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Some relationships between stream benthos and substrate heterogeneity

Abstract—Baskets filled with stones, to give four different degrees of heterogeneity, were placed in a stream and made available for colonization by benthic invertebrates. No difference resulted in the total numbers and weights of animals colonizing; 26% of the species, however, showed clear substrate preferences, 35% showed slight preferences, and 39% showed none. The last group made up most of the benthic invertebrate biomass. A high quantity of detritus accumulated in each substrate, and this may account for the similar densities of detritivores collected on all four substrates.

In an experiment to maximize benthic invertebrate food organisms for salmonids in a seminatural rearing channel, Williams and Mundie (1978) examined the responses of benthos to a range of mean particle size of gravel. Maximum numbers and biomass occurred on a medium-sized uniform gravel (24.2-mm mean diameter), whereas diversity was greatest on a larger-sized uniform gravel (40.8 mm). Some species showed a preference for a particular size, others showed no preference. This study explores the response of running water benthos to substrate physical heterogeneity.

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The experiment was done on the east branch of Duffin Creek (third order stream), about 3 km south of its twin sources and 1 km below their junction in Durham County, Ontario (43°58′N, 79°05′W at 180-m elevation). This area represents a drumlinized till plain with overlying soils that are highly calcareous (Chapman and Putnam 1966), resulting in a pH of the stream water of around 7.5. Part of the watershed is used for mixed agriculture, but much of it is in rough pasture or is wooded (primarily eastern white cedar, *Thuja occidentalis*).

At the study site the stream is about 5 m wide and has a series of riffles each about 15 m long; its gradient is about 1 in 12. Depth on the riffles ranges between 20 cm in midsummer and 60 cm during spring runoff, with corresponding current velocities between 25 and 110 $cm \cdot s^{-1}$. The substrate consists of mixed gravels with some sand and clay patches underlain by clay at a depth of 20 cm. Flat cobbles, up to 20 cm in diameter, are frequently found imbedded near the surface and may be heavily encrusted with travertine in the summer months. Dissolved oxygen is high for most of the year, but may fall during periods of thick ice cover. Water temperature has an annual range of 0.2°–24°C.

Four substrate mixtures were made up by dry-sieving washed, ashed, local gravel from a gravel pit. Each was placed in a wire basket (1-cm^2 mesh), lined with 0.023-cm^2 mesh plastic mosquito screening to prevent escape of the smallest particles (0.2 cm). The baskets measured 30 × 30 × 15 cm deep and held about 20.5 kg of substrate. Three replicates were made of each mixture and the baskets were nestled into the streambed, randomly, in a 4 × 3 pattern in the middle third of a large riffle. Enough distance was left between them to prevent interference. The experiment was started on 18 October 1977 and colonization allowed to proceed for 28 days. During this period, the water temperature ranged between 11.1° and 2.2°C, the depth between 25 and 35 cm, and the current between 40 and 60 $cm \cdot s^{-1}$. Each basket was then loosened from the streambed with a large 53-μm-mesh Nitex handnet inserted under its base to prevent any loss of animals while it was being lifted. Ani-

