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THE VERTICAL DISTRIBUTION OF MACROBENTHOS WITHIN THE SUBSTRATUM OF THE BRAZOS RIVER, TEXAS[1]

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Abstract

Vertical stratification samplers were developed for sampling the grave-sand substrate of a Brazos River, Texas riffle. Fifteen of 25 species recovered, occurred below 10 cm. Mean percentages of total organisms recovered were 66.4%, 20%, 6.1% and 7.5% per 10 cm level, respectively, from the surface down. Dominant insects were *Neochoroterpes mexicanas* naiads and chironomid, *Simulium, Cheumatopsyche* and *Stenelmis* larvae. Seasonal population peaks of these five groups in the top 10 cm correspond with observed emergence peaks. The smaller size classes were generally predominant in the 0-10 cm level. Larvae of *Stenelmis* were the most evenly distributed among the various 10 cm levels in all size classes. A movement of *Cheumatopsyche* and *Neochoroterpes* to lower levels was observed following a large flood, suggesting an escape response to increased silt load and scouring.

Dissolved oxygen ranged from saturation at the surface down to 0.4-0.7 ppm at 30-40 cm, indicating that it was possibly limiting at lower levels. Maximum temperature difference between 10 cm levels was only 3 C. Flow was negligible below 10 cm.

The vertical stratification sampler recovered significantly greater populations in the surface 20 cm, but not in the total 40 cm, than a modified Hess sq. ft. sampler.

Introduction

Quantification of stream macrobenthos populations has remained a difficult problem despite numerous attempts at improvement. Needham & Usinger (1956), Chutter (1969), and others have pointed out the difficulty in obtaining adequate numbers of samples which will yield population estimates with desired statistical confidence, and still maintain some degree of sampling economy. Attempts to improve confidence through improved sampling devices and techniques have led to development of numerous types of samplers (Southwood, 1968; and Hynes, 1970a).

Samplers and techniques described by Surber (1937) and Hess (1941) are probably the most used currently despite their limitations (Waters & Knapp, 1961; Kroger, 1972), and continued suggestions as to their deficiency for quantitative analyses (Hilsenhoff, 1969). Coleman & Hynes (1970), Radford & Hartland-Rowe (1971), and Hynes (1970b) have shown that sampling gravelly-bottomed streams with devices that penetrate only the top few centimeters (e.g., Surber and Hess type samplers) do not include those portions of the macrobenthos present beneath the sampled stratum. Ford (1962) stated that vertical distribution must be considered in quantitative work.

In some instances a more deeply penetrating sampler than is generally employed should be used to obtain accurate estimates of insect populations. The phenomenon of vertical stratification is exhibited in most communities; vertical layers are characterized by certain species at certain times, with vertical movements between strata and horizontal movements within a stratum. Vertical movements could reflect organism responses to environmental changes or adaptive advantage in the life cycle through selection.

In the most recent studies of stratification of macrobenthos, Coleman and Hynes (1970) and Hynes (1974) have designed and used samplers that made use of natural substrate and collected organisms to a depth of 30 and 50 cm, respectively. In the latter study, the „super-pot" samplers were driven into place. Their original 'pot' samplers were composed of four horizontal layers about 7.5 cm deep and 25.5 cm in diameter; they were left in the stream bed for 1-28 days. Colonization of the samplers were possible both vertically and horizontally. Macrobenthos increased steadily with time and only 20% of the total organisms recovered were found in the top 7.5 cm layer. Other overall percentages were 27% in each of two middle layers, and 26% in the lowest layer. Most groups of animals recovered exhibited vertical distributions approximating these percentages.

The present study was undertaken with three objectives: (1) to design an improved sampler for determining populations of aquatic insects at 10 cm intervals down to 40 cm, (2) to determine the nature and extent of vertical stratification of riffle insect communities in the modified Brazos River in Palo Pinto County, Texas, and (3) to compare total populations sampled vertically down to 40 cm with those on the same area sampled with a modified Hess surface sampler. In addition, seasonal periodicity of vertical stratification and its relationship with the emergence and life cycles of selected insect species was of interest.

Materials and methods

The study was made on a large, uniform riffle located above Texas State Highway 4 Bridge on the Brazos River, approximately 20 miles below Possum Kingdom Dam. This stretch of the Brazos River has been under investigation by several workers recently and detailed descriptions of the study riffle were given by Stewart *et al.* (1973), Vaught & Stewart (1974), Cloud (1973), and Rhame (1973).

The Sampler

Ten samplers, modified after Coleman & Hynes (1970), were constructed from 18 ga flat rolled expanded steel, with diamond shaped holes approximately 0.5 cm x 1.5 cm. Eight of the samplers consisted of eight inside pots 10 cm high and 16 cm i.d., with a solid steel bottom. Two other inside pots measured 40 cm high and 16 cm i.d.

