Hydrobiologia vol. 55, 3, pag. 231-249, 1977

MICRODISTRIBUTION OF BENTHIC INVERTEBRATES IN A ROCKY MOUNTAIN (U.S.A.) STREAM

G. WAYNE MINSHALL & JUDY N. MINSHALL

Department of Biology, Idaho State University, Pocatello, Idaho 83209

Received October 26, 1976

Keywords: Streams, Benthic invertebrates, Microdistribution, Substratum, Current

Abstract

A study of the benthic invertebrate community inhabiting a small, foothill trout stream in the Rocky Mountains of Idaho was conducted over a two-year period. Monthly Hess samples and short-term experiments using substratum-filled trays were used to describe the spatial dispersion of the benthos and to examine the response of invertebrate populations to substratum and current. A method was devised for measuring available surface area which involved coating individual stones with latex and measuring the area of the 'print' resulting from inking the impression left on the latex mold.

The dispersion of all populations was clumped throughout the year. Alteration of the cross-sectional pattern of current velocity and stream bed composition changed the pattern of distribution but not the extent of clumping. Collections made in areas of depositing and eroding substrata revealed a more diverse fauna in the latter. Most groups of organisms found in the riffle were scarcer in the pools or absent from them. The pool fauna contained no important additions over those found in the riffles.

After a year's study of invertebrate populations in an otherwise undisturbed riffle, the substratum was altered and the flow made more uniform; an increase in the abundance of most of the benthic invertebrates followed. No single factor was responsible for the increase, but the change in substratum size and degree of compaction accounted for most of the change. Interpretation of the results was aided by findings from experiments using substratum-filled trays.

Two series of stream experiments using the trays were conducted: one to test the relative importance of current and substratum and the other to test the effect of particle size on the distribution of the benthic fauna. In the first series, placement of trays of stones in a pool resulted in an increase in numbers of some but not all of the invertebrates over numbers usually occurring in the pool. Trays filled with stones and placed in a riffle supported fewer animals than found on the adjacent stream bed but more than in the pool. Variations are attributed to differences in current velocity and amounts of imported organic and inorganic debris. Three different relationships of population numbers to current velocity were found for different members of the community (direct, indirect, and parabolic) over the range of 10 to 60 cm/sec. The second series of experiments consisted of two sets of trays filled with stones of medium or large pebbles, respectively. Nine taxa, as well as all of the combined taxa, showed a preference for trays of small stones over the natural stream bed. A few taxa were noticeably more abundant on the small substratum than on the large but most of the fauna showed only slight increases in numbers or remained constant on the two substrata. Only three taxa showed a direct relation of numbers to total surface area presented by the stones.

Number and kinds of organisms found in trays filled with a uniform size of substratum did not correspond to those taken in Hess samples from the natural stream bed. This has important implications in terms of currently recommended pollution monitoring techniques. However, it is suggested that if the substratum composition of the trays more nearly matched that of the stream, the correspondence would be much better. The results of the present study also throw considerable doubt on the adequacy of generalizations derived from earlier studies of responses to substratum size and suggest several reasons for reevaluating current ideas regarding the influence of substratum on invertebrate distribution.

Introduction

In this investigation experimental manipulations based on the use of substratum-filled trays were used in conjunction with the more conventional descriptive approach to study the microdistribution of benthic invertebrates in a small Rocky Mountain stream. As noted by Moon (1939) and Ulfstrand (1967) several of the factors known to influence the abundance and distribution of the benthic fauna, such as temperature and chemical composition of the water, may be disregarded when microdistribution is studied. On the other hand, con-

Dr. W. Junk b.v. Publishers - The Hague, The Netherlands

sideration of the environmental factors likely to affect animal numbers within any small segment of stream suggests that current velocity (Scott, 1958; Ambühl, 1959; Jaag & Ambühl, 1964; Edington, 1968), substratum (Percival & Whitehead, 1929; Wene & Wickliff, 1940; Linduska, 1942; Pennak & Van Gerpen, 1947; Scott, 1958; 1966; Cummins, 1964; Cummins & Lauff, 1969), and food (Scott, 1958; Egglishaw, 1964; 1969) are likely to be of prime importance (see also Williams & Hynes, 1973). Under certain conditions light (Hughes, 1966; Thorup, 1966) and some factor associated with water depth (Lillehammer, 1966; Chutter, 1969) also may play a role, but these are unlikely to vary appreciably across any given section in small streams. Generally, the behavior of the animals will be modified in response to the factors but there also may be other inherent responses that tend to result in non-random distribution (Allen, 1959; Ulfstrand, 1967; Egglishaw, 1969). Although competition may be one of these (P. Enckell, pers. comm.). there is yet no concrete evidence of its importance in the distribution of stream benthic invertebrates.

The purpose of the present study was to describe the microdistribution of benthic invertebrates and to learn more about the factors responsible for it. Based on the information already published, it was decided to concentrate on the effect of substratum and to control or otherwise account for current velocity and food quality and quantity.

The study proceeded along two main lines. The first involved monthly collections of invertebrates along a single transect across the stream. Following a year of sampling the natural stream bed, the substratum was altered and both it and the current velocity patterns were made more uniform. The effects of these changes were followed for another year. The second approach involved the use of substratum-filled trays to explore the effects of current velocity and substratum particle size on microdistribution.

Description of the study area

Mink Creek, Bannock County, Idaho (112° 23′ W longitude; 42° 43′ to 42° 48′ N latitude) is a tributary of the Portneuf and Snake Rivers and joins the Portneuf about 8 km upstream from the city of Pocatello. The stream arises on mountain slopes, the highest of which (Scout Mtn.) reaches 2,657 m. The upper slopes of the mountains are wooded with Douglas fir *(Pseudotsuga menzinsii)* and aspen *(Populus tremuloides)*, but most of the drainage is covered with grasses (*Poa* spp.), sagebrush *(Artemisia tridentata)*, and juniper *(Juniperus utahensis)*. In the valley bottom the stream is overgrown with shrubs: willow *(Salix exigua)*, dogwood *(Cornus stolonifera)*, chokecherry *(Prunus virginiana)*, rose *(Rosa woodsii)*, and birch *(Betula occidentalis)*. A major part of the drainage basin is National Forest land-sparsely inhabited but used for grazing of cattle and sheep. The main study area was located just inside the boundary of the National Forest at an elevation of about 1700 m.

Mink Creek is fed by melting snow and springs. Consequently, the water is cool throughout the year, rarely exceeding 15°C, and the stream is subject to high discharge and scouring during the spring (April-May). Excluding the period of maximum runoff, the stream at the study site varied in mean depth from 7 to 32 cm and in width from 3.6 to 4.1 m; mean current velocity ranged from 15 to 65 cm/sec and discharge from 0.05 to 0.35 m^3/sec.

The water is fairly high in dissolved solids (mean 265 mg/l, range 198-319 for twelve monthly measurements), most of which are in the form of calcium and magnesium carbonate (mean total alkalinity 211 mg/l as $CaCo_3$, range 182-232). Consequently, the water is well buffered and an alkaline pH is maintained over a narrow range of 8.0 to 8.6.

Methods

Current velocity

Current velocities were measured with a small Ott C-l meter with automatic counter and No. 3 propeller. Velocities at the sites of the benthos collections were measured as near the substratum as possible and three measurements were taken across each site sampled. Current velocities over the experimental trays were determined with the propeller shaft held level with the surface of the tray. Current velocity and depth of the water over the trays was measured at the top of each tray corner.

Benthos

Samples from the stream bottom were collected with a modified Hess sampler (Waters and Knapp, 1961), which enclosed an area of 625 cm^2; the mesh size was 263 μm. Collections were made at approximately monthly intervals within a l-m band across the stream beginning in March 1968 and ending in March 1970. But no samples were collected in April 1969 due to high water and the center sample from March 1969 was lost.

Preliminary processing of the samples was carried out in the field to remove the larger stones and most of the sand and gravel. The stones were measured and returned to the spot from which they were obtained. Then three measurements of current velocity were made just ahead of each of the sites circumscribed by the sampler and the depth of the water over each site was measured. Although this procedure is less satisfactory than if the measurement had been made before disturbance of the bottom, it avoided possible loss of animals before they could be encircled by the sampler.

The trays used in the experiments enclosed the same area as the Hess sampler (625 cm^2) and the sides had a height of 9.5 cm. The bottom of each tray was of tempered hardboard, perforated with holes to permit circulation of water when in the stream and to allow for rapid draining off of water when the trays were removed. The upper surface of the hardboard was covered with 263 μm mesh nylon netting before being attached to the 25 cm x 25 cm (ID) square redwood frame.

The stones used in the trays were recently crushed basalt and were coarse textured and angular in shape. Two sizes were used (Table 1). If the width measurements are used as an estimate of mean diameter, the two sizes correspond to 'medium' and 'large' pebbles according to a modification of the Wentworth classification proposed by Cummins (1962).

To obtain the estimates of size, 20 stones from each small substratum tray and 7 from each large substratum tray were measured. Few attempts have been made to determine the surface areas of stones for use in studies of benthic invertebrates. The method described here is a satisfactory alternative to the one described by Calow (1972). Surface area was determined by coating the rocks with liquid latex. Once the latex had dried an opening was made in the mold and the latex was peeled off. The mold was then slit at strategic locations so that it could be pressed flat. The inside (especially along the leading edges) was inked with a large stamp pad and the impression printed on bond paper. Where necessary the mold was cut into pieces to facilitate printing. The surface area of each impression was measured with a planimeter to obtain the surface area of the stone. Length and width of each stone were measured with a vernier caliper (in mm) along the longest axis and, at approximately right angles to this, along the widest axis, respectively. Weight of each stone, to the nearest 0.01 g, was obtained by means of an Ohaus 'Dial-O-Gram' balance.

Table 1. Assessment of the size of stones in each tray. Sample size in group A was 7 per tray and in B it was 20 per tray.

	(cm^2) $\bar{x}$±S.E.	(g) $\bar{x}$±S.E.	Max. Length (mm) $\bar{x}$±S.E.	Max. Width (mm) $\bar{x}$±S.E.
A. "Large" substratum				
Tray I	123.11 ± 8.38	286.56 ± 35.99	91.2 ± 5.8	60.5 ± 3.1
Tray II	142.41 ± 12.08	310.99 ± 36.56	88.8 ± 7.3	64.5 ± 6.5
Tray III	144.21 ± 10.82	335.33 ± 31.50	94.5 ± 6.0	60.9 ± 1.8
B. "Small" substratum				
Tray IV	21.92 ± 0.62	18.94 ± 0.93	33.8 ± 0.7	26.4 ± 0.5
Tray V	20.22 ± 0.77	16.11 ± 0.99	32.9 ± 1.0	24.6 ± 0.8
Tray VI	19.67 ± 0.87	15.66 ± 0.90	32.6 ± 0.9	25.5 ± 0.7

From an estimate of the surface areas of the stones it was possible to calculate the entire area presented in each tray. Since the mean values for the trays in each set were not significantly different, a composite mean was used for each set. Thus the total surface area per tray of small stones ($P = 0.95$) was 11,536.0 ± 504.0 cm^2 and per tray of large stones it was 3,824.2 ± 360.0 cm^2 (= 3.02 : 1).

