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# Colonization of Immature Black Flies (Diptera: Simuliidae) on Artificial Substrates in a Nebraska Sandy River

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## Abstract

Colonization of *Simulium bivoittatum* Malloch and *S. luggeri* Nicholson and Mickel on artificial substrates exposed for two consecutive time periods of 1, 2, or 4 d was contrasted with colonization after continuous exposures of 2, 4, or 8 d. Reduced colonization of early instars of both species during the second 4-d period was attributed to an aging population, whereas reductions in total black flies after 8 d seemed to be caused by substrate changes. Numbers of pupae increased as exposure time increased.

**Keywords:** Insecta, *Simulium*, artificial substrates, colonization

Black flies are abundant in large, shallow rivers draining the Nebraska sandhills. Natural substrates in these shifting sand bottom streams are limited to vegetation, primarily grasses, near the shore. Unregulated upstream portions of these rivers have very constant flows, but continuous bank erosion or siltation or both occurs. As a result, spatial distribution of natural substrates and their suitability for colonization change over time, making quantitative evaluation of larval densities difficult. Many types of artificial substrates have been employed for sampling black flies (Gersabeck & Merritt 1979, Flannagan & Rosenberg 1982, Colbo 1987); a good discussion of advantages and limitations is provided by Rosenberg & Resh (1982). Previous workers have been concerned with exposure times needed to obtain maximum black fly densities (Gersabeck & Merritt 1979) or densities most closely approximating those found on natural substrates (Boobar & Granett 1978). I conducted

experiments to test specific hypotheses about colonization in an attempt to define an optimum colonization period for assessing relative density and age structure of the black fly fauna.

### Materials and Methods

All studies were conducted 18–26 August 1986 in the Calamus River, Loup County, Nebraska, immediately downstream from the Rt. 183 bridge. A relatively uniform stretch of stream 25–35 cm deep with a surface current of about 0.5 m/s was employed. Grasses near the shore were heavily (but unevenly) colonized by *Simulium bivittatum* Malloch and *S. luggeri* Nicholson and Mickel. Other potential substrates were lacking.

Artificial substrates were constructed from yellow plastic strapping tape (Interlake Poly-band 420-yellow, Oak Forest, Illinois). This rather rigid tape (0.7 mm thick, 1.2 cm wide) was quite smooth but had shallow, diamond-shaped depressions. Strips were cut to a length of 0.5 m, and one end was turned back on itself and stapled to form a small loop. This loop was inserted on an aluminum rod bent to hold the strip in a vertical position with the rod pushed into the stream bottom to anchor the upstream end of the strip at chosen depths. Stream current maintained the downstream end at about the same depth.

Depth of substrate anchorage (5 versus 15 cm beneath surface) was studied in two separate experiments. Each experiment consisted of four primary treatments (exposure time) in two consecutive Latin squares for a total of eight replications. Substrates in each row (perpendicular to stream flow) were spaced about 0.35 m apart in columns (distance from shore) with rows separated by 1 m. Strips were exposed for 1, 2, 4, or 8 d. Upon removal, each substrate was replaced with a new substrate to maintain constant substrate density, permitting direct comparison of colonization in the same position over consecutive time intervals of equal duration.

The two depths (5 versus 15 cm) were directly contrasted in another experiment, using random pairing and eight replications. All strips were sampled after 2 d and replaced with new strips; the experiment was repeated for a 6-d period.

Larvae and pupae were scraped from the strips in the field and preserved in ethanol. On each sample date, immatures also were collected from vegetation. Larvae and pupae were recorded by species and size. Results for each species are reported as small larvae (first to fourth instars), large larvae, and pupae.

All data were transformed by  $\log(x + 1)$  and results are reported as geometric means. Statistical testing of hypotheses was by *t* test for paired observations. Identical positions were available for testing equality of colonization over consecutive times of equal duration. Pairing was within rows for contrasting any exposure time with the sum of two shorter times of equal total duration.

Because strips exposed for 1, 2, or 4 d were sampled twice during the experiment but total removal of all strips did not occur until 8 d after initial placement, only totals for the entire experiment could be analyzed for row and column effects. The possibility of position effects of shorter duration was investigated by correlating the paired observations for consecutive times of equal duration.

The relative abundance of small larvae of each species in relation to combined total density of large larvae was analyzed by regression. Data for the 4-d and the 8-d exposures in both experiments were combined, omitting those substrates having < 100 total larvae ( $n = 42$ ). Relative deviation of observed from expected numbers of small larvae were computed from the means for each time period within each experiment.

