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Substrate Selection as a Factor in *Hexagenia* Distribution

by

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ABSTRACT

Our observations demonstrate that in laboratory aquaria *Hexagenia bilineata* (Say) nymphs will leave clay, gravel, and sandy clay and migrate to adhesive mud or fine sandy mud with no response to coarse sandy mud indicated. The nymphs were able to burrow into all substrates offered, but the time required for penetration varied significantly. Burrowing times ranged from 30 s in adhesive mud to 168 s in clay. Ease of penetration (as measured by burrowing time) correlated well with observed substrate preferences. None of the physical or chemical parameters of the substrates which we measured correlated significantly with either selection or burrowing time. Ease of penetration seemed to be dependent upon a combination of factors including particle size and compactness and cohesiveness of the substrates. The characteristics of the two selected substrates include high moisture content (40% for each), low bulk density (0.91 and 0.94 g/cm^3), high organic content (7.1 and 7.9%), and predominantly silt composition (29% clay, 60% silt, and 11% sand for adhesive mud and 26% clay, 51% silt, and 23% sand for fine sandy mud). Our data suggest that substrate selection may play an important role in observed distribution patterns.

Keywords: *Hexagenia bilineata*, substrate selection, substrate characteristics, laboratory tests, egg distribution, migration.

INTRODUCTION

Substrate selection is widely recognized as an important parameter in determining the distribution of marine invertebrates, but it has received relatively little experimental attention by freshwater biologists (Cummins and Lauff, 1969). This probably results from the differences in life cycles characteristic of organisms in the two environments. Marine invertebrates almost

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universally have a pelagic larval stage. The selective advantage for sessile or burrowing species of larval selection of the appropriate substrate for settlement is obvious and thus has formed the focus of many experimental studies (see reviews by Meadows and Campbell, 1972: and Crisp, 1974). Reduction or elimination of the planktonic stage is a common adaptation of freshwater invertebrates. Thus, the *raison d'être* for substrate selection studies is not as obvious. However, in several freshwater insects such as burrowing mayflies and chironomids, the areas of egg deposition do not correspond with the areas in which the burrowing larval stages are found in highest densities. Chironomids have been shown to have pelagic first instar larvae which will swim through the water in search of a suitable substrate (McLachlan, 1969). Thus, substrate selection may be as important to these organisms in determining their distribution as it is to marine invertebrates.

A considerable amount of field data circumstantially suggests that migration and substrate selection also occur in burrowing mayflies of the genus *Hexagenia.* These mayflies are normally found in highest densities in a substrate referred to by several investigators as "mud" or "soft mud" (Hunt, 1953; Lyman, 1956; Carlander et al., 1967; Walker and Burbanck, 1975). This type of substrate is characteristic of many lakes, reservoirs, and slow-moving rivers, but it is usually found away from the shoreline, below depths affected by wave action or water-level fluctuations. However, it has been observed that deposition of mayfly eggs frequently occurs in the nearshore zone which frequently has gravel, sand, or clay substrates (Hunt, 1953). This has been verified by the high densities of small nymphs found in nearshore zones during the autumn following egg laying (Hunt, 1953; Cowell and Hudson, 1967; Swanson, 1967; Craven and Brown, 1969; Horst, 1974). Most of these investigators also noted a decrease in densities of nymphs in nearshore areas and a corresponding increase in density in offshore areas between autumn and the following spring (Hunt, 1953; Swanson, 1967; Horst, 1974). Although these investigators explained their field observations by suggesting or inferring that migration and substrate selection had occurred, other explanations are conceivable. Laboratory substrate selection experiments are necessary to demonstrate the potential significance of migration and substrate selection in the distribution of *Hexagenia* nymphs.

Previous laboratory studies of substrate selection by burrowing mayflies are few and limited in scope. Lyman (1943) demonstrated that *Hexagenia* nymphs could burrow into mud or sandy mud, but not into pure sand. No selection experiments were performed, however. Walker and Burbanck (1975) make brief reference to a laboratory study which indicated that 96% of the *Hexagenia* tested selected a mud or sandy mud substrate in preference to sandy substrate. Tsui and Peters (1974) observed that first instar nymphs of *Tortopus incertus* (Traver) (a normal inhabitant of clay banks of streams and rivers) also demonstrated a preference for mud over sand. All of these experiments were limited to a small range of substrate types.

