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THE EFFECT OF PHYSICAL FACTORS ON COLONIZATION
OF ARTIFICIAL SUBSTRATES BY IMMATURE BLACK FLIES
(DIPTERA: SIMULIIDAE)

presented by

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THE EFFECT OF PHYSICAL FACTORS ON COLONIZATION OF ARTIFICIAL SUBSTRATES BY IMMATURE BLACK FLIES (DIPTERA: SIMULIIDAE)

By

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ABSTRACT

THE EFFECT OF PHYSICAL FACTORS ON COLONIZATION OF ARTIFICIAL SUBSTRATES BY IMMATURE BLACK FLIES (DIPTERA: SIMULIIDAE)

By

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Prosimulium mixtum/fuscum and *Cnephia dacotensis* preferred velocity ranges in the laboratory of 10 to 15 cm/sec and 5 to 10 cm/sec respectively. Both species did not increase their relocation behavior when exposed to depths of 2 to 30 cm and temperatures of 2 to 30°C. *P. mixtum/fuscum* increased relocation activity in the absence of light in the laboratory. The number of larvae attaching to white ceramic tiles and clear plastic tape in the field was influenced by both depth of placement and length of exposure time for both species. *P. mixtum/fuscum* readily attached to either substrate, whereas *C. dacotensis* demonstrated a preference for the white ceramic tiles.

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INTRODUCTION

Many species of black fly adults are important as biting pests of man and animals both as a nuisance and as vectors of disease. Since most control recommendations, whether chemical or biological, are based on the number of larvae collected on different substrates, an accurate means of estimating true population densities of larvae in natural streams and rivers is needed.

Black fly larvae typically occur in the benthos, sediments, periphyton or in the drift. Rather than searching for specific concentrations of larvae on natural substrates, many investigators have utilized artificial substrates to collect or sample the immature stages. Many techniques have been used to determine black fly species composition and to estimate relative larval densities over time. Various devices which have been used are: polythene tapes (Doby et al. 1967, Obeng 1967, Pegel and Ruhm 1976, Williams and Obeng 1962); wooden boards or floats (Grenier 1949, Holbrook 1967, Wanson and Henrard 1945, Carlsson 1962, 1967, Curtis 1968); plastic, metal or concrete cones (Abdelnur 1968, Benfield et al. 1974, Johnson and Pengelly 1966, Wolfe and Peterson 1958, Philipson 1956); ceramic tiles (Lewis and Bennett 1974, 1975, Zahar 1951); cheesecloth and nylon organdy (Tarshis 1968a); construction bricks (Ali et al. 1974); sealed tins and nets (Wanson and Henrard 1945, Elliott 1971b) and vegetative material tied to ropes (Disney 1972, Yakuba 1959).

A major problem in comparing studies is that individual investigators have utilized different materials, different geometric shapes and different placement techniques, and comparative relationships among and between different substrates have only been examined by a few workers (Benfield 1974, Williams and Obeng 1962). This lack of standards has led Lewis and Bennett (1974) to propose the use of a standardized artificial substrate.

The purpose of this study was to examine the effect of physical factors on the behavior of immature black flies colonizing different artificial substrates.

MATERIALS AND METHODS

Field Sites

Mud Creek in the Rose Lake Wildlife Research Area (Clinton - Shiawassee Counties, Michigan) was chosen as the first study site. It is a first order stream modified by its origin in a small lake lying in a deciduous forest/farmland drainage basis. It was approximately 2 meters wide and 25 cm deep at the study site. In the upper reaches of this creek, larvae of the Prosimulium mixtum/fuscum Syme and Davies complex occurred as an isolated population from October through March and was studied for three seasons (1974 - 1977).

The second study site was a small (1st order) drainage stream immediately below the outlet of Lake Lansing in Clinton County, Michigan. The drainage basin consisted mainly of a lowland swamp/residential area and the outlet was approximately 40 cm deep and 300 cm wide at the study area. The highly organic nature of the lake and outlet made the site an ideal breeding area for Cnephia dacotensis Dyar and Shannon which reportedly prefers such an environment (Ezenwa 1974, Jamnback 1969, Stone 1964). Larvae of C. dacotensis, present in the stream from March through April, were studied from 1974 - 1977.

Field Studies

Both species were exposed to white ceramic tiles, 6 cm x 20 cm x 1 cm and clear plastic tapes, 4 cm x 39 cm x 0.5 mm. The size of the plastic tape was designed to have the same surface area as the ceramic tile. Since the tape was tested using a Beckman^R spectrophotometer and shown not to be absorbtive of electromagnetic radiation in the UV and the visible light spectrum, it was considered to be invisible when placed in a stream.

Each substrate was placed at a depth of 0 (on the streambed), 4, 8, 12 and 20 cm above the streambed via a ringstand. Both substrates were exposed for the following intervals at each depth: 0.5 (sunrise to sunset), 0.5 (sunset to sunrise), 1, 2, 3, 5, 7, 9 and 11 days. The exposure period of a particular substrate was randomly chosen to avoid having the test results simply reflect seasonal developments occurring in the field.

Prior to the removal of a sample, a fine mesh net (0.2 mm) was placed immediately downstream of the artificial substrate since it was found that approximately 10% of the larvae released upon the slightest disturbance of the attachment site. After the substrate was removed from the stream, the number of larvae both attached to the substrate and collected in the net were combined to represent a total sample.

At both study sites, stream temperature was monitored during experiments and general stream velocity was measured using a Gurley Pygmy Current Meter.

Laboratory Studies

An artificial linear stream, Fig. 9, (Merritt and Gersabeck, unpublished) was used to examine larval relocation behavior (Appendix). Water flowed through a pivoting channel (3 m x 45 cm x 30 cm), over a foot gate which controlled depth and into a reservoir containing a cooling apparatus. The water then flowed through a recirculating pump to the upstream end of the channel where another gate controlled head pressure. The system enabled the following parameters to be adjusted independently: depth, temperature, slope and velocity. In addition, a series of lights and timers allowed for controlled photoperiods. Velocity in the artificial stream was measured utilizing the dye tracing technique of Wilson (1968a, 1968b).

During each test the physical parameters, except for the one being tested, were held constant at the values given in Table 1. These values were similar to actual field conditions. At the beginning of each experiment, 10,000 to 15,000 larvae were placed in the upstream end of the artificial stream and allowed to acclimate and stabilize for 24 hours. A mixture of brain-heart infusion broth, Purina^R dog chow and brewer's yeast powder (Tarshis 1968b) was added at the rate of 5 grams per 100 gallons of water at time 0 and at 72 hours. The stream bed was divided into a grid and the number of larvae occupying a random subset of the grid was counted. The parameter being tested was reset once every 24 hours and the larvae remaining in the selected grid cells were counted at each 24 hour interval. The number of larvae that left an area were assumed to have found the change in conditions unfavorable. Physical parameters being tested were varied for each species in the following manner: 1) velocity (2, 5, 10, 15, 20 and 25 cm/sec); 2) temperature (2, 6, 12, 18, 24 and 30^{o}C) and 3) depth (2, 4, 8, 16, 24 and 30 cm). In addition, photoperiod for P. mixtum/fuscum was varied at the following light:dark hourly intervals; 8:16, 9:15, 10:14, 11:13, 12:12 and 13:11. Because of the rapid developmental time of C. dacotensis in the field, the photoperiod experiment was not conducted on this species.