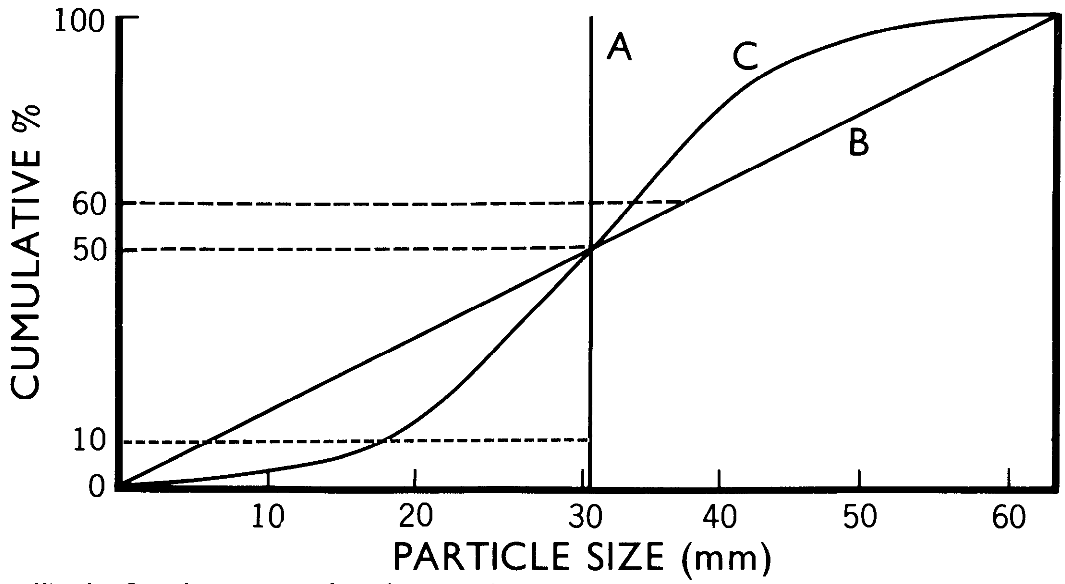

Fig. 1. Cumulative curves for substrates of different heterogeneity. Line A—particle size composition of low heterogeneity substrate; line B—composition of mixed and structured high heterogeneity substrate; line C—composition of medium heterogeneity substrate.

mals and all fine organic and inorganic particles were removed from the baskets by repeatedly washing the gravel with water and preserved in 5% Formalin.

In the laboratory, all the material from each basket was subsampled into six equal units (Mundie 1971)—three for counting and identification and three for dry weight estimates (110°C for 24 h, i.e. to constant weight). Each unit contained at least 200 animals.

The total amount and size distribution of particles <2.0 mm that had collected in the substrates over the colonization period were estimated by drying all material that passed through a 2.0-mm sieve and subjecting this to serial sieving down to 53 μm. Organic matter (weight reduction at 550°C over 24 h) was determined for this material plus the subsample residues from which the animals had been picked. Porosity of each substrate was calculated by the volume displacement method of Pollard (1955).

Because the effect of substrate heterogeneity on colonizing animals was my primary concern, I took great care in preparing the experimental substrates. Ideally the habitat that they presented was to be different in heterogeneity but similar in other characteristics, i.e. mean grain size and range of grain size. Schwoerbel (1961) described a method of calculating the heterogeneity (degree of particle size diversity) of a substrate by making a plot of the cumulative weight percentage of a sample against the grain size (gs). Mean grain size was thus = gs 50% and heterogeneity = gs 60%/gs 10%. If suitable cumulative curves are drawn, it should be possible to construct substrates with known characteristics. This has been done in Fig. 1. I chose 3.2 cm as a suitable mean gravel size and a basket containing this size alone represents a heterogeneity of 1.0—the lowest value possible; its cumulative plot is represented by line A. Line B represents a substrate mixture of relatively high heterogeneity (6.0) and line C, a mixture of intermediate heterogeneity (1.83). Many naturally occurring stream substrates may have heterogeneity values >10; however it was not possible to attain this level within the volume of practically handleable wire baskets. Except for the low heterogeneity substrate, all had identical particle size ranges. All had an identical mean grain size.

To see if the arrangement of particles

Fig. 2. Diagrammatic representation of substrate mixtures as offered for colonization (not actual size).

in identical substrates has any effect on colonizing animals, I set out three baskets containing randomly mixed particles and another three with the same size particles laid in a structured manner with the smallest size class in a layer at the bottom of the basket and each successively larger size class spread directly on top. The four types of substrate presented for colonization are pictorially represented in Fig. 2.

Figure 3 shows the response of benthos to the four substrate types after 28 days of colonization. There was no significant difference ($P < 0.05$) in total numbers and dry weight biomass between any of the substrates. There were, however, significantly more taxa found on the mixed, high heterogeneity substrate (mix-high-H, mean = 25.7) than on the low heterogeneity substrate (low-H, mean = 20.7). There was a possibly significant difference ($P < 0.1$) between mix-high-H and med-H (mean = 21.7); there was no significant difference between low-H and med-H. There was also no significant difference between the numbers of taxa on the mix-high-H and structured, high heterogeneity (struct-high-H) substrates.