The position of the trays was staggered so that one tray did not lie immediately behind another. In the experiment using two different substratum sizes, the positions of the trays of small and large substrata were reversed at the start of each new trial. At the termination of the incubation period, any animals adhering to the outside were removed.

Results and interpretation

Spatial dispersion

The results of the monthly collections from three sites across the stream were analysed to determine the pattern of spatial dispersion in the populations. A Chi-Square test (variance to mean ratio) was used to test for agreement with a Poisson series and hence for a random distribution of animals (see Elliott, 1971; p. 40). It was performed on 22 sets of data for each taxon. Agreement with a Poisson series was rejected ($P < 0.05$) in nearly all sets, indicating that the animals were clumped. The proportion of months in which clumping was detected was smallest for *Paraleptophlebia heteronea* and *Alloperla* and greatest for *Baetis intermedius* (Table 2). A contagious distribution seems to be the most common pattern of spatial dispersion in benthic invertebrates

Table 2. Synthesis of data from the monthly collections for selected taxa, illustrating the spatial dispersion of the animals across the stream (E - east, C - center, W - west) in two different years (I 1968-1969, II 1969-1970).

Taxon	Yr.	No. Months Present	No. Months Clumping Occurred ($x^2 > 7.38$)	Frequency and position where maximum numbers occurred				Total numbers		
				E	C	W		E	C	W
Baetis intermedius	I	11	10	0	5	5		372	983	1759
	II	11	11	2	4	5		1943	2356	2668
Cinygmula mimus	I	11	7	4.5	1.5	1		647	505	331
	II	11	9	1	6	2		1575	2308	1671
Optioservus quadrimaculatus Larvae	I	11	9	2	4	3		515	693	580
	II	11	11	0	5	6		806	2114	2087
Paraleptophlebia heteronea	I	11	6	2	1	3		95	82	149
	II	10	6	2	4	0		987	1301	822
Glossosoma 2 spp.	I	11	9	6	3	0		646	497	190
	II	11	10	3	1	6		1207	862	2065
Ephemerella inermis	I	11	8	1	0	7		299	292	603
	II	11	9	2	5	2		891	1830	1164
Alloperla 3 spp.	I	11	8	7	1	0		310	155	63
	II	10	6	4	2	0		211	168	111
Capnia 5 spp.	I	7	6	1	1	4		127	137	217
	II	9	5	1	4	0		1377	1921	887
Total (excl. Chironomidae)	I	11	11	3	4	4		3722	4365	4819
	II	11	11	0	7	4		10603	16240	17349
Velocity (cm/sec)	I	11	11	5	5	1	$\bar{X}$	39.8	37.3	20.2
							S^2	(571.1)	(481.5)	(79.7)
	II	10	6	3	3	0	$\bar{X}$	35.0	32.8	32.0
							S^2	(132.1)	(261.3)	(287.9)
Depth (cm)	I	9	2	0	2	0	$\bar{X}$	10.1	16.7	15.0
							S^2	(8.8)	(17.6)	(8.8)
	II	9	0	0	0	0	$\bar{X}$	12.9	12.4	13.3
							S^2	(6.5)	(4.5)	(4.5)

(Mottley, Rayner & Rainwater, 1938; Needham & Usinger, 1956; Hales, 1962; Chutter & Noble, 1966; Egglishaw, 1969; Elliott, 1971; Paterson & Fernando, 1971) and the data require special treatment for application of most of the usual statistical tests (Elliott, 1971). In the present study a logarithmic transformation was used to normalize the data.

Examination of the abundance of selected taxa collected from the three sites provides some insight into the spatial variations across the stream (Table 2). There was considerable difference in the two years, apparently due to changes in the riffle imposed during the second year (see later). In 1968-1969 the current velocity generally was higher in the center and along the east side and the water was slightly deeper in the center and on the west side. In 1969-1970 the mean velocity and depth were uniform for all three locations.

Spatial distribution of *Baetis intermedius* and *Alloperla* was similar in the two years. *Paraleptophlebia heteronea, Ephemerella inermis,* and *Capnia* all were most abundant along the west side during 1968-1969 but showed a distinct preference for the center in the following year. *Cinygmula mimus* and *Glossosoma* were most abundant along the east side during the first year; in the second year *Cinygmula* seemed to prefer the center and *Glossosoma* the west side. *Optioservus quadrimaculatus* larvae shifted from a relatively even distribution in the first year to a greater abundance in the center and on the west side in 1969-1970.

Occurrence in erosional versus in depositional areas

Comparison of distribution in riffles and pools

All of the routine collections were taken in an area of eroding substratum (riffle), but in order to give the results greater generality, a few collections (both qualitative and quantitative) were made in an area of depositing substratum (pool). Data in Table 3, for a date on which three Hess samples each were taken in the pool and riffle areas, illustrate kinds of faunal differences found in the two areas. Most groups of organisms found in the riffle were

Table 3. Differences in the invertebrate fauna of eroding and depositing areas of Mink Creek, Idaho, illustrated by collections taken February 26, 1970. Numbers are the totals of three Hess samples from each area expressed as ratios. The factors for calculating the actual numbers are given in the final column.

		Riffle	Pool	Factor
I.	Less abundant in pool than in riffle.			
A.	Absent from pool collection			
	Glossosoma 2 spp.	474	0	1
	Pteronarcys 2 spp.	56	0	1
	Epeorus 2 spp.	36	0	1
	Rhithrogena morrisoni	16	0	1
	Narpus concolor	11	0	1
	Rhyacophila 3 spp.	9	0	1
	Physa	8	0	1
	Nemoura 4 spp.	3	0	1
	Nematoda	2	0	1
B.	A few in pool collection			
	Cinygmula mimus	117	1	7
	Alloperla 3 spp.	90	1	1
	Paraleptophlebia heteronea	83	1	2
	Flumincola nuttaliana	51	1	1
	Ephemerella inermis	32	1	9
	Optioservus quadrimaculatus	20	1	43
	Ephemerella grandis	8	1	1
	Arcynopteryx - Isogenus - Isoperla	5	1	8
	Antocha	3	1	4
	Acarina	2.3	1	60
	Neothremma prob. *alicia*	2.2	1	5
	Capnia 5 spp.	2.0	1	28
	Limnephilidae	2.0	1	1
	Tipulidae other than *Antocha*	1.9	1	35
II.	About equally abundant in riffle and pool.			
	Baetis intermedius	1.01	1	281
	Pericoma	0.8	1	98
	Simulium	0.6	1	10
	Tubificidae	1.5	1	18
III.	More abundant in pool than in riffle.			
	Pisidium	1	6	32
	Chronomidae	1	5	633
	Heleidae	1	3	4
	Ameletus oregonensis	1	2	1
	Stratiomyidae	0	2	1

scarce in the pool collections or absent from them. Only a few taxa were equally abundant in both areas or more abundant in the pool. Only one taxon that occurred in the pool was absent from the riffle. The taxa which showed the greatest increases in the 'pool' areas, *Pisidium* and *Chironomidae*, are animals commonly associated with fine sediments. Likewise many of the taxa most reduced in numbers in the pool (e.g., *Glossosoma, Epeorus, Cinygmula,* and *Alloperla*) are animals usually associated with stony substrata.

Effect of introducing a stony (erosional) substratum into a depositional area

Four trays were filled with an equal number of similarly-sized ('large pebble') recently quarried stones; two trays were placed in the pool and two in an adjoining riffle and all were allowed to become colonized. The assumption was that if substratum particle size were the primary factor responsible for the greater diversity of invertebrates in the riffle than in the pool then provision of larger stones would enhance diversity in the pool. Three such trials were performed, in which the colonization periods were from 4 to 10 weeks (Table 4). Trials 1 and 2 were conducted early in the year when conditions in the stream were stable and changes in numbers of animals were not affected by emergence. Trial 3, of longer duration, encompassed a period of snow-melt runoff and high sediment transport and included the emergence period of a number of the insects. Some of the results of the latter trial may appear anomalous for these reasons.

In this set of experiments analysis is complicated by the fact that although the depth at which the trays were

Table 4. Results of experiment where stone-filled trays (625 cm^2) were placed in areas of eroding and depositing substrata. Results for only selected taxa are given (as number of animals per tray). Taxa are arranged in the same manner as in Table 3.

Trial 1 1-20 to 2-24-69

Substratum		Eroding		Depositing	
Tray		I	II	III	IV
Depth (cm)	$\bar{X}$	7.5	7.8	5.3	x 9.0
	C.L.	± 4.4	± 4.4	± 3.7	÷ 7.1
Velocity (cm/sec)	$\bar{X}$	50.15	37.23	32.00	16.53
	C.L.	± 11.26	± 9.70	± 6.36	± 6.46
I A					
Glossosoma 2 spp.		12	25	1	0
Pteronarcys 2 spp.		2	2	0	0
Epeorus 2 spp.		2	0	0	0
Nemoura 2 spp. (with gills)		0	1	0	0
I B					
Cinygmula mimus		113	127	192	75
Alloperla 3 spp.		1	4	7	2
Paraleptophlebia heteronea		4	5	0	11
Fluminicola nuttaliana		0	0	0	0
Ephemerella inermis		22	42	44	40
Optioservus quadrimaculatus		1	3	0	1
Ephemerella grandis		6	7	1	1
Capnia 5 spp.		16	32	113	222
II					
Baetis intermedius		388	204	174	91
Pericoma		0	0	0	0
Simulium		73	15	2	1
III					
Pisidium		0	1	0	0
Chironomidae		95	211	193	0

Trial 2 2-24 to 3-28-69

Substratum		Eroding		Depositing	
Tray		I	II	III	IV
Depth (cm)	$\bar{X}$	21.0	22.0	17.5	14.3
	C.L.	± 7.3	± 7.5	± 6.7	± 6.0
Velocity (cm/sec)	$\bar{X}$	43.63	x 28.43	x 20.38	x 13.73
	C.L.	± 10.50	÷ 1.86	÷ 1.95	÷ 1.47
I A					
Glossosoma 2 spp.		27	80	0	2
Pteronarcys 2 spp.		7	10	0	1
Epeorus 2 spp.		0	0	0	0
Nemoura 2 spp. (with gills)		0	0	0	0
I B					
Cinygmula mimus		183	206	71	88
Alloperla 3 spp.		3	8	7	2
Paraloptophlebia heteronea		21	27	81	45
Fluminicola nuttaliana		1	1	1	0
Ephemerella inermis		102	147	303	225
Optioservus quadrimaculatus		5	9	2	4
Ephemerella grandis		1	8	0	1
Capnia 5 spp.		19	18	92	83
II					
Baetis intermedius		204	111	91	64
Pericoma		0	0	25	20
Simulium		1	4	0	0
III					
Pisidium		0	0	6	2
Chironomidae		1767	1731	3329	2882

Table 4 cont.

