The influence of density and behavioural type on the active entry of two mayfly species (Ephemeroptera) into the water column

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Laboratory experiments were conducted in still water and under lighted conditions to induce maximal departure rates of two mayfly species from a substrate. A larger proportion of *Baetis vagans* McDunnough nymphs left the substrate than of *Paraleptophlebia mollis* (Eaton) at densities of up to 750 nymphs per 330 cm². Differences in the tendency of nymphs of each species to leave the substrate were related to behavioural traits rather than density. Overt aggression between interacting *P. mollis* nymphs served as a spacing mechanism on substrate surfaces. Interactions among *B. vagans* nymphs were never observed. Rather than developing a spacing mechanism on the substrate, these nymphs are adapted to swim into the water column. A comparison of drift–density relationships for mayflies used in this and other studies revealed a gradation among behavioural groups within the Ephemeroptera in their tendency to leave the substrate and enter the water column.

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Des situations expérimentales ont été créées en eau stagnante, à la lumière, de façon à déclencher des taux maximaux d'abandon du substrat chez deux espèces d'Ephémèroptères. A des densités allant jusqu'à 750 larves/330 cm², une plus grande proportion de larves de *Baetis vagans* McDunnough quittent le substrat comparativement aux larves de *Paraleptophlebia mollis* (Eaton). Les tendances différentes à quitter le substrat sont reliées à des aspects du comportement plutôt qu'à la densité. L'aggressivité manifeste entre les larves de *P. mollis* sert de mécanisme d'espacement à la surface du substrat. Chez les larves de *B. vagans*, de telles interactions ne se produisent pas; plutôt que de recourir à un mécanisme d'espacement sur le substrat, ces larves se sont adaptées à nager en eau libre. La comparaison des relations dérive–densité chez ces espèces et chez les éphémères utilisées lors d'autres travaux a révélé l'existence d'une gradation entre les groupes classés selon leur comportement et leur tendance à quitter le substrat pour nager en eau libre.

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Introduction

Waters (1961, 1962, 1966) suggested that drift rates of benthic insects could be used to predict stream productivity in those circumstances where the carrying capacity of the stream was exceeded. Many workers (Dimond 1967; Elliott 1967; Pearson and Franklin 1968; Hughes 1970; Pearson and Kramer 1972; Reisen and Prins 1972; Hildebrand 1974; McKone 1975) have investigated whether the relationship between drift and benthic density was linear (density independent) or exponential (density dependent) for a variety of organisms in numerous streams.

Lehmkuhl and Anderson (1972) questioned whether certain organisms drifted more because they occurred at higher densities in the stream bed or because they had a greater tendency to drift. Earlier, Bailey (1966) had stated that "creeping and burrowing ephemeropteran nymphs are much less frequent in the drift than free-swimming *Baetis rhodani* (Pict.) nymphs." Minshall and Winger (1968) observed that the mayfly nymphs *Baetis* sp. and *Ephemerella* sp. actively left the stream bed and entered the water column after a sudden reduction in the stream discharge. Elliott (1968) found that mayfly nymphs in still water with oxygen saturations greater than 82.5% and in the presence of light frequently occupied the upper substrate surface and that many were seen swimming in the water column. This contrasted to flowing-water situations where the nymphs showed strong positive thigmotaxis and negative phototaxis.

This present study utilizes the phenomenon of increased activity among mayfly nymphs in still-water situations to study the upper limits of nymphal departure from a substrate into the water column for the two species *Baetis vagans* McDunnough and *Paraleptophlebia mollis* (Eaton). Knowing these upper limits, one could determine the potential contribution of each species to the drift fauna in field situations.

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Materials and Methods

Mayfly nymphs were selected for this study because they are common components of the drift. Nymphs may be easily grouped behaviourally as swimmers, clingers, crawlers, or burrowers, based on mode of locomotion and morphological features (see Berner 1959). Swimming nymphs such as *Baetis* spp. have a streamlined body with a round head. Crawling nymphs, represented by *Paraleptophlebia* spp., have a slightly depressed body with a somewhat dorsoventrally flattened head. *Baetis vagans* and *P. mollis* were both present in abundance at the sample site, near the Forks of the Credit River, Peel County, Ontario.

Since the behaviour of a nymph may change with size, its particular developmental stage is an important variable in experimental work. Consequently, I have adopted Clifford's (1970) four standardized stages of mayfly groupings based on wing-pad development. Nymphs selected for experiments possessed mesothoracic wing-pad lengths which were greater than the distance between them, excluding those with black wing pads (Clifford's stage III).

