lengthened the life cycle. The time required for the development of the first pupae of *S. nigritarse* in the laboratory corresponded to the finding of Chutter (1972) that the duration of the larval stages was approximately 3 weeks in the Bloukrans River.

Both sexes of *S. nigritarse* and *S. adersi* spent equal periods of time as larvae. However, twice as many S. *adersi* males pupated successfully compared with the number of females of the same species (Fig. 1 & 2). Owing to the turbulent conditions in the rearing jars, the tubes were more effective in retrieving live adult flies. There were high mortalities amongst young pupae, and consequently not all the pupae that were formed could be sexed.

THE EFFECT OF TEMPERATURE ON THE DURATION OF THE PUPAL STAGE OF *S. nigritarse*

Materials and Methods

Mature larvae were collected for use from the Modder River at Glen, transported to the laboratory and kept as described above. Newly-formed pupae were removed, transferred to 5 mℓ glass vials containing damp absorbent paper and placed in incubators with a 12 hour day-night cycle at 6, 10, 15, 20, 25 and 30 °C. They were examined at least twice daily till adult black flies emerged and these were kept in the incubators until a total of 30 flies had emerged at each temperature. The minimum and maximum times spent in the pupal stage were recorded and the mean time spent in this stage calculated for each temperature. As it was found that 30 °C was fatal to pupae, aerated jars with mature larvae were kept in an incubator at 30 °C and the effect of this temperature on them noted.

TABLE 1 The egg incubation period and the duration of the larval stages of *S. nigritarse* and *S. adersi*

Egg incubation period (days) at 25 °C	Development from eggs to pupae (days) at 20 °C		Number of jars containing *S. nigritarse* larvae	Number of jars containing *S. adersi* larvae
	Min.	Max.		
3	45	69	3	1
4	30	42	5	2
5	23	56	4	3
6	31	34	2	—
8	21	—	1	—
9	30	42	2	—
13	20	31	2	1

* Yokogawa, Tokyo, Japan

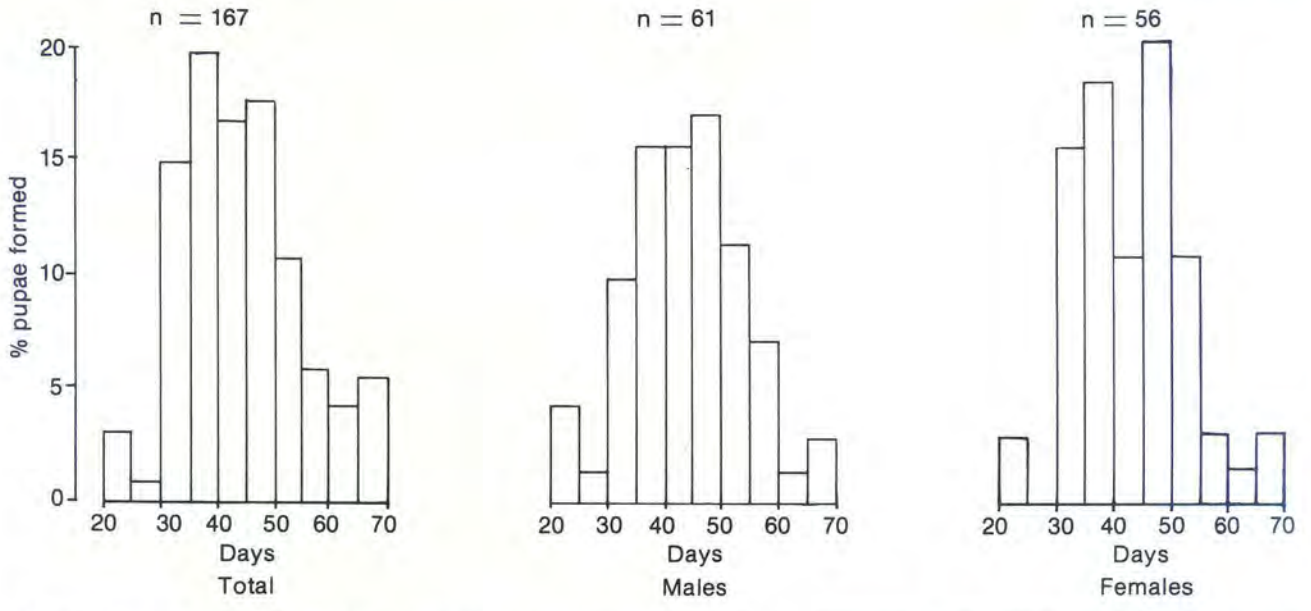

FIG. 1 *Simulium nigritarse:* time taken by larvae to pupate (each vertical column represents the percentage of pupae formed during a period of 5 days)