Trial 3 3-28 to 6-7-69

Substratum		Eroding		Depositing	
Tray		I	II	III	IV
Depth (cm)	$\bar{X}$	33.5	35.5	30.8	21.5
	C.L.	± 9.2	± 9.5	± 8.8	± 7.4
Velocity (cm/sec)	$\bar{X}$	65.80	61.88	34.48	27.73
	C.L.	× ÷ 3.04	± 12.51	× ÷ 1.78	± 8.37
Detritus (g)		17.0	14.6	66.2	53.1
Volume (cm^3) inorganic sediment (sand)		200	225	750	500
I A					
Glossosoma 2 spp.		69	46	4	0
Pteronarcys 2 spp.		35	11	12	8
Epeorus 2 spp.		76	61	8	6
Nemoura 2 spp. (with gills)		68	39	54	22
I B					
Cinygmula mimus		107	103	55	42
Alloperla 3 spp.		4	3	19	5
Paraleptophlebia heteronea		22	37	28	16
Fluminicola nuttaliana		0	1	0	13
Ephemerella inermis		7	8	27	0
Optioservus quadrimaculatus		194	252	139	81
Ephemerella grandis		12	17	18	0
Capnia 5 spp.		0	0	0	0
II					
Baetis intermedius		452	635	173	61
Pericoma		0	0	5	1
Simulium		0	0	1	1
III					
Pisidium		0	28	99	146
Chironomidae		0	0	284	141

placed was reasonably similar in each case, the velocity over the trays was not. However, in Trials 1 and 2, it was possible to obtain at least two trays, one from each area, that had similar current velocities. These data have been placed together (two center columns of Table 4) for easier comparison.

The expected effect, that the provision of a stony substratum in the pool would enhance conditions there and bring about an increase in numbers over those normally found, occurred only with some of the invertebrates. Most of the animals showing a positive response were from group IB, notably *Cinygmula mimus, Paraleptophlebia heteronea, Ephemerella inermis,* and *Capnia.* (In Trial 3 the numbers of *Ephemerella inermis* and *Capnia* were depleted by emergence.) Interestingly, the animals from group IA *(Glossosoma, Pteronarcys, Epeorus)* were not attracted to the pool area by the availability of a stony substratum.

Colonization of identical stony substrata in a pool and a riffle

The response of animals in group IA (Table 4) to trays placed in riffle and pool was almost identical to that found in samples from the stream bed (Table 3), showing marked reduction in the number of individuals in the pools. However, in Trial 3 a few individuals from this group did appear on the stones in the pool trays and this is attributed to the high flows which preceded and continued until the removal of the trays.

In Trial 1, when trays with similar current velocities are compared, the number of animals found in the pool and riffle were similar except for group IA, *Capnia* and *Simulium.* In Trial 2 the ratio of organisms in the pool to those in the riffle was greater than expected for *Paraleptophlebia heteronea, Ephemerella inermis, Capnia, Pericoma,* and Chironomidae and smaller than expected for *Cinygmula.* The high numbers of Chironomidae in the 'pool' trays suggest that there may have been a build-up of fine sediments in those trays. In Trial 3 analysis is complicated by the lack of comparable current velocities and a large accumulation of coarse sediment. While not conclusive, the findings tend to suggest that substratum and not current was the factor preventing the riffle fauna from invading the pools.

Occurrence of organisms in substratum-filled trays compared to occurrence on the stream bed

In both the pool and the riffle, most taxa were less abundant in the trays than in the Hess samples. However, a total of ten taxa were more abundant in the trays than on the stream bed of the riffle in one or more trials; and these accounted for about a third of the total number of comparisons. Most taxa in this group were the same as those which were more abundant on stones in the pools than on fine sediments in the same area (*Baetis intermedius, Cinygmula mimus, Ephemerella inermis,* and *Paraleptophlebia heteronea).* It was particularly striking that two others (*Glossosoma* and *Pteronarcys* in Trials 2 and 3) were more abundant in the trays than on the stream bottom. This cannot be explained on the basis of differences in current velocities over the trays (Table 4) compared to those over the Hess collection sites (54 ± 11.7 cm/sec and 31.9 $\overset{\times}{\div}$ 1.3 cm/sec for Trials 2 and 3, respectively).

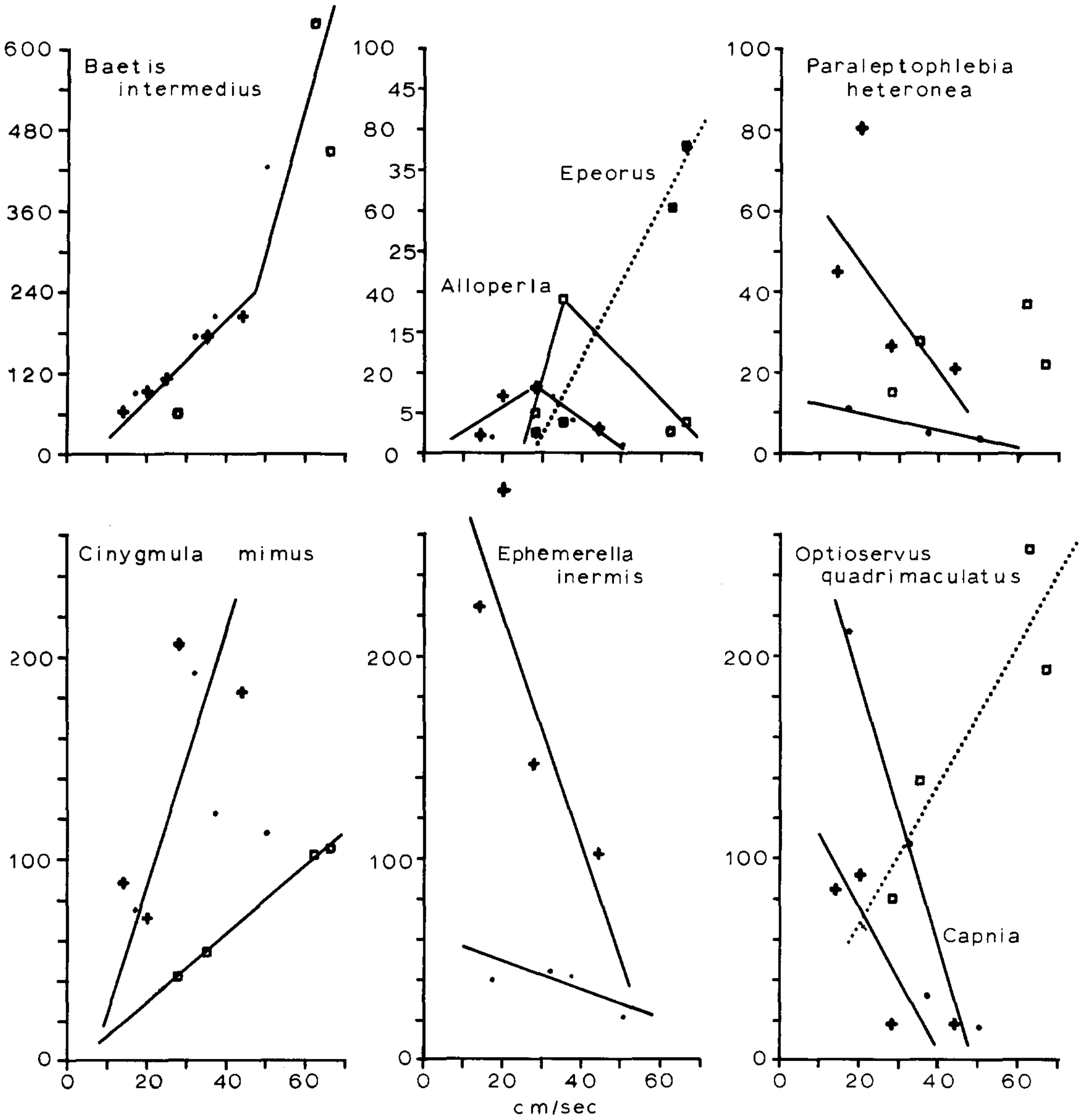

Fig. 1. Numbers of selected benthic invertebrates in relation to current speed. Values are from three separate times ● Trial 1; + Trial 2; □ Trial 3. Note that the scales on the ordinates vary from one figure to another; in the top center figure the outer scale is for *Epeorus* and the inner scale is for *Alloperla.*

Relation of abundance to current velocity

Variation in placement of the trays with regard to current velocity permitted examination of the relationship between velocity and abundance of some of the more common members of the invertebrate community (Fig. 1). Although the data points are limited, they allow some preliminary conclusions to be reached which aid in the interpretation of the results of the tray experiments and serve as a guide to further research.

The results show three different relationships of invertebrate populations to current velocity. *Baetis intermedius, Epeorus, Cinygmula mimus,* and *Optioservus quadrimaculatus* (Trial 3) increased in numbers as velocity increased; those of *Capnia, Paraleptophlebia heteronea,* and *Ephemerella inermis* decreased. The third type of response, seen in *Alloperla,* showed an optimum in mid-range, with the numbers tapering off on either side. Similar responses to current have been documented by Ambühl (1959).

The data (Fig. 1) indicate that the smaller number of *Baetis intermedius* in the pool trays than in the riffle trays was due to the reduced current over the former. In Trials 1 and 2 *Cinygmula mimus* showed a wide variation in response to current, but as maturity approached (Trial 3) the relationship became more clearly defined. In Trial 3 the numbers of *Optioservus quadrimaculatus* varied directly with current. The very low numbers of *Optioservus* in Trials 1 and 2 compared to the high numbers in Trial 3 suggest that the occurrence of finer substrata is important in their distribution. Visual observation showed much greater amounts of fine particles in trays in Trial 3 than in 1 and 2.

