With my compliments,
Dudley Williams

Lewis Berner

The occurrence of benthos deep in the substratum of a stream

D. DUDLEY WILLIAMS *and* H. B. N. HYNES *Department of Biology,
University of Waterloo, Waterloo, Ontario, Canada*

Summary

(1) The vertical distribution of the benthic fauna of the Speed River, Ontario, was studied over a 13-month period from October 1970 to October 1971. Various physical and chemical parameters of this interstitial environment were also measured.

(2) Several new techniques for sampling the interstitial environment of rivers were devised. These methods and their relative efficiencies are considered.

(3) The validity of the terms 'hyporheal' and 'hyporheic' are discussed and the term 'hyporheos' is offered to replace the former.

(4) A brief résumé of interstitial sampling methods is given with comments on their limitations for sampling deep heterogeneous substrates.

(5) Chemical parameters are thought to be more important in the control and distribution of the fauna than physical parameters.

(6) It is suggested that many larvae of stream-dwelling chironomids have over-wintering stages when they penetrate deep into the substrate to: (a) actively feed on the trapped organic detritus; (b) follow an optimum temperature for development.

(7) It is suggested that the shape of an organism determines its success as a hyporheic form and examples are given.

(8) The numbers of animals occurring in the sub-benthic populations are shown to be very large indeed. For the Speed River, estimates of between 184,760 and 797,960 animals/m^3 are made for different times of the year. Dry weight biomass is estimated to vary between 30·9 g and 253·2 g/m^3 throughout the year.

(9) Sub-benthic or hyporheic populations are shown to exist in at least three other Canadian rivers. Some of the animals found are shown to be common to two or more of these rivers.

(10) The inefficiencies of many conventional benthic samplers in sampling the total biomass of certain streams with hyporheic populations is discussed.

Introduction

Interstitial spaces have long been regarded as refuges for small animals (Angelier, 1953), some of which have become very specialized to this type of environment. Such

Correspondence: Dr D. D. Williams, Department of Biology, University of Waterloo, Waterloo, Ontario, Canada.

habitats occur in marine, brackish and fresh waters, and in the soil, and some of the truly interstitial animals have been shown to have wide geographic distributions (Cook, 1969), suggesting that they are survivors of ancient faunas (Delamare-Deboutteville, 1957).

In fresh water we find two basic types of interstitial environments, namely, lentic, where the flow is negligible, and lotic, where the interstices are continually permeated by flowing water. Sassuchin, Kabanov & Neiswestnowa-Shadina (1927) were perhaps the first to examine fluvial deposits, but it was not until 1942, when Chappuis initiated a reasonably successful sampling technique, that any impetus was given to this area of study. Both Kühtreiber (1934) and Aubert (1959) suggested that stream fauna could occur elsewhere than on the stream bed. Aubert, for example, noted that the larvae of some Leuctridae were found far less often than the adults, and Berthèlemy (1966) showed that these larvae could be found in specialized areas deep within the stream-bed.

The interstitial environments of rivers can be classed into two somewhat distinct groups. The first is the sand along the sides of, and in sandy areas of the potamon region of rivers. The former resembles the psammon of lakes, and the latter is a thin difficult habitat where small highly specialized animals occur (Neiswestnowa-Shadina, 1935). The second, is the 'hyporheic' region of the rithron, where the interstices are formed in a mixture of coarse sand, gravel and rocks.

The term hyporheic was coined by Orghidan (1953, 1959) and has since been used by various researchers (Angelier, 1962; Coman, 1961; Ruffo, 1961; Schwoerbel, 1961; Coleman & Hynes, 1970). Motas (1962), however, considers it to be superfluous, because he claims that this biotope is a part of the groundwater, or the 'nappe-phréatique' of Daubrée (1887). He further considers that use of the term erroneously implies the existence of two distinct faunal groups in the groundwater zone, one occurring in underground wells and rivers and the other in the interstitial water beneath streams. Chappuis (1950) and White (1960) seem to support this concept in that they used the term 'phreatic' alone, moreover Delamare-Deboutteville (1957) considers the fauna of the water beneath rivers to be the true phreatic fauna. Orghidan (1959) and Schwoerbel (1961) on the other hand believe the hyporheic to be a middle zone, bordered by the epigeic water of the stream above and by the true groundwater below. Both state that its physical and chemical characters fluctuate because of considerable flushing by surface water. It cannot, therefore, be included in the definition of groundwater, in which these parameters remain fairly constant. We support the view that this intermediate zone be known by a separate term, but we prefer to continue to use the term hyporheic rather than to follow the suggestion of Husmann (1966, 1970) who attempts a unification of terms for ecosystems, biotopes and biocoenoses, and suggest that the hyporheic zone is equivalent to his newly-coined 'rithrostygon'.

Similar disagreements exist with regard to nomenclature for the animals of the hyporheic zone. Angelier (1953, 1962) divides the fauna into three distinct groups: the psammoxenes—whose presence in the substrate is accidental; the psammophiles which occur in the interstices for only part of their lives; and the psammobites which are restricted to this zone.

A similar classification is put forward by Schwoerbel (1961) who divides the 'hyporheal' into two groups. The first consists of animals which invade the substrate as protection against the current, is made up primarily of Cladocera, insect larvae and Harpacticoida, and occurs mostly in the upper levels of the substrate. The second,

represented by the Porohalicaridae and the Nematoda, increases in number with depth into the substrate.

Motas (1962) again challenges the term hyporheal to describe the fauna, claiming that it has already been used in another context (Kraus, 1959).

We support the need for a separation of terms and we favour the division made by Schwoerbel. However, since hyporeal seems to have been wrongly applied, we would suggest that it be replaced by 'hyporheos'; we also suggest that this fauna be sub-divided into the 'occasional' and the 'permanent', the former to refer to the larvae of the surface benthos which seek out this habitat during part of their life cycle, and the latter to refer to forms such as copepods and mites which complete their entire life cycle there.

Interstitial sampling methods

Sampling methods for the interstitial habitats of streams can be classed under four main headings.

Ditch digging. This is perhaps the simplest method and was used by Sassuchin in 1930. The limitations are that it is not quantitative and it cannot be used in mid-stream.

Frozen cores. These were used by Efford (1960) to sample shallow slow-flowing streams, but the method is inapplicable to the hyporheic zone where particle sizes are large and very mixed. The method described by Ryan (1970) may be more suited to this type of substrate but it has not been used to sample interstitial fauna.

Mechanical corers. These may be designed to sample to considerable depths but they work only in soft homogeneous substrata, and so are not useful for work in streams. Box samplers such as those of Cummins (1961) and Mundie (1971), which can be used on hard heterogeneous substrata, can be made to penetrate to only shallow depths, and thus can give little information on the hyporheos.

Colonization of substrates. This was first used by Moon (1935) and involves placing a cleaned portion of the natural substrate into a perforated container sunk into the stream bed. The container is removed after it has become colonized. The problems arising from this method are numerous. Firstly, much of the fine silt and organic detritus is lost when the material is initially removed from the stream bed. This is important as both Schwoerbel (1961) and Egglishaw (1964) have shown the amount of organic detritus to be a major factor controlling the number of animals present. Secondly, the pore space is inevitably altered and this may lead to unnatural recolon-ization. Thirdly, there is little agreement about the time necessary for complete recolonization (21–28 days, Crowe, 1970; 28, days Mason, Anderson & Morrison, 1967; more than 28 days, Coleman & Hynes, 1970). Lastly, the time factor involved prohibits quick on-the-spot sampling such as would be needed to determine, for example, the redistribution of animals after abnormal stream conditions such as floods or droughts.

Sampling programme

With the various failings of the above techniques in mind, we designed a standpipe corer to sample the hyporheos. It was used in conjunction with a more conventional surface sampler in the hope that we could make an accurate description of the total benthic biomass and its seasonal distribution and variation. Faunal samples were taken, each month for 13 months (October 1970–October 1971), together with various physical and chemical measurements of both the surface and interstitial waters.