Nymphs used in laboratory experiments were collected by hand from the Credit Forks on the day of an experiment. Up to 170 nymphs were added to 450 ml of river water in wide-mouth thermos bottles to minimize changes in water temperature during transportation to the laboratory. Within an hour of collection, the nymphs were transferred to enamelled pans containing aerated river water and stones.

Experiments were conducted in a Plexiglas tank of dechlorinated water located in a walk-in, temperature-controlled environmental room. Water temperatures corresponded to those at the sample site. Four, 40-W fluorescent tubes provided a light intensity of 2150 lx measured beneath 10 cm of water. To insure saturated oxygen levels, the water was well aerated between experiments. All experiments were conducted in still water since previous work had indicated that maximal departure rates of *B. vagans* and *P. mollis* nymphs occurred under these conditions (Corkum 1976).

Temporal Pattern

The initial experiments were designed to examine the temporal pattern of nymphs leaving the substrate and entering the water column in still-water conditions. Three trials, each of which entailed 24 h of continuous observation of a sample population of the summer generation of *B. vagans* nymphs, were conducted on August 1–2, 3–4, and 9–10, 1973, starting at 1600, 0920 and 1750 hours Eastern Standard Time, respectively. Water temperature was 15°C. An electrical timer was used to provide photoperiod control which corresponded to that in the field. Nighttime observations were conducted using a 40-W red bulb for illumination. One hundred *B. vagans* nymphs were pipetted onto a brick (surface area: 330 cm², excluding the bottom) submersed in 10 cm of dechlorinated water. The brick was positioned flat against the stream bottom, to prevent nymphs from occupying its lower surfaces. Prior to each experiment, the brick was autoclaved to eliminate food as a variable.

Those nymphs swimming into the water column or crawling away from the vicinity of the brick were immediately removed by pipette to prevent their return to the brick surface. Total numbers leaving the brick were tallied at 10-s intervals.

Increased Initial Densities

Populations with initial densities of 50, 100, 150, 200, 300, 400, 500, and 750 nymphs per 330 cm² were observed for 300-min periods in still water under lighted conditions. The maximum density of 750 nymphs per 300 cm² (about 22 500 nymphs per square metre) greatly exceeds or is comparable with maximum densities recorded for mayfly species on the surface of the

stream bed in field studies (Waters 1966; Elliott 1967; Pearson and Franklin 1968; Lehmkuhl and Anderson 1972; Pearson and Kramer 1972).

In the 300-min trials, nymphs were added to the brick surface by pouring them through an inverted funnel (surface area: 73.9 cm²) positioned on top of the brick. After a settling period of 50 min, the funnel was carefully removed and 300 min of continuous observations in the light begun. Verbal tallies were made on tape recordings during the first 60 min of an experiment when the frequency of departure was high and exact times when nymphs left the brick were later determined from a playback of the tape. Thereafter, nymphal departure was scored directly. Nymphs leaving the brick were immediately removed by pipette and placed in a dish of aerated water. One density series was conducted with the winter generation of *B. vagans* (water temperature = 10°C) and two were conducted with the summer generation in 1974 (water temperature = 12.5 ± 2°C). For *P. mollis* nymphs, two trials of the density series were conducted in the spring of 1974 and a third in the spring of 1975 (water temperature = 15 ± 2°C). *Paraleptophlebia mollis*, unlike *B. vagans*, has only one generation per year.

Activity Levels

Finally, it was decided to determine whether nymphs which left the brick early have different or similar activity levels than those remaining on the substrate at the end of the 300 min. In the previous experimental set, nymphs were segregated into those which left during the first 60 min and those which had remained on the substrate at the end of the 300-min period. On the next day, an examination of the nymphal numbers leaving the brick was repeated for each group, over a 150-min period. The 60-min and 300-min groups were randomly examined. Sample sizes were unequal.

Additional experiments were conducted in which equal initital densities from the two time groups were also examined. To provide a subsample of 30 nymphs from both the 60- and 300-min groups, an initial density of 300 nymphs was selected. Four trials were conducted for each species in the spring of 1975 in which each subsample was examined for 150 min.

Results

The mean number of *B. vagans* nymphs remaining on the brick in still water decreased rapidly during the first 50 min of each 24-h period (Fig. 1). Many nymphs either swam into the water column or crawled away from the brick. After this initial burst of activity, the constant slope of the line illustrates that regardless of density, the proportion of nymphs which left the brick was constant.