Thus the data confirmed that the absence of a suitable substratum was the main factor preventing the invasion of pools by certain of the riffle fauna and further suggested that the size of the particles may play an important role in either pool or riffle. However, it also is seen that in other cases current velocity is important, sometimes operating as the sole factor and in other cases working in conjunction with a suitable substratum. Still other factors, unmeasured in this study, appear to be important in regulating the abundance of species such as *Glossosoma* and *Pteronarcys.* The most likely of these are food and silt.

Effect of altering the natural substratum composition on the riffle fauna

After a year's study of the invertebrate populations in an otherwise undisturbed riffle of Mink Creek, the bottom of the riffle was plowed, the particle-size distribution of the substratum altered, and the flow pattern made more uniform. This was done by removing all of the boulder- and cobble-sized stones, loosening and redistributing the remainder, and introducing additional pebble- and gravel-sized stones and by installing a log weir across the stream. A Mann-Whitney U-test performed on the Chi-square values confirmed that the manipulation significantly reduced the variability in water depth and current velocity from that found during the first year.

Most of the taxa found during the first half of the study increased in abundance following the manipulation (Table 5). Only a few remained about constant or decreased in numbers and of these only three *(Alloperla, Ephemerella grandis, Simulium)* were common enough to warrant attention. Many taxa showed an increase of from two to fourfold but several *(Ameletus, Capnia, Nemoura,* and *Paraleptophlebia)* showed increases of 8 to 16 times. Most of the increased numbers occurred in the autumn and winter and likely were due to enhanced survival of newly hatched young or their retention in the area.

Since the study area had been made more homogeneous by the alteration, it might be expected that the distribution of invertebrates across the stream would also become less variable. If this hypothesis were confirmed it would give support to the belief that the increase in numbers was a result of the manipulation and not due to some extraneous factor.

A Mann-Whitney U-test (Elliott, 1971) was performed on the Chi-square values of most of the abundant taxa for eleven of the collection dates in each of the two years. The null hypothesis, that there was no significant difference in clumping between the two years, was sustained ($P > 0.05$) for all species tested *(Capnia, P. heteronea, N. cinctipes, Alloperla, C. mimus, E. inermis, Glossosoma, B. intermedius)* except *Optioservus quadrimaculatus.* However, since total numbers for the two periods were significantly different ($P < 0.01$) and a concomitant increase in spatial homogeneity could be demonstrated and since the populations colonizing trays of identical substrata located in regions of comparable depth and current velocity showed clumping in spite of the apparent uniformity of environmental conditions (see later), it seems most likely that the size of the sample quadrat was too large to detect any differences in the dispersion patterns (see Elliott 1971, p. 68-71). Normally as the numbers of animals increase, their distribution becomes more clumped.

Table 5. Total number of benthic invertebrates collected during 11 month periods *before* (April 1968 - February 1969) and *after* (May 1969 - March 1970) *manipulation* of the stream bottom and flow pattern. Values are expressed as ratios but the factor for obtaining actual numbers is given. The taxa are arranged according to their abundance for the two periods combined.

	Before (1968 - 1969)	After (1969 - 1970)	Factor
I. Taxa which increased			
A. Abundant (1,000 - 10,000)			
Capnia 5 spp.	1	10.6	482
Paraleptophlebia heteronea	1	9.5	326
Cinygmula mimus	1	4.0	1483
Ephemerella inermis	1	3.3	1194
Glossosoma 2 spp.	1	3.1	1339
Pericoma	1	2.6	256
Chironomidae	1	3.1	1368
Baetis intermedius	1	2.2	3138
Optioservus quadrimaculatus	1	2.2	2517
B. Common (400 - 950)			
Nemoura 2 spp. (w/cervical gills)	1	16.0	37
Acarina	1	9.1	94
Arcynopteryx - Isogenus - Isoperla	1	4.7	140
Fluminicola nuttaliana	1	4.0	80
Tipulidae (excluding *Antocha*)	1	2.8	151
C. Infrequent (40 - 260)			
Ameletus oregonensis	1	8.4	9
Physa	1	6.0	7
Neothremma prob. *alicia*	1	4.8	11
Heleidae	1	4.3	9
Pteronarcys 2 spp.	1	3.9	29
Hydropsyche 2 spp.	1	3.8	13
Nematoda	1	3.7	12
Rhithrogena morrisoni	1	3.6	24
Antocha	1	3.0	42
Tubificidae	1	2.6	66
Epeorus 2 spp.	1.	1.7	94
Narpus concolor	1	1.7	23
D. Rare (1 - 20)			
Limnephilidae	1	1.6	7
Ephemerella doddsi	0	12	1
Lymnaea	0	11	1
Zaitzevia parvula	0	14	1
Brachycentrus americanus	0	2	1
Lepidostoma	0	2	1
Hydroptilidae	0	2	1
Dytiscidae	0	1	1
Lara	0	1	1
II. Taxa which remained about constant			
Ephemerella grandis	1	1.2	315
Pisidium	1	1.2	134
Simulium	1	1.1	204
Alloperla 3 spp.	1	0.9	528
Rhyacophila 4 spp.	1	0.9	74
III. Taxa which decreased			
Nemoura 2 spp. (without gills)	3.5	1	6
Lumbriculidae	2.3	1	3
Sialis	4	0	1
Stratiomyidae	3	0	1
Tabanidae	2	0	1
Acroneuria californica	2	0	1
Pacifastacus prob. *gambelli*	1	0	1

Response to substrata of two different sizes
Due to the manner in which this experiment was conducted, it was not possible to separate the effects of current and substratum or to be absolutely certain that the changes observed in the second period were due to the imposed conditions and not to some extraneous factor. Therefore, another tray experiment was carried out in conjunction with the stream sampling program to test the effect of different substratum sizes on the benthos. Two sets of three trays were filled with medium or large pebbles, respectively. Each set was arranged across the riffle, just upstream from where the stream (Hess) samples were obtained and roughly in line with them. The experiment was repeated three times. The first trial lasted about 6 weeks, the other two lasted about 12 weeks each. The results are summarized in Table 6 for all of the abundant and common taxa which increased following the manipulation (see Table 5) and for a few others which seemed interesting.

In spite of attempts at uniformity, some variability between the trays was introduced by differential accumulation of sand and detritus in the trays. Although no significant differences between the two sets of trays could be demonstrated for either factor by means of Students' t-test ($P < 0.05$), the possible effects were examined more closely because of the presumed importance of these materials to the distribution of invertebrates. From inspection of the numerical data for each tray the taxa most likely to show a positive relationship to either the amount of detritus or the amount of sand were selected as well as all of the other abundant taxa. The data for these were plotted in a manner similar to that done previously for current velocity. Of the 14 taxa examined, only four (*Paraleptophlebia, Pericoma,* Chironomidae,

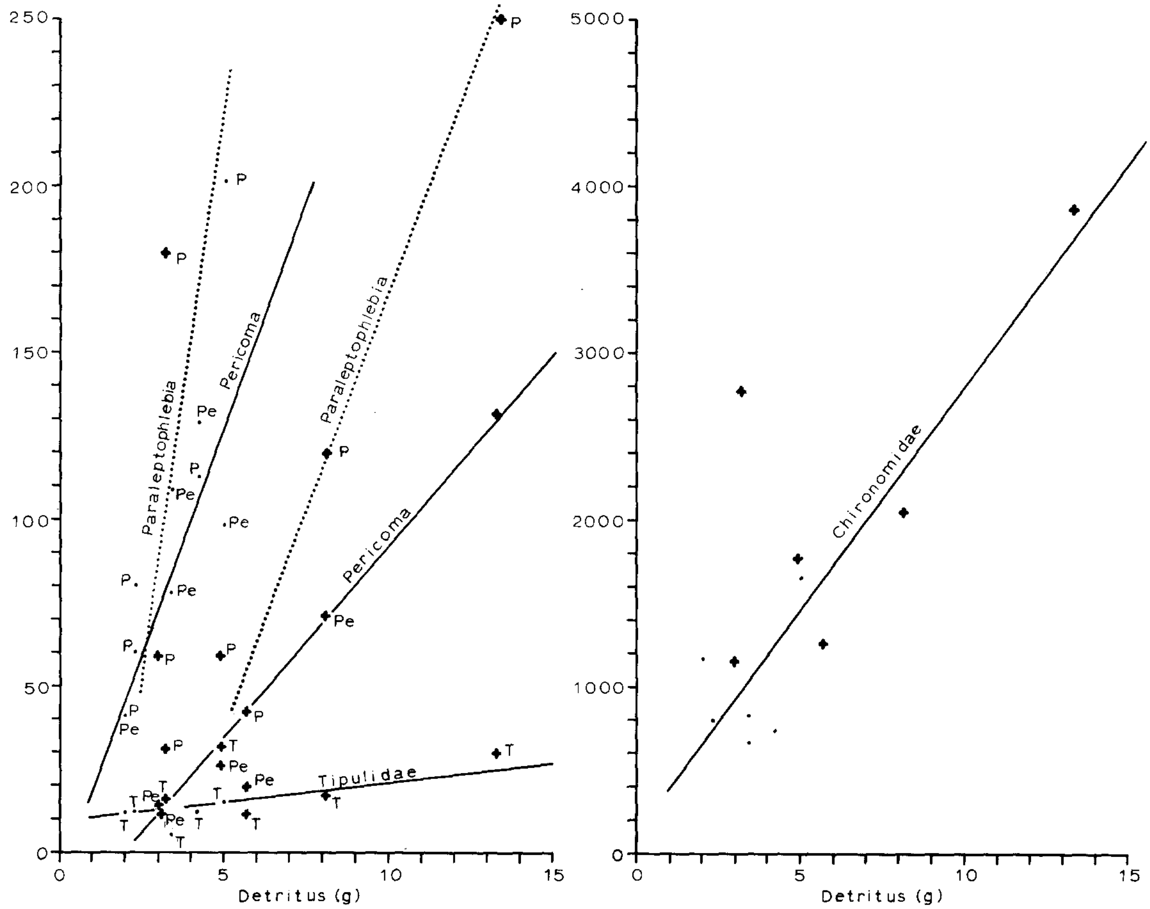

Fig. 2. Numbers of benthic invertebrates showing a direct relationship to amount of detritus (out of 14 taxa examined). Points for *Paraleptophlebia heteronea* (P), *Pericoma* (Pe) and Tipulidae (T) identified by letters. Data from January 20, 1970 (●) and April 23, 1970 (+).