Each inside pot fitted into a corresponding outside cylinder open at both ends, 21 cm i.d. and measuring 10, 20, 30, 40 cm high, depending on the depth of sample for which it was designed. All the metal parts of the samplers were coated with green epoxy paint for protection.

Nets were constructed from parachute nylon (33 threads/cm; 10 um opening), fitted to a copper ring approximately 17 cm i.d., which fit between the inner pot and the outside cylinder. All nets were 15 cm in length so that they extended 5 cm past the top of the inside pot when the samples were retrieved.

The handle used to raise the net around the inside pot, in 10-20, 20-30 and 30-40 cm samples ran through two holes in a partition which fitted against the top of the inner pot *in situ*. The partition was a sandwich arrangement of two species of metal holding a rubber gasket which fitted tightly against the walls of the outside cylinder.

Installation of the Sampler

The outside cylinders were buried in the riffle with their tops level with the top of the substrate, and remained in place throughout the study. The inside pots were filled with dry rubble and sand from the stream bank in proportions that closely match those of the substrate at the level from which the sample was to be taken.

The filled inside pot of the 0-10 cm and 0-40 cm samplers were placed in the buried outside cylinder with the net ring at the bottom of the samplers. The inside pots placed below 10 cm were similarly placed inside the buried outer cylinder and the partition was positioned against the top of the inside pot. The space between the partition and the surface was filled with rubble in a plastic bag.

The net at the bottom of the sampler allowed access to the inside pot laterally from the surrounding substrate (Figure 1). The top few centimeters of the in-place samplers were camouflaged with sand and rubble from the stream. The 10 samplers were placed along two transects across the riffle. The order of placement by different levels and distance between samplers were determined randomly.

Retrieval of Samples

Retrieval consisted of: (1) removal of the camouflage and plastic bag of rubble and lifting the handle which pulled the net-ring up against the metal partition (except the 0-10 and 0-40 cm samples), and (2) pulling the bag-enclosed sample (inner pot) up out of the stream. The metal partition acted as a top for the bag-enclosed pot and prevented washout of the sample.

The sample and insects clinging to the inside of the net

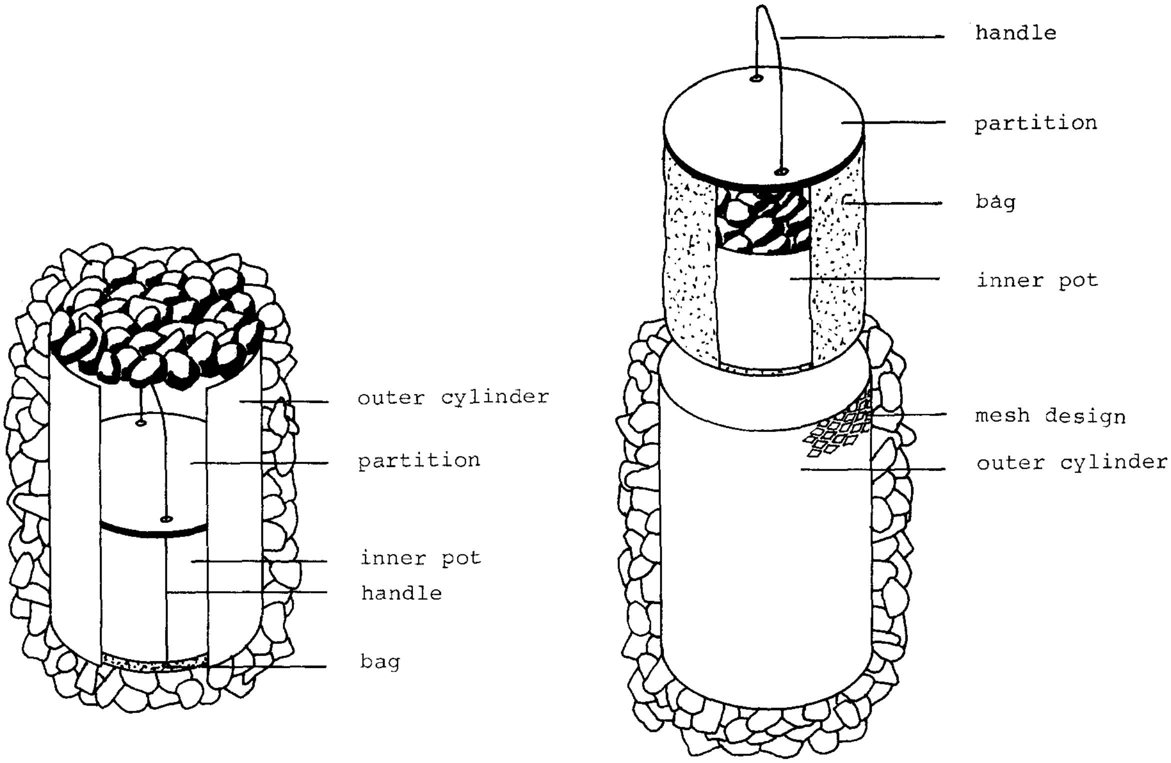

Fig. 1. Vertical sampler (in place and retrieved, 10-20 cm).