Our studies significantly contribute to the understanding of substrate selection behavior of burrowing mayflies in several ways: (1) we included a broad range of substrate types in our substrate selection experiments; (2) we sought evidence for rejection of poor substrates and migration as well as for selection of preferred substrates; (3) we quantified several substrate parameters and analysed the correlation of these parameters to substrate selection; and (4) we included field data to show how our substrate selection results relate to actual field distributions.

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METHODS

Substrate samples were collected from a cove on Watts Bar Lake near the Kingston Steam Plant, where field studies are concurrently being conducted (Auerbach et al., 1978). The samples were chosen to represent the range of substrate types found in the cove, including those nearshore which are exposed during winter by water-level drawdown. The substrates were qualitatively described as clay, adhesive mud, fine sandy mud, sandy clay, coarse sandy mud, and gravel. Multiple samples of each type were combined and autoclaved at 120°C (250°F) for about 5 min to remove all existing mayflies and other macrobenthos.

Tests to determine the occurrence of movement and selection were conducted in three aquaria (30 x 60 cm), each containing 12 plastic containers, approximately 10 cm square x 10 cm deep, filled to the top with substrate. Two containers of each substrate type were randomly positioned in four rows of three containers in each of the aquaria. This left a space equivalent to slightly more than three containers open at one end of the aquarium. A similar container with the bottom cut out was placed on top of each substrate container to restrict the mayflies during introduction. Ten mayflies (10- to 14-mm body length) were introduced, one at a time. This bottomless container was removed after all mayflies had burrowed into the substrate or had settled into a crevice or depression. Substrate containers were removed after one week, and mayflies in each container were counted after the sediment was sieved with a 0.425-mm (No. 40 mesh) screen. The null hypothesis that equal numbers of nymphs would be found in each container (i.e., that inter-container movement either did not occur or was random) was examined with a goodness-of-fit test using the chi-square statistic.

Comparison of substrate characteristics was made on the basis of mayfly behavioral reponses as well as chemical and physical measures. During introduction of mayflies, a behavioral estimate of penetrability was obtained by measuring the time required for the first two to four mayflies to burrow into the substrate in each of the containers. A total of 14 measurements were made for each substrate type. Burrowing time was defined as the interval between the first digging movements and disappearance of the tip of the abdomen. Some of the nymphs did not burrow in the clay and sandy clay substrates, but merely settled into crevises or depressions in the substrate surface; these individuals were not included in calculating mean burrowing time. Differences in burrowing times between substrate types were analyzed using one-way analysis of variance and Duncan's Multiple Range Test. The relationship between mean burrowing time and substrate selection was determined using correlation analysis.

The chemical and physical parameters of the substrates measured included percent loss on ignition, particle size composition, bulk density, and water content. The percent loss on ignition which gives an approximate estimate of organic content was estimated by weighing two subsamples of each substrate before and after ashing at 450° C (850° F) for 24 h. Particle size composition was determined by dividing two subsamples into four size classes; gravel (> 2 mm), sand (0.0625 - 2 mm), silt (0.0039 - 0.0625 mm), and clay (< 0.0039 mm). After separation of the sand and gravel by wet seiving, the remaining silt and clay fractions were separated by centrifugation at 750 rpm. Runs of approximately 2.5 min were repeated until the clay fraction was completely removed (Jackson, 1956). All components were washed into preweighed evaporating dishes and dried at 100° C (212° F). Weight of each size fraction was reported as percent of total sample weight. These data were used to describe the soil texture based on the U.S.D.A. soil texture triangle (Buckman and Brady, 1969). Median particle size of each substrate type was determined by plotting cumulative percent by weight of the gravel, sand, silt and clay, size fractions against the phi (∅) scale (Cummins, 1962). Bulk density was obtained by determining the wet weight of a measured volume of sample (EPA, 1979). Percent water content was determined by: wet weight — dry weight x 100/wet weight (EPA, 1979). Single estimates of each of these chemical and physical parameters were obtained from each substrate type. Correlation analysis was used to evaluate the relationship of burrowing time and substrate selection to each other and to all other physical and chemical parameters of the substrates.

Field distributions of *Hexagenia* were determined by monthly transect sampling in three coves of Watts Bar Lake over a 12-month period. Samples were collected with a petit ponar grab (15 x 15 cm) and mayflies were separated from the substrates by sugar flotation (Anderson, 1959). The total number of samples collected over the year was 252 in the reference and intake coves and 300 in the discharge cove. Substrate classes were designated only qualitatively at the time of collection, but they correspond to the substrate classes used in the laboratory experiments except as noted in the results.