Table 6. Mean number (per 625 cm^2), variance, and chi-squared values (N = 3) for selected taxa colonizing small (SS) and large (LS) substrata and those occypying the adjacent stream bed (Hess). The Hess collections were obtained at the end of each trial except for Trial 3, where collection from March 30, 1970 was used. Chi-squared values <0.05 indicate regular distribution, those between 0.05 and 7.38 indicate random (*) distribution, and those >7.38 show a clumped (**) distribution.

	Trial 1 (8-14 to 10-24-69)			Trial 2 (10-24-69 to 1-20-70)			Trial 3 (1-21 to 4-23-70)		
	$\bar{X}$	S^2	X^2	$\bar{X}$	S^2	X^2	$\bar{X}$	S^2	X^2
Capnia 5 spp.									
SS Tray	226.7	4497.3	39.7**	129.7	8696.3	134.1**	1.0	3.0	6.0*
LS Tray	12.0	16.0	2.7*	36.7	417.3	22.7**	0.0	0.0	0.0
Hess	205.0	2352.0	22.9**	269.7	29284.3	217.2**	2.0	1.0	1.0*
Paraleptophlebia heteronea									
SS Tray	656.3	5104.3	15.6**	464.7	14085.3	60.6**	183.7	4300.3	46.8**
LS Tray	304.0	1413.0	9.3**	131.3	3912.3	59.6**	53.3	96.3	3.6*
Hess	185.3	5841.3	63.0**	97.7	450.3	9.2**	60.0	57.0	1.9*
Cinygmula mimus									
SS Tray	326.7	18732.3	114.7**	953.7	48662.3	102.0**	596.7	15358.3	51.5**
LS Tray	160.7	2921.3	36.4**	188.0	2836.0	30.2**	159.7	7658.3	95.9**
Hess	205.7	7272.3	70.7**	293.3	18937.3	129.1**	292.7	8997.3	61.5**
Ephemerella inermis									
SS Tray	499.3	9112.3	36.5**	181.3	1946.3	21.5**	56.3	1704.3	60.5**
LS Tray	355.3	9994.3	56.3**	237.0	10261.0	86.6**	81.3	2046.3	50.3**
Hess	286.0	43771.0	306.1**	165.3	19854.3	240.2**	128.3	408.3	6.4*
Glossosoma 2 spp.									
SS Tray	3.7	12.3	6.7**	16.3	265.3	32.6**	51.3	108.3	4.2*
LS Tray	4.7	22.3	9.5**	3.0	4.0	2.7*	53.7	233.3	8.7**
Hess	291.3	35769.3	245.6**	144.7	8465.3	117.0**	139.3	1941.3	27.9**
Pericoma									
SS Tray	189.0	39277.0	415.6**	69.7	1192.3	34.2**	81.3	3160.3	77.7**
LS Tray	184.3	20736.3	225.0**	101.3	684.3	13.5**	19.7	42.3	4.3*
Hess	49.0	1033.0	42.2**	69.7	3301.3	94.7**	19.0	244.0	25.7**
Chironomidae									
SS Tray	205.3	34126.3	332.4**	936.3	42233.3	90.2**	2954.7	796281.3	539.0**
LS Tray	141.3	2336.3	33.1**	1020.3	298842.3	585.8**	1445.3	121256.3	167.8**
Hess	91.0	4908.0	107.9**	125.3	2726.3	43.5**	266.0	4681.0	35.2**
Baetis intermedius									
SS Tray	852.0	444916.0	1044.4**	170.3	9194.3	108.0**	208.0	637.0	6.1*
LS Tray	486.3	35004.3	144.0**	158.3	35224.3	445.0**	188.3	18282.3	194.2**
Hess	299.3	18105.3	121.0**	69.0	457.0	13.2**	104.7	1686.3	32.2**
Optioservus quadrimaculatus									
SS Tray	60.3	1100.3	36.5**	111.0	931.0	16.8**	225.7	6226.3	55.2**
LS Tray	80.3	320.3	8.0**	65.0	283.0	8.7**	78.7	17.3	0.4*
Hess	290.3	64986.3	447.7**	112.7	8005.3	142.1**	154.7	8234.3	106.5**
Nemoura 2 spp. (with gills)									
SS Tray	18.0	52.0	5.8*	32.3	2.3	0.1*	0.7	1.3	3.8*
LS Tray	13.7	22.3	3.3*	20.7	64.3	6.2*	0.7	0.3	1.0*
Hess	13.7	312.3	45.6**	0.3	0.3	2.0*	0.0	0.0	0.0
Arcynopteryx - et al.									
SS Tray	69.7	126.3	3.6*	187.0	5956.0	63.7**	39.3	562.3	28.6**
LS Tray	24.7	281.3	22.8**	59.7	133.3	4.5*	17.0	49.0	5.8*
Hess	29.0	403.0	27.8**	36.3	310.3	17.1**	8.3	5.3	1.3*
Ameletus oregonensis									
SS Tray	97.0	457.0	9.4**	17.3	152.3	17.6**	0.0	0.0	0.0
LS Tray	64.0	2223.0	69.5**	42.3	592.3	28.0**	2.7	14.3	10.6**
Hess	7.3	49.3	13.5**	4.3	10.3	4.8*	0.0	0.0	0.0
Epeorus 2 spp.									
SS Tray	0.0	0.0	0.0	26.3	86.3	6.6*	59.0	1213.0	41.1**
LS Tray	0	0.0	0.0	18.3	34.3	3.7*	64.3	8.3	0.3*
Hess	0.3	0.3	2.0*	2.7	9.3	6.9*	21.7	41.3	3.8*
Ephemerella grandis									
SS Tray	22.3	169.0	15.2**	13.7	26.0	3.8*	12.3	5.3	0.9*
LS Tray	19.7	54.0	5.5*	8.3	10.0	2.5*	15.7	186.3	23.7**
Hess	12.3	122.3	19.9**	7.0	27.0	7.7**	2.7	8.3	6.1*
Simulium									
SS Tray	1.3	5.3	8.2**	1.7	8.3	9.8**	2.0	4.0	4.0*
LS Tray	0.0	0.0	0.0	3.7	30.3	16.4**	19.7	1046.3	106.2**
Hess	2.3	1.3	1.1*	3.0	1.0	0.7*	1.0	0.0	0.0
Alloperla 3 spp.									
SS Tray	4.7	2.0	1.0*	10.0	1.0	0.2*	37.0	1204.0	65.1**
LS Tray	5.7	52.0	18.4**	7.0	9.0	2.6*	6.3	10.3	3.0*
Hess	9.3	0.0	0.1*	19.3	46.0	4.8*	34.0	337.0	19.8**
Total (exclud. Chironom.)									
SS Tray	3287.3	690608.3	420.2**	2509.0	20176.0	16.1**	1756.7	46630.3	53.1**
LS Tray	1809.7	173192.3	191.4**	1245.0	220204.0	353.7**	944.0	23893.0	50.6**
Hess	2030.0	751647.0	740.5**	1373.0	170031.0	247.7**	1088.3	29444.3	54.1**

and Tipulidae) showed a relationship to the amount of detritus (Fig. 2) (it may be assumed that the quality of detritus would be similar in all cases) and none showed a relation to the amount of sand.

Distribution of uniformly small and large stones in relation to that on the stream bed

The numbers of abundant taxa taken in the Hess samples at the time that the trays were retrieved were compared with the numbers found in the two sets of trays (Table 6). Most of the taxa listed were more abundant in either of the sets of trays than they were on the stream bed. Analysis of the results (Table 7) using Students' t-test on the transformed data (log x + 1) indicates that many of the differences are statistically significant ($P < 0.05$). Nine taxa (notably *Paraleptophlebia heteronea, Cinygmula mimus,* and *Arcynopteryx*), as well as the total number of all taxa collected (excluding Chironomidae), preferred trays of small stones to the stream bed; four of these (Chironomidae, *Nemoura, Ameletus, Epeorus*) also preferred large stones on occasion. In contrast, *Glossosoma* and *Alloperla* were significantly more abundant in the stream collections than in either set of trays and a similar response was found on two occasions for *Capnia,* but only with respect to the large substratum. Only *Ephemerella inermis, Pericoma, Optioservus quadrimaculatus,* and *Simulium* showed no statistically significant preference for either the natural or the introduced substrata. The results indicate that for most of the taxa examined, the trays of substrata provided enhanced conditions for the fauna independent of current velocity and depth. This finding suggests that the changes in substratum effected by the manipulation of the stream were in some way responsible for the observed increase in the numbers of benthic invertebrates.

The failure of *Glossosoma* to increase on the introduced substrata suggests that a factor found on stones exposed for different times may be involved. The most logical explanation is that the 'mature' stones in the stream support a more abundant source of food (periphyton) than do the recently introduced ones in the trays (see Scott, 1958; Ulfstrand, 1968). The reason for the preference of *Alloperla* and *Capnia* for the natural instead of the introduced substratum is not known, but the greater availability of small (< 2 mm) inorganic and organic particles is thought to be involved.

In an attempt to define more clearly which aspects of substratum alteration were likely to be responsible for the increase in the benthos, Students' t-test was used to

Table 7. Comparison of the mean number of animals (Students' t values) from (a) trays of small and of large stones (S vs L), (b) collections on the stream bed and trays of small stones (H vs S), and (c) collections from the stream bed and trays of large stones (H vs L). Asterisks denote conventional probability levels (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$). The analysis was performed on transformed data (log X + 1).

	Trial 1	Trial 2	Trial 3
Capnia 5 spp.			
S vs L	15.53***	2.03	1.00
H vs S	–0.39	1.02	0.24
H vs L	11.96***	3.11*	1.86
Paraleptophlebia heteronea			
S vs L	7.98**	4.19*	4.97**
H vs S	–4.58*	–7.43**	–4.79**
H vs L	–1.92	–0.80	0.93
Cinygmula mimus			
S vs L	1.95	7.60**	3.74*
H vs S	–1.25	–4.31*	–3.28*
H vs L	0.80	1.31	1.72
Ephemerella inermis			
S vs L	1.72	–0.83	–0.80
H vs S	–1.36	–0.61	2.46
H vs L	–0.76	–1.03	1.57
Glossosoma 2 spp.			
S vs L	1.19	2.08	–0.13
H vs S	5.90**	3.57*	4.77**
H vs L	9.94***	6.73**	3.86*
Pericoma			
S vs L	–0.20	–1.36	2.64
H vs S	–1.49	–0.27	–1.67
H vs L	–1.93	–1.17	–0.64
Chironomidae			
S vs L	1.07	1.12	3.20*
H vs S	–1.07	–8.00**	–10.72**
H vs L	–1.19	–4.68**	–8.77***
Baetis intermedius			
S vs L	0.44	0.63	0.63
H vs S	–1.24	–2.44	–3.00*
H vs L	–1.37	–0.54	–0.93
Optioservus quadrimaculatus A & L			
S vs L	–0.98	2.31	4.40*
H vs S	2.36	–0.38	–1.06
H vs L	2.16	0.59	1.44
Nemoura 2 spp. (with gills)			
S vs L	0.90	2.14	–0.22
H vs S	–1.01	–13.95***	–1.00
H vs L	–0.74	8.39***	–2.00
Arcynopteryx et al.			
S vs L	3.14*	4.04*	1.41
H vs S	–4.46*	–4.88**	–2.16
H vs L	–2.55	–1.91	–1.20

compare the mean numbers found on the two sizes of substrata (Table 7). Total numbers of invertebrates (excluding chironomids) were significantly greater (p. < 0.05) on the small substratum than on the large one in all three trials, but *Paraleptophlebia heteronea* was the only individual taxon for which this was so. Numbers of most individual taxa showed only slight differences on the two substrata. These findings suggest that the observed differences in the two years were not entirely due to changes in substratum *size*. They do not discount the possibility that other substratum related factors were involved such as interstitial flow, degree of compaction, or amount of inhabitable surface area.