A one and two-way analysis of variance was used to test for significant differences in the laboratory and field data respectively (Elliott 1971a). In laboratory experiments, nine replicates were utilized for *P. mixtum/fuscum* and seven replicates were used for *C. dacotensis*. In the field, seven replicates were conducted for both species in all trials.

RESULTS

Laboratory Data

The results of changes in velocity with P. mixtum/fuscum and C. dacotensis are shown in Figures 1 and 2. Larvae of P. mixtum/fuscum showed minimum changes in location between 10 and 15 cm/sec and those of C. dacotensis between 5 and 10 cm/sec. However, at either end of the velocity range studied, there was an increase in the number of larvae of both species that released from their initial position. As velocity decreased, for both groups the percentage of the population relocating was represented almost equally by members of all age classes with a slight dominance by early instars. As velocity increased, the number of larvae relocating significantly ($P \leq .01$) increased and there was a marked predominance of early instars observed in the relocating population of both species. With increasing velocity, P. mixtum/fuscum relocated at a greater rate than did C. dacotensis.

Table 2 shows the effect of changes in depth, temperature and photoperiod on relocation behavior. There were no significant differences in the number of larvae relocating at tested depths with P. mixtum/fuscum or C. dacotensis. At lower temperature, the numbers of larvae of both species relocating remained fairly constant; however, as temperature increased beyond 18°C for P. mixtum/fuscum and beyond 12°C for C. dacotensis, a rapid decrease in the total number of larvae detaching occurred. The response of P. mixtum/fuscum to various intervals of light and dark exposure showed under all conditions that more larvae relocated during the dark phase than the light phase.

Field Data

The results of exposing tiles and tapes to *P. mixtum/fuscum* larvae at different depths for varying lengths of time in Mud Creek are shown in Figures 3 through 6. The experiments were conducted when stream discharge was both high (0.55 to 0.65 m^3/sec, Figures 3 and 4) and low (0.45 to 0.55 m^3/sec, Figures 5 and 6). A general trend was evident in which the total number of larvae attaching to substrates increased with increasing exposure time followed by a post-peak decline. A vertical distribution of larvae attached to substrates in the stream was also evident, whereby peak numbers for both tiles and tapes occurred at 4 and 8 cm above the streambed.

Peak larval colonization on tiles placed on the streambed occurred two days sooner and in significantly ($P \leq .05$) greater numbers at high stream discharge rates than at low stream discharge rates. Peak larval density on tapes occurred at day 5 for both the low and high discharge rates, but peak numbers were greatest at the high discharge rate. At 0 depth, a significantly ($P \leq .05$) greater number of larvae attached to the tiles than the tapes at both high and low rates of stream discharge. Following peak larval densities, there was a decline in total numbers for all treatments at the above depth.

At 4 cm above the streambed, peak numbers occurred on both the tiles and the tapes two days sooner at high discharge rates than at low discharge rates. Unlike the tapes, there was no significant difference ($P \leq .05$) in the peak number of larvae that occurred on tiles at both high and low rates of discharge. Peak numbers were statistically similar for the tiles and tapes at high discharge, but the successful colonization of the tapes (in terms of total numbers) decreased at lower discharges. In all treatments at 4 cm above the streambed, a post-peak decline in total numbers was evident. The total number of larvae colonizing substrates at this depth was approximately double the number colonizing the substrates when placed on the streambed (0 depth).

Artificial substrates placed at 8 cm above the streambed were also colonized significantly ($P \leq .05$) faster at high discharge rates than at low discharge rates. Both tiles and tapes at high discharge were colonized to comparable numbers by day five. Larval colonization at 8 cm above the streambed was lower on tapes than tiles at low discharge rates. The total number of larvae colonizing both substrates at this depth was similar to larval numbers at 4 cm and was significantly ($P \leq .01$) greater than the numbers found when the substrates were placed on the streambed. At both rates of discharge, the tiles at 8 cm above the streambed took approximately two days longer to be colonized to peak densities than at 4 cm. In all treatments at the 8 cm placement, a post-peak decline in total numbers was also observed.

At 12 cm above the streambed, both tiles and tapes showed an extended time period needed to achieve peak numbers at both high and low discharge rates. At this depth, the number of larvae colonizing the tapes was significantly ($P \leq .05$) greater than the number of larvae colonizing the tiles at high discharge. For all treatments at this depth, peak numbers of larvae occurred several days later than the peaks at 8, 4 or 0 cm above the streambed. Peak numbers were also significantly ($P \leq .01$) less than the peaks at 4 and 8 cm depths and comparable to those numbers observed at 0 depth.

With the tiles and tapes placed at 20 cm above the streambed, both total numbers and rates of colonization were greatly reduced when compared to any other treatment depth or time.

The response of _C_. _dacotensis_ larvae to artificial substrates placed at varying depths for different exposure times is shown in Figures 7 and 8. There was a general increase in the number of larvae attaching to tiles and tapes over time; however, the number of larvae colonizing the tapes was significantly ($P \leq .001$) less than the tiles.

With the substrates placed on the streambed (0 depth), the number of larvae on both tiles and tapes increased until the exposure period was terminated at eleven days. The number of larvae attached to the tiles was approximately 18 times the number attached to the tapes.

At 4 cm above the streambed, tiles showed an increase in total numbers to day seven with a slight decrease from 7 - 11 days of exposure. The peak number of larvae colonizing the tiles at this depth were over twice the peak numbers observed with the tiles placed on the streambed. Peak number of larvae on the tapes represented only 2.5% of the peak number of larvae that colonized the tiles.

At 8 cm above the streambed, a general increase in total numbers of larvae colonizing tiles began to approach 0 at day five. From day 5 - 11, a slight increase in peak numbers was found which was contrary to the post-peak decrease in total numbers recorded at 4 cm above the streambed. Peak numbers occurred on tiles approximately two days sooner than at the 4 cm depth. As was shown for the 4 cm depth, the total numbers colonizing the tiles were over twice the peak numbers recorded when the tiles were placed at 0 depth. An increase in total numbers on the tapes was observed until day seven; however, they were only colonized to approximately 2% of the numbers that colonized the tiles. There was no statistical difference in the numbers colonizing tapes at 8 cm above the streambed when compared to the peak numbers colonizing tapes placed at 0 and 4 cm above the streambed.