RESULTS

After one week, the distribution of mayflies among the six substrate types was found to be nonrandom. Comparisons of the goodness of fit between observed and expected numbers of mayflies per substrate resulted in a highly significant chi-square (Table 1). The expected number per container was determined by dividing the total sum of mayflies recovered from all containers within each aquaria by the number of containers (12). That figure was found to be: 8.5, 8.3, and 8.2 in aquaria 1, 2, and 3, respectively. Our calculation of expected number was based only on mayflies found in containers, even though in one aquarium several nymphs were found alive on the bottom outside the substrate containers. More than the expected number of mayflies (~ 8.3) were found in 11 of the 12 containers filled with fine sandy mud and adhesive mud. More than the original number (10) of nymphs introduced were found in 10 of these containers, indicating that intercontainer migration had occurred. Substrates containing high and low numbers were generally consistent among aquaria, indicating that selection of substrates took place. Mean numbers of mayflies in each substrate type used for correlation analysis are tabulated in Table 1.

Table 1. Observed numbers of mayflies recovered from containers compared with expected numbers per container estimated from total survivorship per aquarium after one week.* Ten mayflies were initially introduced into each container with two replicate containers of each substrate type per tank. Mean numbers are used in subsequent correlation analysis.

	Number per container						
	Expected	Observed					
Aquaria	All substrates	Fine sandy mud	Adhesive mud	Coarse-sandy mud	Gravel	Sandy clay	Clay
1	8.5	16	12	12	8	7	0
		11	13	5	7	7	4
2	8.3	14	10	9	7	3	5
		12	15	8	5	9	2
3	8.2	20	12	14	6	6	1
		17	4	6	6	4	2
Mean		15.0	11.0	9.0	6.5	6.0	2.3

*Total chi-square equaled 98.11, $P < 0.005$ foor 33 degrees of freedom.

Comparison of mean burrowing times among the six substrates by one-way analysis of variance demonstrated significant differences ($P < 0.001$). Mean time required to burrow into clay was a factor of five greater than that for adhesive mud. The results of Duncan's Multiple Range Test (Table 2) indicated that the substrates may be separated into several overlapping groups.

Table 2. Mean burrowing time ($n = 14$) for each of the six substrates

	Time (s)					
	Adhesive mud	Fine sandy mud	Coarse sandy mud	Gravel	Sandy clay	Clay
Mean (Standard deviation)	30 (11)	46 (39)	59 (52)	92 (67)	127 (71)	168 (83)
Duncan's Multiple Range Test*					———	———
				———	———	
		———	———	———		
	———	———	———			

*Means enclosed by the range of any one line do not differ significantly ($P = 0.05$).

Mean burrowing time for each substrate correlated well with mean number of mayflies per container for each substrate. Correlation analysis, over all substrates, of the number of mayflies per container with mean burrowing time for that container (33 containers) produced a plot with considerable scatter (r = —0.51), but a highly significant correlation coefficient (P = 0.003) (Fig. 1). The number of mayflies per container was not significantly correlated with burrowing time for that container for any of the six substrates (P = 0.70, 0.23, 0.08, 0.34, 0.65, 0.22). Thus, the correlation over all substrates could be attributed to differences among substrate types. The plot of mean number of mayflies versus mean burrowing time for each substrate smoothed out the container differences and indicated a significant correlation between burrowing time and mean number of mayflies (Fig. 2).