Importance of substratum surface area to macroinvertebrate distribution

In order to determine if increased surface area were the factor responsible for the differences between the trays and the stream bottom, the results for the two sizes of substrata were expressed on the basis of the total surface area of the stones in each tray and compared (Fig. 3). The surface area presented by the small stones was about three times that of the large stones. Therefore, the scale of the two axes has been adjusted in the ratio of 3 : 1 and the line with the 45° slope describes the expected relationship if total surface area is the main factor responsible for the differences in absolute numbers in the two sets of trays. The values for a few taxa *(Capnia, Cinygmula mimus, Paraleptophlebia heteronea)* lie close to the line, indicating a positive relation to total surface area. Therefore, it is likely that the 'preference' for small stones by these taxa, noted earlier, is simply an outcome of the greater surface area available for colonization. The numbers of a few others (e.g., *Baetis intermedius, Ephemerel-*

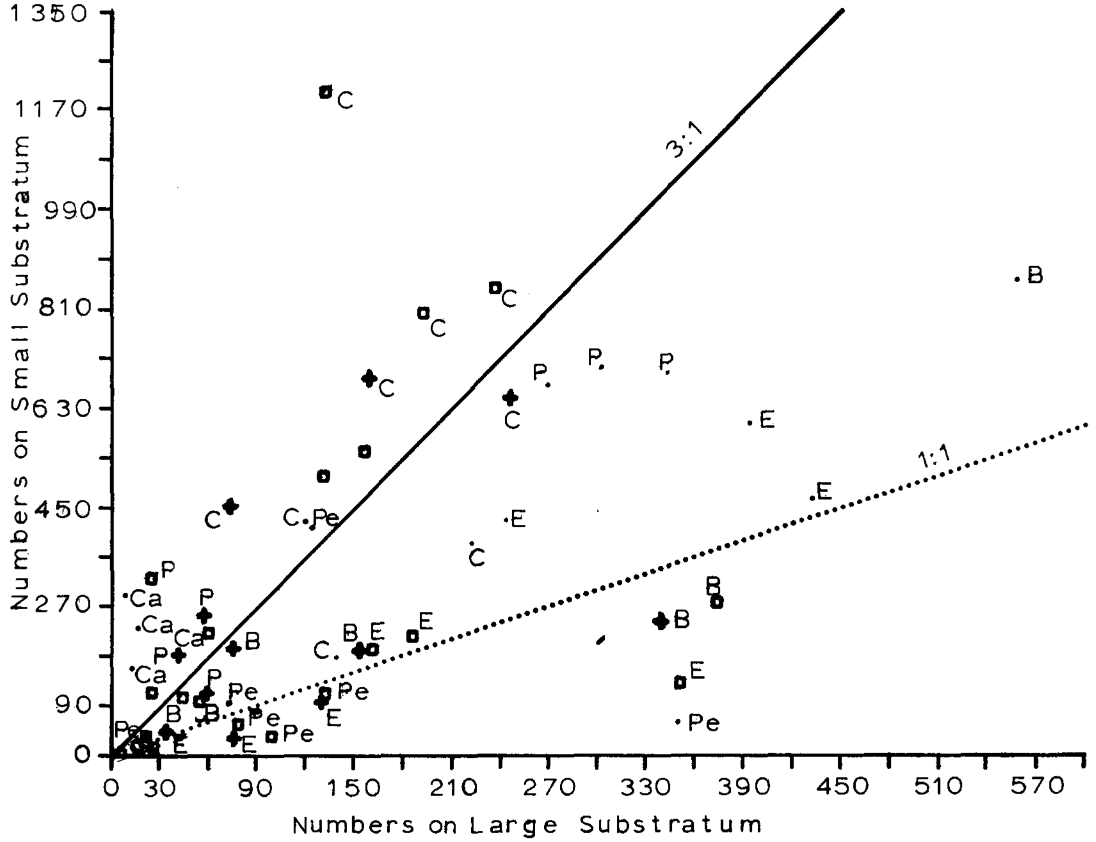

Fig. 3. Numbers of selected benthic invertebrates collected in trays of small stones as a function of numbers collected in adjacent trays of large stones. The scale of the ordinate is three times that of the abscissa to compensate for the difference in surface area between the two sizes of stones. Data from three dates were used ● Oct. 24, 1969; □ Jan. 20, 1970: + April 23, 1970. Data points for individual taxa are identified by letters: *Baetis intermedius* (B), *Cinygmula mimus* (C), *Capnia* spp. (Ca), *Ephemerella inermis* (E), *Paraleptophlebia heteronea* (P), *Pericoma* spp. (Pe).

la inermis, and *Pericoma*) appear to be independent of total surface area but show a consistent relationship between the paired trays. This suggests that current velocity or the amount of food or silt present within the stones may have been important in determining the distribution of the animals. A few others *(Alloperla, Ephemerella grandis, Optioservus quadrimaculatus)* showed no relation between the two sets of trays.

Discussion

Interplay of environmental factors regulating microdistribution

When considered together, the results of this study suggest that the microdistribution of benthic invertebrates is the outcome of a series of responses to a set of interacting variables (current, substratum, food, competition, etc.). The influence of each of these variables seems to be imposed in a hierarchial fashion but their order of operation varies from one species to another. The differential responses of various species to the microenvironment emphasizes the need to consider each species individually. The multiplicity of responses to variations in the micro environment shown by members of the benthic community reveals some of the subtle complexities involved in even relatively straight-forward environmental changes. This emphasizes both the difficulties and advantages of adopting a field experimental approach in attempting to discover the causes of the microdistribution of stream invertebrates.

Several kinds of responses to current velocity were found, but within the range of conditions normally measured in the study area current velocity probably was not of primary importance in the microdistribution of the animals. Within the rather wide limits of current velocity found (approx. 15 to 70 cm/sec.), manipulation of the substratum evidently improved conditions for most of the resident fauna. Furthermore, it was possible to show experimentally that the provision of stones in sections of the stream normally lacking them (pools) can cause a marked increase in the abundance of invertebrates, bringing the numbers to near levels found in natural riffles. The latter results confirm and amplify similar findings by Wene & Wickliff (1940).

The responses of a number of taxa to particular factors varied from one time to another. Many of these responses cannot be explained by the present data and warrant further study. However, the variations may in fact be accurate reflections of changing responses associated with different stages in the life history (Cummins, 1964; Lillehammer, 1966; Elliott, 1971; Williams & Hynes, 1973). For example, Cummins (1964) showed pronounced shifts in substratum selection, case type, and food habits and corresponding changes in microhabitat distribution with different stages in the larval life history of *Pycnopsyche lepida,* a limnephilid caddisfly.

Effects of alteration of the natural stream substratum

The increases in abundance of *Capnia, Paraleptophlebia heteronea,* and *Cinygmula mimus,* species which showed the greatest changes in numbers following manipulation of the stream bottom, appear to be due mainly to an increase in the amount of available surface area produced by the alteration. The results further suggest that these animals occupy different locations on the stream bottom; the first two showed a negative response to current velocity while the latter showed a positive one. *Nemoura,* which showed the greatest *relative* increase in abundance following the manipulation, responded inconsistently to the factors tested and requires additional study.

Increases in numbers of the remaining taxa following alteration of the stream were less pronounced and the specific reasons for the differences are less clear. *Baetis intermedius, Optioservus quadrimaculatus,* and *Epeorus* all were rather indifferent to experimental changes in substratum but did show a positive response to increased current velocity. Although the mean current velocity did not increase significantly in the second year, it did become less variable and this may have resulted in an increase in the number of suitable positions with respect to current velocity. Also, there simply were more stones available for occupation the second year.

Ephemerella inermis, along with *Paraleptophlebia heteronea* and *Capnia,* was more abundant on stones placed in pools than on those placed in riffles and also showed a positive relation to decreasing current velocity. Unlike the other two, *E. inermis* did not show a direct correlation with surface area. It is thought that *E. inermis* was favored by the increase in the number of interstices provided by loosening of the substratum during the manipulation; this would provide shelter from the current while still affording a solid substratum on which to cling and an improved supply of food.

Only *Glossosoma, Alloperla,* and *Capnia* showed a preference for the natural substratum over that of the introduced stones. For *Capnia* this relation held only for the large stones and not for the small ones and seems to indicate a preference for smaller substrata. The other two preferred the natural bottom over either size of introduced stones. The most widely accepted (e.g., see Scott, 1958) explanation for the microdistribution of *Glossosoma* is that it is able to find a more suitable source of food on 'mature' stones in the stream than on introduced stones. However, it also was observed that *Glossosoma* tended to aggregate on the outside of the trays. These larvae were removed and not included in the counts but their presence there suggests a possible preference for smooth substrata. This provides an alternative hypothesis to the food explanation and may be especially important in their distribution immediately prior to pupation. Either explanation could account for their negative response to depositing conditions.

Alloperla, Ephemerella grandis, and *Simulium* were the only taxa of the 14 considered here that did not show an increase following alteration of the stream bed. However, there is no clear indication as to why this was so. *Alloperla* seems to prefer intermediate current velocities (approx. 25-35 cm/sec.), showed no preference for either of the particle sizes used, but like *Glossosoma* was more numerous on the stream bottom than in either set of trays. *E. grandis* and *Simulium* did not react positively to any of the factors examined. In nature, *E. grandis* is found associated with large substrata – the very sort that were removed as part of the manipulation. *Simulium* is found in areas of rapid flow, also a condition not enhanced by the alteration.

