Hydrobiologia **288**: 65–78, 1994.

Microhabitat of *Anthopotamus verticis* (Ephemeroptera: Potamanthidae)

Y. J. Bae & W. P. McCafferty
Department of Entomology, Purdue University, West Lafayette, IN 47907, USA

Received 24 September 1992; in revised form 10 August 1993; accepted 14 September 1993

Key words: Anthopotamus verticis, burrowing mayflies, Ephemeroptera, microhabitat, substrate particle size

Abstract

A field and laboratory study was designed to elucidate the microhabitat of the North American riverine mayfly *Anthopotamus verticis* (Say). Previous reports have suggested that these mayflies are surface sprawlers or clingers; but despite a flattened body shape that is often associated with sprawling or clinging benthos, our investigation showed that larvae of *A. verticis* burrow and inhabit the hyporheic biotope. Substrate particle size was found to be a primary limiting factor in the microdistribution of the larvae. When homogeneous substrates (either fine, medium, or coarse gravel, or small or large pebbles) were provided, small, mid-sized, and large larvae significantly preferred coarse gravel or small pebbles. Small larvae were better represented in coarse gravel, and large larvae were better represented in small pebbles. Vertical distribution was deeper in coarse gravel than in medium gravel, and smaller larvae were generally found deeper than large larvae. In the field, larvae occur in gravel or pebbles (excluding coarse sand or smaller particles) or mixed substrates, often at the interface of large rocks and finer substrates. Videomacroscopic examination also indicated larvae to be interstitial dwellers. Although relatively crude burrowers, larvae do use their tusks to excavate substrate, and their gills to generate interstitial current. We distinguish the fossorial behavior of *Anthopotamus* from most other ephemeroid mayflies because tube burrows are not formed. Minimum-sized interstices may be required for filter feeding and/or adequate ventilation, and may explain a deeper penetration of smaller larvae in finer substrates.

Introduction

Detailed studies of habitat, along with biogeographic considerations, are the primary means of understanding the distribution of organisms. With regard to lotic zoobenthos, studies of habitat, whether conducted at the population level (e.g., Cummins & Lauff, 1969; Lamberti & Resh, 1979; McClelland & Brusven, 1980; Wright & Mattice, 1981; Osborne & Herricks, 1987) or community level (e.g., Brusven & Prather, 1974; Crisp & Crisp, 1974; Allan, 1975; Ward, 1975; DeMarch, 1976; Williams, 1978, 1980; Reice, 1980; Hawkins, 1984, Erman & Erman, 1984; Fuller & Rand, 1990) indicate that presence and absence of particular species or assemblages of species can be strongly correlated with habitat parameters of substrate composition and configuration. Functional adaptations of zoobenthos also may be expected to be closely related to substrate habitat, and species of aquatic insects, for example, often demonstrate morphological traits predictive of their specific microhabitat (e.g., Stuart, 1958; Hynes, 1970; Nachtigall, 1974; McCafferty, 1981;

Statzner & Holm, 1982; McShaffrey & McCafferty, 1987; Craig, 1990).

Mayflies whose larvae are typically found within substrates (silt, marl, clay, fine to coarse sand, wood, sponges) historically have been known as the burrowing mayflies. Most, including Potamanthidae, are phylogenetically related (see McCafferty, 1991). Potamanthidae, however, commonly have been categorized as sprawlers or clingers, primarily because their depressed body form and outspread legs and gills (Fig. 1) are more characteristic of sprawling and clinging mayflies. Burrowing mayflies typically have cylindrical bodies, with legs adapted for digging. Ecological notes and summaries by Morgan (1913), Argo (1927), Berner (1959), McCafferty (1975), Edmunds *et al.* (1976), Bartholomae & Meier (1977), and Cummins *et al.* (1984) reiterated this assumption, but offered no substantial observational or experimental data to corroborate it.