Because of this constant slope, the duration of experiments with varying initial densities was reduced to 300 min. A linear regression was performed for each species to examine the relationship between the numbers of nymphs which had left the substrate after 300 min and their initial density (Fig. 2). All values were independent. The resultant equations explained 98.2% and 97.2% of the variation in the winter and summer generations of *B. vagans*, respectively, and 54.7% of the variation in *P. mollis*. Thus, the nymphal number leaving the substrate was independent of density over the particular range which was examined.
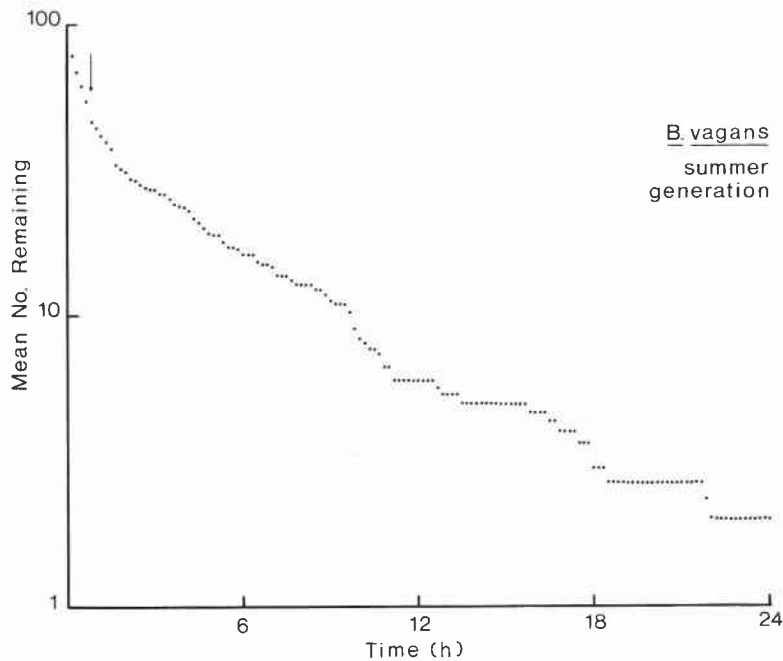
FIG. 1. The relationship of the number of nymphs remaining on the substrate over time, based on the mean of three trials from samples of the summer generation of *B. vagans* nymphs. The vertical arrow indicates the first 50 min, during which time there was an initial burst of nymphs into the water column.

An analysis of covariance (Snedecor and Cochran 1967) indicated that the slopes representing the two generations of *B. vagans* did not differ; however, there was a significant difference ($p < 0.05$) in elevation between the two regression lines. This difference might be due to the larger size of stage III nymphs in the winter (head capsule width (HCW): 0.760–1.173 mm) than in the summer generation (HCW: 0.632–1.014 mm). Water temperatures at the two different times were similar.

The analysis of covariance also indicated that there was a significant difference in slope ($p < 0.001$) between *P. mollis* and each generation of *B. vagans*. With each increase in density, a significantly larger proportion of each generation of the swimming species left the substrate and entered the water column than that of the crawling species, *P. mollis* (HCW of stage III nymphs: 0.767–1.388 mm). This occurred despite the larger surface area occupied by *P. mollis* nymphs.

The experiment designed to examine the differences in activity levels within a sample population gave consistent results whether or not equal subsamples were used. There was no significant difference in activity levels between the two time groups of *B. vagans* nymphs. There was, however, significantly less time required ($p < 0.05$) for one-half of the sample population of *P. mollis* nymphs to leave the substrate and enter the water column

from the 60-min sample than from the 300-min sample (Mann-Whitney U ranking test) (Elliott 1971). Although all nymphs were categorized in stage III, it might have been possible for more than one population to be present within this size range. To determine if two populations were present, I measured the head capsule width of each nymph used in the experimental series. There was no significant difference ($p > 0.05$) in nymphal size between the two time groups for either males or females. This would seem to imply that the difference in time for nymphs leaving the substrate is related to differences in activity level within one population.

Nymphal movement on the brick surface was observed for both species during the 1st h of every experiment. After this initial active period, *B. vagans* nymphs remained stationary until they swam away from the substrate. In contrast, *P. mollis* nymphs actively crawled about the brick surface throughout an entire experiment. Those *P. mollis* nymphs which left the brick were not necessarily those which had previously been most active.