As in the previous experiment on substrate size selection (Williams and Mundie 1978), I tried to determine if the major taxa in Duffin Creek showed any preference for a particular substrate. For each taxon, analysis of variance was done on the mean numbers of animals found on each substrate type. Table 1 summarizes these data, as well as giving the food guilds to which the various taxa belong (according to Merritt and Cummins 1978). A definite preference is defined as the presence of significant numbers of animals (*see* Cassie 1971) on one or two substrate types only (of adjacent heterogeneity); e.g. *Paragnetina media* (Walker) was found only on the struct-high-H substrate, and *Dolophilodes distinctus* (Walker) was found only on the low-H and med-H substrates with no significant difference in its density on these two types. Some preference is defined as presence on all four substrate types but

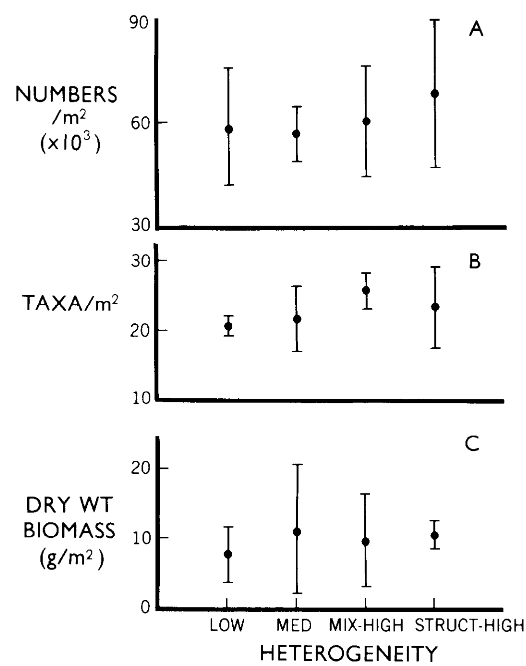

Fig. 3. Responses of benthos to substrates of different heterogeneity. A—Number of animals; B—number of taxa; C—dry weight biomass (means of nine subsamples, together with 95% confidence limits).

Table 1. Significant relationships between taxa and substrate type ($P < 0.05$).

Definite preference	Some preference	No preference
Phasgonophora capitata	*Nais simplex*	*Gammarus pseudolimnaeus*
Paragnetina media	*Bryocamptus* sp.	*Brachyptera* sp.
Rhyacophila sp.	*Nemoura* sp.	*Baetis* sp.
Dolophilodes distinctus	*Stenonema vicarium*	*Ephemerella subvaria*
Thienemannimyia gp.	*Hydropsyche* sp.	*Stenelmis* sp.
Ablabesmyia sp.	*Eukiefferiella* sp.A	*Polypedilum* sp.
	Eukiefferiella sp.B	*Rheotanytarsus* spp.
	Eukiefferiella sp.C	*Corynoneura* spp.
		Thienemanniella sp.
5 Predators	0 Predators	0 Predators
1 Collector-filterer	1 Collector-filterer	1 Collector-filterer
	1 Shredder-detritivore	1 Shredder-detritivore
	6 Collector-gatherers, scrapers	7 Collector-gatherers, scrapers

with significant differences between the mean number of animals on two to four types; e.g. densities of *Nais simplex* Piquet were only different between med-H and mix-high-H substrates although it occurred on all four types. No preference is indicated by a species' presence on all four substrate types at densities that are not significantly different from one another, e.g. *Gammarus pseudolimnaeus* Bousfield.

Characteristics of the substrates are given in Table 2. As there was negligible loss of original particles from any of the baskets in the stream, mean size, size range, and heterogeneity theoretically remained as they were at the start of the experiment. Porosity measurements at the start of the experiment showed the low-H and struct-high-H substrates to have the highest values, while the med-H substrate had a significantly lower value and the mix-high-H substrate had the lowest porosity. All the above characteristics will have been altered slightly however, because during the colonization period, particles suspended in the water column settled in the interstitial spaces and some compaction may have occurred. The six high heterogeneity baskets collected the most sediment, a lesser amount was collected by the low-H substrate and an even smaller amount by the med-H substrate. These amounts of sediment are not correlated with the initial porosity values of the substrates. The total organic matter content of the sediments remained relatively constant at between 3 and 4%. Particle size analysis of the collected sediments (Fig. 4) showed little differential settling caused by characteristics of the various substrates or their secondary consequences, e.g. microcurrents.