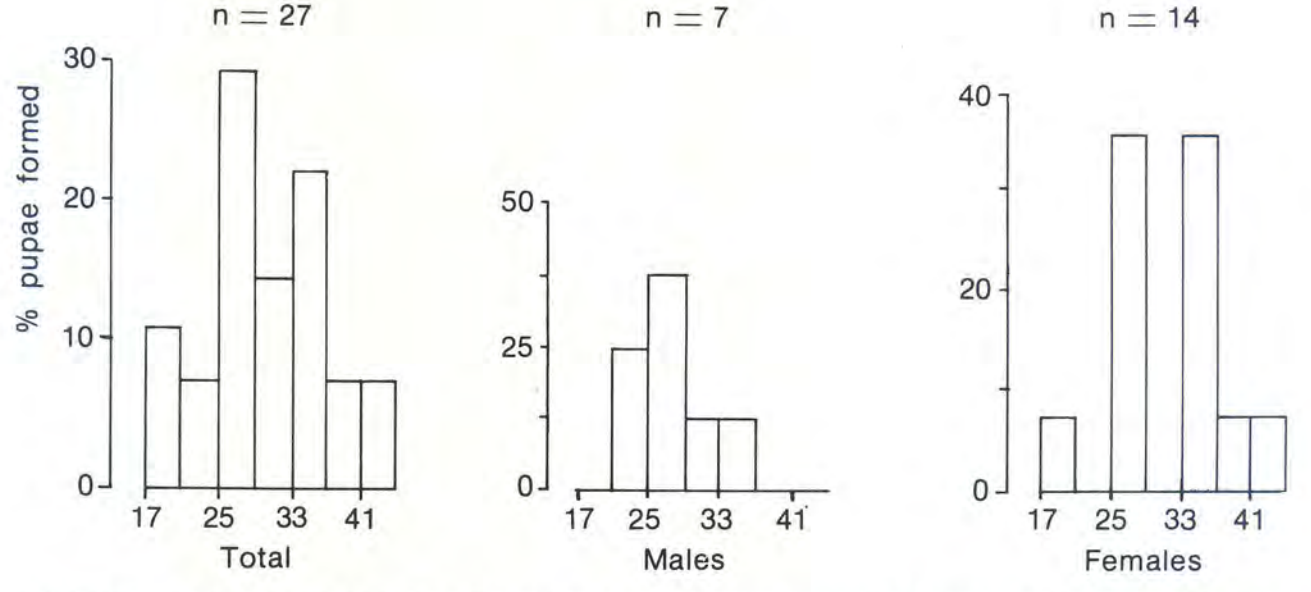

FIG. 2 *Simulium adersi:* time taken by larvae to pupate (each vertical column represents the percentage of pupae formed during a period of 5 days)

Results and Discussion

The results are summarized in Table 2.

Pupal mortalities were extremely high, possibly as the result of injury when they were removed from the rearing jars. Fungal attack may also have contributed to the mortalities. At a water temperature of 30 °C all the pupae died. Pupation occurred in aerated jars kept at this temperature but no mature larvae lived longer than 3 days and none of the pupae produced adults. The minimum time taken by pupae to complete their development was 47 hours at 25 °C and the maximum 569 hours (23,7 days) at 6 °C. Variation in the time taken by the pupae to complete their development was less at 20 °C than at any other temperature used in this study.

ECLOSION RHYTHMS IN ADULT *S. nigritarse*

Materials and Methods

Flat shale stones with *S. nigritarse* pupae attached were removed from the Modder River at Glen and were transported to the laboratory in plastic bags to prevent desiccation. Practically all the pupae found in the river were attached to the underside of those stones under which there was water movement, even when they were completely submerged. The maximum depth of the stream from which the pupae were collected was approximately 100 mm and the water was clear throughout.

In experiments carried out under daylight conditions, the stones with pupae attached were transferred to gauze cages (300 mm × 300 mm × 300 mm), which

TABLE 2 The length of the pupal stage of *S. nigritarse* as calculated for both sexes

Temp. (°C)	No. of pupae used	% mortality	Time taken by the pupae to develop into adults (hours)			Coefficient of variance (%)
			Min.	Max.	Mean	
30	45	100	—	—	—	—
25	40	25,0	47	124	93,7	15,8
20	36	16,7	79	120	106,9	8,2
15	38	21,1	72	267	162,5	23,8
10	42	28,6	271	360	307,9	19,2
6	94	68,1	296	569	457,5	37,6