Tiles placed at 12 cm above the streambed had the largest number of larvae attaching of all depths tested during a five day exposure. Post-peak numbers of larvae on tiles did not change significantly from day five through eleven. Larvae colonizing tapes increased in number through day five and then decreased through day eleven. At this depth, _C_. _dacotensis_ colonized the tapes to only 2% of the density found on the tiles.

At 20 cm above the streambed, there was a progressive increase in the numbers of larvae attaching to both tiles and tapes until day eleven when the exposure period ended. Again, tiles were significantly ($P \leq .001$) more effective than the tapes in collecting total numbers of larvae. Peak numbers observed on the tiles were not as high as tiles placed at 4, 8 and 12 cm, but were much higher than was observed when the tiles were placed on the streambed. Peak larval density occurred four to six days later at 20 cm with tiles than was recorded at previous depths. Peak numbers of larvae occurring on the tapes were similar to those occurring on tapes at other depths.

DISCUSSION

Laboratory Data

C. dacotensis and *P. mixtum/fuscum* showed different velocity range preferences with *P. mixtum/fuscum* tolerating a higher velocity range than did *C. dacotensis* (Figures 1 and 2). At low velocities, the population of moving larvae was represented almost equally by all instar groups. This suggested that the larvae of both species perceived a limit occurring on one or more of their resources due to low velocity and responded by attempting to move to a new location. As velocity increased, *P. mixtum/fuscum* had a significantly ($P \leq .05$) greater rate of increase in the number of larvae relocating than did *C. dacotensis*. This suggested that although *C. dacotensis* preferred a lower velocity, it was more tolerant of a wider range of velocities than *P. mixtum/fuscum*.

As velocity increased beyond 15 cm/sec for *P. mixtum/fuscum*, drifting larvae began to be dominated by early instars. Larvae of *P. mixtum/fuscum* were observed in the laboratory to periodically scrape a circular area around their base of attachment. If an encounter was made between a fourth or larger instar and a smaller larva, the smaller one was generally disloged. An encounter between two larger instars usually resulted in no change in position. At higher velocities, it appeared that the earlier instars were not physically able to cope with the stress of holding on while being disturbed by larger instars. However, at lower velocities, the smaller larvae may have been able to tolerate contact without causing detachment. The behavioral interactions of *P. mixtum/fuscum* appeared to be an important factor accounting for the predominance of younger larvae in the drift at higher velocities. "Fighting" by *Prosimulium* sp. larvae in laboratory studies has been observed by Kurtach (1973). Also, Anderson and Dicke (1960) have observed larvae obtaining food by scraping around their attachment site.

The scraping behavior of *P. mixtum/fuscum* was not observed with *C. dacotensis*, although smaller larvae also predominated in the population of moving larvae at higher velocities. The larvae relocating in this experiment may have been showing a direct velocity or space preference rather than a dislogement phenomenon as was observed with *P. mixtum/fuscum*. *C. dacotensis* larvae did not appear to be as affected by crowding as was *P. mixtum/fuscum*, as it was not uncommon to find smaller larvae attached to larger larvae.

In a natural stream, changing velocities are known to induce black fly larvae to migrate (Carlsson 1967) and younger instars have been shown to be predominant in the populations of drifting larvae (Kureck 1969). This may be due to: 1) the limiting of some resource, 2) increased territorial activity as was observed with *P. mixtum/fuscum* or 3) simply having a poor attachment for the velocity range encountered. The number of larvae and instar distribution on an artificial substrate was shown to be partially due to the velocity of the water column it intercepts. In a mixed species stream, species isolation on an artificial substrate may be favored if the preferred velocity ranges are distinct enough between species and the substrate could be placed in a position to intercept different velocity ranges.

Changes in depth, independent of velocity, did not cause an increase in the rate of relocation for either species. In natural streams, a change in discharge would be accompanied by both a change in velocity and a change in depth. Black fly larval response to changing rates of stream discharge could be based on either velocity or depth. Data in this study indicated that for P. mixtum/fuscum and C. dacotensis, the changing column of water above the larvae did not in itself induce the larvae to change position.

With increasing temperature in the artificial stream, both species reached a point where the numbers of larvae relocating dropped sharply. For P. mixtum/fuscum, this represented a thermal threshold above which most of the larvae died. However, C. dacotensis responded to increasing temperature by increasing its rate of development, and the reduction in numbers relocating represented pupation rather than mortality. P. mixtum/fuscum normally developed over several months at Mud Creek and may be physiologically unable to cope with conditions at higher temperatures over short time periods. Ross (1977) and Ross and Merritt (1978) observed high mortality in this species at temperatures above 8.5°C. C. dacotensis normally passed from the egg to adult stage in four to six weeks at the Lake Lansing outlet. Its ability to develop from early instars to pupae in slightly less than one week in the laboratory implied that the rapid development of this species was temperature dependent, as has been observed with other species of black flies (Mokry 1976, Becker 1973). In a natural situation, nutrition would not be a concern since this species typically occurs below outfalls of eutrophic lakes where food is plentiful during their larval developmental period in the spring (Ezenwa 1974, Jamnback 1969, Stone 1964). Water temperature regime is a concern in sampling because it limits the time period that larvae are available for study. Species such a *P. mixtum/fuscum* with a long developmental time may be sampled over several months; whereas a population like *C. dacotensis*, which develops quickly with an increase in temperature, may be accidently missed by the investigator in a study stream.

Increased downstream drift of simulid larvae in natural streams at night was reported by Pearson and Franklin (1968) and Disney (1972) with the latter attributing it to increased activity of other stream organisms. When *P. mixtum/fuscum* larvae were exposed to changing photoperiods, there was a significantly greater change in location during the dark period than during the light. The fact that the number of larvae relocating over varying intervals of light and dark remained approximately the same would suggest that the larvae may be responding to the light-dark interface rather than the length of the light or dark period. This also suggests that the increased relocation behavior is not necessarily due to the activity of other organisms. Since more activity occurred during the dark phase, an investigator could maximize substrate exposure time by initially placing a substrate at sunset and recovering it at some later sunrise.

Field Data

With tiles and tapes at both high and low discharge, *P. mixtum/fuscum* showed a general increase in numbers to some peak density and then a post-peak drop off in total numbers over time. The post-peak decline occurring when the substrate was placed on the streambed was due to fouling of the substrate by material moving along the stream bottom. The fouling was progressive with time and after approximately two weeks exposure, the substrate was totally covered with debris, thereby rendering the substrate unsuitable for attachment.

When substrates were placed above the streambed, the post-peak decline in total numbers observed with P. mixtum/fuscum did not appear to be mainly due to substrate fouling but rather territorial behavior among the larvae. As was observed in the laboratory, some older larvae had scraped circular areas around their attachment points on the substrates and presumably smaller larvae became disloged in the field as they had in the laboratory. Characteristic of this period of decline, was a predominance of fourth or larger instars attached to the substrate. This did not simply reflect normal larval growth and development in the field, since an increasing pattern of colonization by younger instars would reoccur if new substrates were placed at the same site when the decreasing trend in larval numbers on the old substrates was observed.