The study area

Most of the work was done on the Speed River, Wellington County, Ontario approximately 3 km from its source in East Garafraxa Township. The exact area chosen was a uniform riffle some 100 m long, Rowan's Farm (80° 16′ 24″ W., 43° 43′ 54″ N.). A detailed description of this area is to be found in Bishop & Hynes (1969). A few sets of samples were also taken from other rivers for comparison.

Materials and methods

(a) *Water chemistry of the Speed River*

Water samples were taken from both the surface and the interstitial waters. The former were taken directly and the latter with a standpipe.

Many techniques have been developed for the extraction of sub-surface water. Standpipes were used by Borden (1931); Gangmark & Bakkala (1958); McNeil (1962) and Brafield (1964), and other methods are reviewed by Creaser (1971). Most are, however, unsuited to the coarse substrate of stony streams, so we designed a heavy-duty steel standpipe which functioned adequately in this environment.

The standpipe (Fig. 1), which has an internal diameter of 2·5 cm and a solid conical tip, has four sets of holes near its apex. These were closed by a plug rod 'A' whilst the pipe was being driven into the substrate. During this procedure the top was protected with a metal cap 'C' from the blows of a small hand operated, sleeve-shaped stake driver 'D' (see Stocker & Williams, 1972 for details of this). The cap transmitted the force to a ring welded to the outside of the pipe as shown. Once the pipe had been driven to the appropriate depth, as indicated by the change in position of the water level on the body of the pipe, the cap and plug were removed and the close-fitting Perspex tube 'B' was inserted in the open position and pushed down to the bottom of the pipe. After a few minutes, the interstitial water had risen in the tube which was then closed by pulling on the rod 'X' to seal the bottom end of the tube with a rubber bung 'Y'. Next the top end of the tube was closed with a rubber bung 'Z'. This enabled the tube to be removed from the standpipe without loss of water through the glass tube 'W'. The first two columns of water obtained were always discarded, as it was possible that they had been contaminated by surface water as the pipe was driven in. The third and later tubefulls were regarded as true samples of interstitial water and were released into bottles by releasing the bung 'Z'. When water was collected for oxygen determinations a piece of rubber tubing 'T' was fitted over the end of the glass tube so that the sample could be released directly under paraffin oil in a conical flask. It did not therefore come in contact with the atmosphere.

Oxygen, carbon dioxide, pH and total alkalinity were measured once every 2 months and B.O.D. and interstitial organic matter were measured twice during the 13-month period. pH was measured in the field using a portable Metrohm (E280A) meter. Oxygen was measured by the modified Winkler method for waters rich in organic matter (Alsterberg, 1926), and alkalinity by titrating against 0·01 N HCl; free carbon dioxide was calculated from this value and the pH. The B.O.D. was invariably high and so samples were incubated over a 24-h period rather than the standard 5 days.

Organic matter was estimated by three different methods. The concentration in the interstitial sediment was found using the oxidation by hydrogen peroxide method (Judd & Weldon, 1939) and a wet oxidation technique (modified after Maciolek,
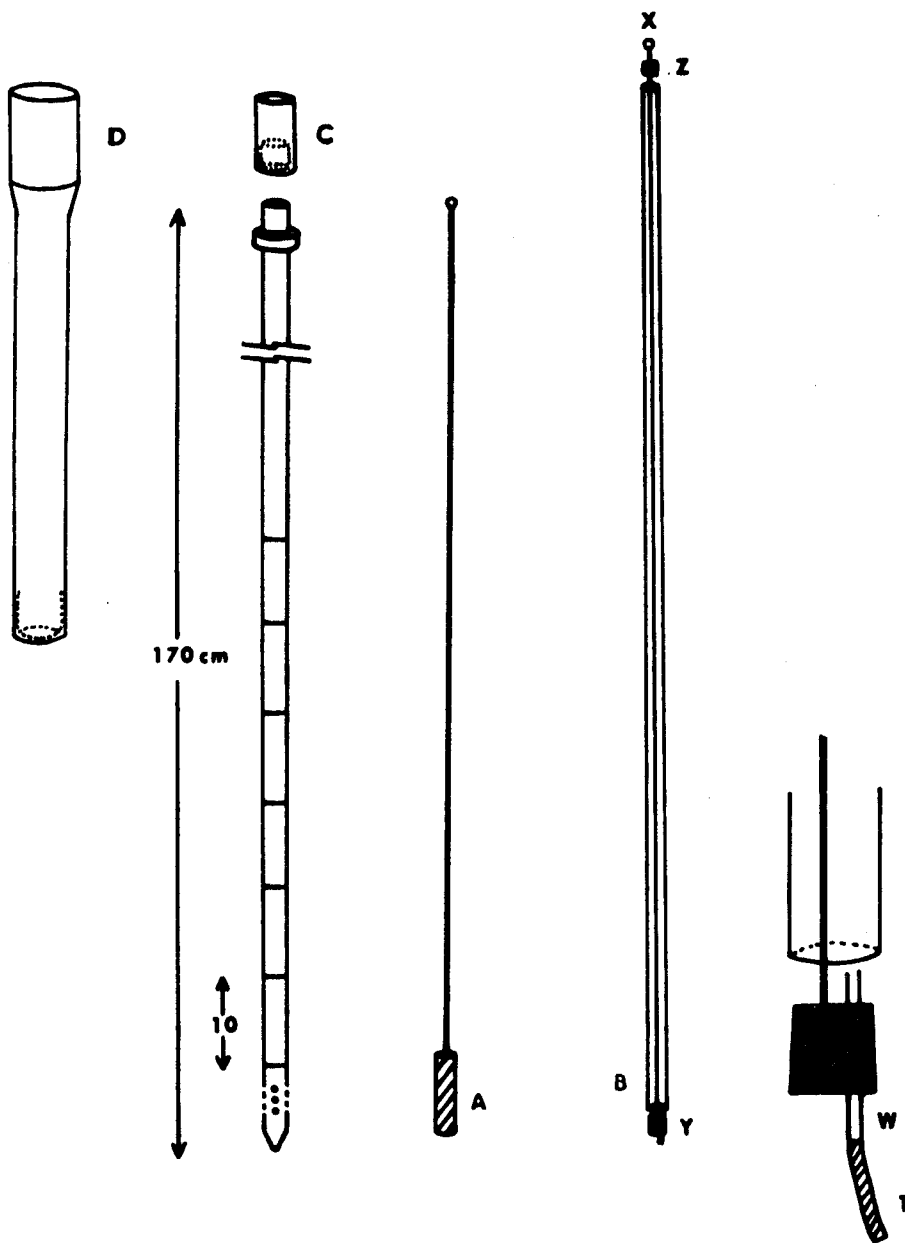
Fig. 1. The interstitial water sampler and stake driver.

1962). These gave values approximately ten times those obtained using a high temperature (450°C for 24 h) weight reduction method on entire gravel samples.

(b) *Physical characters of the Speed River*

Water height and current velocity were measured whenever work was done on the river. The height was measured with a metre stick at a fixed position, and current

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was measured with a direct reading current meter at several points across a reference board placed flush with the substrate. Temperature was monitored throughout the year with a multi-channel Scanning Telethermometer and a laboratory recorder (Y.S.A. models 47 and 80 respectively). Thermistor probes were buried at 10-cm intervals in the stream-bed from 60 cm to the surface. Air temperature was also recorded.

The flow of water through the gravel is difficult to measure, but it is obviously important to sub-benthic animals. Several methods have been devised for measuring it, mostly with reference to salmonid eggs, and most are based on standpipes, although electrolytic baths (Vaux, 1968) and manometers (Stuart, 1953) have been used in laboratory situations. Basically the standpipe methods are of two types, those that measure dilution of a salt solution by intragravelar water (Gangmark & Bakkala, 1958) and those that trace the path of an introduced dye (Pollard, 1955).

In this study we traced the movement of fluorescein dye, following Doyle (1906) and Sheridan (1962). The standpipe shown in Fig. 1 was used in conjunction with a similar 'fluoro-probe' standpipe of smaller diameter (1 cm I.D.).