were placed in a bucket having mesh openings of 0.5 mm; larger pieces of substrate were removed after carefully washing clinging insects back into the sample. The remaining substrate and insects constituting the condensed sample were placed in one liter jars of 70% isopropanol. The inside pot was then refilled with substrate and replaced in its outside cylinder, in the manner already described, to allow colonization for the next sample period.

Sampling Schedule

Original installation of samplers was made on July 7, 1972. Two samples for each 10 cm depth were retrieved seasonally on five subsequent dates: October 12, 1972, January 15, April 2, June 1, and August 1, 1973. The original intent was to take six semi-monthly samples, since preliminary studies on the riffle indicated that colonization of the samples stabilized within approximately eight weeks. However, prolonged floods on the river in September and December 1972 prevented retrieval according to schedule. It was decided that sampling for seasonal effect might be more desirable than adhering to a temporal schedule.

Three surface samples were taken between the two transects with a modified Hess sq. ft. sampler (Hess, 1941) on the same dates as retrieval of vertical layer samples. Dissolved oxygen and temperature were recorded in each 10 cm sampling level on January, April, and June, 1973 retrieval dates with a YSI Model 54 Oxygen Meter. Access to each 10 cm level was achieved through use of a perforated steel pipe. The pipe was driven to the appropriate level, allowed to remain a minimum of 1-hour, and then the probe was lowered to the level of the perforations and agitated by hand for DO and temperature measurements.

An attempt was made to determine if any water flow occured within the substrata, by use of preweighed salt tablets. Two-minute dissolution rates of salt tablets at the surface with known flow rates, and in graduated cylinders of standing river water (at the same levels as measured experimentally) were used as controls. Dissolution rates were determined by lowering preweighed salt tablets, in a stainless steel clip into the perforated pipe, driven to the appropriate level where they were allowed to remain for two minutes. Surface flow immediately above the substratum was measured with a Kahl Pigmy Flow Meter.

Laboratory analysis of each vertical and surface sample included separation of the insects by hand picking, identification, counting, drying at 80 C and weighing. Prior to drying, the head capsule width of randomly subsampled individuals of the five predominant taxa were measured. Dry weights were expressed as totals per taxa per level sampled.

Results and discussion

Fifteen of the total 25 taxa recovered in vertical sampling devices exhibited stratification below 10 cm (Table 1). Low numbers of *Tabanus, Tricorythodes,* Hirudinea, *Corydalus, Heptagenia* and *Erpetogomphus* were collected in only the top 10 cm of substrate, leaving open to question whether they migrate below 10 cm. *Palopmyia, Chimarra, Neoperla clymene* and *Elophila* also appear to inhabit only the surface 10 cm (Table 1).

The mayfly *Neochoroterpes mexicanus* Allen, the larvae of the beetle *Stenelmis,* adult *Stenelmis bicarinata,* nematodes and oligochaetes exhibited a marked degree of stratification. Dominant insects recovered in vertical samples, indicated by % frequency occurrence (Table 1) were chironomid larvae, *Simulium* larvae, *Cheumatopsyche* larvae, *Neochoroterpes mexicanus,* and *Stenelmis* larvae. Because of sampler design, colonization by all organisms at various levels occurred through horizontal movements.

Coleman & Hynes (1970) found that in the Speed River, Wellington Co., Ontario, about 20% of total recovered organisms sampled occurred in the 0-7.5 cm level; about 26% occurred in the 22.5-30 cm level and the remainder were about equally distributed in the 7.5-15 and 15-22.5 cm levels. Mean percentages of total organisms in the Brazos River were 66.4%, 20.0%, 6.1% and 7.5%, respectively, in the 10 cm levels, from top to bottom. These differences in stratification between the two studies are probably accounted for by differences in substrate characteristics and organism adaptations to the sub-

Table 1. Total Catch/m^2 and % Frequency Occurrence of Organisms by Period Recovered Using Multi-level Residual Sampler, Brazos River, Texas, 1972-1973.