Our casual observations of the behavior and habitat of Potamanthidae larvae in eastern North America and Korea did not agree with published accounts of microhabitat. We therefore designed a study to examine the microdistribution of *Anthopotamus verticis* (Say), a locally abundant species and perhaps the most common of the four species of the North American genus (see Bae & McCafferty, 1991). We were interested in knowing the degree to which larvae may live or burrow within substrate, the degree to which substrate particle size may be related to microdistribution, and the degree to which fossorial behavior differs from that of other related mayflies.

Methods

Field investigations

Field data were collected in June, 1988, on the Tippecanoe River (White County, Indiana, USA), a sixth order stream in the upper Wabash River drainage system. The river at the study area was *ca* 50 m wide with water depth *ca* 0.5 m in summer, with discharge varying because of a regulated dam 10 km above the area. Diel maximum water temperature in 1988 ranged from 0–30 °C,

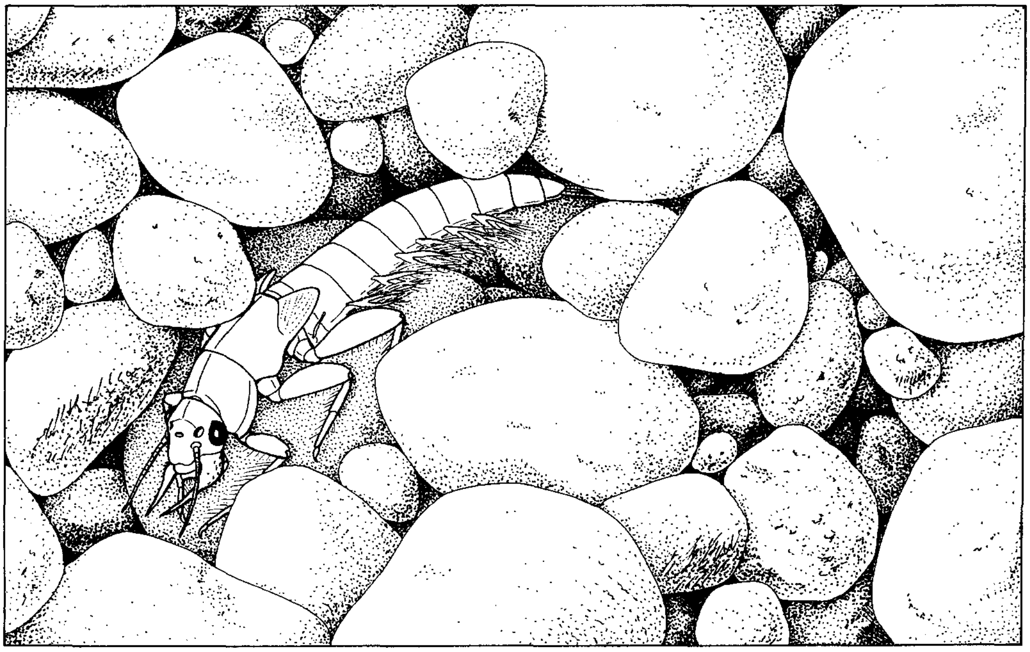

Fig. 1. A. verticis larva, diagrammatic in-substrate position.

and from 22–28 °C in June. The pH was 8.0–8.6 throughout the year. Substrate ranged from sand to large rocks, and current was moderately swift (0.1–0.8 m s^{-1}). *Anthopotamus verticis* is one of the most abundant benthic macroinvertebrates in the area.

Field distribution data were taken on 7 June when a broad range of larval instars and body size classes are present. Larvae were classified into three size classes: small (<5.1 mm length including tusks but not caudal filaments), mid-sized (5.1–10.0 mm), and large (>10.0 mm). These classes reflect major larval developmental stages, with the large class including mature, penultimate, and ultimate instars. Other studies of *Anthopotamus* (Ide, 1935; Bartholomae & Meier, 1977; Munn & King, 1987a, Bae & McCafferty, unpublished) have shown a similar simultaneous occurrence of this broad range of larval size classes in early summer.