The spatial and temporal ranges tested in the field indicated that there was a definite depth range at which P. mixtum/fuscum larvae were capable of attaching to an artificial substrate. This may reflect the population distribution of the larvae moving in the stream drift. For this species, the depth at which the substrates were placed in the stream was a major factor in determining how many larvae colonized the substrates over time.

At high stream discharges, both tiles and tapes were colonized by the same numbers of P. mixtum/fuscum larvae. Since the tapes were essentially invisible when suspended in the water, P. mixtum/fuscum apparently did not require a visible cue in order to discover the substrate. It is true that as the tapes were colonized, they became visible due to material accumulating on the surfaces i.e., larvae, silk, debris, etc. However, if it was necessary for the tapes to be visible before being colonized, one would expect a much lower initial rate of colonization until a coating was formed, and then a rapid rate of colonization by the larvae, this was not observed.

C. dacotensis larvae also showed a general increasing trend over time to some peak number, but unlike P. mixtum/fuscum, they did not exhibit a significant post-peak period of decline. Clear plastic tape proved very ineffective for sampling this species when compared to ceramic tiles. After eleven days of exposure, there was a 20 - 60% greater colonization of the tiles than the tapes. It was possible that the tapes were not seen by the C. dacotensis larvae. However, in just a few hours of exposure, the tapes were completely coated with a greenish-brown slime making them highly visible. Considering that C. dacotensis still did not colonize the tapes to any great extent, it would appear that the tape surface, once coated with a micro flora and fauna, offered a poor surface for larval attachment, a condition which has been noted for other species of black flies (Carlsson 1962, Zahar 1951). Tiles did not become slime coated for approximately thirteen days and it would appear that this coating accounted for the differences in colonization of the two substrates. Flexibility of the tape was not considered to be a contributing factor since adjacent flexible vegetative material was readily colonized by C. dacotensis.

Both substrates collected significantly fewer C. dacotensis larvae when they were placed on the streambed. As was true for P. mixtum/fuscum, this was due to substrate fouling. When colonization densities on the tiles were compared between P. mixtum/fuscum and C. dacotensis, a significantly ($P \leq .001$) greater peak number of *C. dacotensis* larvae was observed. This apparently was due to the scraping behavior exhibited by *P. mixtum/fuscum* which resulted in a spatial requirement for the larvae.

CONCLUSIONS

This study has shown that when sampling black fly larvae with artificial substrates, the following points should receive consideration: 1) individual species may have a preference for the type of material to which they will attach, 2) the placement depth of the substrate in the stream and exposure time will greatly affect larval colonization, 3) stream velocity may affect both the number of larvae attaching to and the instar distribution on artificial substrates and 4) physical characteristics of the stream itself may affect larval colonization.

This study also has implications for control techniques: 1) knowledge of the microenvironmental needs of a species; i.e. velocity, temperature, etc., may enable one to induce migration during inopportune periods, 2) intraspecies competition may limit the number of available sites for development within a stream and 3) interspecies competition may allow the replacement of a target species by a non-pest species.

APPENDIX

APPENDIX

Artificial Stream Construction

METHODS

Figure 9 shows a schematic diagram of the artificial stream. A steel frame was constructed from 1/8 inch x 2 inch standard angle iron stock. This pivoted at 2/3 of the total length measured from the head end. At 1/3 of the length from the head, a pair of scissor jacks were used to raise or lower the head end of the stream. This allowed a variable control of the slope.

Unless otherwise stated, all plastic used was standard 3/8 inch stock.

The trough measured 240 cm long x 45 cm wide x 30 cm high. This was set on 3.5 cm of styrofoam insulation which in turn rested on the steel frame. The insulation minimized heat loss and helped maintain a constant temperature.

Head pressure was produced by a reservoir attached to the head end of the stream. It measured 45 cm x 25 cm x 75 cm and had the water supply entering from the bottom. A fibreglass screen on a 45^{o} angle over the inlet broke the energy of the water inflow. A verticle sliding headgate measuring 45 cm x 50 cm placed at the interface of the head reservoir and the stream channel controlled the depth of the water in the reservoir which in turn controlled the amount of head pressure obtainable. Depth was controlled by a pivoting footgate measuring 45 cm x 15 cm at the downstream end of the channel.

Water, after passing over the footgate, free fell through a channel (90 cm x 45 cm x 10 cm) into a reservoir (75 cm x 75 cm x 120 cm) constructed of 3/4 inch exterior plywood stock. This was lined with three layers of fibreglass and reinforced along the edges. Within the reservoir was a cooling coil driven by a 1/4 horsepower refrigeration compressor for temperature control. The reservoir was covered with 5 cm of styrofoam insulation on all sides to reduce heat loss.

A pump with the inflow pipe through the reservoir wall returned water to the head reservoir via a flexible hose. Our pump was custom built, but a similar unit can be obtained from an irrigation supply company. We specified the pump impeller to be ground to supply maximum volume with minimum pressure and also required a continuous duty motor. Pump output was controlled by the insertion of a valve between the output pipe of the pump and the flexible hose.

DISCUSSION

The stream has been in use for three years to study black fly larvae under flowing water conditions. Over time, the plastic showed a tendency to warp if water temperature was changed over a short time period, as would be encountered during filling or addition of water to maintain volume. Besides making slow temperature adjustments, it would appear desirable to use thicker plastic.

The 1/4 horsepower refrigeration compressor proved to be inadequate to cool the stream below $10^{\circ}C$ and a supplemental cooler had to be used. We found that the cooling unit had to compensate for heat imput from both ambient air temperature and the heat generated by the circulating water. This condition was aided by the addition of insulation, but a larger compressor was needed to maintain the temperature below $10^{\circ}C$.

In use, with increasing velocity, an area of turbulence was generated at the head gate. It would be desirable to increase the length of the stream to get a more constant usable area.

In use, the bed can be lined with any material (rocks, tiles, etc.) and subdivided into smaller areas by the addition of baffles along the length of the bed. A diversity of habitat might be needed to prevent the test organisms from being washed over the foot gate during long term studies. Food must be added only in small amounts to prevent unwanted scum formation and occasionally distilled water must be added to prevent concentration of dissolved materials due to evaporation. When in operation, a great deal of noise was generated and a site should be chosen that is either isolated or sound deadened.

Table 1. Control conditions in the artificial stream.

Species	Velocity cm/sec	Temperature ^{o}C	Depth cm	Photoperiod light:dark
P. mixtum/fuscum	15	1	10	9:15
C. dacotensis	10	4	10	11:13

Table 2. The effect of changes in depth, temperature and photoperiod in the artificial stream represented as the percent of the initial population that relocated.