Both pipes were plugged and driven to the same depth in the substrate, the fluoro-probe being a known distance directly upstream of the standpipe. Both plugs were then removed and the Perspex tube inserted into the standpipe. Fluoroscein was introduced into the fluoro-probe and water samples were taken from the standpipe at 10-sec intervals until the colour appeared. The experiment was repeated for different horizontal distances which, when graphed, showed a linear relationship between the time taken and the distance travelled by the dye. The intragravelar current velocity was then determined from the slope of the graph. This procedure was repeated for various depths at 10-cm intervals. Surface current was also recorded with a portable velocity meter.

The study of the composition of the substrate involved liquid nitrogen to freeze the substrate, in situ, around a standpipe driven into the stream bed, and has already been described (Stocker & Williams, 1972).

(c) *The fauna of the Speed River*

As none of the sampling methods reviewed earlier was without drawbacks a new method was designed, with which it was possible to sample to depths of 1 m or more in streams of mixed substrate. The method involved the use of the standpipe corer shown in Fig. 2. The pipe itself has an internal diameter of 2·5 cm, a solid conical tip, a notch 'N' at the top and two openings (10 cm in length) shielded by two welded wings near the tip. The core-rod 'A' fits inside the pipe so that when in the open position 'O' (shown by the stop 'B'), its diametrically opposite windows coincide with the two openings in the standpipe. In the closed position 'C' (again shown by the stop 'B') the windows of the core-rod are opposite the closed walls of the pipe. When taking a sample, the core-rod was placed in the closed position and a lock 'K' was placed over the top to prevent the apparatus from opening whilst being driven in. The tip of the core-rod has a rubber bung 'X' set in it to cushion it from blows applied to the protective cap 'P'. The heavy sleeve stake driver, previously described, was then used to pound the standpipe into the substrate. It is almost certain that the pounding has little effect on the fauna as the whole process is over in a few seconds. Once the desired depth was reached the cap 'P' and the lock 'K' were removed and the core-rod was turned to the open position by means of a turnkey 'T'. An ordinary heavy duty
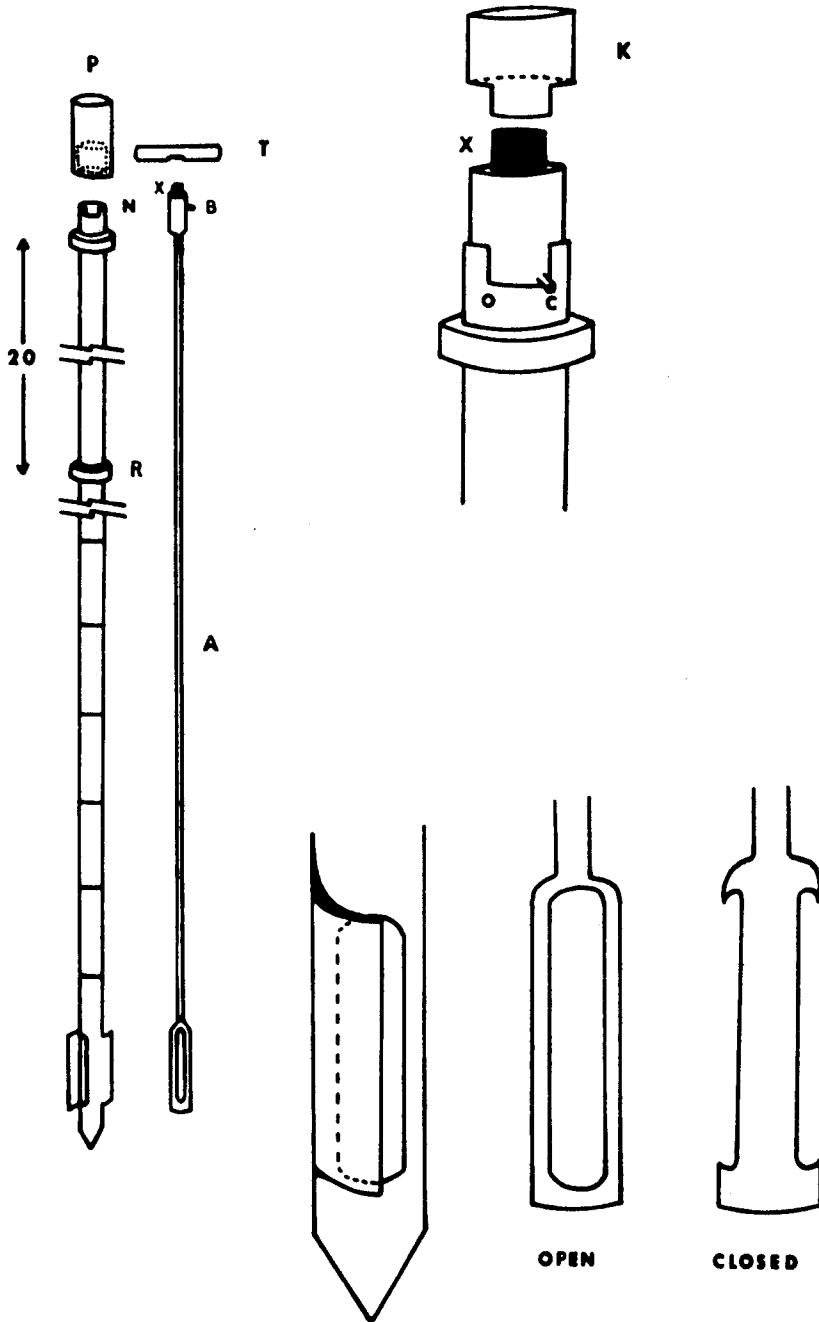
Fig. 2. The standpipe corer, with details of the sampling chamber and locking device.

metal pipe-wrench was then locked around the lower welded ring 'R', which has two flattened faces, and the whole was turned in an anti-clockwise direction. This causes the wings to scoop gravel into the chamber of the core-rod.

The possibility arose that animals from the surface could be dragged downwards by a vortex caused by a combination of the turning of the pipe and waterflow against the pipe. However, tests made by placing coloured sand on the substrate surface around the pipe showed less than 1 % of objects on the surface to be collected in the subsurface cores.

After the requisite number of turns (which can be gauged with practice) the core-rod was rotated to the closed position thus sealing off a volume of 25 ml of substrate, and the pipe was pulled out of the stream-bed. Its tip was quickly placed into a poly-ethylene bottle containing 5 % formalin solution, the core-rod was rotated and removed and the gravel was washed out into the bottle. Five such 25-ml replicate samples were taken, at 10-cm intervals from 10 cm to 70 cm depths, each month from October 1970 to October 1971.

Obviously, comparative surface samples could not be taken with this corer so the surface benthos was collected by means of kick samples (Hynes, 1961). This method was chosen initially because of its simplicity. Two kick samples were taken each month, each was halved and then two of the opposite halves were recombined to give one whole sample. In this way a larger area could be sampled with a minimum amount of analysis. In order to make these data comparable to those obtained with the corer, it was necessary to express the kick sample area as a volume. Each kick sample disturbed an area of approximately 30 cm by 30 cm to a depth of 5 cm, and thus involved 4500 ml. This is thirty-six times that of the five core unit of 125 ml, and so we can make a reasonable comparison between surface and subsurface benthos by dividing the number of animals obtained in the surface samples by thirty-six.

In the laboratory, the samples were strained through a net with sixty-three meshes per cm and then floated with saturated calcium chloride solution (Hynes, 1961). The animals were then preserved in 70 % alcohol and sorted under a binocular microscope.

Results and observations

The stream-bed as an interstitial environment

Physical conditions. Sets of two frozen cores were taken at depths of 0–30 cm and 30–60 cm, respectively, in the river bed. From these, particle size distributions, porosity, mean grain size and heterogeneity of grain size were calculated for 10-cm intervals over the 0–60-cm depth range. These results, which are given in detail by Stocker & Williams (1972), show a definite transition zone at a depth of approximately 30 cm in the gravel. In this region, the mean grain size and the heterogeneity of the grain size decrease and the porosity increases.

The fact that the sediments are layered below 30 cm suggests that they are undis-turbed by the surface water. Similarly, the mixed nature of the upper 30 cm suggests that aerated surface water regularly penetrates to this depth, but no further. This is borne out by a lack of detectable oxygen and a build-up of carbon dioxide below 30 cm. Cooper (1965) has similarly recorded surface water penetrating to a depth of 46 cm in a clean homogeneous gravel bed. pH also shows a change at this level.