Use of substratum-filled trays for representing conditions on the stream bottom

Use of introduced substrata in the study of benthic invertebrates was first employed by Moon (1935a, b; 1940) and Wene & Wickliff (1940). Recently the technique has begun to receive critical examination and wider use (Henson, 1965: Lillehammer, 1966; Mason, Anderson & Morrison, 1967; Ulfstrand, 1968; Hilsenhoff, 1969; Mason *et al.,* 1973; Minshall & Andrews, 1973; Crossman & Cairns, 1974; Ulfstrand, Nilsson, & Stergar, 1974). However, it has been used to advantage as an experimental tool in stream ecology in only a few instances (Cianficconi & Riatti, 1957, Ulfstrand, 1968). Recently the use of artificial substratum devices has been advocated as a means of monitoring environmental conditions in streams (Slack *et al.,* 1973). The technique is still in a developmental stage and needs further testing under a variety of conditions. The limited results obtained thus

Table 8. Comparison of the mean number of animals per square meter ($\bar{X}$) collected in the Hess and tray samples. Values are given for the predominant orders as well as for the more abundant species or genera. Hess samples were collected at the end of each period given, except for Trial 3, where a collection from March 30, 1970 was used.

Taxon	Trial 1 (8-14-69 to 10-24-69)						Trial 2 (10-24-69 to 1-20-70)						Trial 3 (1-21-70 to 4-23-70)					
	Hess		SS Tray		LS Tray		Hess		SS Tray		LS Tray		Hess		SS Tray		LS Tray	
	$\bar{X}$	CL	$\bar{X}$	CL	$\bar{X}$	CL	$\bar{X}$	CL	$\bar{X}$	CL	$\bar{X}$	CL	$\bar{X}$	CL	$\bar{X}$	CL	$\bar{X}$	CL
Ephemeroptera	15968 ×÷	3.5	42224 ×÷	1.7	22256 ×÷	2.0	10288 ×÷	2.8	29328 ×÷	1.1	12592 ×÷	2.9	9840 ×÷	1.5	17920 ×÷	1.2	9088 ×÷	1.8
Baetis intermedius	4784 ×÷	2.9	13632 ×÷	15.3	7776 ×÷	3.0	1104 ×÷	2.1	2720 ×÷	3.6	2528 ×÷	18.2	1680 ×÷	2.8	3328 ±	1584	3008 ×÷	6.5
Cinygmula mimus	3280 ×÷	2.6	5232 ×÷	3.5	2576 ×÷	2.2	4688 ×÷	3.0	15264 ×÷	1.7	3008 ×÷	2.1	4688 ×÷	2.2	9552 ×÷	1.7	2560 ×÷	4.6
Ephemerella inermis	4576 ×÷	13.7	7984 ×÷	1.6	5680 ×÷	2.2	2640 ×÷	7.1	2896 ×÷	1.9	3792 ×÷	2.7	2048 ±	800	896 ×÷	5.3	1296 ×÷	4.4
Paraleptophlebia heteronea	2960 ×÷	3.4	10496 ×÷	1.3	4864 ×÷	1.4	1552 ×÷	1.8	7440 ×÷	2.0	1648 ×÷	3.2	960 ±	304	2944 ×÷	2.5	848 ±	384
Coleoptera	4688 ×÷	7.6	976 ×÷	5.6	1328 ×÷	1.7	1808 ×÷	9.7	1792 ×÷	2.0	1040 ×÷	2.0	2528 ×÷	5.1	3616 ×÷	2.7	1264 ±	160
Optioservus quadrimaculatus	4320 ×÷	7.8	896 ×÷	5.6	1232 ±	640	1720 ×÷	9.9	1696 ×÷	2.1	1008 ±	576	2336 ×÷	4.3	3200 ×÷	2.6	1008 ±	80
Plecoptera	4160 ×÷	1.7	5136 ×÷	1.6	976 ±	528	5216 ×÷	6.1	5160 ±	976	2016 ×÷	1.8	880 ×÷	2.5	1504 ±	720	496 ×÷	3.2
Capnia 5 spp.	3280 ×÷	1.7	3632 ×÷	2.2	192 ±	160	4320 ×÷	9.4	2080 ×÷	7.8	592 ×÷	3.7	32 ±	48	16 ±	64	0 ±	0
Alloperla 3 spp.	144 ±	16	80 ±	64	96 ×÷	29.9	304 ±	272	160 ±	48	112 ±	112	544 ×÷	3.5	592 ×÷	9.0	96 ±	128
Diptera	2784 ×÷	8.9	6784 ×÷	8.5	5616 ×÷	2.4	3488 ×÷	3.3	16320 ×÷	1.6	18288 ×÷	3.1	5024 ×÷	1.4	49056 ×÷	2.1	24064 ×÷	1.7
Chironomidae	1456 ×÷	8.8	3280 ×÷	9.3	2320 ×÷	2.1	2000 ×÷	2.6	14976 ×÷	1.7	16320 ×÷	3.4	4256 ×÷	1.8	47280 ×÷	2.1	23120 ×÷	1.8
Pericoma	784 ×÷	11.9	3024 ×÷	13.0	2944 ×÷	6.4	1120 ×÷	7.6	1120 ×÷	3.4	1616 ×÷	1.9	304 ×÷	119.0	1296 ×÷	6.6	320 ±	256
Trichoptera	4720 ×÷	4.9	192 ±	160	176 ±	112	2384 ×÷	4.1	432 ×÷	5.8	160 ±	144	2368 ×÷	2.0	1024 ±	400	1056 ±	576
Glossosoma 2 spp.	4640 ×÷	4.9	64 ±	144	80 ×÷	17.5	2320 ×÷	4.4	256 ×÷	11.9	48 ±	80	2288 ×÷	2.0	816 ±	416	864 ×÷	2.1
Total invertebrates	33920 ×÷	3.7	55888 ×÷	2.0	31216 ×÷	1.8	23968 ×÷	2.1	55120 ×÷	1.2	36240 ×÷	2.9	21664 ×÷	1.3	75376 ×÷	1.6	38224 ×÷	1.5

far suggest that the method does not provide an accurate assessment of actual standing crops found on the adjacent stream bed but that it does provide a reasonably good measure of relative abundance and biomass of the predominant groups (Hilsenhoff, 1969; Mason *et al.*, 1973).

The data summarized in Table 8 provide a convenient means of examining the adequacy of the substratum-filled trays in the present study for representing conditions on the stream bottom. It is evident that in this study as well the trays did not provide a good measure of the predominant taxa found in the Hess samples. If it is assumed that the Hess collections provide reasonable estimates of the numbers actually occurring on the stream bottom, then it is clear that the numbers collected in either set of trays are not a true reflection of the numbers actually present in the stream. Furthermore, the trays even failed to provide a suitable measure of the relative abundance of the animals on the stream bed. Comparisons of Hess samples with small substratum samples and Hess samples with large substratum samples were tested by means of the Spearman rank correlation coefficient. A significant correlation ($P < 0.05$) in the ranking was found only in Trial 3. (In contrast, comparison between the large and small substratum trays showed very significant correlation in all three trials, ($P < 0.01$). Comparisons by means of the Chi-square goodness-of-fit test showed that the proportions of animals in the trays and on the bottom were significantly different ($P<0.05$) in all of the trials.

However, these comparisons are between trays filled with a uniform-size substratum (as has been suggested by those interested in stream monitoring) and a stream bottom of mixed sizes. Analyses given earlier for variations between trays of a given substratum type suggest that reasonably close agreement could be obtained if material identical to that on the stream bed in composition and packing were used in the trays. Furthermore, as demonstrated by the present study, the lack of agreement between the trays and the bottom need not detract from the value of the technique as an experimental device. In fact, the method has been shown to hold considerable promise.

Need for re-evaluation of present concepts regarding substratum

It has been thought that the abundance of benthic invertebrates might be related in some way to the surface area of the individual particles or to the total surface area contained within a given (three-dimensional) section of stream bottom (Allen, 1959; Scott & Rushforth, 1959; Scott, 1960; Hynes, 1970). Methods for predicting the numbers of invertebrates per unit of stream bottom have been suggested on the basis of this premise but they have met with only limited success. Scott (1966) demonstrated that the abundance of some New Zealand stream animals bore no relationship to the area of the river bed covered by the upper layer of stones. Allen (1959) found no correlation with abundance of the fauna and either the total area of exposed surface of stones or size (kg) of the largest stones. In the present study, the abundance of only three taxa was found to be directly related to the total surface area present in the trays; most of the fauna responded to some other factor or to a set of factors of which surface area may have been only a part. The results of this study indicate that part of the problem lies in the fact that substratum is itself a multi-factor variable. This suggests that components such as texture and degree of compaction (or extent of interstitial spaces) as well as particle size and surface area may act to regulate species composition and abundance.

Egglishaw (1964, 1969) found a correlation between the amount of detritus and the microdistribution of a number of benthic invertebrates. The presence of detritus was not measured routinely in the present study because it was thought that it would not vary appreciably within the relatively small area (approx. 1 x 6 m) being sampled. A somewhat belated attempt to examine the distribution of detritus revealed that in fact some variation did occur. Rabeni & Minshall (1977, in press) have since demonstrated that appreciable amounts of detritus tend to accumulate in the substratum-filled trays and that the smaller substratum particle sizes tend to accumulate more detritus than the larger-sized substratum particles and support a greater abundance of invertebrates. A similar condition would be expected to occur on the natural stream bed. In the present study only *Paraleptophlebia heteronea, Chironomidae,* and *Pericoma* increased in numbers with increasing detritus. But even the minimum amounts found (48 g dry wt/m^2) may have been beyond the limiting threshold for most of the other detritivores. Likewise, although Barber and Kevern (1973) found no consistent relation between different amounts of detritus and numbers of invertebrates, the levels of detritus used (121, 484, 847 g dry wt/m^2) greatly exceeded those generally found under natural conditions. While the importance of detritus to the benthic consumers is not questioned, the results of the present study suggest that the roles of current velocity and substratum many have been under-rated in the past (see e.g., Egglishaw, 1969). Since the amount of detritus which accumulates results from the interplay of current and substratum, the correlation between amount of detritus and invertebrate numbers may, in many cases, be coincidental. Furthermore, in a grazer community the production of algae and fungi is important; the quality of the detritus is similarly important. Both factors must be considered in the future in examining the distribution of invertebrates in relation to detritus.