Species	Parameters					
	Depth - cm					
	2	4	8	16	24	30
P. mixtum/fuscum	10.5	10.6	10.4	10.7	10.3	10.6
C. dacotensis	8.8	8.6	8.1	9.2	8.6	8.1
	Temperature - °C					
	2	6	12	18	24	30
P. mixtum/fuscum	8.2	8.4	8.2	7.3	1.8	0.0
C. dacotensis	7.7	7.8	7.1	1.3	0.6	0.0
	Photoperiod - hours $\frac{\text{light}}{\text{dark}}$					
	$\frac{8}{16}$	$\frac{9}{15}$	$\frac{10}{14}$	$\frac{11}{13}$	$\frac{12}{12}$	$\frac{13}{11}$
P. mixtum/fuscum	$\frac{4.3}{6.3}$	$\frac{4.5}{5.9}$	$\frac{3.7}{5.6}$	$\frac{4.4}{5.9}$	$\frac{4.2}{5.8}$	$\frac{3.8}{6.1}$

Figure 1. The percentage of the initial population of P. mixtum/fuscum larvae that relocated with changes in the artificial stream velocity.

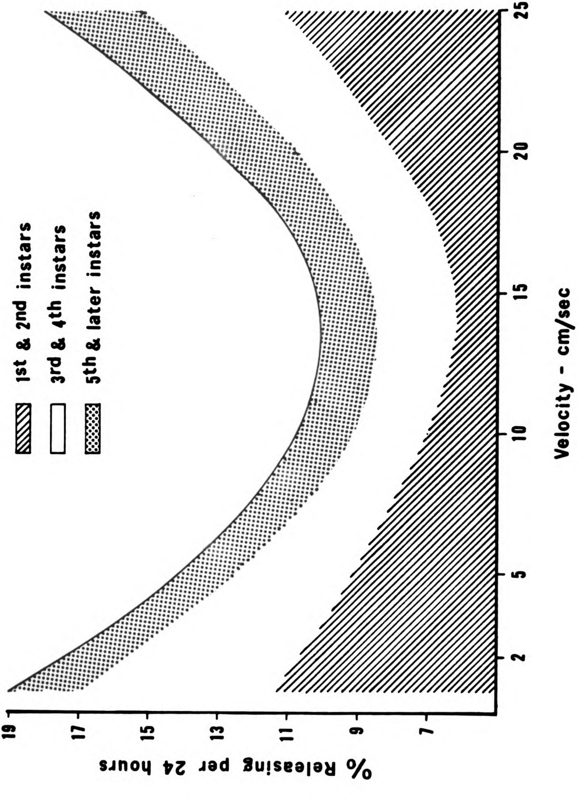
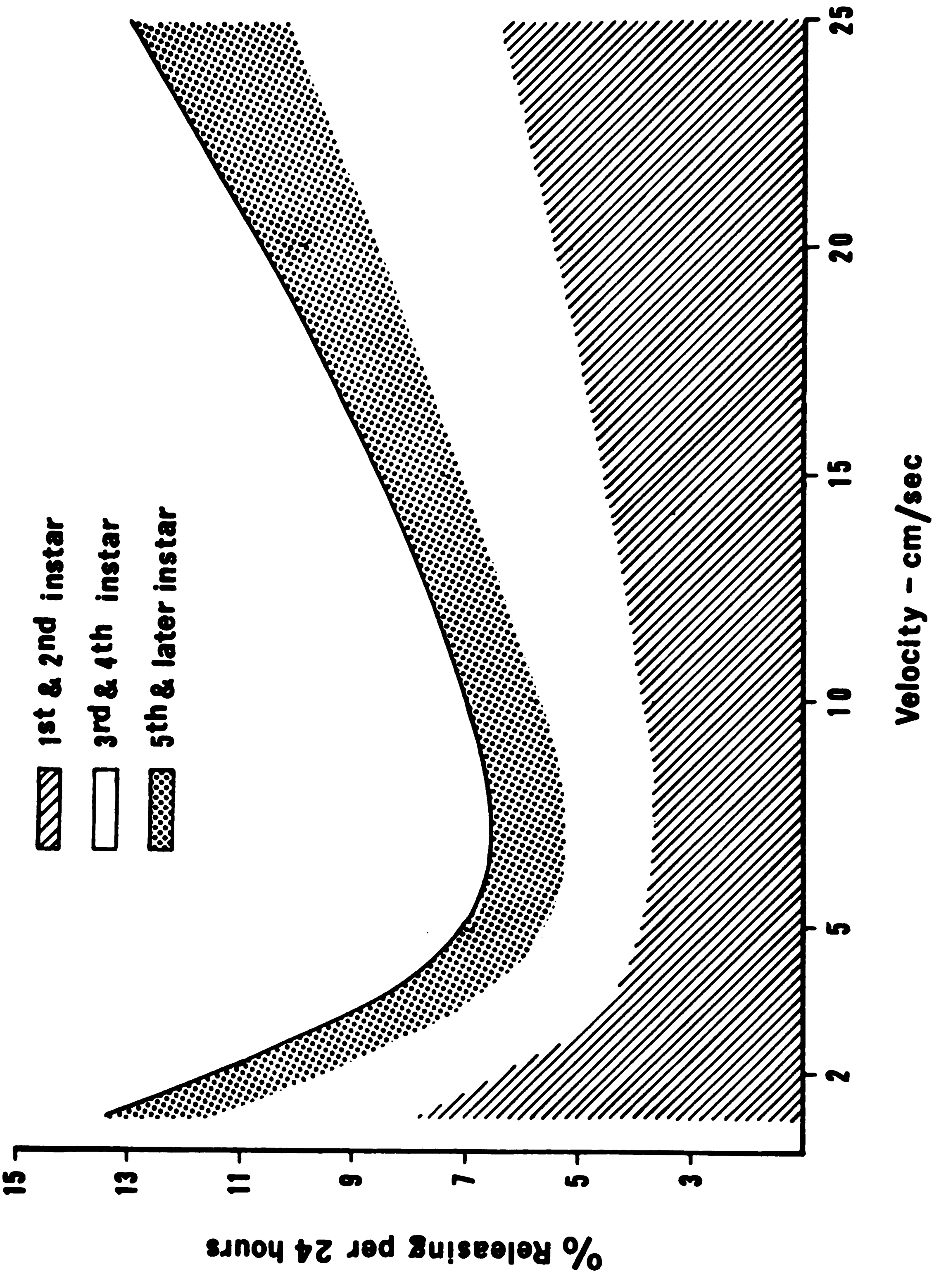

1st & 2nd instars
3rd & 4th instars
5th & later instars
% Releasing per 24 hours
19
17
15
13
11
9
7
Velocity - cm/sec
2
5
10
15
20
25

Figure 2. The percentage of the initial population of *C. dacotensis* larvae that relocated with changes in artificial stream velocity.