Examples of measurements of intragravelar flow of fluorescein at various depths are shown in Fig. 3(a) where the horizontal distance travelled is plotted against time. The intragravelar velocities are shown by the slopes of the graphs, and two such series together with the corresponding surface water velocities are shown in Fig. 3(b). The intragravelar flow is very much less than the surface flow, and it seems to decrease with depth until at 50 cm it is negligible. It also appears that even though the range
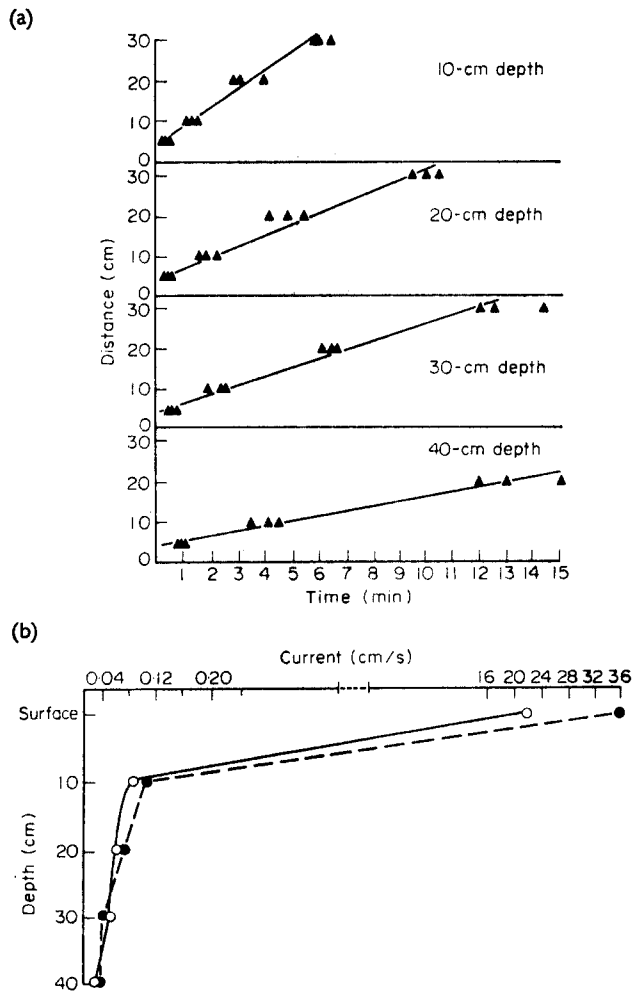
(a)

(b)

Fig. 3. (a) Graphical determinations of intragravelar water velocities to 40 cm. (b) Two plots of water velocity *vs.* depth for the Speed River.

of the surface velocities may be large the resulting changes in the interstitial velocities are small indeed.

Although temperature data were obtained at 10-cm intervals down to 60 cm, for the sake of clarity, only those for substrate depths of 10 and 60 cm and surface water over a period of 12 months are shown in Fig. 4. As might be expected, whilst the air temperature fluctuated widely over the year (−20·0–28°C) the surface water did so to a much lesser extent. The substrate temperature varied even less, i.e. between 0·5°C and 18·0°C at 60 cm as compared with 0·0–24·0°C for the surface water. The upper 20 cm of substrate tended to follow the surface water temperature more closely than did the lower layers. Daily fluctuations of the surface water temperature were higher than those of the substrate temperature, especially during the spring. This was probably due to the buffering effect of the groundwater and the high specific heat of the substrate. Generally speaking, the substrate temperature was on the average 3·0–4·0°C lower than the surface water temperature during the summer and 0·5–1·0°C warmer in mid-winter.
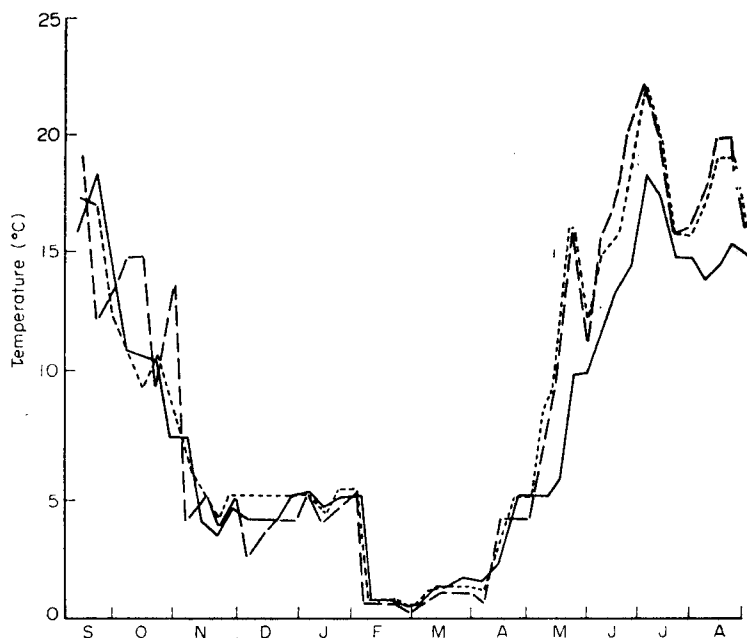
Fig. 4. Surface and interstitial water temperatures for the Speed River (1970–71). – – –, Surface water; . . . ., 10 cm; ————, 60 cm.

Chemical conditions. Except on one occasion, oxygen was not detected in the substrate below a depth of 30 cm. Fig. 5(a) shows that the amount decreased in an almost linear way with depth to this point (the various symbols represent separate sets of samples). Usually there was a drop of from 20 to 60% saturation from the surface to the interstitial water at a depth of 10 cm. From 10 to 20 cm, there was a further drop of between 20 and 30% saturation, until at 30 cm the oxygen saturation is only about 5% of that found in the surface water. These values differ quite markedly from those obtained by Schwoerbel (1961), who found only a slight decrease with depth, and values of between 45 and 75% saturation at 30 cm for the German river he worked on, and Husmann (1971), who obtained an oxygen decrease of more than 50% for the river Lautenthal in the first 3 cm of substrate, with a sharp increase back to approximately 80% saturation at 6 cm, the level remaining high down to 30 cm.

Carbon dioxide measurements showed similar amounts in the surface water of the first 30 cm of substrate (Fig. 5b), but below 30 cm, concentrations were three to four times as great. Similar values were obtained by Husmann (1971), but Schwoerbel (1961) found that hardwater streams exhibit the smallest increase in carbon dioxide with depth. This does not appear to be supported by the above results.

The pH of the surface water ranged from 7·85 to 8·2 throughout the year and it declined slowly down to 30 cm whence it remained fairly constant down to 70 cm at between 7·4 and 7·6 (Fig. 5c). This decrease in pH with depth has been shown by many workers, but the amount of decline reported varies from 0·1 to over one unit (Sassuchin, 1930; Angelier, 1953; Ruffo, 1961; Schwoerbel, 1961; Husmann, 1971).