## Results

### *Depth*

In the direct comparison of depth of substrate, more pupae of *S. luggeri* occurred at 15 versus 5 cm after 6 d of exposure (30 versus 18,  $P < 0.05$ ). All other differences for either the 2- or 6-d exposures were nonsignificant.

### *Position Effects*

Row and column effects were nonsignificant in both experiments involving colonization over time, based on totals for each position where the same position was sampled more than once.

### *Colonization over Time*

Results of experiments at 5- and 15-cm depths are presented in Figure 1. If colonization were uniform over time, and if previous colonization did not interfere with subsequent colonization, there should be a linear increase in density with exposure time, and density for any colonization time should be the same as the sum of the component parts of that time. Numbers of black flies and time are graphed on a logarithmic scale in Figure 1 to facilitate comparison with the expected linear response.

Through 4 d, colonization of all larval instars of both species increased linearly in both experiments as predicted by uniform colonization. But in both experiments, the number of small larvae of either species present after 8 d was less than the sum of two consecutive 4-d colonizations. At the 15-cm depth, the sum after 8 d was less than the number colonizing new strips during the second 4-d period.

At the 5-cm depth, large larvae continued to increase through 8 d such that the sum of two 4-d exposures did not differ from 8 d. However, at the 15-cm depth, not only was the number present after 8 d less than the sum of two 4-d exposures, the number present also was less than the number colonizing during the second 4-d period.

Pupae increased with increasing exposure time at both depths such that the sum of any two periods was usually significantly less than the number present after a single exposure of equal duration.