Taxa	Period 1		Period 2		Period 3		Period 4		Period 5	
	Total Catch/m^2	% Freq. Occur.	Total Catch/m^2	% Freq. Occur.	Total Catch/m^2	% Freq. Occur.	Total Catch/m^2	% Freq. Occur.	Total Catch/m^2	% Freq. Occur.
Diptera - larvae										
Chironomidae	431	4.0	513	3.0	4,994	22.6	1,025	8.3	163	3.3
Chironomidae pupae			6	0.04	281	1.3				
Simulium sp	231	2.1	563	3.3	4,169	18.9	119	1.0		
Simulium sp pupae	19	0.2	63	0.36	706	3.2			6	0.1
Tabanus sp	19	0.2	6	0.04						
Palpomyia sp	156	1.4			44	0.2	6	0.05		
Trichoptera - larvae										
Cheumatopsyche sp	1,369	12.7	594	3.4	481	2.2	1,506	12.2	531	10.7
Cheumatopsyche sp pupae					31	0.1				
Hydropsyche simulans					13	0.06	13	0.1	19	0.4
Chimarra obscura	25	0.23							13	0.3
Hydroptilla icona	25	-			13	0.06				
Pycnopsyche sp					19	0.08				
Ephemeroptera - naiads										
Neocoroterpes mexicanus	138	1.3	1,800	10.1	2,700	12.2	1,738	14.0	325	6.5
Tricorythodes sp	6	0.06			13	0.06	6	0.05		
Heptagenia sp										
Isonychia sicea manca									6	0.1
Plecoptera - naiads										
Neoperla clymene	13	0.10	6	0.4	19	0.08	56	0.5		
Lepidoptera - larvae										
Elophila sp							44	0.4	13	0.3
Odonata										
Erpetogomphys sp							6	0.05		
Coleoptera - larvae										
Stenelmis sp	8,338	77.3	13,606	78.82	8,469	38.3	7,756	63.0	3,856	77.4
Coleoptera - adults										
Stenelmis bicarinata	6	0.06			31	0.1			25	0.5
Stenelmis mexicanus					38	0.02	13	0.1		
Berosus sp	6	0.06			6	0.03				
Megaloptera - larvae										
Corydalus cornutus					6	0.03				
Turbellaria										
Dugesia sp			13	0.07					13	0.3
Oligochaeta			38	0.22	50	0.2	25	0.2	13	0.3
Nematoda			50	0.3	6	0.03				
Hirudinea			6	0.04						
Total Organisms	10,782		17,264		22,089		12,313		4,983	

strate. Coleman & Hynes (1970) described the Speed River substrate as coarse and fine silty gravel, intermixed with stones ranging up to 10 cm diameter; apart from a few larger stones at the surface, the gravel did not vary greatly with depth. The Brazos substrate on the other hand is composed basically of two layers: (1) the top 10-20 cm composed of coarse gravel with a few scattered larger stones, and (2) 20-40 cm of scattered gravel and larger stones surrounded by fine, packed sand and silt.

If insects have a behavioral tendency to migrate within the substrate, then one would expect the more even distribution observed by Coleman & Hynes (1970) in the less-layered substrate. In the Brazos, restriction of such movements by the packed sand and silt would be consistent with the observed predominance of insects in the surface 0-10 and 10-20 cm layers.

Stratification characteristics indicated in Table 1 probably were not related to temperature, since a maximum of only 3°C difference existed from the surface to the lowest 10 cm layer, on dates measured. During warmer months, water was colder in subsurface layers and the converse was true during the one winter measurement.

Oxygen and flow characteristics may have limited colonization of lower layers. Dissolved oxygen characteristically dropped from saturation (8.5-12.9 ppm) at the surface, progressively to as low as 0.4 ppm in the 30-40 cm level on January 16 (Figure 2). Flow, based on the two-minute dissolution rate of salt tablets, became virtually non-existent in the subsurface strata (10-40 cm).

Immediately prior to the removal of the June-August sample, a very heavy rain (approximately 14 cm) on the watershed below Possum Kingdom Dam, introduced a heavy silt load into the river and created flooding conditions. The subsequent sample indicated that insects, especially *Chematopsyche* and *Neocoroterpes mexicanus,* were reduced in the surface strata but numerous in the 10-20 cm layer (Table 1), possibly an escape response to heavy silting and scouring effects. Numerous conventional surface samples, taken after spates on the study site in previous years have indicated a depleted fauna, suggesting scouring and catastrophic drift. However, rapid recovery, not accountable by drift (because of the Dam's location with even greater expected scouring upstream and a strictly sand substrate river with meager fauna above the reservoir) remained a mystery. This observed movement into deeper strata during unfavorable surface conditions could explain such recovery.