were then submerged in containers of tap water to keep the lower halves of the stones moist. Pupae which were exposed to a day-night cycle or continuous darkness were placed in containers of water on the bottom of cages (2 000 mm × 1 000 mm × 2 000 mm) fitted with a zip so that one could enter from one side. Since eclosed flies had to be traced in the dark by means of dim torch-light so as to avoid exposing the pupae to light, these cages were more suitable than the smaller ones in which flies could be caught at arm's length. The temperature was maintained at 25 °C and the humidity at 70%. Eclosed flies were removed before sunrise and thereafter at hourly intervals. Night observations were limited to a single count before sunrise, and the mean hourly hatching rate at night was calculated from this observation. Flies kept under day-night conditions were exposed to an artificial day-night cycle (Philips "day-light") which corresponded to the prevailing natural day-night cycle. Two experiments, each lasting 2 days, were conducted with a light gradient of between 42 and zero lux, and 185 and 2 lux respectively. In the first experiment both sunrise and sunset lasted 1 hour. During the second experiment, sunrise took 2 hours and sunset 2,5 hours. Another 2 experiments were carried out, one in total darkness and the other in continuous light (185 lux).

Results and Discussion

Eclosion reached a peak at sunrise (Fig. 3 a–d) which, according to Disney (1969), is typical of tropical *Simulium* species. In contrast Colbo (1977) found that *Austrosimulium bancrofti* reached a peak in the afternoon and that a much higher percentage of flies emerged during daytime. Similar results were obtained by Edwards & Trenholme (1976) with *S. damnosum* in the Ivory Coast, where up to 98% of the flies emerged during the day. Males and females emerged simultaneously, unlike *S. ornatipes* in Australia, where the females emerge a little earlier than the males (Hunter, 1977; Colbo, 1977). In all experiments a peak in

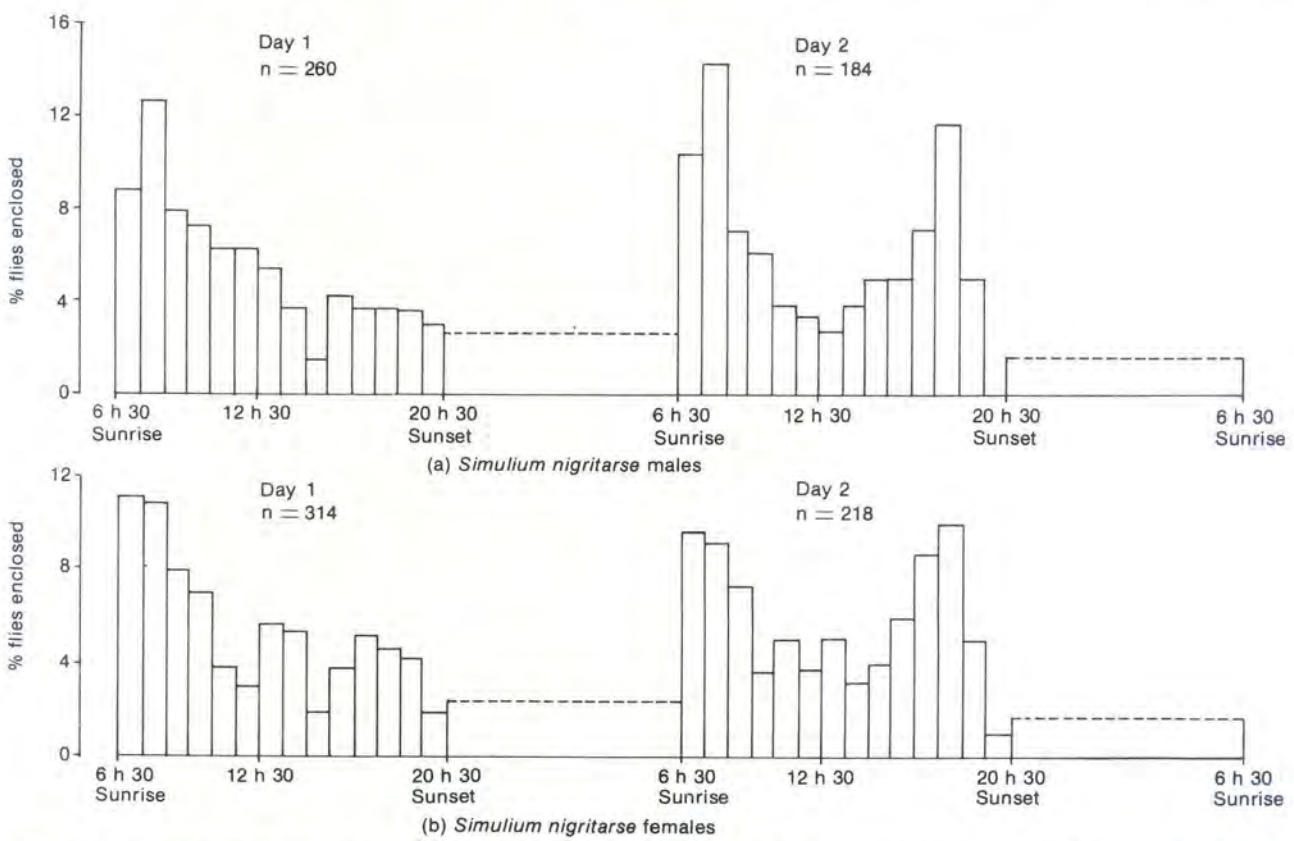

FIG. 3a, b *Simulium nigritarse:* eclosion rhythms during a day-night cycle of 12 hours with a light intensity between nil and 42 lux (each vertical column represents the percentage of flies eclosed during a period of 1 h)