Table 2. Substrate characteristics.

	Low-H	Med-H	Mix-high-H	Struct-high-H
Mean particle size (mm)	32	32	32	32
Range in particle size (mm)	none	3.5–64.0	3.5–64.0	3.5–64.0
Heterogeneity	1.0	1.83	6.0	6.0
Initial porosity	0.46	0.41	0.38	0.45
Total sediment collected (dry wt, $g \cdot m^{-2}$)	12,807	10,324	17,899	19,035
Total organic matter collected (dry wt, $g \cdot m^{-2}$)	421	513	513	605
% organic content of sediment (by wt)	3.3	3.5	2.9	3.2

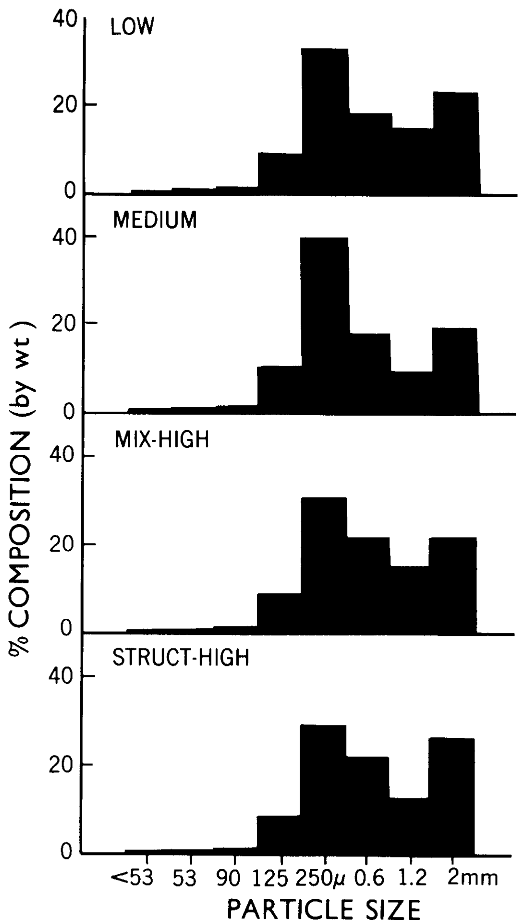

Fig. 4. Size analyses of sediment collected by each substrate after 28 days in stream.

An increase in species diversity along a gradient from simple, uniform microhabitats to complex, diverse microhabitats would be expected (e.g. Hynes 1970). This is borne out in Fig. 3B which shows more taxa on the mix-high-H substrate than on the low-H substrate. The fact that there was no significant difference between the taxa found on the low-H and med-H substrates suggests that the difference between them (H = 1.0 and 1.83) was not great enough to affect the animals. Arrangement of particles within substrates of identical heterogeneity (mix-high-H = struct-high-H = 6.0) had no significant effect on the numbers of taxa on each, although a few species, for example, the stonefly *P. media,* were found only on the struct-high-H type.

It is perhaps surprising that there was no difference in the total numbers and weight of animals found on all four substrates. This suggests that available resources, in terms of habitable space, food, and predation, were similar in all the baskets, while initial porosity and total organic matter values on the other hand suggest otherwise. Perhaps, standing crop did not correlate with the amount of organic matter, in constrast to the results of other studies (e.g. Egglishaw 1969; Rabeni and Minshall 1977; Williams and Mundie 1978) because the total amount of organic matter collected by the substrates in Duffin Creek was relatively very large (e.g. up to 10× that found in the British Columbia experiment) (*see also* Winterbourne 1978). It is possible, therefore, that detritus for food was always present in excess of the requirements of the carrying capacity of each basket (assuming that that accumulated in all baskets was of equal quality).

Although the site of our previous experiment on substrate size selection is a great distance from the site of this one, many of the taxa involved in the two experiments are similar; in fact, many of the genera are identical. If, as has been suggested (Merritt and Cummins 1978; Wiggins and Mackay 1978), the generic rank in aquatic insects can be considered an expression of a theme in terms of broad habitat requirements (and species may be variations on that theme), we may be justified in comparing the faunal responses to substrate in these two experiments.