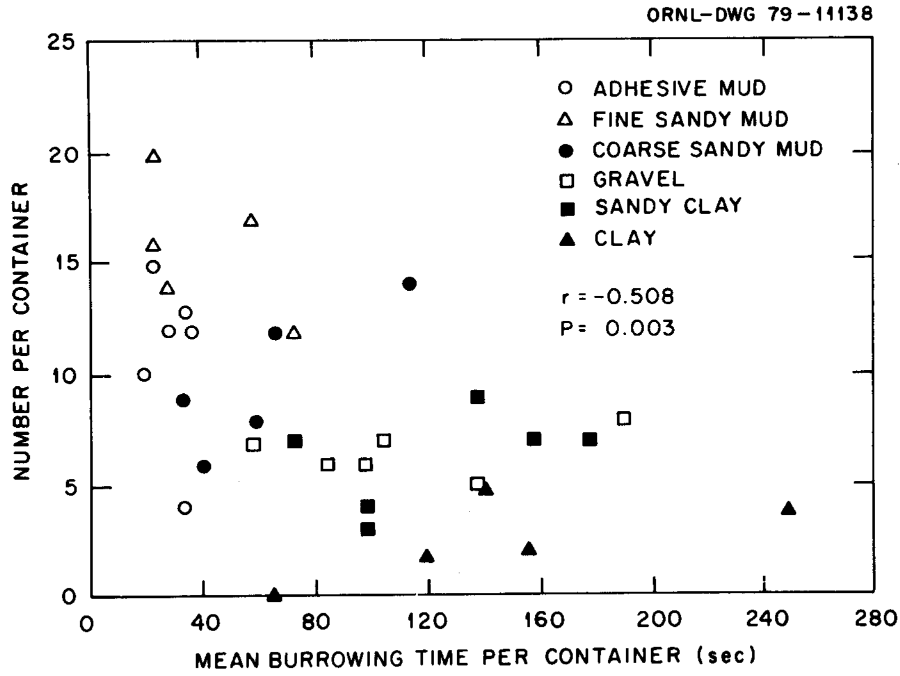

Figure 1. Mean burrowing time versus number of mayflies remaining in the same container one week after introduction.

The results of the analysis of particle size distribution, expressed as median phi (MDϕ), organic content bulk density, percent moisture and soil class designation are tabulated in Table 3 with the mean value of burrowing time and mean number of mayflies per container after selection had occurred. Correlation of the four physical and chemical parameters to number of mayflies and to burrowing time indicates no significant correlation of any physical or chemical

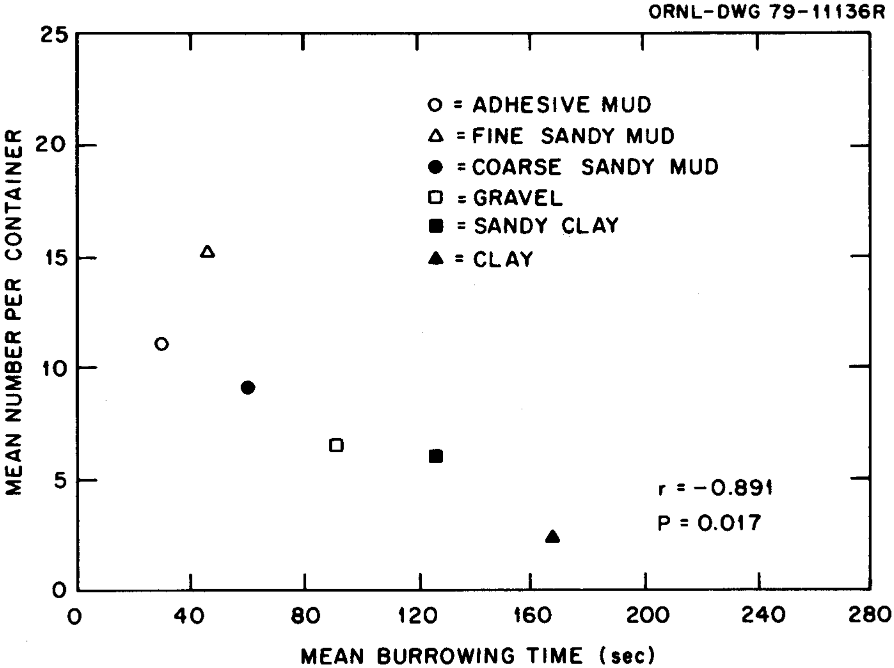

Figure 2. Mean burrowing time per substrate versus mean numbers of mayflies per container for the six substrate types.

Table 3. Summary of substrate physical and chemical characteristics and preferences as indicated by substrate selection

Substrate	Mean No. mayflies per container	Burrowing time (s)	Soil* class	Particle size (MD ϕ)	Organic† content (%)	Bulk density (g/cm^3)	Percent moisture (%)
Clay	2.3	168	Silty clay	7.8	4.2	1.19	32
Adhesive mud	11.0	30	Silt loam	6.6	7.9	0.94	40
Fine sandy mud	15.0	46	Silt loam	6.1	7.1	0.91	40
Sandy clay	6.0	127	Silt loam	6.1	5.8	1.49	22
Coarse sandy mud	9.0	59	Sandy loam	3.0	9.1	1.23	29
Gravel	6.5	92	Gravelly sand	—0.9	3.8	1.73	16

*Based on the United States Department of Agriculture Soil texture triangle (Buckman and Brady, 1969).
†Actually percent loss on ignition.