eclosion was evident on the 2nd day before sunset, whether in constant light or constant darkness. The minimum time taken by flies to complete their pupal development was 47 hours at 25 °C. The pupae used in the experiments under day-night conditions were collected from water with a temperature of 13 °C (pH 8,4 and conductivity 950 micro-ohms per centimetre). In the experiments where constant darkness or constant light prevailed, the pupae used were collected from water with a temperature of 14,5 °C (pH 8,6 and conductivity 950 micro-ohms per centimetre). The peak in eclosion found during the 2nd day at sunset (Fig. 3, a–d) could thus be the effect of synchronization during the time spent as pupae because of the abnormal raising of the pupal environmental temperature.

Where the normal day-night cycles were replaced by constant light or darkness, an eclosion pattern similar to the one under normal day-night conditions was found. This was particularly evident during the morning of the 2nd day of the experiments (Fig. 3, e–h). Hunter (1977) found that normal daytime rates of eclosion of *S. ornatipes* continued under conditions of total darkness. This was not the case, however, under conditions of continuous light.

TABLE 3 The percentages of *S. nigritarse* adults that eclosed during daytime

	Day 1		Day 2	
	Males	Females	Males	Females
Constant light	57,9	45,3	56,1	52,3
Constant darkness	57,9	45,0	58,3	55,8
Daylight intensity 42 lux, day-length 12 hours	67,7	70,4	73,4	78,9
Daylight intensity 185 lux, day-length 14 hours	65,9	77,7	86,2	88,5