Organic matter determinations are shown in Fig. 5d, the values for the fine interstitial sediments being ten times those for the total substrate (the different analytical
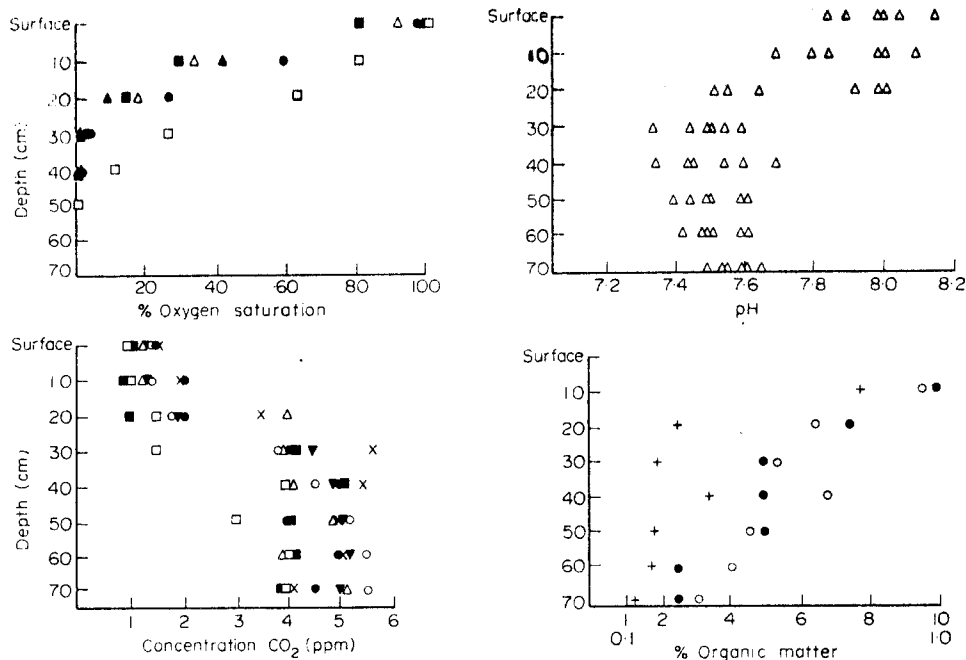
Fig. 5. Plots of various chemical parameters *vs.* depth in the substrate. (a) Percentage oxygen saturation; (b) dissolved carbon dioxide; (c) pH; (d) percentage organic matter.

methods indicated by the symbols $+$, $\bullet$, and $\bigcirc$ respectively). There also seems to be a general almost linear decrease in the amount of organic matter with depth.

Table 1 shows the mean B.O.D. of three replicate samples at various depths for August and October 1971, when the temperatures were approximately 16° and 8°C respectively. Apart from a slight peak at 20 cm, the B.O.D. of the substrate decreases with depth and is greater at the higher temperature.

The numbers of animals. The total numbers of animals found during 13 months and the percentages found at the various depths sampled are shown in Table 2. It should be noted that some were regularly found to a depth of 70 cm, although their numbers generally decreased to this depth. A second point is that the maximum number of animals occurred at a depth of 10 cm in the substrate. Few were ever found at

Table 1. Relationship between B.O.D. and depth in substrate for two different temperatures

Depth (cm)	24 h B.O.D.	
	October 1971 (at 8°C) (mg O_2/l)	August 1971 (at 16°C) (mg O_2/l)
10	3·80	4·08
20	3·91	4·15
30	3·75	4·05
40	3·32	3·75
50	3·05	3·62
60	2·68	3·70
70	2·70	3·70

Table 2. Percentage depth distribution of total numbers of animals

Date	Surface	10 cm	20 cm	30 cm	40 cm	60 cm	60 cm	Depth 70 cm	Total number of animals*
1970									
Oct.	—	35·3	30·4	13·3	10·0	2·9	3·8	4·3	368·0
Nov.	1·6	32·4	24·8	16·6	5·7	10·6	5·3	3·0	696·6
Dec.	2·4	29·7	30·4	19·3	6·2	3·3	3·5	5·2	904·6
1971									
Feb.	1·5	53·8	20·8	16·7	2·8	1·8	1·8	0·8	931·0
Mar.	3·5	34·8	21·4	19·7	6·6	2·1	11·3	0·6	798·9
Apr.	9·1	35·4	19·4	16·4	8·0	8·7	1·9	1·1	262·9
May	2·8	39·6	21·1	13·2	16·1	2·9	1·5	2·9	659·4
Jun.	3·8	15·7	21·5	32·5	4·8	7·3	8·3	3·3	688·9
Jul.	2·7	51·9	18·6	5·9	8·0	6·1	3·8	2·9	522·1
Aug.	3·5	40·3	22·1	9·7	8·9	7·1	5·0	3·4	756·4
Sep.	2·7	28·3	22·3	17·9	13·0	6·7	4·6	4·6	1142·4
Oct.	2·4	31·4	28·3	18·9	5·1	6·1	6·1	1·7	1028·9

* Fractions obtained are the result of scaling down the kick sample volume to fit the core sample unit.

80 cm and so for the Speed River, 70 cm was considered a sufficient maximum sampling depth. Another obvious feature is that the numbers varied quite markedly from month to month, with maxima occurring in the winter (December, possibly January, and February) and autumn (August–October). The peaks in mid-winter and autumn were perhaps caused by young larvae hatching from eggs laid in late summer and mid-summer, respectively, as many of the insects, particularly the chironomids, were very small. The numbers of animals occurring on the surface of the substrate seem almost insignificant as compared with those found beneath it. The percentages of animals occurring at each depth remained fairly constant throughout the year. Exceptions to this are February and July, when more than 50% of the total were found at 10 cm, and June, when the majority occurred at 30 cm.

Figure 6 represents a plot of the biomass (mg dry weight) against depth for months with the lowest and highest total numbers (April and October, respectively).

Notes on selected groups

Ephemeroptera. Caenis was recorded commonly throughout the year. Occasionally very small specimens were found to 70 cm but their usual maximum depth was about 40 cm. The numbers were minimal in October and April and maximal during the summer.

Several species of *Ephemerella* were found but mainly between November 1970 and August 1971. *E. deficiens* appeared as very small individuals in November and gradually increased in size until June when it presumably emerged. *E. excruciens* on the other hand, was still present as large individuals until early August when it, too, emerged. A few small nymphs, probably of this species, were found between October and December suggesting that eggs laid in early August hatched after only a few weeks, but those of *E. deficiens* were definitely identified only in November implying that it has a longer period of egg diapause. The maximum penetration depth for both these species appears to be 30 cm. Tsuda (1966) has also found the young larvae of *Ephemerella* to occur in the beds of sandy streams.
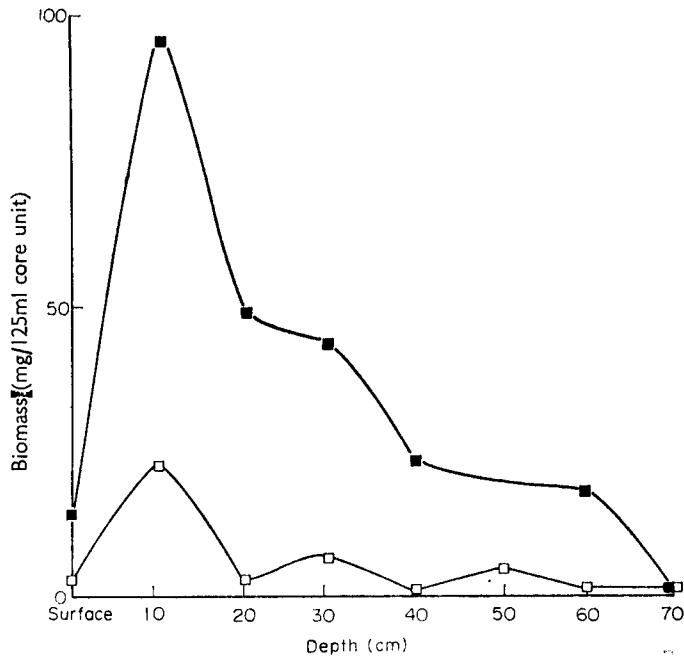
Fig. 6. Dry weight biomass (70% alcohol preserved) of animals *vs.* depth in substrate for 2 months in 1971 (Speed River). ■, April; □, October.

Nymphs of *Paraleptophlebia* were found on the surface for much of the year, but during the summer and early fall (June–October) some occurred as deep as 30 cm, although its slender and leaflike gills would seem to preclude the genus entirely from any interstitial penetration.

Trichoptera. Cheumatopsyche is well known for its wide tolerance range (Ross, 1944), so its occurrence in the interstitial habitat, where pore space permits, would therefore seem a natural progression. The average penetration was 20 cm but with occasional deeper excursions (Fig. 7a). It is possible that its position is correlated with the amount of organic matter.

The snail-like cases of *Helicopsyche borealis* are perhaps the most adapted of all the Trichoptera to an interstitial existence. Their spherical shape is probably easily moved in this habitat, and it can withstand being crushed. They occurred down to 30 cm and numbers were greatest both on the surface and below during May, August, September and October suggesting a quick succession of generations (Ross, 1944) followed by a long egg diapause of 5–6 months.