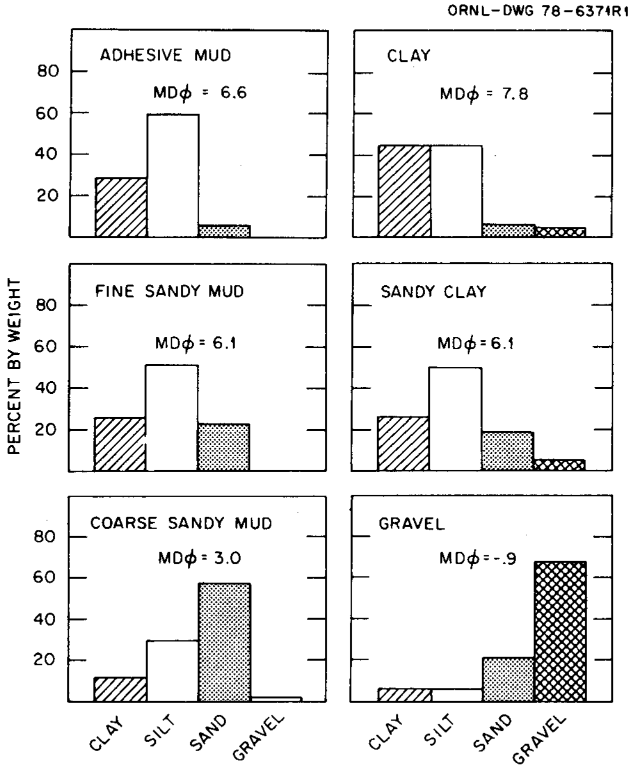

Figure 3. Composition of experimental substrates as defined by percent by weight of fractions of gravel (> 2 mm), sand (0.0625-2 mm), silt (0.0039-0.0625 mm), and clay (< 0.0039 mm), and by the median phi value (MD ϕ). MD ϕ is the negative log to the base two of the diameter of the median particle size.

parameter with either behavioral measure at the 95% confidence level (Table 4). The parameter showing the greatest tendency for correlation was organic content. The lack of significant correlation may be due to the small number of degrees of freedom. Because only one estimate of each parameter was made for each substrate type, there were only six correlate pairs in each case.

The distribution of *Hexagenia* among substrates in the field are summarized in terms of yearly mean number per ponar grab in each of six substrate types (Table 5). The substrates do not correspond exactly with those used in the laboratory. Gravelly mud was not investigated in the laboratory because it is so infrequently sampled in the field. The fine sandy mud which appears in the laboratory study is normally considered a subset of adhesive mud in the field. The coarse sandy mud of the laboratory experiment, is the same as the sandy mud in the field.

Table 4. Summary of correlation coefficients (r) and probability levels (P) resulting from correlation of number of mayflies per substrate and burrowing time with physical and chemical characteristics of the substrate

		Particle size	Organic content	Bulk density	Percent moisture
Number of mayflies	(r)	0.02236	0.64713	—0.59811	0.58760
	(P)	0.9665	0.1648	0.2098	0.2200
Burrowing time	(r)	0.19057	—0.73324	0.46980	—0.46422
	(P)	0.7176	0.0972	0.3471	0.3537

Table 5. Summary of the distribution of *Hexagenia* nymphs with regard to substrate type in coves of Watts Bar Lake. Numbers represent the mean number per ponar grab (15 x 15 cm) averaged over one year of monthly samples.

Cove	Adhesive mud	Sandy mud	Gravelly mud	Sandy clay	Gravel	Clay
Intake	10.7	13.2	8.3	.2	1.9	1.9
Reference	9.3	7.4	6.4	3.4	3.0	.5
Discharge	6.1	5.7	4.6	3.3	.6	.2

DISCUSSION

Our data demonstrate that *Hexagenia bilineata* in the 10- to 14-mm size range will reject some substrates and move to others. Movement was away from substrates typical of the nearshore area such as gravel, clay, and sandy clay, and toward substrates we defined as adhesive mud and fine sandy mud, which are generally found in deeper water. No selection or rejection of coarse sandy mud can be postulated based on our data. The laboratory selection data agree very well with the distribution patterns of *Hexagenia* found in our field study sites. It also supports the hypothesis by Hunt (1953) and others that observed density shifts from nearshore to offshore areas which occur as the nymphs increase in size are a result of migration and substrate selection.