▨ 1st & 2nd instar
☐ 3rd & 4th instar
▩ 5th & later instar
% Releasing per 24 hours
15
13
11
9
7
5
3
2
5
10
15
20
25
Velocity – cm/sec

Figure 3. Mean number of P. mixtum/fuscum larvae colonizing tiles during high discharge (D = sunrise to sunset, N = sunset to sunrise).

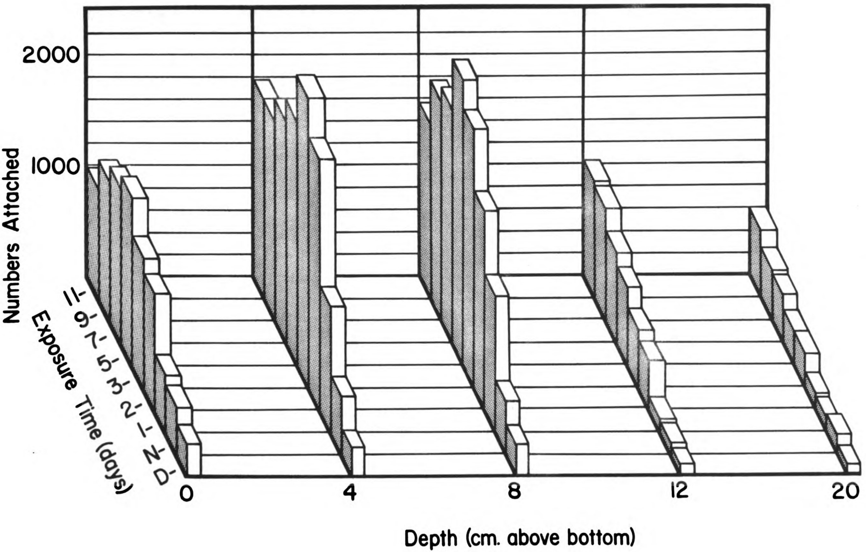

Numbers Attached
2000
1000
Exposure Time (days)
11
9
7
5
3
2
1
N
D
0
4
8
12
20
Depth (cm. above bottom)

Figure 4. Mean number of P. mixtum/fuscum larvae colonizing tapes during high discharge (D = sunrise to sunset, N = sunset to sunrise).

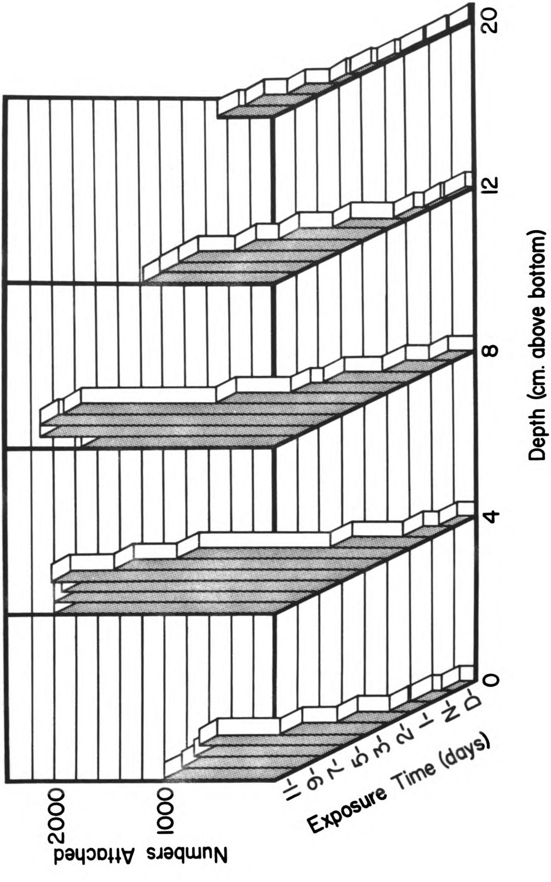

Numbers Attached
2000
1000
Exposure Time (days)
11
9
7
5
3
2
1
N
D
Depth (cm. above bottom)
0
4
8
12
20

Figure 5. Mean number of _P_. _mixtum/fuscum_ larvae colonizing tiles during low discharge (D = sunrise to sunset; N = sunset to sunrise).

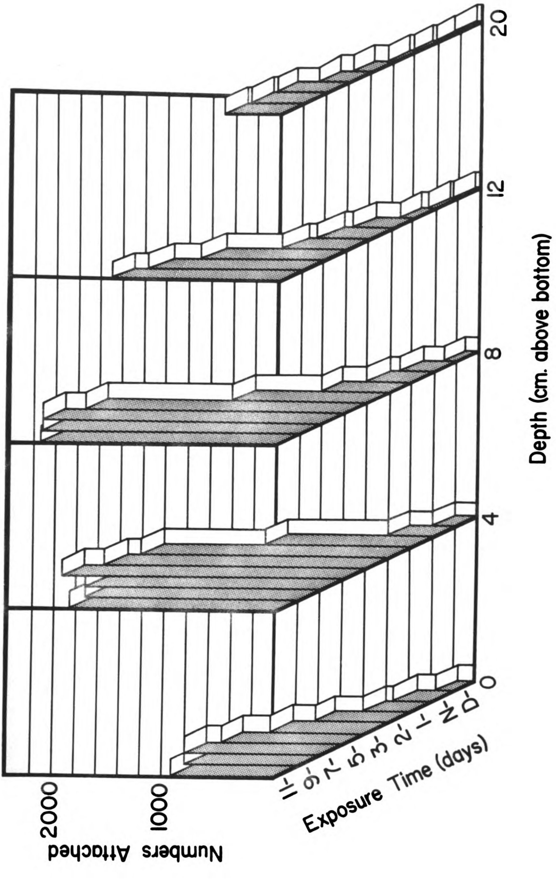

Numbers Attached
2000
1000
Exposure Time (days)
11
9
7
5
3
2
1
N
D
Depth (cm. above bottom)
0
4
8
12
20

Figure 6. Mean number of _P_. _mixtum/fuscum_ larvae colonizing tapes during low discharge (D = sunrise to sunset, N = sunset to sunrise).