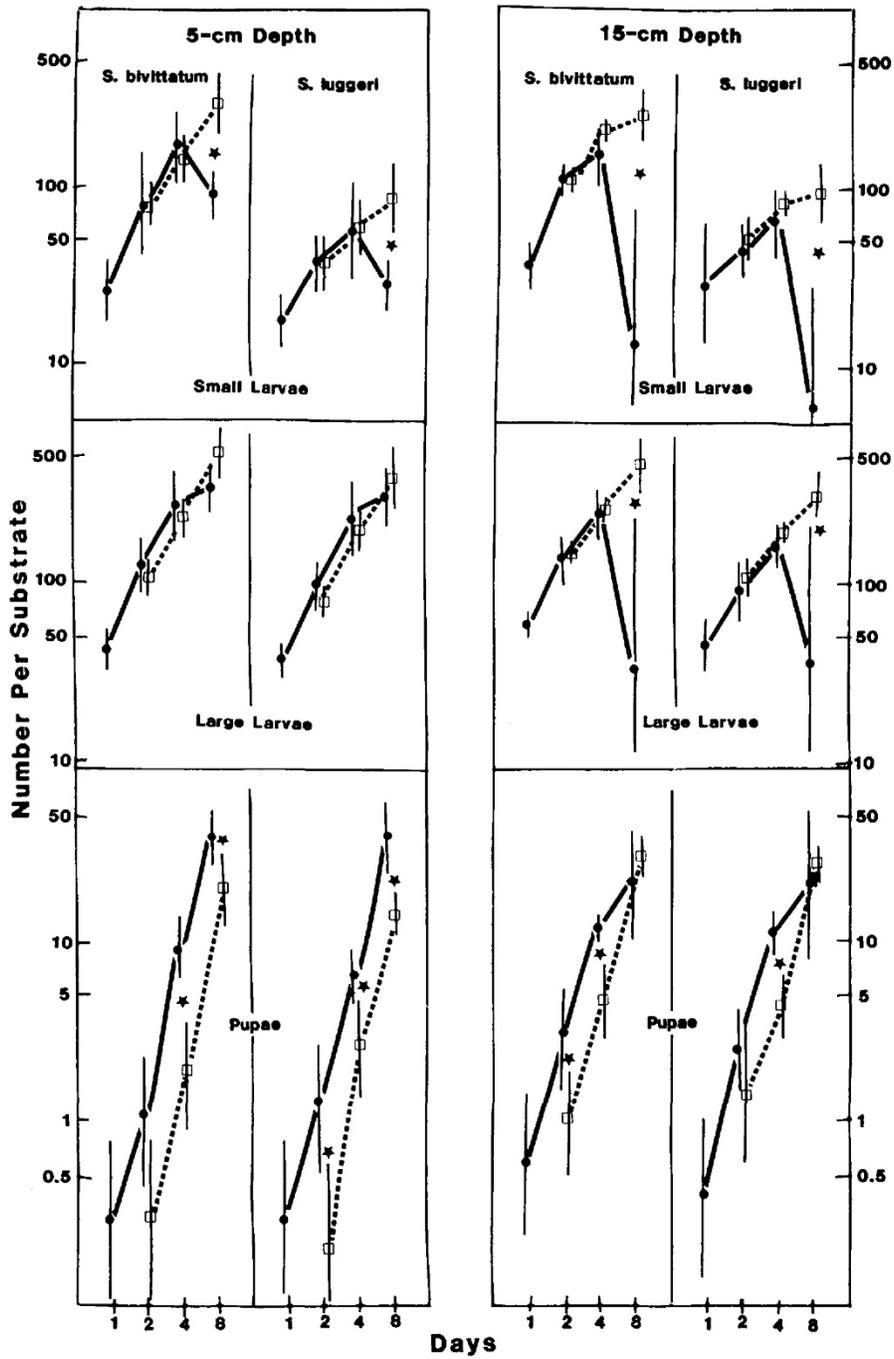


Figure 1. Mean numbers of *S. bivittatum* and *S. luggeri* colonizing artificial substrates at two depths for different exposure times. Solid lines are numbers after continuous exposure, dashed lines the sum of two equal exposures of same total duration. Vertical bars are 95% confidence intervals, stars indicate significant ( $P < 0.05$ ) differences between means by paired  $t$  test.

## Discussion

### *Position Effects*

The Latin square design is appropriate for conditions under which some uniform gradient might occur which persists for the duration of the experiment. Although statistically significant row or column effects were not found, there was visual evidence of a row effect which developed at the 15-cm depth between days 4 and 8, during which three rows had bases of the substrates embedded in sand and reduced densities of black flies. Their omission would not have changed the conclusions, but their inclusion increased the variance of estimates and illustrated the increased variance which might be expected on either natural or artificial substrates exposed for extended times in such streams.

Only the correlation for the two 1-d exposures was significant ( $r = 0.507$ ,  $n = 15$  for combined experiments), suggesting possible position effects which remained relatively constant for only short time spans. For longer exposures, any slight gain in precision by pairing was negated by the loss in degrees of freedom for statistical testing (7 for paired versus 14 had independent observations been used).

I anticipated that the high density of substrates employed might result in reduced colonization downstream. Such was not the case, indicating drift must have been in excess of the capacity of substrates and available attendant vegetation to intercept potential colonizers.

### *Colonization over Time*

Other workers (Boobar & Granett 1978, Gersabeck & Merritt 1979) generally have found peak larval densities to result 5–7 d after substrate placement and noted a reduction in small larvae on older substrates. Previous experimental designs, however, have not permitted a direct separation of effects because of changes in substrate suitability versus changes in population density or age structure that also may occur over time. This problem was addressed by using new substrates for consecutive time periods of equal duration.

Large larvae followed a uniform colonization pattern throughout the 8-d period except for the second 4-d period at the 15-cm depth, where siltation rather than a reduction in available colonizers seemed to be the logical explanation. However, small larvae of both species colonized new strips at a lower rate for the second 4-d period, indicative of an aging population. A similar change in age structure noted on natural substrates cannot be considered supporting evidence, inasmuch as no new substrates were added to this section of stream during the experiment.

Pupae rarely drift, and the increase with increasing exposure times must have been in response to maturing larvae from previous colonization.

### *Substrate Suitability over Time*

There is adequate evidence in the literature (although based on observational rather than experimental data) that periphyton is detrimental to black fly colonization and accumulates over time on all types of artificial substrates. Gersabeck & Merritt (1979) noted that small larvae, scarce on older substrates, again colonized new substrates.

I found that not only were there fewer small larvae available for colonization during the second 4-d period, but that the number present on strips after 8 d was less than the number added to new strips during the second 4-d period. However, the design I used did not permit separation of substrate suitability from possible competition by large larvae for available space. In other trials (unpublished data), strips exposed for 14 d were often heavily coated with periphyton and lacked black flies of any stage. The possible differential response of different instars or of different species to periphyton remains to be investigated experimentally.

#### ***Competition between Small and Large Larvae***

Small larvae predominate in drift (Kureck 1969), and laboratory studies conducted by Gersabeck & Merritt (1979) indicated that small larvae can be dislodged by larger larvae. Hart (1987) provides an excellent review of competition.

I found that the relative abundance of small larvae declined as the number of large larvae increased. Significant ( $P < 0.05$ ) regressions resulted, accounting for 38% of the variation in the relative density of small larvae of *S. bivittatum* and 16% for *S. luggeri*. Although suggestive that *S. bivittatum* might be more adversely affected by the presence of large larvae, neither the slope nor intersects of the two regressions differed significantly. Analyses including data for 1 or 2 d of colonization indicated only random variation in number of small larvae at lower total densities of large larvae.