The 0-40 samples were not retrieved. Silting in between the outer and inner cylinders and the unforseen weight of

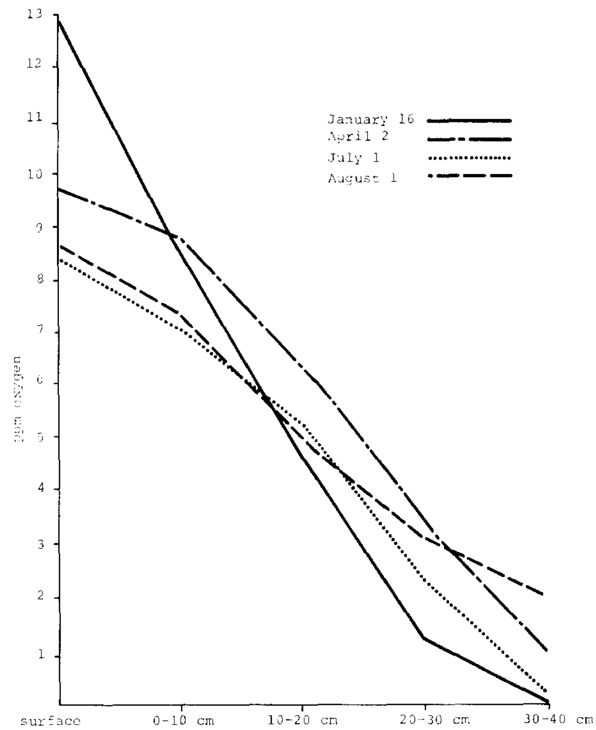

Fig. 2. Oxygen measurements per 10 cm level on four dates, Brazos River, Texas, 1972-1973.

the inner pot in relation to the bail strength precluded removal of an undisturbed sample.

The species list of insects collected during the course of the study is presented in Table 1 with catch/m^2 and % frequency occurrence. Organisms occurrence in percentages by level is presented in Table 2. The five most abundant insects removed from the vertical sample, Chironomidae, Simulium sp., *Cheumatopsyche* sp., *Neochoroterpes mexicanus,* and *Stenelmis* sp. were examined more closely to determine possible relationship between vertical stratification and life cycles. Distribution of size class by level for each period is presented in Table 3.

Stenelmis larvae were the most prevalent insects recovered from deeper strata. Density varied seasonally at different levels with largest populations always occurring in the 0-10 cm layer. Smallest numbers in the 20-30 and 30-40 cm layers were recovered in June and August, probably reflecting migration into upper strata during summer emergence. Largest numbers occurred in these deeper strata during winter and early spring (January, April). Smaller larvae, measuring 0.17-0.28 mm and 0.34-0.45 mm head capsule width (size class I and II, respectively), were most numerous in the 0-10 level. The

Table 2. Percent of Each Taxa Recovered By Level and Period Using Multi-Level Residual Sampler, Brazos River, Texas, 1972-1973 (continued).

Taxa	Period 1 % at each level				Period 2 % at each level				Period 3 % at each level				Period 4 % at each level				Period 5 % at each level			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Diptera - larvae																				
Chironomidae	94.2	1.4	1.5	2.9	89.0	6.0	1.4	3.6	93.8	1.3	1.5	3.4	92.6	4.3	0.06	3.1	100.0	-	-	-
Chironomidae pupae					-	-	-	100.0	95.5	-	-	4.5								
Simulium sp	75.6	18.3	6.1	-	81.0	15.5	-	3.5	90.2	5.0	1.2	3.4	86.8	15.8	10.6	36.8				
Simulium sp pupae	100	-	-	-	60.0	40.0	-	-	98.2	-	-	1.8					100.0	-	-	-
Tabanus sp	100	-	-	-	100.0	-	-	-												
Palpomyia sp	100	-	-	-					100.0	-	-	-	100.0	-	-	-				
Trichoptera - larvae																				
Cheumatopsyche sp	94.9	1.82	1.82	1.46	81.0	14.7	-	4.3	89.6	1.3	-	9.1	97.5	0.4	-	2.1	37.6	42.3	5.8	14.3
Cheumatopsyche sp pupae									100.0	-	-	-								
Hydropsyche simulans									100.0	-	-	-	100.0	-	-	-	33.3	33.3	-	33.3
Chimerra obscura	100	-	-	-													100.0	-	-	-
Hydroptila icona	75.0	-	25.0	-					100.0	-	-	-								
Pycnopsyche sp									66.6	33.4	-	-								
Ephemeroptera - naiads																				
Neocoroterpes mexicanus	81.8	13.6	4.6	-	90.6	7.3	1.7	0.4	73.4	15.7	8.6	2.3	97.8	0.7	0.7	0.8	1.9	90.3	1.9	5.9
Tricorythodes sp	100	-	-	-					100.0	-	-	-								
Heptagenia sp													100.0	-	-	-				
Isonychia sicea manca																	100.0	-	-	-
Plecoptera - naiads																				
Neoperla clymene	100	-	-	-	100.0	-	-	-	100.0	-	-	-	100.0	-	-	-				
Lepidoptera - larvae																				
Elophila sp													100.0	-	-	-	100.0	-	-	-
Odonata																				
Erpetogomphys sp													100.0	-	-	-				
Coleoptera - larvae																				
Stenelmis sp	55.6	28.2	10.6	5.6	58.3	21.3	7.5	12.9	46.4	14.4	12.3	26.9	72.3	17.9	3.2	6.6	55.5	34.0	7.2	3.3
Coleoptera - adults																				
Stenelmis bicarinata									40.0	20.0	-	40.0					-	100.0	-	-
Stenelmis mexicanus	-	100	-	-					100.0	-	-	-	50.0	50.0	-	-				
Berosus sp	-	100	-	-					100.0	-	-	-								
Megaloptera - larvae																				
Corydalus cornutus									100.0	-	-	-								
Turbellaria																				
Dugesia sp					50.0	-	-	50.0									50.0	-	-	50.0
Oligochaeta					66.6	16.7	16.7	-	-	12.5	25.0	62.5	100.0	-	-	-	-	-	-	100.0
Nematoda					-	25.0	25.0	50.0	-	-	-	100.0								
Hirudinea					100.0	-	-	-												
Total Organisms	63.9	22.7	8.9	4.5	64.0	19.0	6.4	10.6	72.3	8.8	6.4	12.5	80.6	12.0	2.3	5.1	51.2	37.5	6.3	5.0