Two river sites similar in depth, current velocity, and temperature but differing primarily in substrate composition were chosen for sampling. One site had mainly coarse-gravel substrate (Phi = −3: 8–16 mm), the other mainly small-pebble substrate (Phi = −4: 16–32 mm). Fifteen years of collecting in the area had shown that *A. verticis* larvae were most abundant in these substrates, as opposed to finer or coarser substrates. Particle size was measured by median diameter [the Phi value (-log particle diameter in mm) at the 50th percentile on a cumulative curve by weight], using the Wentworth classification system (Wentworth, 1922; Cummins & Lauff, 1969; Minshall, 1984). Each site had a surface area of 80 m^2. The substrates were relatively homogeneous (QD = 0.5) at each field site. This heterogeneity parameter was measured by quartile deviation [QD = (25th–75th)/2, where 25th and 75th are Phi values from the cumulative curve on those percentiles] (DeMarch, 1976; Lamberti & Resh, 1979; Erman & Erman, 1984; Minshall, 1984).

Fifteen different samples of substrate, each circumscribed by a surface area of 0.093 m^2 and to a depth of 0.4 m (substrate volume of *ca* 0.037 m^3), were sampled within each study site. Stones and other sediment were removed by hand and trowel and placed in buckets, and a fine mesh bag was held immediately downstream to capture any dislodged macrobenthos. Collected substrate was variously brushed and washed to remove any additional larvae. Numbers of each size class of *A. verticis* larvae for each sample at each site were recorded. When possible, the position of larvae in the substrate was noted. The collected substrate was quantified with respect to particle size and heterogeneity. T-tests were calculated to compare the abundance of larvae between the field sites.

Supplementary samples were taken at the study area in the summers of 1989–90, when scuba gear was used for underwater behavioral observations. In addition to the study sites described above we sampled slow current/depositional reaches and swift current/homogeneous cobble reaches in the study area to check for the occurrence of *A. verticis* larvae.

Lab experiments

Lab experiments were conducted from 24 July–19 September, 1991. Previous unpublished pilot studies were conducted in the summers of 1988–91. Larvae of *A. verticis* (1.5–15.7 mm length) were recruited from the field study area. Individuals were sorted to size classes, as described above, and held with stream water and natural substrate in aerated aquaria. Substrate was washed to remove extraneous material, then sorted to size by appropriate sieves.

For the first experiment, transparent polyethylene experimental boxes (280 × 200 × 100 mm) were filled with substrate to a depth of 50 mm. To test for particle size preference, five homogeneous (QD = 0) substrate types [fine gravel (Phi = −1: 2–4 mm), medium gravel (Phi = −2: 4–8 mm), coarse-gravel (Phi = −3: 8–16 mm), small pebbles (Phi = −4: 16–32 mm), and large pebbles (Phi = −5: 32–64 mm)] were arranged sequentially in each of these experimental boxes (Fig. 2). Transparent polyethylene plates (200 × 43 × 2 mm) partitioned the substrates,