Abdomen bending frequently occurred between closely associated *P. mollis* nymphs. In these situations, the abdomen of each nymph bent side to side in a horizontal plane striking the adjacent individual. When nymphs faced each other, both forelegs and antennae were repeatedly raised and lowered in direct contact with the other nymph.
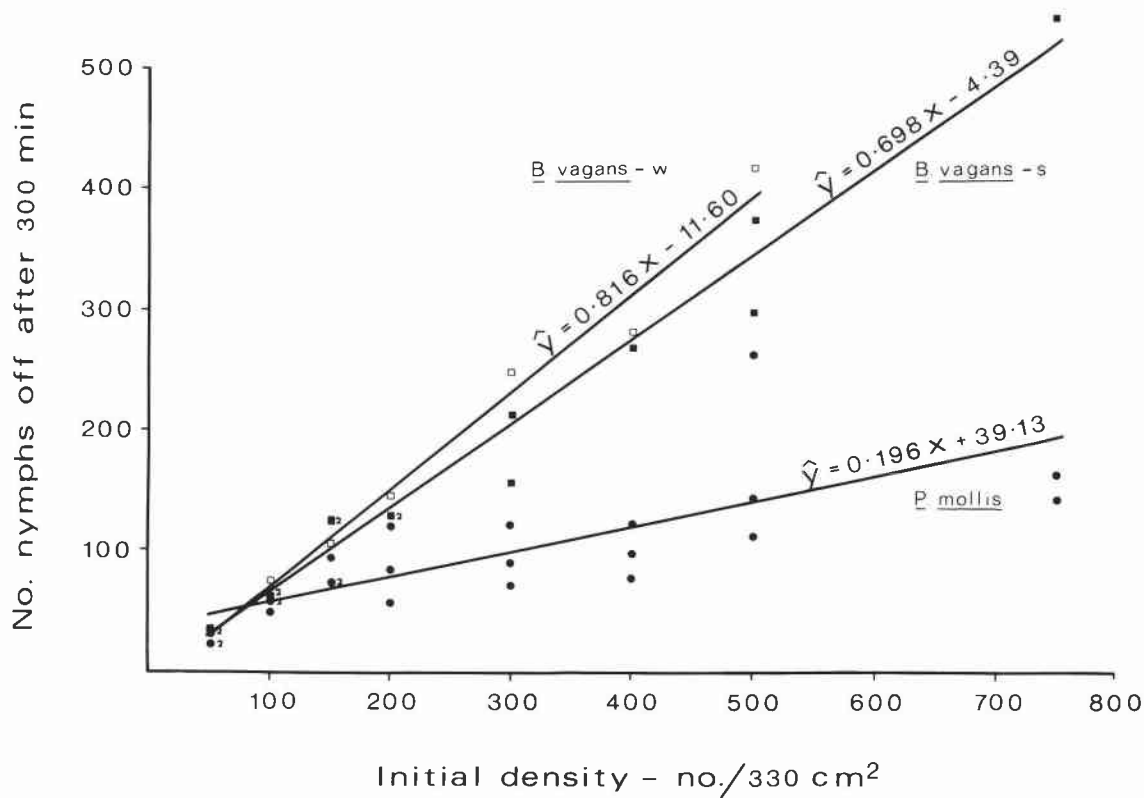
FIG. 2. The total number of *B. vagans* (winter (*w*) and summer (*s*) generations) and *P. mollis* nymphs which left the substrate after 300 min, in relation to their initial density.

Following any interaction, nymphs either withdrew a few millimetres or one would 'chase' another over the brick. Few nymphs ever left the brick as a result of an interaction. In contrast with *P. mollis*, interactions were never observed between *B. vagans* nymphs.

Discussion

The findings of these experiments suggest that an increase in nymphal density results in no change in the proportion of nymphs leaving the substrate under still-water conditions over the density range examined in this study. Although both species actively enter the water column, *B. vagans* nymphs (swimmers) did so continuously, whereas *P. mollis* nymphs (crawlers) tended to remain on the substrate after the initial disturbance period. Thus, it seems that swimmers must contribute more to the drift fauna than crawlers.

There was no difference between subsamples in the time required for one-half of the sample population of *B. vagans* nymphs to leave the substrate and enter the water column. In contrast, less time was required for *P. mollis* nymphs to enter the water column from the first 60-min group than for those

nymphs which had previously remained on the brick. This indicates either that there were two populations present or that there was an actual difference in activity level within the sample population of *P. mollis* nymphs. If the latter is true, as previously suggested, some nymphs within a species are more active or have a greater tendency to leave the substrate than others.