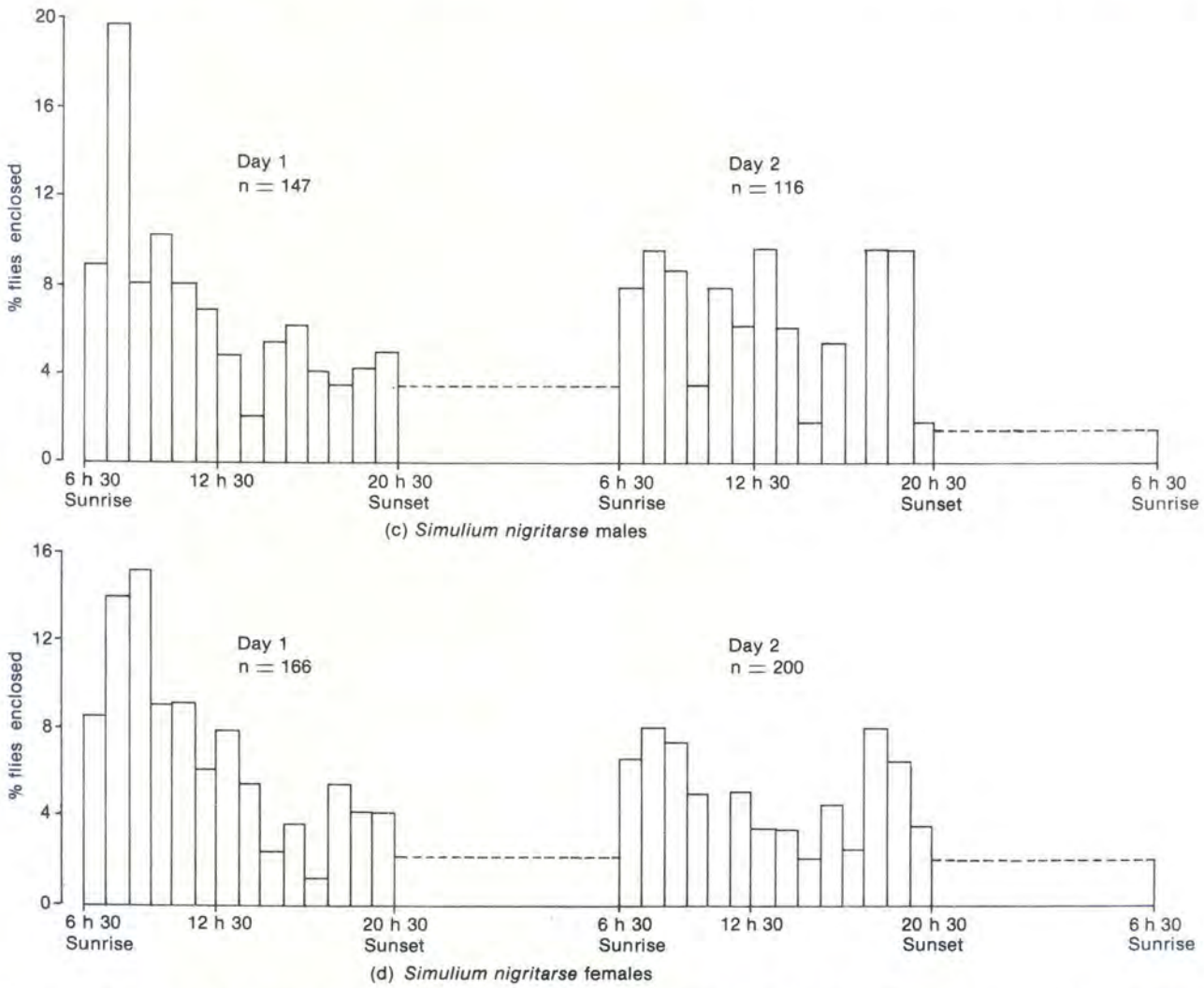

FIG. 3c, d *Simulium nigritarse:* eclosion rhythms during a day-night cycle of 12 hours with a light intensity of between 2 and 185 lux (each vertical column represents the percentage of flies eclosed during a period of 1 h)

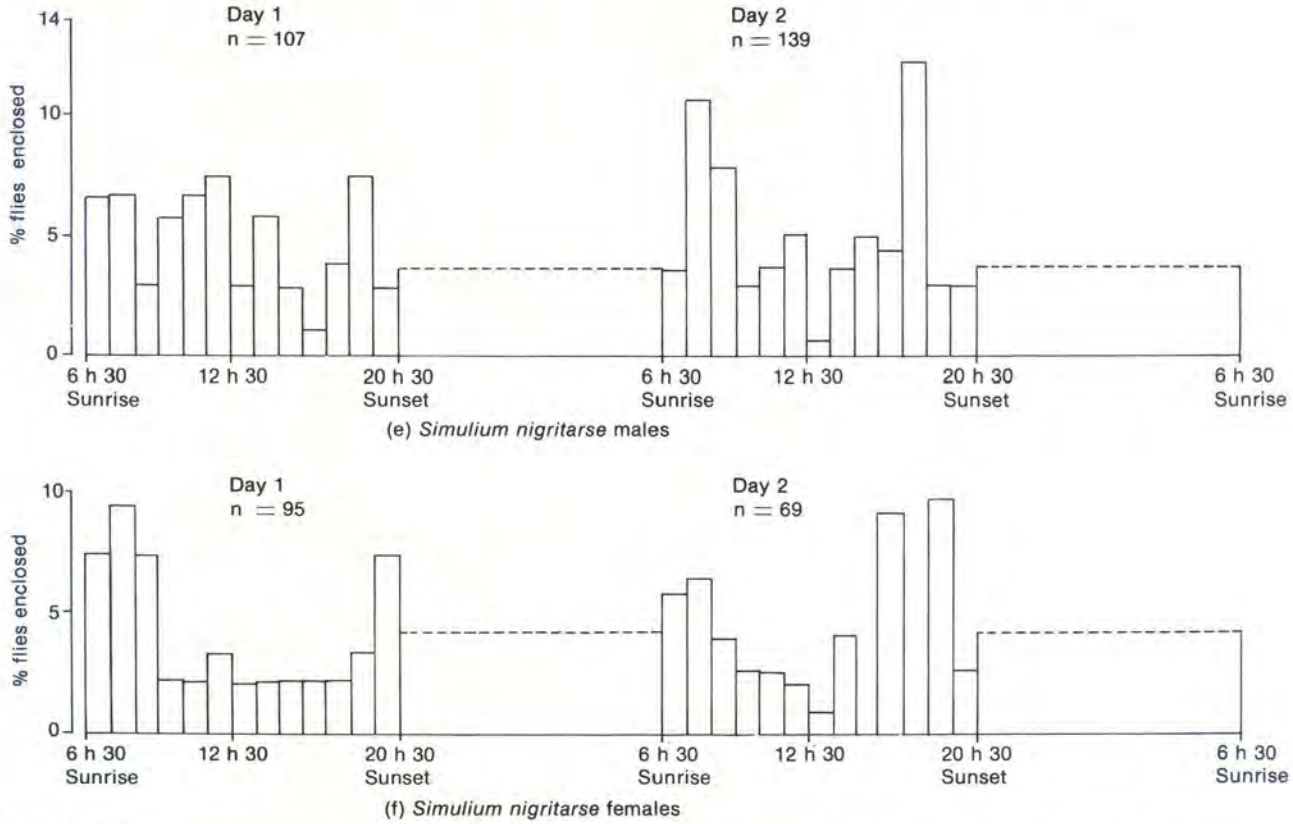

FIG. 3e, f *Simulium nigritarse:* eclosion rhythms during constant light of intensity 185 lux (each vertical column represents the percentage of flies eclosed during a period of 1 h)