Hydropsyche, Rhyacophila, Hydroptila and *Chimarra* were on the surface for parts of the year, but were never found down in the substrate. A few small specimens of *Limnophilus, Oecetis* and *Leptocerus* were occasionally found to 20 cm.

Plecoptera. Few stonefly nymphs were found, but of these the majority seemed to be able to live in the substrate, particularly in their early stages. For example, *Allocapnia pygmaea* was found to 30 cm with the nymphs in characteristic diapause position (Harper & Hynes, 1970).

Coleoptera. The larvae of three genera of Elmidae were found. Both *Stenelmis* and *Macronynchus* larvae occurred in equally large numbers in the substrate, with a few
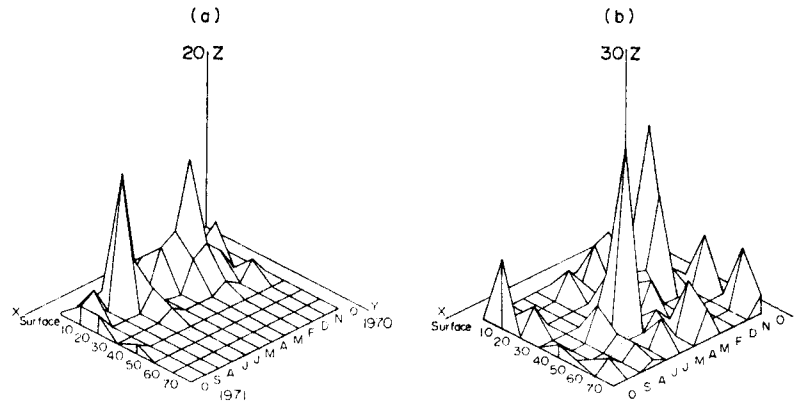
Fig. 7. Computer plots of the number of animals *vs.* depth in the substrate *vs.* time. (a) *Cheumatopsyche* larvae; (b) Harpacticoida. x-scale = 13 months; y-scale = 70 cm; z-scale = 20 animals (a); 30 animals (b).

individuals on the surface. *Dubiraphia* larvae were rarer and were never found below the surface. Adults of all the genera were found on the surface and down to 30 cm.

Two genera of *Psephenidae* occurred as larvae throughout the year and penetrated to 30 cm, the smaller *Ectopria* penetrating more frequently than *Psephenus*.

Oligochaeta. Most of the oligochaetes were Tubificidae and Naididae, the latter being mostly *Nais behningi* (a first record for Canada) and a species from the *Nais bretscheri-pardalis* complex. These two constituted approximately 80% of the total oligochaetes and were found down to 70 cm at all seasons. The tubificids were *Limnodrilus claparedeanus* and *L. hoffmeisteri* and were most numerous during the spring (February–April) but only down to 10 cm.

Mollusca. Only two genera of molluscs were at all common, and the thicker-shelled *Pisidium* penetrated to 50 cm, 20 cm deeper than *Sphaerium*.

Acari. The genera *Hygrobates*, *Torrenticola*, *Lebertia* and *Aturus* were found. The first three are typical running-water forms with rounded bodies and long legs and were primarily limited to the surface and upper 30 cm of the substrate. *Aturus*, however, occurred more deeply and was rare on the surface. Its body, in contrast to the others, is small and oval with short stout legs which would seem to favour it for an interstitial existence, be it soil water or hyporheic (D. R. Cook, personal communication).

Copepoda. Harpacticoida. The distribution of *Bryocamptus zschokkei* Schmeil 1893, the only harpacticoid found in the Speed River, was mostly interstitial with very few individuals on the surface (Fig. 7b). This small species has a wide distribution, being found in the silt of small springs and in wet moss, but usually in acid media (H.C.Yeatman, personal communication). The hyporheic zone of the Speed River, while not acid, is almost 1 pH unit less alkaline than the surface water (i.e. around 7·3).

The small elongated harpacticoid *Parastenocaris starretti* Pennak 1939, was found in quite large numbers (eight in a single 25-mm core) down to 100 cm in samples taken from the hyporheic zone of the Matamek River, Quebec.

Cyclopoida. In the Speed River *Cyclops* was most numerous from August to December and regularly penetrated to 30 cm with infrequent excursions to 60 cm.

Diptera (non chironomid). Generally, the larger larvae such as *Tipula* were restricted to the surface with a few individuals at 10 cm, whereas the smaller *Antocha* larvae were more common to 30 cm, especially during the summer. Larvae of the *Bezzia–Probezzia* group (Ceratopogonidae) were mostly limited to the surface with a few penetrations to 20 cm. Also, some *Hemerodromia* (Empididae) were found on the surface during July.

Chironomidae. Large numbers of chironomid larvae occurred at 10, 20 and 30 cm depths for most of the year, but with a minimum during the summer months (April–August) (Fig. 8a). No pupae were found deeper than 10 cm. Some of the more common genera are discussed below, but the comments made are only preliminary suggestions as to the life cycles, as little confirmatory literature is available.
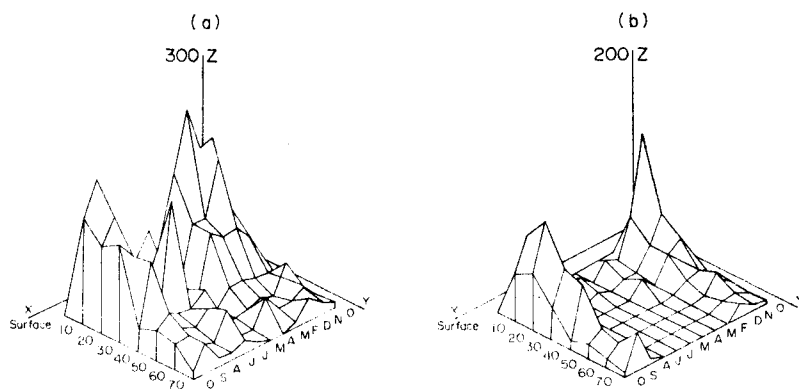
Fig. 8. Computer plots of the number of animals *vs.* depth in the substrate *vs.* time. (a) Total Chironomidae larvae; (b) *Cladotanytarsus* larvae. x-scale = 13 months; y-scale = 70 cm; z-scale = 300 animals (a); 200 animals (b).

Subfamily Chironominae. Figure 8(b) suggests that *Cladotanytarsus* is univoltine with adults appearing from April to early August, and eggs beginning to hatch around mid-August. There then appears to be a downward migration of small larvae into the substrate coinciding with a drop in the surface water temperature. The larvae overwinter at various depths in the substrate with an upward migration in March when the surface water begins to warm up. It would seem, therefore, that possibly the majority of larval growth takes place in the substrate during the winter months.

Microtendipes was present in the substrate in quite large numbers from September to March (Fig. 9a) and also appears to be univoltine with adults emerging from April to August when there were many large larvae near the surface. The eggs seem to hatch from August onwards, and small larvae overwinter in the substrate until the following March or April when their growth acclerated and they began to pupate and emerge.

Subfamily Orthocladiinae. Figure 9(b) suggests that *Orthocladius* is univoltine with a diapausing egg stage lasting from July to November. A few small larvae appeared in November and there was a steady increase in their numbers until March. They then apparently grew rapidly to pupate and emerge in May.

Subfamily Diamesinae. Fairly large specimens of *Prodiamesa* were found for most of the year to depths of 20 and 30 cm. The carnivorous larvae were large in comparison with others in the family and were frequently found with prey.
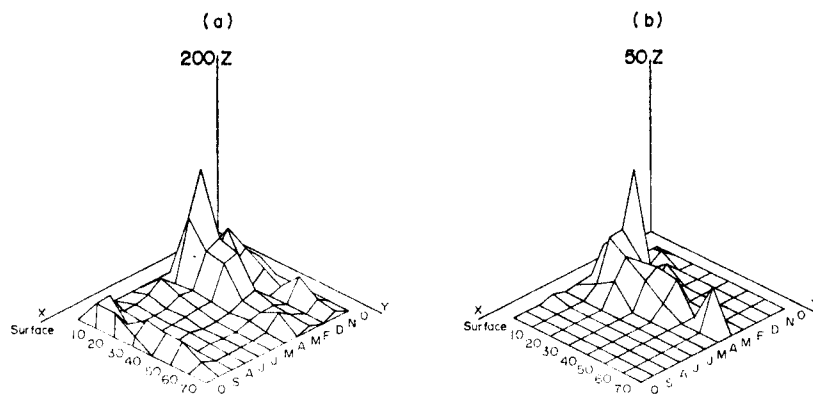
Fig. 9. Computer plots of the number of animals *vs.* depth in the substrate *vs.* time. (a) *Microtendipes* larvae; (b) *Orthocladius* larvae. x-scale = 13 months; y-scale = 70 cm; z-scale = 200 animals (a); 50 animals (b).