Table 3. Distribution of Selected Organisms Per Meter^2 by Size Class and Level for Each Sampling Period.

TAXA	Period 1			Period 2			Period 3			Period 4			Period 5			LEVEL
	Size Class			Size Class			Size Class			Size Class			Size Class			
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	
Stenelmis sp	85	105	35	55	160	85	90	110	45	40	185	100	65	155	10	1
	5	45	195	10	115	155	40	60	105	20	105	100	20	185	30	2
	25	65	40	20	55	125	65	70	85	30	20	90	10	130	40	3
	25	130	95	15	70	85	10	90	125	10	85	80	20	65	10	4
	140	345	365	100	400	450	205	330	360	100	395	370	115	535	90	
Cheumatopsyche sp	155	45	5	25	80	110	5	15	145	120	55	30	0	15	45	1
	10	10	0	10	30	30	0	0	5	0	5	0	80	85	15	2
	10	10	0	0	0	0	0	0	0	0	0	0	10	10	5	3
	5	10	0	0	10	10	0	0	35	5	10	5	20	30	50	4
	180	75	5	35	120	150	5	15	185	125	70	35	110	130	115	
Neocoroterpes mexicanus	45	30	15	150	60	0	130	120	30	120	115	10	0	5	0	1
	0	10	5	40	50	5	95	90	20	10	0	0	35	75	20	2
	0	5	0	15	5	0	60	100	5	0	5	5	0	5	0	3
	0	0	0	5	0	0	20	30	0	5	5	0	5	5	5	4
	45	45	20	220	115	5	305	340	55	135	125	15	40	90	25	
Simulium sp	25	80	5	15	135	75	35	95	95	10	25	0	0	0	0	1
	0	35	0	0	25	45	20	105	25	5	10	0	0	0	0	2
	0	5	0	0	0	0	20	20	0	5	5	0	0	0	0	3
	0	0	0	10	5	0	10	65	25	0	35	0	0	0	0	4
	25	120	5	25	165	120	85	285	145	20	75	0	0	0	0	
Chironomidae	110	90	0	60	135	5	40	205	10	110	95	10	70	60	0	1
	0	0	5	15	10	0	10	40	0	20	10	5	0	0	0	2
	5	0	0	0	5	0	15	35	5	5	0	0	0	0	0	3
	10	0	0	5	10	0	25	85	0	0	15	0	0	0	0	4
	125	90	5	80	160	5	90	365	15	135	120	15	70	60	0	

largest larvae, size class III, (0.51-0.62 mm) were most numerous in the three deeper strata. *Stenelmis* larvae were the most evenly distributed among the various levels in all size classes.