THE POSITION OF LARVAL ATTACHMENTS AND PUPATION OF *S. nigritarse*

A. THE EFFECT OF WATER-DEPTH

Materials and Methods

An attempt was made to determine the preference, if any, for the position and the maximum depth of water in which *S. nigritarse* would pupate. An upright glass tube (70 mm × 1 200 mm), plugged at the bottom with a perforated rubber stopper, was divided lengthwise into 2 halves by means of a perspex sheet stretching upwards from an aeration stone to a point 200 mm from the top of the tube (Fig. 4). When the tube was filled with water, air was forced into it with an aquarium pump via the perforated stopper and the aeration stone. This stone was positioned so that the air bubbles escaped up one side only of the lumen, thus causing a vertical current. *S. nigritarse* larvae of all ages, washed directly from stones in the Modder River into 300 mℓ glass jars full of water and transported to the laboratory, were liberated into the column while it was gradually filled with de-ionized water to a depth of 1 100 mm. The column was evenly illuminated by means of neon tubes at a light intensity of 1 700 lux. The water temperature was 20 ± 1 °C and the average water speed 110 mm per second. The numbers of larvae and pupae present in 100 mm zones, measured from the water surface downwards, were recorded every 24 hours and the positions of the pupae were marked with a felt pen on the outer surface of the column. Recordings were taken until most adult larvae had pupated. Three replicates were done, giving a total of 10 readings (Table 4).

In a further experiment larvae were added to the bottom of the original tube which was then extended by means of a 10 mm diameter glass tube to give a water depth of 2 m (Fig. 4). Compressed air was forced through the column with a large commercial compressor. Because the pressure supply was erratic, a constant watch had to be kept to prevent the column from overflowing or aeration from being terminated.

TABLE 4 The effect of water-depth on the position of larval attachment and pupation sites

Water-depth (cm).....	10	20	30	40	50	60	70	80	90	100	110
Total larvae.....	210	159	548	325	269	244	239	127	218	200	300
Total pupae.....	132	24	110	42	28	12	32	19	18	24	43
Ratio of the No. of larvae to the No. of pupae formed at the different depths.....	1,6†	6,6†	5*	7,7	9,6	20,3	7,5*	6,7	12	8,3	7*