Discussion

The only physical parameter which appears to have any direct relationship with the total numbers of animals present in the hyporheic zone is the porosity, but even this does not hold for the depths below 30 cm. Mean grain size and heterogeneity of grain size show no correlation with total numbers, although it is likely that a certain minimum size would restrict the penetration of certain animals.

As we have seen, even though the surface water velocity may vary a great deal, the intragravelar flow remains relatively constant at a very low level. Therefore, since the variation in this subsurface component is very small, it is likely that it, too, has little seasonal effect on the fauna. This opposes Angelier's idea (1962) that the distribution of the hyporheic fauna is dependent on the surface currents.

Likewise, variations in normal stream height seem to have little effect on the fauna, unless we consider the indirect effect of a combination of surface water velocity and water height through the control of oxygen penetration into the substrate. However, extremes of water velocity and height may have a considerable effect on the fauna (as in cases of floods and droughts). For example, Clifford (1966) has shown that the surface fauna of a stream may move deeper into the substratum during spates— presumably as a protective mechanism against the increased current. The results shown in Fig. 10 of the vertical distribution of the fauna of the Speed River appear to confirm this. The curve for May follows the normal monthly pattern but in June, when the samples were taken 24 h after a very large spate, the peak in numbers had shifted to 30 cm. The curve for July again conforms to the norm even though these samples were taken only 48 h after another large spate. This might indicate that this protective migratory movement can be very quickly reversed once the spate has passed.

The temperature of the interstitial water, although generally a few degrees warmer in winter and lower in summer than the surface water, appears to have little effect on the total numbers of animals present. This is contrary to Angelier's idea (1953) that temperature is the true regulator of this community. The obvious objection here is that taking the total number of animals precludes any consideration of individual stenothermic species (as possibly in the case of some of the chironomid larvae).
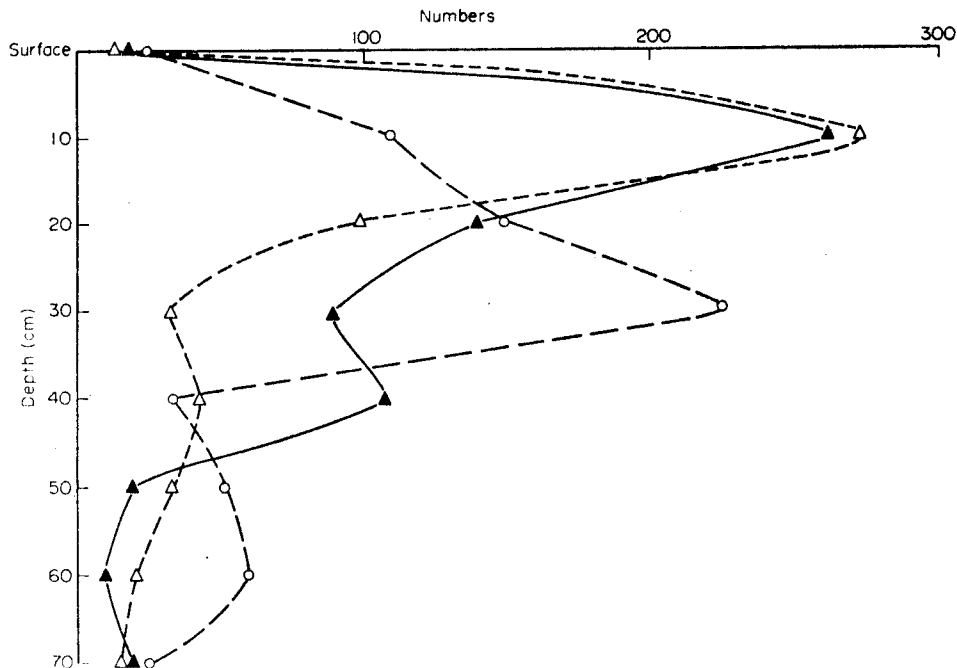
Fig. 10. Plot of the total number of animals/125 ml core unit *vs.* depth in the substrate for May (▲——▲), June (○————○) and July (△- - - △) 1971. (Speed River).

The relationships between total numbers and the interstitial chemical environment seem to be a little more obvious than for the physical environment. For example, dissolved oxygen and total numbers, and dissolved carbon dioxide and total numbers have correlative coefficients (*r*) of 0·698 and −0·768 respectively.

Apart from this the fact remains that below 30 cm no oxygen was detected but yet animals were found. This may be due to one or more of the following reasons. Firstly, the amount of oxygen present was too small to be detected by the modified Winkler technique. Secondly, the animals below 30 cm were in a state of reduced activity or even diapause; a few chironomid larvae were found in the remains of what might have been cocoons (see Buscemi, 1957), but the majority of the fauna was obviously active when examined soon after collecting. Thirdly, certain of the species may respire anaerobically, see Lindeman (1942).

Finally, Cole (1921) claimed that decomposing plant tissues give off small amounts of atomic oxygen even under anaerobiosis and that many invertebrates are able to use this. If this is true, then quite significant amounts of oxygen could be produced in the substrate from the large quantities of plant detritus trapped there.

The percentages of interstitial organic matter correlated with total numbers of animals give '*r*' values of between 0·761 and 0·921 depending on the method of organic matter analysis used. This indicates a close relationship between the two, as has already been shown for the interstitial fauna by Schwoerbel (1961) and for the surface benthos by Egglishaw (1964). The former author claims that the large proportion of organic matter which gets trapped in the interstices of stream beds may well be the most important single factor leading to the invasion of this habitat by surface detritivores.

The shape of an organism may also be important in determining if it is suited for an interstitial existence. There are perhaps two basic shapes. The first is long and slender with a flexible body which allows easy passage between the substrate particles. The second is small and blunt with a hard protective shell or exoskeleton which will withstand being crushed as the animal bludgeons its way through the pore spaces. Of the animals studied, the former seems to be a preadaptation of larvae of some Diptera, Ephemeroptera, Trichoptera, Plecoptera, Elmidae, Chironomidae and the Nematoda and the Oligochaeta. The latter group, similarly, is a preadaptation of the bivalve Mollusca, cased Trichoptera, Elmidae adults and the Oribatei. Perhaps, such very small animals as the Copepoda and the Ostracoda should be included in yet a third group, the characteristic of which would be extreme smallness of size, enabling them to actually swim in the interstitial water.

It is unlikely that any one of the above adaptations to, or characters of, the interstitial environment will alone control the number of animals living in it. More likely it is the combined effect of many of these parameters, together with others not mentioned, that determines the qualitative and quantitative nature of these sub-benthic populations.