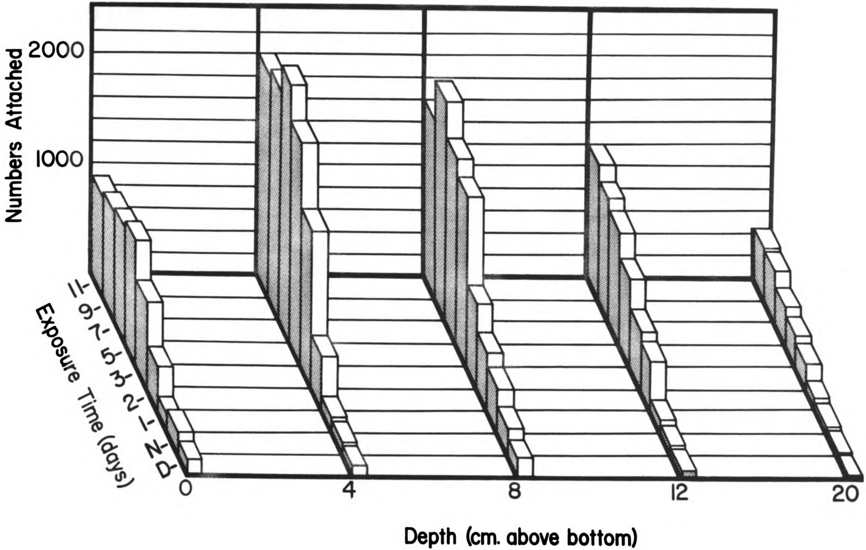

Numbers Attached
2000
1000
Exposure Time (days)
11
9
7
5
3
2
1
N
D
0
4
8
12
20
Depth (cm. above bottom)

Figure 7. Mean number of *C. dacotensis* larvae colonizing tiles (D = sunrise to sunset, N = sunset to sunrise).

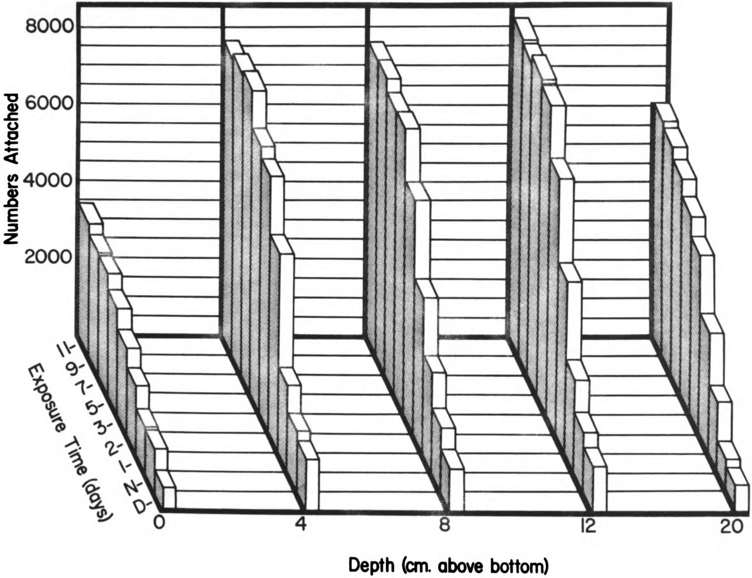

8000
6000
4000
2000
Numbers Attached
11
9
7
5
3
2
1
N
D
Exposure Time (days)
0
4
8
12
20
Depth (cm. above bottom)

Figure 8. Mean number of *C. dacotensis* larvae colonizing tapes (D = sunrise to sunset, N = sunset to sunrise).

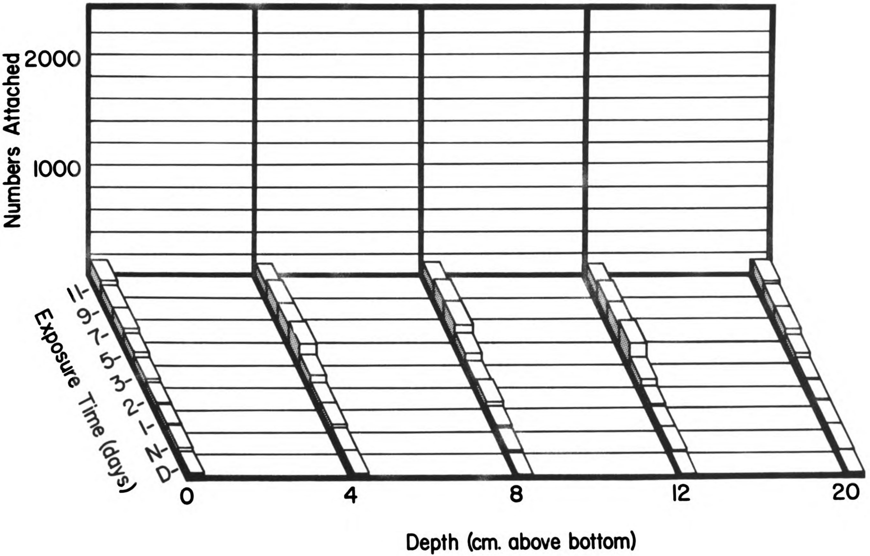
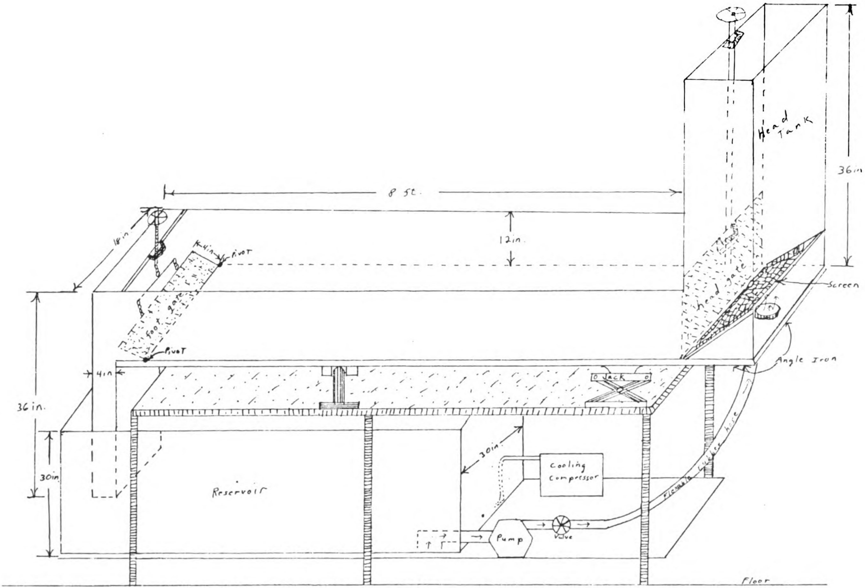

Numbers Attached
2000
1000
Exposure Time (days)
11
9
7
5
3
2
1
N
D
0
4
8
12
20
Depth (cm. above bottom)

Figure 9. Schematic diagram of the Artificial Stream.



Head Tank
36in
8 ft.
12in.
18in.
4in
Pivot
foot gate
Pivot
head gate
Screen
Angle Iron
Jack
36 in.
4in
30in
30in.
Reservoir
Cooling compressor
Pump
Valve
Flexible rubber hose
Floor

LITERATURE CITED

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Abdelnur, O. M. 1968. The biology of some black flies (Diptera: Simuliidae) of Alberta. Quaes. Ent. 4: 113-174.