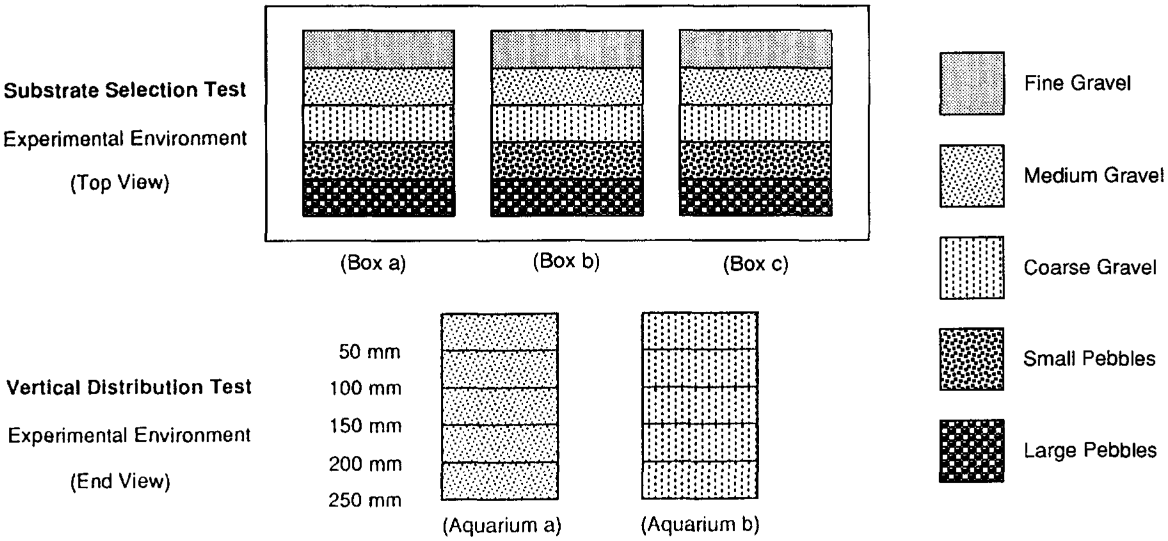

Fig. 2. Laboratory experimental designs.

except for 7 mm high gap left at the bottom so that larvae could move between the substrates as well as at the upper surface. Three such boxes (see boxes a, b, and c in Fig. 2), all containing the five partitioned substrates, were placed in a larger aquarium (750 x 300 x 300 mm, with final water depth at 200 mm). Water temperature was maintained at 22–24 °C, and air stones were placed medially at the sides of each experimental box so that DO was evenly distributed in the aquarium. Because Bartholomae & Meier (1977) and Meier & Bartholomae (1980) reported some negative phototaxis in *Anthopotamus*, low light (*ca* 50 Lux) was maintained in the laboratory during the times larvae were allowed to burrow.

Initially, five larvae were positioned randomly on the surface of each substrate type in each box, giving 25 larvae per experimental box and a total of 75 larvae used. This density of larvae was based on values commonly encountered during field sampling. A different size-class of larvae was used in each of the three boxes. Thus, 25 each of small, mid-sized, and large larvae were used. The experimental boxes were individually covered with 1 mm mesh screens, allowing movement between substrate types within the experimental boxes, but preventing movement between experimental boxes, and randomly distributed each time. Larvae were recaptured and their frequencies per substrate type were recorded after 24 hours. Our pilot study, although not quantified, had indicated no indicative differences in larval frequency among selected substrate types in 24, 48, or 72 hour tests. There also were no apparent differences related to proximity of substrate types to each other when sequential (Fig. 2) or random positioning of substrate types were compared. This experiment was repeated five times, for a total of five independent replicates and a total of 125 larvae used of each of the three size classes.

To recapture larvae after each 24-hour period, each experimental box was carefully removed from the aquarium and water was immediately siphoned out of the box with a large pipette. Our pilot study also showed that larvae cannot effectively move within substrate in the absence of water, and thus this method ensured that they did not change position upon removal. Each substrate type was then quickly removed from the experimental box and placed in separate white pans (400 x 220 x 65 mm). Each substrate was washed 3–5 times with water to dislodge all larvae. To prevent larvae that were being enumerated from moving around or back into substrate, cooled water (< 15 °C) was used to immobilize them. Since a few of the larvae were lost or died during the experiment (see Results), percentages compared are based on live recaptured larvae (see Fig. 3).

The second experiment was designed to determine depth of vertical distribution in two homogeneous (QD = 0) substrate types (Fig. 2) relative to the size class of the larvae. Although our pilot studies and results from the previous experiment indicated that larvae were most prevalent in coarse gravel, a less preferred substrate of medium gravel was compared. Other substrate types, e.g., fine gravel and small pebble, were additionally examined. For each of the substrate types, five 10 mm mesh wire baskets (300 x 150 x 50 mm deep) were filled with substrate and stacked on top of each other in an opaque, 380 mm deep polyethylene aquarium (300 x 150 mm). The five substrate baskets together filled each of the three aquaria to the 250 mm level. Water was added to the 350 mm level, and a strong current of air was pumped into each aquarium at 10 mm above the substrate surface to maximize penetration and circulation of aerated water. Temperature and light were maintained as described for the previous experiments. Twenty individuals each of small, mid-sized, and large larvae were placed into the experimental aquaria (see aquarium a and b in Fig. 2), respectively. This number was based on maximum larval densities found in field samples. Larvae were recaptured after 24 hours. Our pilot study also had shown no apparent differences in larval frequency among 24, 48, or 72 hour tests, and after 72 hours DO became somewhat depleted towards the bottoms of the aquaria. This procedure was repeated 15 times, so that there were five replicates of independent data for each size class in each substrate type.