The greatest numbers of *Cheumatopsyche* sp. larvae were recovered from the 0-10 cm layer in all periods except the last two which corresponded with known April-May, July-August emergence of *Chematopsyche* on the study site (Rhame, 1973). During the last sample period (June, August), the 10-20 cm layer contained the greatest number of organisms. This sudden increase in numbers during the June-August sample probably resulted from high water and heavy silting conditions brought about by the earlier described flood. The relatively large winter populations at this level could reflect migration to lower strata for overwintering, and low populations during summer could indicate migration to upper strata for emergence. Populations were the lowest in the 20-30 cm range, and on some sample dates, no *Cheumatopsyche* at all were recovered from this level. Smaller larvae, size class I, (0.228-0.487 mm) were most abundant in the 0-10 cm layer, as were size class II, (0.572-0.801 mm) and III (0.858-1.144 mm). In the deeper strata, especially in the 10-20 and 30-40 cm levels, the larger size classes predominated.

Simulium was recovered in greatest abundance from the 0-10 cm level. Population densities in the upper stratum follow a trend that would be expected with observed early spring emergence. The absence of *Simulium* from any sample in August would suggest that all individuals were in the egg or very early larval stage; apparent emergence and long egg dormancy on the Brazos correspond with life cycle notes on *Prosimulium vernale* Shervell by Tarshis & Stuht (1970). *Simulium* found in the lower strata, especially the 575/m^2 at 30-40 cm in April, are puzzling, when the generally accepted filter-feeding habit is considered. Coleman & Hynes (1970) found that *Simulium* that colonized their samplers disappeared from lower strata after 7 days. The largest numbers of all three size classes of *Simulium* were present in the 0-10 cm level (Table 1). The smallest larvae, size class I (0.172-0.342 mm) were least abundant in all levels and decreased markedly from level I to the other three 10 cm levels. Size class II (0.400-0.629 mm) was the most abundant encountered at all levels. The presence of size class III (0.686-0.858 mm) decreased with depth.

The largest populations of *Neochoroterpes mexicanus* were found in the upper level although the numbers at the different levels varied with season. Highest numbers in all levels occurred in the January-April sample followed by a drop in the 10-40 cm levels, possibly indicating a movement to the surface for feeding and in preparation for a summer emergence. This is substantiated by the drop in the surface populations between the June and August samples. The great increase in *Neuchoroterpes* in the 10-20 cm layer in August is probably an escape response to avoid unfavorable surface conditions brought about by the large flood already described. The smaller larvae, size class I (0.229-0.515 mm) were present in large numbers in the 0-10 cm stratum and decreased progressively with depth down to 40 cm. Size class II (0.572-0.915 mm) was present in moderate numbers in the upper layer and also decreased progressively with depth. The third size class (0.972-1.315 mm) varied little with depth but the two upper levels 0-10 and 10-20 cm contained more than the lower two layers 20-30 and 30-40 cm.

The 0-10 cm layer contained the greatest populations of chironomid larvae in each sample, and was consistently high compared to the three lower levels. The population increase during the January-April sample is probably in preparation for summer emergence. This explains the drop in population in all levels during the April-June and June-August samples. The 0-10 cm level contained the greatest number of all the size classes. Size class I (0.057-0.172 mm) decreased steadily with depth except for the 30-40 cm level which showed a slight increase over the 10-20 and 20-30 cm levels. Size class II (0.228-0.400 mm) had the highest numbers in the 0-10 cm level of any of the size classes and decreased with depth, with the exception of the 30-40 cm level which also showed an increase in numbers. The third size class (0.458-0.515 mm) was in low numbers in every level and absent in the 30-40 cm level.

A comparison of Student's 't' test of both biomass and numbers of the total benthic fauna recovered from the different levels, from all levels combined, and from the modified Hess sq. ft. sampler is presented in Table 4. Level 1 (0-10 cm) contained significantly more organisms than any other single level or all levels combined, although it did not generally contain significantly greater biomass. This is owing to the predominance of smaller size classes in the upper layers. Levels 2 (10-20 cm), 3 (20-30 cm) and 4 (30-40 cm) showed no significant difference among themselves, except the weight of animals in level

Table 4. Comparison by Student's 't'[1] of Mean Weights and Numbers of Organisms Recovered in Vertical and Surface Samples.

	Mean ± sd	N	All Levels	Level 4	Level 3	Level 2	Level 1
Hess	2.96 ± 1.42 / 15,198 ± 14,570	15 / 15	0.09 / 0.33	2.28*[2] / 2.14*	3.50**[3] / 2.33*	0.87 / 1.31	2.57* / 3.07**
Level 1	7.14 ± 6.10 / 37,105 ± 21,198	5 / 10	1.95 / 3.62**	2.08 / 4.66**	2.36* / 4.68**	1.72 / 4.08**	. . / . .
Level 2	2.35 ± 1.07 / 8,820 ± 5,549	5 / 10	0.29 / 0.81	1.36 / 1.62	3.42** / 1.72	. . / . .	. . / . .
Level 3	0.65 ± 0.47 / 3,235 ± 2,710	5 / 10	1.24 / 1.76	1.02 / 0.48	. . / . .	. . / . .	. . / . .
Level 4	1.30 ± 1.34 / 4,786 ± 5,575	5 / 10	0.87 / 1.52	. . / . .	. . / . .	. . / . .	. . / . .
All Levels	2.86 ± 3.91 / 13,486 ± 17,138	20 / 40	. . / . .	. . / . .	. . / . .	. . / . .	. . / . .