Several workers have observed spacing among aquatic immature insects in species where nets, cases, or tubes physically delimit a space on a substrate (Edington 1965; McLachlan 1969). This present study demonstrates that it is the aggressive behaviour among *P. mollis* nymphs which maintains intragroup distance. Thus, drift would become dependent on density at some point *before* the entire substrate surface was covered. For *P. mollis* nymphs, substrate space must be available for a nymph and for corridors between its neighbours. Corridor space is evidently not required for those immature species which are anchored by a case or tube since there is already a living space between the organism and its enclosure.

Interactions among *B. vagans* nymphs were not

TABLE 1. A comparison of slopes from regression lines calculated from nymphal numbers drifting over a time period versus density (numbers per 0.03 m²) for representative mayfly species exposed to low food sources. Values are from Hildebrand (1974)[a] and this study

Species	n	Slope	Nymphal behaviour
Baetis vagans			
Winter generation	7	0.816	Swimmer
Summer generation	14	0.698	Swimmer
Ephemerella needhami[a]	8	0.548	Clinger
E. serrata gp.[a]	12	0.370	Clinger
Paraleptophlebia mollis	23	0.196	Crawler
Tricorythodes spp.[a]	12	0.133	Crawler

[a]Time of Hildebrand's (1974) study involved drift throughout the night. In this study, nymphs were recorded as they left the substrate over a 5-h light period.

evident. This observation is consistent with Elliott's (1968) activity study with *Baetis rhodani* (Pictet). Rather than developing a spacing mechanism on a substrate, these nymphs are adapted to actively swim away from the substrate and drift downstream to another site.

Hildebrand (1974) examined the drift–density relationship among mayfly species using an artificial stream. Table 1 incorporates Hildebrand's data and my own and indicates the slopes of numbers of mayfly nymphs drifting versus benthic density at low food levels (where numbers drifting refer to numbers leaving the substrate and entering the water column). The swimmer (*B. vagans*) drifts most, followed by the two clingers (*Ephemerella needhami* and *Ephemerella serrata* gp.), and then by the crawlers (*P. mollis* and *Tricorythodes* spp.). Using this gradation pattern, one could, by knowing either a nymph's mode of locomotion or its morphological characteristics, predict how prevalent any mayfly species would be in the drift fauna.

Lehmkuhl and Anderson (1972) calculated mean drift:benthos ratios over 10 months for the mayfly species *Baetis tricaudatus* (5.31), *Baetis parvus* (0.32), *Paraleptophlebia temporalis* (0.47), and *Cinygmula reticulata* (0.24) in an Oregon stream. These authors suggest that the drift:benthos ratio for *B. parvus* may be low since their sampling schedule did not include the summer months when this species is expected to have higher drift rates.

From the behavioural groups represented by these species, I would have predicted that the swimmers, *B. tricaudatus* and *B. parvus*, would have had the highest tendency to drift. Since *C. reticulata* has a much more flattened form than *P. temporalis*, I would expect the former to be less likely to drift than the latter even though they are both crawlers. With the exception of *B. parvus*,

which has previously been explained by low drift estimates, the behavioural ranking of these mayfly species agrees with the drift:benthos ratios of Lehmkuhl and Anderson (1972).

This study is consistent with field studies in the literature indicating that the contribution of *Baetis* spp. to the total drift is greater than that of *Paraleptophlebia* spp., except during hatching periods (Anderson and Lehmkuhl 1968; Bishop and Hynes 1969; Clifford 1972). Furthermore, the difference in drift patterns between *B. vagans* and *P. mollis* is consistent with their differences in behavioural characteristics.

Some workers may doubt the value of the behavioural grouping among immature insects, since many species have been shown to have unique drift patterns. In fact, this study even illustrates differences in drift levels between generations of one species. I feel, however, that the advantage in illustrating general trends in drift patterns, based on the behaviour of the insect, presents the possibility of comparing faunal groups in a wide variety of running waters.

An extension of this present work should include a more realistic representation of field situations in laboratory streams. Conditions of still water, constant light, and the absence of food were used in this study to create the maximum behavioural drift patterns for the two species examined. Additional studies (Corkum 1976) show that departure patterns for *B. vagans* and *P. mollis* are maintained under changing current velocities and substrate types. Hildebrand (1974) demonstrated that the addition of a food source to the substrate decreased the drift levels of representative taxa. The importance of vegetation not only as a food source, but also as a cover should also be investigated.

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