† Perspex division absent
* Protruding perspex fastening

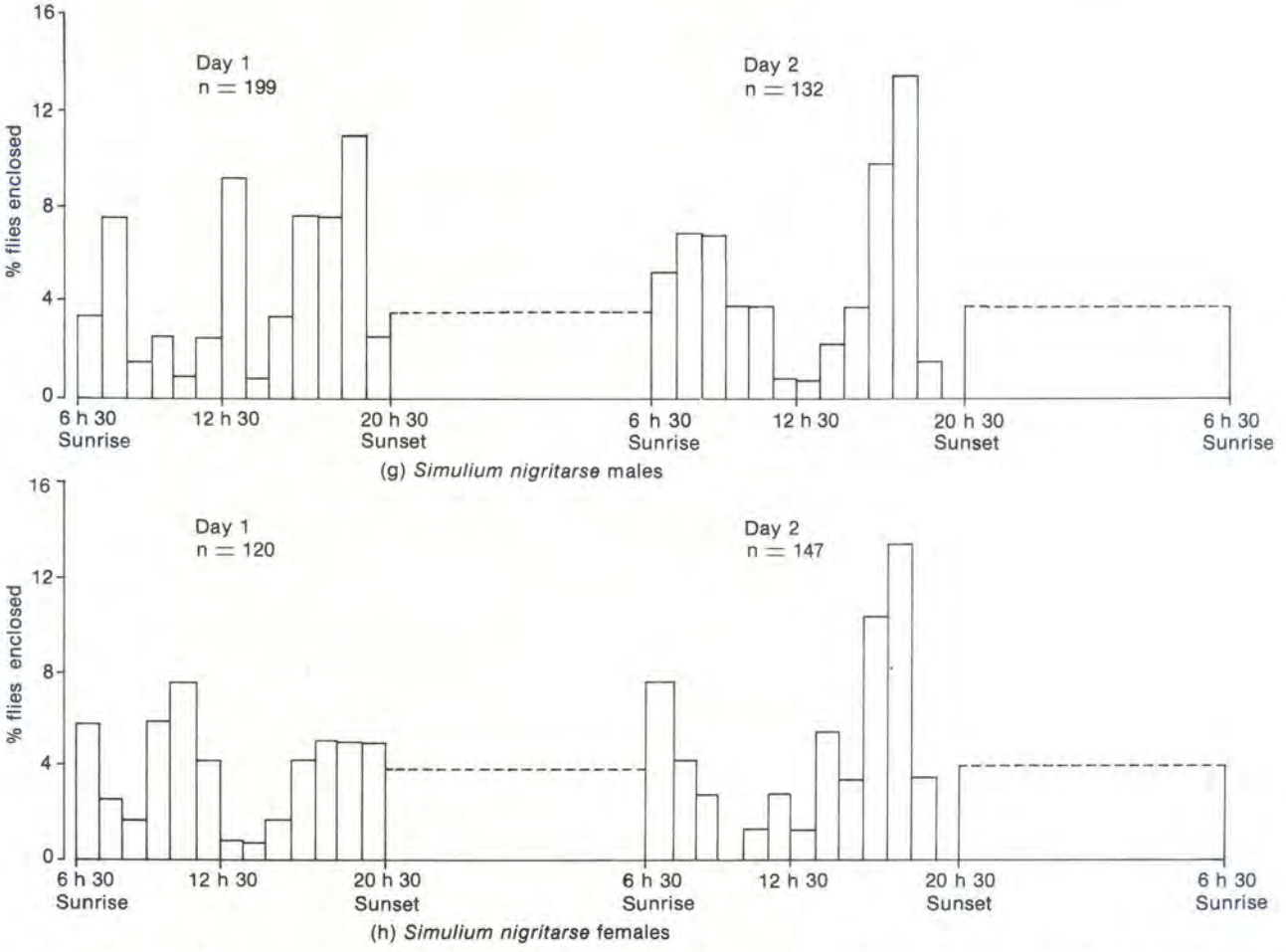

FIG. 3g, h *Simulium nigritarse:* eclosion rhythms during constant darkness (each vertical column represents the percentage of flies eclosed during a period of 1 h)

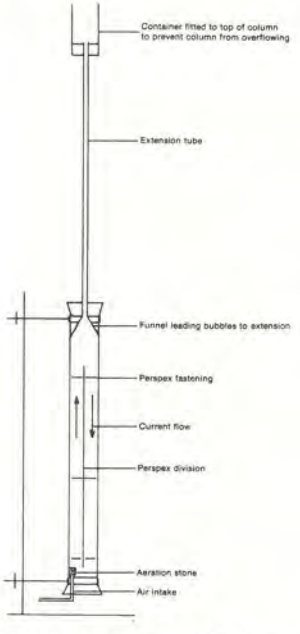

FIG. 4 Structure of glass column used to determine the position of larval attachment and pupation of *Simulium nigritarse*

Results and Discussion

The final positions taken up by the larvae in the tube were influenced by the fact that their silk threads became entangled in protruding objects in the current. The largest number of pupae, in relation to the number of larvae, was found in the upper 300 mm of the tube where the water current was slowest and had changed direction and where protruding objects were present (Table 4). Pupation also took place, however, right down to a depth of 1 100 mm.

After the aeration of the water in the tube was stopped, no general migration by the remaining larvae towards the water surface could be detected. Larvae within approximately 20 mm of the water surface tended to select the surface periphery of the tube, probably because of the higher oxygen content of the water there.

After 2 hours aeration in the lengthened column the first pupa was formed at the bottom of the tube. At this stage the experiment was discontinued, though it was clear that pupation could take place at a depth of 2 m.

B. THE EFFECT OF LIGHT INTENSITY AND CURRENT SPEED

Materials and Methods

Larvae were placed in round, transparent, plastic containers (210 mm × 170 mm), filled to a depth of 60 mm with de-ionized water. Magnetic stirrers were used to create a water current in these containers, as was done by Colbo & Thompson (1978). The containers were evenly illuminated from above at 1 100 lux (neon light) and the water temperature was kept at 20 ± 1 °C. The centrifugal action of the spinning magnet produced a maximum water current speed on the

bottom of the containers. After 6 hours the positions and numbers of larvae were recorded, then half the surface area of each container was covered with black plastic sheeting, thus creating a light gradient in the water ranging from 1 100 lux to 5 lux. The positions of the larvae were divided into 6 zones (Fig. 5). Eighteen and 42 hours later the positions of all the larvae and pupae in the 6 zones were noted. The water movement in the containers was subsequently stopped and the positions of the larvae recorded again 6 hours later. Six replications of this experiment were done (Table 5).