It is interesting that species of the same genera show no preference in the Ontario experiment and a definite preference for a particular substrate type in the British Columbia experiment. For example, *Brachyptera, Polypedilum, Rheotanytarsus,* and *Thienemanniella* showed no preference here for substrate heterogeneity, although they selected certain sizes of homogeneous substrate over others. Conversely, three of the four species of the chironomid *Eukiefferiella* showed at least some degree of preference for mixed gravels, whereas two of the three west coast species showed no preference when presented with uniform sizes of gravels.

Ranges in amounts of total sediment and organic matter collected by the different types of gravel were much larger here than in the B.C. experiment (up to 8× the 95% confidence limits around the mean), as indeed were the ranges in values of total numbers, number of taxa, and biomass (up to 2.5× the 95% C.L.). They may be directly attributable to the degree of uniformity of conditions under which each experiment was done. The B.C. experiment was done on a flow-controlled rearing channel where conditions on the riffles were uniform across both length and width, and discharge was constant over the experimental period. Materials being transported in the water column would, therefore, have been distributed evenly, and microcurrents would have been relatively uniform. In Duffin Creek, however, a stream subject to natural variations over short periods, the increased heterogeneity of all these parameters would create greater variations in the responses of the animal communities (Egglishaw 1969). That such high variability plagues all quantitative work in stream ecology has been well documented (Cummins 1975; Sheldon 1977). Despite these larger variations, certain taxa in the present experiment did preferentially select substrates of different heterogeneity, although in terms of numbers and biomass they were less important than those species that showed no preference.

The lack of differences in the fauna with substrates of different physical substrate heterogeneities may indicate that the effects of heterogeneity are less important than is usually thought. Patchiness in benthos may be evident only in the presence of much greater heterogeneity, or selection of a suitable substrate mixture may be secondary to selection of suitable organic heterogeneity or microcurrent heterogeneity. Egglishaw (1964), for example, noted a correlation between numbers of benthos and leafy detritus; Mackay (1977) noted that, in the laboratory, three species of *Pycnopsyche* responded positively to organic substrates rather than to mineral substrates; Phillipson (1956) observed that black fly larvae moved around on substrates in response to changes in current. However, species that do not feed on accumulated detritus—predators and filter feeders—should not be affected by this (unless the detritus is overwhelming and threatens to suffocate them) and their preference for substrate and, or, microcurrent heterogeneity should still be evident. All those taxa showing a definite substrate mixture preference (Table 1) are either predators or filter feeders. Further, the wide distribution of their prey (no substrate mixture preference) in this case would not force them to show a secondary distribution.

The results of these colonization experiments suggest that association with a particular substrate mixture may be primary in some species (e.g. substrate may provide the necessary physical environment for a species) but secondary (e.g. substrate may provide the necessary physical environment for a predator's prey species), or even tertiary (e.g. substrate may provide the necessary physical environment for the prey of a secondary predator), in others. The influence of these various parameters may be imposed, as Minshall and Minshall (1977) suggest, in a hierarchial way, which varies from one taxon to the next. Consequently, as the results of this and our previous study (Williams and Mundie 1978) show, in many experimental studies it is imperative to seek these relationships at the species level, wherever taxonomic knowledge makes this possible (Wiggins 1966; Hynes 1970).

Although those species showing a definite preference for a particular substrate type were not dominant, some (e.g. Plecoptera and Trichoptera) may be useful as food for salmonids because of their large size. Selection for these animals through manipulation of substrate heterogeneity in a fish-rearing facility would seem desirable.

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Radioactively labeling of natural assemblages of bacterioplankton for use in trophic studies[1]

Abstract—Natural assemblages of marine bacteria are labeled with [^{3}H]thymidine, most of which is incorporated into the bacterial DNA. The method circumvents the problem of loss of label during short term feeding experiments. Highly labeled assemblages of bacteria thus obtained allow the measurement of grazing by microzooplankton with clearance rates as low as 1 $\mu l \cdot$individual$^{-1} \cdot d^{-1}$.

The development of a new method to measure the numbers and biomass of bacteria in plankton communities (the acridine orange direct counting—AODC—technique: Hobbie et al. 1977) has revealed that bacteria are both far more abundant and much smaller than was formerly supposed (Watson et al. 1977). Other workers (Ferguson and Rublee 1976; Azam and Hodson 1977) have shown that most bacterioplankton are not

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