[1] "t" values are expressed as weight/number

[2] Significant at the 5% level

[3] Significant at the 1% level

2 was significantly greater than that in level 3 (Table 4). This was expected, since level 3 generally showed consistently lower numbers of all size classes than other levels. In most samples, the greater numbers of benthos at level 4 compared to level 3, while not significantly different, were probably due to greater numbers of *Stenelmis* in the lowest level.

Comparison of the mean organism/m^2 collected by the modified Hess sampler with those from the various 10 cm levels indicated that it took significantly more organisms than the multi-level samplers at 20-30 and 30-40 cm, but not more than the samplers at the 0-10, 10-20 cm levels. This is in part due to there being more animals available to the Hess at the surface than to the multi-level samplers at 20-30 and 30-40 cm. However, in view of the significant difference in numbers between levels 1 and 2, the lack of significance between the Hess sampler and the 10-20 cm samples indicates that use of the Hess omits a significant proportion of the population per unit of area sampled. Further, the significantly greater number/m^2 collected by the 0-10 cm vertical sampler over the Hess suggests that the multi-level sampler is the most efficient. However, addition of a 5-10 cm metal ring (Waters & Knapp, 1961) around the bottom of a Hess sampler might increase its efficiency by allowing deeper penetration. There was no significant difference between Hess samples and the combined 10 cm levels because of the large variance for the different levels of the multi-level sampler.

In agreement with Ford (1962), these data suggest that on the Brazos River, either a vertical sampler or a further modified surface Hess-type sampler that would effectively census the 0-20 cm level would give population estimates with acceptable accuracy while taking into account economy of sampling effort.

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Summary

1. Vertical stratification samplers modified after those of Coleman and Hynes (1970) were used to sample macrobenthos at 10 cm levels down to 40 cm in the substrate of the Brazos River, Texas, from July 1972-August 1973. Observations concerning insect emergence, reaction to physical parameters, and life histories were recorded. Sampling efficiencies of a modified Hess sampler and the vertical samplers were compared.

2. Of the 25 species recovered during the study, 15 were found in samples below 10 cm. Mean percentages of the total organisms per 10 cm level showed 66.4% in the 0-10 cm level, 20% in the 10-20 cm level, 6.1% in the 20-30 cm level, and 7.5% in the 39-40 cm level.

3. Frequency analysis showed the dominant insects in the vertical samples were chironomid larvae, *Simulium* larvae, *Cheumatopsyche* larvae, *Neochoroterpes mexicanus* naiads, and *Stenelmis* larvae.

4. Population peaks in the top 10 cm of substrate corresponded with observed emergence of the five major groups. Head capsule measurements indicated that the surface level 0-10 cm contained the smaller insects. The larvae of *Stenelmis* were the most evenly distributed among the various levels in all size classes of the five predominant groups.

5. Migration to a lower level following a large spate on the Brazos River immediately prior to removal of the last sample, especially by *Cheumatopsyche* and *Neochoropterpes mexicanus,* was possibly a reaction by the insects to escape the adverse conditions of swift flood water and a heavy silt load.

6. Measurements of oxygen and temperature within the substratum were recorded on four sampling dates (January 16, April 2, July 1, August 1) and oxygen appeared to be the most limiting factor to vertical stratification. Oxygen measurements in the substratum showed a drop from saturation (8.5-12.8 ppm) at the surface to a minimum (0.4-0.7 ppm) at 30-40 cm. Temperature differences ranged a maximum of only 3°C between the surface and the 30-40 cm level on the dates measured. Flow measurements using an indirect salt tablet dissolution method on the last sample date suggested that little flow was present below 10 cm.

7. A students 't' analysis of the benthic populations recovered by the modified Hess sampler and the vertical stratification sampler, indicated that the latter recovered significantly greater populations in the 0-20 cm level. A comparison of the Hess sample and 0-40 cm combined vertical sampler showed no significant difference between the two.

8. The results of these studies indicate that the most efficient sampler for riffle studies on the Brazos River would be an *in situ* sampler which penetrated from 0-20

cm, giving an accurate assessment of the population and an economy of sampling.

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