Ali, S. H., P. P. Burbutis, W. F. Ritter and R. W. Lake. 1974. Black fly (Simulium vittatum Zetterstedt) densities and water quality conditions in Red Clay Creek, Pa. Del. Environ. Ent. 3: 879-881.

Anderson, J. R. and R. J. Dicke. 1960. Ecology of the immature stages of some Wisconsin black flies. Ann. Ent. Soc. Amer. 53: 386-404.

Becker, C. D. 1973. Development of Simulium (Psilozia) vittatum Zett. (Diptera:Simuliidae) from larvae to adults at thermal increments from 17.0 to 27.0 C. Am. Midl. Natur. 89: 246-249.

Benfield, E. F., A. C. Hendricks and J. Cairns. 1974. Proficiencies of two artificial substrates in collecting stream macroinvertebrates. Hydrobiologia. 45: 431-440.

Carlsson, G. 1962. Studies on Scandinavian black flies (Fam. Simuliidae Latr.). Opus. Ent Supp. XXI: 280 pp.

Carlsson, G. 1967. Environmental factors influencing black fly populations. Bull. Wld. Hlth. Org. 37: 139-150.

Curtis, L. C. 1968. A method for accurate counting of blackfly larvae (Diptera:Simuliidae). Mosq. News 28: 238-239.

Disney, R. H. L. 1972. Observations on sampling pre-imaginal populations of blackflies (Dipt., Simuliidae) in West Cameroon. Bull. Ent. Res. 61: 485-503.

Doby, J. M., B. Rault et F. Beauccurnu-Saguez. 1967. Utilisation de rubans de plastique pour la recolte des oeufs et des stades larvaires et nymphaux de Simulies (Dipteres Parane matoceres) et pour l'etude biologique de ceux-ci Ann. Parasit. Hum. Comp. (Paris) 42: 651-657.

Elliott, J. M. 1971a. Some methods for the statistical analysis of samples of benthic invertebrates. Freshw. Biol. Assoc. Sci. Publ. No. 25: 144 pp.

Elliott, J. M. 1971b. Upstream movements of benthic invertebrates in a lake district stream. J. Anim. Ecol. 40: 235-252.

Ezenwa, A. O. 1974. Ecology of Simuliidae, Mermithidae and Microsporida in Newfoundland freshwaters. Can. J. Zool. 52: 557-565.

Grenier, P. 1949. Contribution a letude biologique des Simuliidne de France. Physiol. Comp. Oecol. 1: 165-330.

Holbrook, F. R. 1967. The blackflies (Diptera: Simuliidae) of western Massachusetts. Ph.D. Thesis, Univ. of Mass., Amherst, Mass.

Jamnback, H. A. 1969. Blood sucking flies and other outdoor nuisance arthropods of New York State. N. Y. State Mus. Sci. Serv. Mem. 19.

Johnson, A. F. and D. H. Pengelly. 1966. A cone trap for immature blackflies (Diptera: Simuliidae). Proc. Ent. Soc. Ont. 96: 120.

Kureck, A. 1969. Tagesrhythmen lapplandischer Simuliiden (Diptera). Oecologia 2: 385-410.

Kurtak, D. C. 1973. Observations on filter feeding by the larvae of black flies (Diptera:Simuliidae). Ph.D. Thesis, Cornell Univ., Ithaca.

Lewis, D. J. and G. F. Bennett. 1974. An artificial substrate for the quantitative comparison of the densities of larval simuliid (Diptera) populations. Can. J. Zool. 52: 773-775.

Lewis, D. J. and G. F. Bennett. 1975. The blackflies (Diptera: Simuliidae) of insular Newfoundland. III. Factors affecting the distribution and migration of larval simuliids in small streams on the Avalon Peninsula. Can. J. Zool. 53: 114-123.

Mokry, J. E. 1976. Laboratory studies on the larval biology of Simulium venustum Say (Diptera: Simuliidae). Can. J. Zool. 54: 1657-1663.

Obeng, L. E. 1967. Life-history and population studies on the Simuliidae of North Wales. Ann. Trop. Med. Parasit. 61: 472-487.

Pearson, W. D. and D. R. Franklin. 1968. Some factors affecting drift rates of Baetis and Simuliidae in a large river. Ecology. 49: 75-81.

Pegel, V. M. and W. Ruhm. 1976. Versuche zur Besiedlung kunstlicher substrate durch praimaginale Stadien von Simuliiden unter besonderer Berucksichtigung von Boophthora erythrocephala de Geer (Simuliidae, Dipt.). Z. ang. Ent. 82: 65-71.

Philipson, J. 1956. A study of factors determining the distribution of the larvae of the blackfly, Simulium ornatum MG. Bull. Ent. Res. 47: 227-238.

Ross, D. H. 1977. The ecology and distribution of immature black flies (Diptera: Simuliidae) of the Rose Lake Wildlife Research Area, Michigan. M. S. Thesis, Mich. State Univ., Michigan.

Ross, D. H. and R. W. Merritt. 1978. The larval instars and population dynamics of five species of black flies (Diptera: Simuliidae) and their response to selected environmental factors. (In prep.)

Stone, A. 1964. Guide to the insects of Connecticut. Part VI. The Diptera or true flies of Connecticut. Ninth Fascicle. Simuliidae and Thaumaleidae. Conn. State Geol. Nat. Hist. Surv. Bull. 97.

Tarshis, I. B. 1968a. Use of fabrics in streams to collect black fly larvae. Ann. Ent. Soc. Amer. 61: 950-961.

Tarshis, I. B. 1968b. Collecting and rearing black flies. Ann. Ent. Soc. Amer. 61: 1072-1083.

Wanson, M. and C. Henrard. 1945. Habitat et comportement larvaire du Simulium damnosum Theobald. Rec. Trav. Sci. Med. Cong. Belg. 4: 113-121.

Williams, T. R. and L. Obeng. 1962. A comparison of two methods of estimating changes in Simulium larval populations, with a description of a new method. Ann. Trop. Med. Parasit. 56: 359-361.

Wilson, J. F. 1968a. Time-of-travel measurements and other applications of dye tracing. Int. Ass. Sci. Hydrol. 76: 252-262.

Wilson, J. F. 1968b. Fluorometric procedures for dye tracing, techniques of water-resource investigation of the United States Geological Society. Applications of Hydraulics, U. S. Govern. Print. Office. No. 10473.

Wolfe, L. S. and D. G. Peterson. 1958. A new method to estimate levels of infestations of blackfly larvae (Diptera: Simuliidae). Can. J. Zool. 36: 863-867.

Yakuba, V. N. 1959. On the migration of black fly larvae. Ent. Rev. 38: 379-387.

Zahar, A. R. 1951. The ecology and distribution of blackflies (Simuliidae) in south-east Scotland. J. Anim. Ecol. 20: 33-62.