Ease of penetration into the substrate appears to be the primary factor affecting substrate selection. Mean burrowing time, which is our best index to penetrability of the substrates, was the only measurement which showed a strong corrrelation with selection (Figs. 1 and 2). This agrees with observations made by Erickson (1963) who found that oxygen consumption of both *Ephemera simulans* Walker and *Hexagenia limbata* (Serville) varied significantly according to substrate particle size. When *Ephemera* nymphs were given an opportunity to select substrates, preference was inversely correlated with oxygen consumption. Erickson (1963) concluded that the oxygen consumption levels increased or decreased as a result of the difficulty or ease of penetration into the substrates. Our measurements of burrowing time provide a more direct estimate of ease of penetration.

Factors affecting penetrability appear to be complex. Substrate texture (quantified by median particle size) is a poor indicator of ease of penetration as evidenced by the differences in burrowing time between fine sandy mud and sandy clay which had the same median particle size (Fig. 3). Substrate compactness (quantified by bulk density) appeared to account for the differing responses to fine sandy mud and sandy clay, but it did not account for the responses to all six substrates. Gravel substrate, even though highest in bulk density, had pores of sufficient size between the gravels into which the small (10-14 mm) mayfly nymphs could crawl. The clay substrate which was medium in bulk density was the most difficult to penetrate. Because soils of high clay content are known to be more cohesive (Buckman and Brady 1969), the results suggest that cohesion might be a significant factor in penetrability. However, if substrate cohesion can be assumed to vary with clay content, it is readily apparent by inspection (Fig. 3 and Table 3) that cohesion alone also does n ot explain the differences in penetrability among the substrates. Our conclusion is that penetrability is dependent upon a combination of factors including particle size, compaction, and cohesion of the substrate. Burrowing time appears to be an integrating response which reflects the net effect of these substrate characteristics.

Because burrowing time is a behavioral response, it could have been affected by the organic matter and associated bacteria in the substrate. Marzolf (1966) found that burrowing amphipods were attracted to substrates to which organic material had been added regardless of particle size. If attraction to the organic content of the substrates affected the burrowing response of *Hexagenia,* it did not dominate over physical characteristics, because organic content did not correlate strongly with burrowing time.

The possibility that organic content plays a role in substrate selection by *Hexagenia* cannot be entirely discounted based on our data. While our correlations did not demonstrate a significant relationship between organic content and selection, there was a tendency for selected substrates to have higher organic contents. Although the simplest explanation is that an increase in organic content tends to increase the penetrability of the substrate, the level of organic content (or food availability) could have more effect on the choice to leave a substrate than on the selection of a new burrowing site.

In order for selection to be expressed by the nymphs in our experiment, the nymph had to perform three tasks: (1) leave the burrow already established in the substrate to which they were introduced, (2) swim through the water, and (3) burrow into a different substrate. Leaving the substrate and swimming in the water column appears to be a normal response of *Hexagenia* and other burrowing organisms such as chironomids, particularly under unfavorable conditions. Both *Hexagenia* and *Chironomus* have been observed to leave the substrate when oxygen levels are depleted (Bay et al., 1966; Fremling, 1967). When held in overcrowded conditions in the laboratory or on hard substrates, chironomids have been noted swimming in the water (Hilsenhoff, 1966; McLachlin, 1969). Hudson and Swanson (1972) speculated that observed migrations of *Hexagenia* in Lewis and Clark Lake were a result of overcrowding. It is our hypothesis that *Hexagenia* frequently leave the substrate;

reasons may include overcrowding, low food level, or difficulty in constructing burrows. Redistribution of the nymphs into primarily soft mud substrates then occurs simply as a result of the relative ease of penetration into the soft mud substrates.

In summary, our evidence supports Hunt's (1953) hypothesis that mayflies migrate from unsuitable substrates. As long as oxygen or other conditions are not limiting, substrates which are most likely to be selected (or reinhabited) by *Hexagenia* are those which are easiest to penetrate and which have a high organic content. Ease of penetration does not depend upon percent by weight of any one particle size such as sand or clay, but upon the substrate compaction and cohesion, and relative combinations of the different particle size groups. Our data show that migration and selection could significantly contribute to the occurrence of highest densities in soft adhesive mud substrates and could explain density shifts noted by other investigators in the field.

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