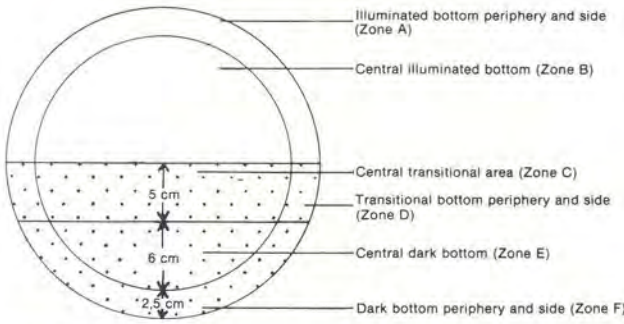

FIG. 5 Zones defined in round plastic containers used to determine the position of pupation and larval attachment of *Simulium nigritarse*

Results and Discussion

Throughout the experiment most larvae were present in the area of the highest water speed. The vast majority of the pupae (89,5%) were formed in the dark area, and, again, most of them where the water velocity was highest (Table 5). These observations explain the fact that the majority of larvae and pupae were attached to the undersides of the shale stones in the Modder River. In the previous experiment on the effect of water-depth on the larvae and pupae, pupal attachment took place in slower moving water, but this was most probably due to the physical conditions created when the vertically moving current slowed down and changed direction. Overwintering *S. ornatum* larvae in Scotland tend to form colonies on the undersides of stones and on their more protected surfaces where there must be less light and certainly where the current is not at its maximum (Smart, 1934). In the Modder River, however, *S. nigritarse* larvae and pupae remained on the underside of stones throughout 1978, even during the summer. There were no floods in the river during this study period; had there been, they might have made *Simulium* alter its position.

After the stirrers had been switched off for 6 hours, 70,1% of the larvae were found in the illuminated area and 41,6% of them attached to the periphery of the bottom and the sides of the containers (Table 5). Positive phototropism in standing water was also shown by *S. ornatum* (Smart, 1934). At this stage of the experiment the dark area contained 17,7% of the larvae, which proved to be mainly adult larvae with well-developed pupal respiratory histoblasts. This might be the result of negative phototropism exhibited by the adult larvae prior to the shedding of their larval skin.

THE OVERWINTERING OF *S. nigritarse*

Materials and Methods

Twenty-three mature *S. nigritarse* larvae were kept in a breeding jar at 6 °C and treated as described in the first experiment in this paper. Enough water had to be siphoned out of each jar every week to allow for the addition of 300 mℓ homogenized aged water.

Results and Discussion

Two out of the 23 mature *S. nigritarse* larvae kept in a breeding jar at 6 °C pupated after 1 month and the rest of the larvae still appeared to be in good condition.

During February 1978, a higher population of *S. adersi* than of *S. nigritarse* was found in the Modder River at Glen. On 4 June 1978, eggs, mostly of *S. nigritarse*, were collected from the river, but on 26 June none could be found. Of the mature *Simulium* larvae collected on 26 June 8,8% were *S. adersi* and 91,2% *S. nigritarse*. On 6 July and again on 10 August only *S. nigritarse* larvae and pupae could be found in the river. On 7 September, however, *Simulium* eggs were again found and *S. nigritarse* females were seen laying eggs under the water surface on partly submerged stones.

Thus a period of 2½ months had elapsed during which apparently no *Simulium* eggs had been laid in the river. In the laboartory the mean time spent in the pupal stage at 6 °C was 23,7 days (Table 2). The fact that mature larvae could be kept for 1 month at 6 °C

Table 5 The effect of light intensity and water current speed on larval migration and the position of the pupation site shown as the percentage larvae and pupae per cm²

Zone	Moving water							Still water
	Water speed	No light gradient		Light gradient				Light gradient
		6 hours		18 hours		42 hours		48 hours
		L	P	L	P	L	P	L
A. Illuminated area	slow	9,2	11,1	5,4	7,6	4,7	4,2	41,6
B. Illuminated area	fast	23,9	—	31,5	7,6	30,0	6,3	28,5
C. Transitional area	fast	25,0	—	33,2	16,2	34,3	14,8	6,3
D. Transitional area	slow	5,3	—	5,3	9,9	2,4	12,0	5,9
E. Dark area	fast	29,8	55,6	19,6	28,2	25,3	40,4	6,9
F. Dark area	slow	6,8	33,3	5,0	30,5	3,3	22,3	10,8
Total number of larvae and pupae		2 442	9	1 966	142	1 511	370	1 123

without pupating suggests that both larvae and pupae of *S. nigritarse* could overwinter at the low water temperatures (0–8 °C) recorded during the winter months.

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