#### ***Substrate Suitability***

The variety of substrates employed for evaluating black fly densities probably exceeds the number of workers using such methods. Unglazed tiles, proposed by Lewis & Bennett (1974) as a possible standard, are perhaps the most widely used. However, tiles are unsuitable for even short exposures in the shifting sand bottom streams of Nebraska and were found unsuitable by Boobar & Granett (1978) for some species that normally colonize only vegetation. The tapes I used were readily colonized by all black fly species occurring in this stream, were inexpensive (< 4 cents each), and were simple to construct and handle. However, tapes may be unsuitable for species that colonize rocks (Gersabeck & Merritt 1979).

#### ***Optimum Colonization Time***

Time to achieve maximum densities, employed by some workers, seems to be a poor criterion. More valid is that time required to achieve densities per unit area of substrate the same as densities occurring on natural substrates (e.g., Lewis & Bennett 1974). However, where vegetation is the primary natural substrate not only are densities difficult to determine but different observed densities are just as likely because of local or temporal changes in the substrate.

This study did not permit an estimate of actual density per unit area of natural substrate. Four-day substrate exposure most closely approximated the age distribution of larvae observed on natural substrates, whereas 1-d exposures had a proportionately greater number of small larvae. However, since no new natural substrates were added to this section of stream during the study, 1-d exposures may have provided a reasonable estimate of actual

age structure and relative density while minimizing factors that contribute to variability in estimates. My conclusion is that the minimum exposure time to provide an adequate sample is best.

### Observations

The reduced colonization by early-instar black flies on substrates 4–8 d old, accompanied by increased colonization by chironomids, supports many published observations that black fly colonization is negatively correlated with periphyton accumulation. This observation, although suggesting that early instars are most adversely affected, is confounded by the observation that early instars also are negatively correlated with abundance of later instars.

Mature larvae were sometimes so firmly attached to substrates that they were severed during removal. Yet an occasional newly formed pupa was found after only 1 d. This suggests that drift of late last-instar larvae is reduced, but that this nondrifting period may be such a small part of total larval development that no serious bias will result in estimating population age structure by use of artificial substrates.

An occasional pupal exuvium was found after 4 d colonization and many after 6 d, suggesting a duration of about 4 d for the pupal stage. This was supported by rearing in which almost all adults emerged within 4 d of pupal collection.

Many black flies have been reported to move, primarily to deeper or more sheltered habitats, for pupation. In the direct comparison of depth, I found more pupae of *S. luggeri* at the 15-cm depth. After 8 d of exposure, a greater proportion of the total population of both species were pupae in the 15- than 5-cm-depth experiment. Analysis by  $\chi^2$  ( $P < 0.05$ ) is invalid because these were separate experiments, and the 15-cm depth was adversely affected by siltation. Rather than larvae changing location for pupation, it is equally possible that less mature larvae simply abandoned an unfavorable position.

### References Cited

- Boobar, L. R. & J. Granett. 1978. Evaluation of polyethylene samplers for black fly larvae (Diptera: Simuliidae), with particular reference to Maine species. *Can. J. Zool.* 56: 2245–2248.
- Colbo, M. H. 1987. Problems in estimating black fly populations in their aquatic stages, pp. 77–89. *In* K. C. Kim & R. W. Merritt [eds.], *Black flies: ecology, population management, and annotated world list*. Pennsylvania State University Press, University Park.
- Flannagan, J. F. & D. M. Rosenberg. 1982. Types of artificial substrates used for sampling freshwater benthic macroinvertebrates, pp. 237–266. *In* J. Cairns, Jr. [ed.], *Artificial substrates*. Ann Arbor Science, Ann Arbor, Mich.
- Gersabeck, E. F. & R. W. Merritt. 1979. The effect of physical factors on the colonization and relocation behavior of immature black flies (Diptera: Simuliidae). *Environ. Entomol.* 8: 34–39.
- Hart, D. D. 1987. Processes and patterns of competition in larval black flies, pp. 109–128. *In* K. C. Kim & R. W. Merritt [eds.], *Black flies: ecology, population management, and annotated world list*. Pennsylvania State University Press, University Park.
- Kureck, A. 1969. Tagesrhythmen lappländischer Simuliiden (Diptera). *Oecologia* 2: 385–410.

- Lewis, D. J. & 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.
- Rosenberg, D. M. & V. H. Resh. 1982. The use of artificial substrates in the study of freshwater benthic macroinvertebrates, pp. 175-235. *In* J. Cairns, Jr. [ed.], *Artificial substrates*. Ann Arbor Science, Ann Arbor, Mich.