It generally is believed that benthic invertebrates increase in numbers over the sequence of increasing particle sizes from sand through large stones (boulders) (Tarzwell, 1936; Needham, 1938; Sprules, 1947; Bell, 1969). The increase in particle size sometimes is equated with an increase in the complexity of the substratum (Hynes, 1970), but this has yet to be established. However, the results of the present work demonstrate that the situation is not likely to be as simple as has been intimated. Furthermore, many studies on the effect of substratum on the distribution of stream invertebrates have not dealt with taxonomic levels lower than order even where the information was available (e.g., Tarzwell, 1936; Pennak & Van Gerpen, 1947; Bell, 1969). These studies have served largely as the basis for our understanding of benthic invertebrate-substratum interaction up to the present time. Examination of the results obtained in the present study for lower taxonomic levels (genera and species) throws considerable doubt on the adequacy of generalizations derived from these earlier works.

Where only a few taxonomic representatives of an order are abundant (as in the Coleoptera, Plecoptera, Diptera, and Trichoptera in the present study), the total numbers found on different types of substrata reflect the responses of those few taxa (Table 8). Thus, possibly significant changes by less abundant taxa (e.g., *Alloperla* or *Pericoma*) will be masked if they are not examined individually. More important perhaps is the case where several taxa within an order are nearly equally abundant (e.g., as with Ephemeroptera in the present study). In these situations analysis at the ordinal level is likely to give inconsistent and frequently inaccurate indications of the responses of the constituent populations to different substrata. Thus, the need for a careful examination of the responses of different species populations over a wide range of particle sizes clearly is indicated.

Acknowledgments

We wish to thank Drs. J. M. Elliott and T. T. Macan for critically reviewing the manuscript. Dr. Elliott provided help on statistical procedures and made a number of suggestions for improving presentation of the data. The manuscript was prepared while the authors were on Sabbatical leave at the Freshwater Biological Association, Windermere Laboratory, England.

References

Allen, K. R. 1959. The distribution of stream bottom faunas. Proc. New Zealand Ecol. Soc. 6: 5-8.

Ambühl, H. 1959. Die Bedeutung der Strömung als ökologischer Faktor. Schweiz. Z. Hydrol. 21: 133-264.

Barber, W. E. & Kevern, N. R. 1973. Ecological factors influencing macroinvertebrate standing crop distribution. Hydrobiologia 43: 53-75.

Bell, H. L. 1969. Effect of substrate types on aquatic insect distribution. J. Minn. Acad. Sci. 35: 79-81.

Calow, P. 1972. A method for determining the surface areas of stones to enable quantitative density estimates of littoral stone-dwelling organisms to be made. Hydrobiologia 40: 37-50.

Chutter, F. M. 1969. The distribution of some stream invertebrates in relation to current speed. Int. Revue ges. Hydrobiol. 54: 413-422.

Chutter, F. M. & Noble, R. G. 1966. The reliability of a method of sampling stream invertebrates. Arch. Hydrobiol. 62: 95-103.

Cianficconi, F. & Riatti, M. 1957. Impiego di pietre artificiali per l'analisi quantitativa delle colonizzazioni faunistiche dei fondi potamici. Boll. Pesca Piscic. Idrobiol. 12: 299-335.

Crossman, J. S. & Cairns, J. Jr. 1974. A comparative study between two different artificial substrate samplers and regular sampling techniques. Hydrobiologia 44: 517-522.

Cummins, K. W. 1962. An evaluation of some techniques for the collection and analysis of benthic samples with special emphasis on lotic waters. Amer. Midl. Nat. 67: 477-504.

Cummins, K. W. 1964. Factors limiting the microdistribution of larvae of the caddisflies Pycnopsyche lepida (Hagen) and Pycnopsyche guttifer (Walker) in a Michigan stream. Ecol. Monogr. 34: 271-295.

Cummins, K. W. & Lauff, G. H. 1969. The influence of substrate particle size on the microdistribution of stream macrobenthos. Hydrobiologia 34: 145-181.

Edington, J. M. 1968. Habitat preferences in net-spinning caddis larvae with special reference to the influence of water velocity. J. Anim. Ecol. 37: 675-692.

Egglishaw, H. J. 1964. The distributional relationship between the bottom fauna and plant detritus in streams. J. Anim. Ecol. 33: 463-476.

Egglishaw, H. J. 1969. The distribution of benthic invertebrates on substrata in fast-flowing streams. J. Anim. Ecol. 38: 19-33.

Elliott, J. M. 1971. Some methods for the statistical analysis of samples of benthic invertebrates. Freshwat. Biol. Assoc. Sci. Publ. No. 25. 148 p.

Henson, E. B., Jr. 1965. A cage sampler for collecting aquatic fauna. Turtox News 43: 298-299.

Hilsenhoff, W. L. 1969. An artificial substrate device for sampling benthic stream invertebrates. Limnol. Oceanogr. 14: 465-471.

Hughes, D. A. 1966. Mountain streams of the Barberton area, Eastern Transvaal. Part 2. The effect of vegetational shading and direct illumination on the distribution of stream fauna. Hydrobiologia 27: 439-459.

Hynes, H. B. N. 1970. The ecology of running waters. Univ. Toronto Press. 555 p.

Jaag, O. & Ambühl, H. 1964. The effect of the current on the composition of biocoenoses in flowing water streams. pp. 31-49. In Int. Conf. Wat. Pollut. Res. Lond. Pergamon Press, Oxford.

Lillehammer, A. 1966. Bottom fauna investigations in a Norwegian river. The influence of ecological factors. Nytt. Mag. Zool. 13: 10-29.

Linduska, J. P. 1942. Bottom type as a factor influencing the local distribution of mayfly nymphs. Can. Ent. 74: 26-30.

Mason, W. T., Jr., Anderson, J. B. & Morrison, G. E. 1967. Limestone-filled, artificial substrate sampler. Float unit for collecting macroinvertebrates in large streams. Progr. Fish Cult. 29: 74.

Mason, W. T., Jr., Weber, G. I., Lewis, P. A. & Julian, E. C. 1973. Factors affecting the performance of basket and multiplate macroinvertebrate samplers. Freshwat. Biol. 3: 409-436.

Minshall, G. W. & Andrews, D. A. 1973. An ecological investigation of the Portneuf River, Idaho: a semiarid-land stream subjected to pollution. Freshwat. Biol. 3: 1-30.

Moon, H. P. 1935a. Methods and apparatus suitable for an investigation of the littoral region of oligotrophic lakes. Int. Rev. ges. Hydrobiol. 32: 319-333.

Moon, H. P. 1935b. Flood movements of the littoral fauna of Windermere J. Anim. Ecol. 4: 216-228.

Moon, H. P. 1939. Aspects of the ecology of aquatic insects. Trans. Soc. Brit. Ent. 6: 39-49.

Moon, H. P. 1940. An investigation of the movements of freshwater faunas. J. Anim. Ecol. 9: 76-83.

Mottley, C. M., Rayner, H. J. & Rainwater, R. H. 1938. The determination of the food grade of streams. Trans. Amer. Fish. Soc. 68: 336-343.

Needham, P. R. 1938. Trout streams. Comstock Publ. Co., Inc., Ithaca, N.Y. 233 p.

Needham, P. R. & Usinger, R. L. 1956. Variability in the macrofauna of a single riffle in Prosser Creek, California, as indicated by the Surber sampler. Hilgardia 24: 383-409.

Paterson, C. G. & Fernando, C. H. 1971. Studies on the spatial heterogeneity of shallow water benthos with particular reference to the Chironomidae. Can. J. Zool. 49: 1013-1019.

Pennak, R. W. & Van Gerpen, E. D. 1947. Bottom fauna production and physical nature of the substrate in a northern Colorado trout stream. Ecology 28: 42-48.

Percival, E. & Whitehead, H. 1929. A quantitative study of the fauna of some types of stream-bed. J. Ecol. 17: 282-314.

Rabeni, C. F. & Minshall, G. W. 1977. Factors affecting the microdistribution of benthic insects. Oikos (in press).

Scott, D. 1958. Ecological studies on the Trichoptera of the River Dean, Cheshire. Arch. Hydrobiol. 54: 340-392.

Scott. D. 1960. Cover on river bottoms. Nature 188: 76-77.

Scott, D. 1966. The substrate cover-fraction concept in benthic ecology. pp. 75-78. In K. W. Cummins, C. A. Tryon, and R. T. Hartman (eds.) Organism-substrate relationships in streams. Publ. Pymatuning Lab. Ecol., Univ. Pittsburg. No. 4. 145 p.

Scott, D. & Rushforth, J. M. 1959. Cover on river bottoms. Nature 183: 836-837.

Slack, K. V., Averett, R. C., Greeson, P. E. & Lipscomb. R. G. 1973. Methods for collection and analysis of aquatic biological and microbiological samples. U.S. Geol. Surv. Tech. Water-Resources Inv. BK 5, Chap. A4 165 p.

Sprules, W. M. 1947. An ecological investigation of stream insects in Algonquin Park, Ontario. Univ. Toronto Stud. Biol. No. 56. Publ. Ont. Fish. Res. Lab. 69: 1-181.

Tarzwell, C. M. 1936. Experimental evidence on the value of trout stream improvement in Michigan. Trans. Amer. Fish. Soc. 66: 177-187.

Thorup, J. 1966. Substrate type and its value as a basis for the delimitation of bottom fauna communities in running waters. pp. 59-74. In K. W. Cummins, C. A. Tryon, and R. T. Hartman (eds.). Organism-substrate relationships in streams. Publ. Pymatuning Lab. Ecol., Univ. Pittsburg. No. 4. 145 p.

Ulfstrand, S. 1967. Microdistribution of benthic species (Ephemeroptera, Plecoptera, Trichoptera, Diptera: Simuliidae) in Lapland streams. Oikos 18: 293-310.

Ulfstrand, S. 1968. Benthic animal communities in Lapland streams. A field study with particular reference to Ephemeroptera, Plecoptera, Trichoptera, and Diptera: Simuliidae. Oikos Suppl. 10: 1-120.

Ulfstrand, S., Nilsson, L. M. & Stergar, A. 1974. Composition and diversity of benthic species collectives colonizing implanted substrates in a South Swedish stream. Ent. Scand. 5: 115-122.

Waters, T. F. & Knapp, R. J. 1961. An improved stream bottom fauna sampler. Trans. Amer. Fish. Soc. 90: 225-226.

Wene, G. & Wickliff, E. L. 1940. Modification of a stream bottom and its effect on the insect fauna. Can. Ent. 72: 131-135.

Williams, N. E. & Hynes, H. B. N. 1973. Microdistribution and feeding of the net-spinning caddisflies (Trichoptera) of a Canadian stream. Oikos 24: 73-84.