The total number of animals occurring in a 70-cm column of stream-bed has already been dealt with, but we propose now to try and relate these figures to the total number per cubic metre of stream-bed. If we take an area of 1 m^2 on the surface, then the converted kick sample unit (125 ml) would have to be multiplied by 400 to give a value of the number of animals to a depth of 5 cm. Similarly, the 125 ml core unit at the 10-cm depth level would have to be multiplied by 800 to give the total number per sq. metre between 5 and 15 cm. The number in the core unit at 20 cm, when multiplied by 800 would give the total number per square metre between 15 and 25 cm, and so on down to 70 cm. Single samples taken from 80 to 100 cm during October 1971 showed that barely 2% of the total fauna occurs here and so a rough estimate of the total density can be obtained by summing the above calculations. This has been done in Table 3 for the months with the highest (October 1971) and

Table 3. Total numbers and dry weight estimates per m^3 of stream bed for Speed River

Depth (cm)	Lowest month (April 1971)		Highest month (October 1971)	
	Total numbers	Dry weight (g)	Total numbers	Dry weight (g)
Surface	9,560	0·88	9,960	52·0
10	58,400	17·68	258,000	76·8
20	40,800	2·00	216,800	38·4
30	34,400	5·04	139,200	35·2
40	16,800	0·08	41,600	18·4
50	18,400	4·00	50,400	16·8
60	4,000	1·12	51,200	15·2
70	2,400	0·16	13,600	0·2
80	—	—	8,000	—
90	—	—	8,000	—
100	—	—	0	—
Total per m^3	184,760	30·96	797,960	253·0

the lowest (April 1971) animal numbers. The total number of animals per cubic metre of stream-bed thus varied between 184,760 and 797,960. These are very large numbers indeed as compared with those of Mundie (1971) who obtained values of between 24,075 and 52,271/m² (to an unspecified depth) for the riffle area of a small creek. However, they do agree more with those of Coleman (1967) who estimated an average of 227,530 animals/m² (to a depth of 30 cm) for the Speed River. The estimated dry weight biomass calculated on a similar basis, ranged from 30·9 to 253·2 g for the lowest and the highest total numbers (Table 3).

Efficiency of the sampling method

Since the standpipe corer used in this study is new it seems worthwhile, here, to discuss its efficiency in sampling the interstitial biotope. The sampler works well in uniform and mixed gravels up to 10·0 mm in diameter, however, in substrates with a large mean grain size the corer may be selective in that it does not collect larger pebbles which possibly would have animals clinging to them. It is feasible, therefore, that in the upper gravel layers of the Speed River—where the mean grain size varies from 10·5 mm to 17·2 mm, the estimates of total animals may be a trifle high. One way of overcoming this would be to increase the diameter of the corer, but this would lead to more effort being needed to drive it into the substrate.

The similarities of replicate samples taken with the corer and analysed using Sørenson's Index of Similarity are shown in Table 4. These results show reasonable replication between the samples with regard to both the percentage composition and the percentage of numbers. As might be expected, the samples become more alike the deeper they are taken due to the decrease in the types of animals which can penetrate the lower substrate levels and their more uniform distribution due to the increased homogeneity of the substrate.

On reflection it now seems that a more accurate method than kick sampling could have been chosen for collecting surface samples. Initially, it was selected for its speed and simplicity as the main body of the work was to be concerned with the interstitial fauna, however, subsequent work showed the link between surface and hyporheic populations to be stronger than was first thought. Frost, Huni & Kershaw in a recent paper (1971) evaluated several of the kick sample methods and found them to be only 20% efficient in collecting all the surface animals present. This is quite possibly true, but they do not specify the depths to which their kicks went and thus this may have

Table 4. Analysis of similarity of five replicate samples which make up the core unit

Depth (cm)	Range in similarity of five samples with regard to	
	% composition	% numbers
10	63·5–88·2	48·2–81·2
20	53·3–84·9	55·0–72·5
30	38·5–75·0	30·6–73·5
40	62·5–70·7	42·9–85·7
50	42·3–71·2	45·1–87·2
60	69·1–82·4	66·9–90·2
70	71·3–88·3	71·3–87·2

led to their inconsistencies with the results of other workers. In the present study, the numbers of animals collected on the surface (averaging 3·3% of the total fauna) does seem remarkably small in comparison with the sub-surface component. Thus, the authors might well agree with Frost *et al.* and allow a correction factor of × 5 to be used for the kick sample results, but even with this correction the surface fauna would still be only a maximum of 17% of the total fauna.

Hyporheic populations in other rivers

Results from the examination of the hyporheos of three other rivers are shown in Fig. 11. They show similar distributions (with the possible exception of the St Anne's River, Quebec) for all the rivers, with a peak in numbers near 10 cm in each case. The only differences are in the densities of the animals present which vary with the different types of rivers. Examination of the composition of the hyporheos of all four rivers showed a few major groups to be common to two or more rivers and some genera to be identical, notably *Microtendipes* and *Micropsectra* amongst the chironomids and the *Aturus* oribatid mites.
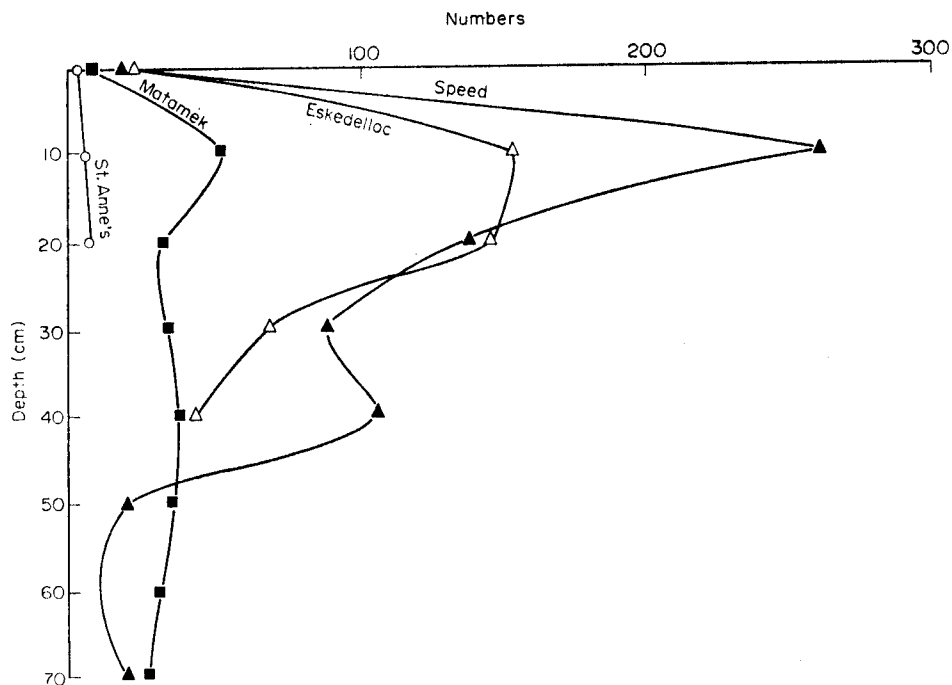

Fig. 11. Plot of the total number of animals/125-ml core unit *vs* depth in the substrate for four Canadian rivers. ▲, Speed River; △, Eskedelloc River; ■, Matamek River; ○, St Anne's River.

The variety and number of animals penetrating the interstices of the Matamek River, Quebec and the St Anne's River were markedly less than for the other two rivers although quite varied faunas existed in their surface benthos. This was probably due to these particular zones lacking one or more of the basic essentials such as food or oxygen and hence not encouraging mass penetration. Nevertheless, hyporheic

populations occurred in all these rivers which suggests that a large proportion of rivers with deep gravel beds may have their total biomass shared between the conventional benthos and the hyporheos. This brings to light the inefficiencies of many of the benthic samplers in use today, as although most of these samplers are quite accurate in their quantitative and qualitative estimates of surface benthos populations, they all with very few exceptions sample to a shallow depth. In fact, the majority of them are limited to a depth of only up to 5 cm. Thus, if as it now seems, the surface faunas of many streams extend down to depths of 1 m or more, and in quite large numbers, then most of these samplers fail to give an accurate picture of the total biomass. This does not mean, however, that these methods are obsolete in this respect, but perhaps to be comparable, the exact depth to which they sample should be stated. Further, for any kind of accurate production work, they should be used in conjunction with a more suitable quantitative interstitial sampler.

Conclusion

The hyporheic fauna itself consists of a variety of different taxa which can perhaps be divided into two types, the occasional and the permanent hyporheos. The former consists of the larvae of most of the surface benthos, and the latter consists of many specialized forms of copepods, mites, ostracods, tardigrades and syncarids. It is conceivable that this zone originated as a refuge for surface animals when conditions on the stream surface were unfavourable, and subsequently, many of these animals have become adapted to an interstitial existence. During the course of time, the hyporheic biotope may well have played an important role in populating other sub-terranean environments by acting as the 'Vorraum des unterirdischen Raumes' (Orghidan, 1959), as well as acting as a faunal reserve should the surface benthos be destroyed.

Acknowledgments

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