To recapture larvae, water was drained by unplugging a 10 mm diameter drain hole near the bottom of each of aquaria. As mentioned above,

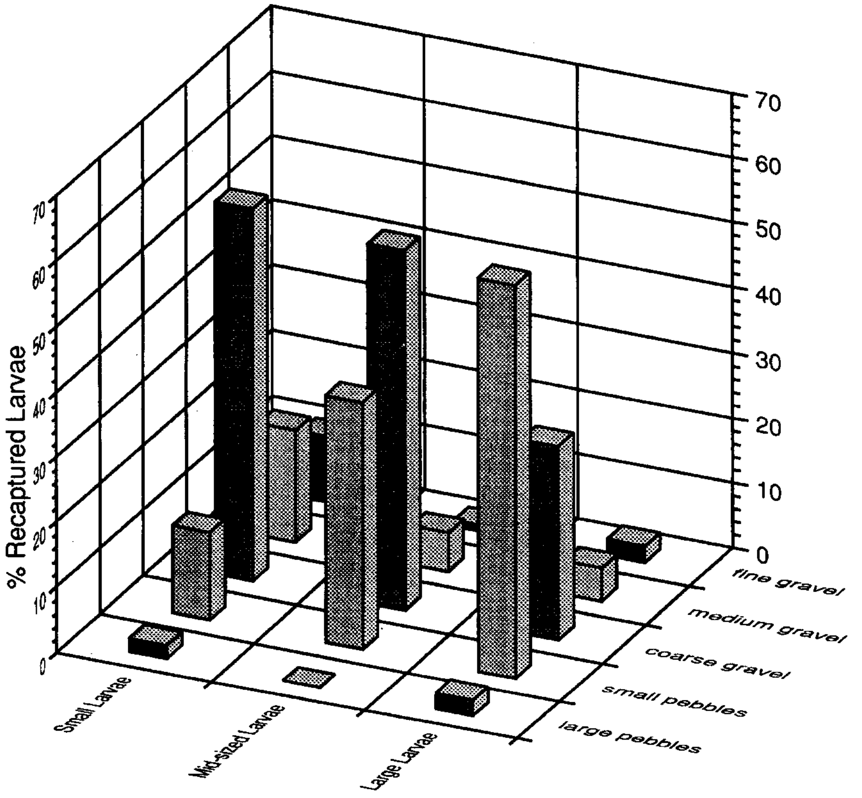

Fig. 3. Experimental distribution of *A. verticis* larvae among five substrate types.

the absence of water effectively prevents larvae from changing their position. After the surface of the substrate of the top basket was carefully examined for the possible presence of any larvae, the substrate baskets were sequentially removed from the aquarium and transferred to separate pans and washed as described previously. All grades of gravel, when moist after draining, could be conveniently handled in these wire baskets without crumbling. Even small grains do not fall through the larger mesh when moist and cohesive. Because of mortality and loss as explained for the previous experiment, percentages used in analysis are based on live recaptured larvae only (Figs 4, 5).

Only descriptive statistics were provided for analyses of the lab experiments because of their relatively small sampling sizes. Voucher specimens from experiments were preserved in 80% ethanol and deposited in the Purdue Entomological Research Collection.

Lab observations

Videomacroscopic observations for analyzing microhabitat orientation and burrowing behavior were performed using an artificial stream and a flow-through observation cell (after McShaffrey & McCafferty, 1988). The VCR and camera systems used were largely based on the techniques outlined by Keltner & McCafferty (1986) and McShaffrey & McCafferty (1986, 1988, 1990, 1991). All three size classes of larvae were studied. Scanning electron micrographs were taken with a JEOL JSM-840 microscope.

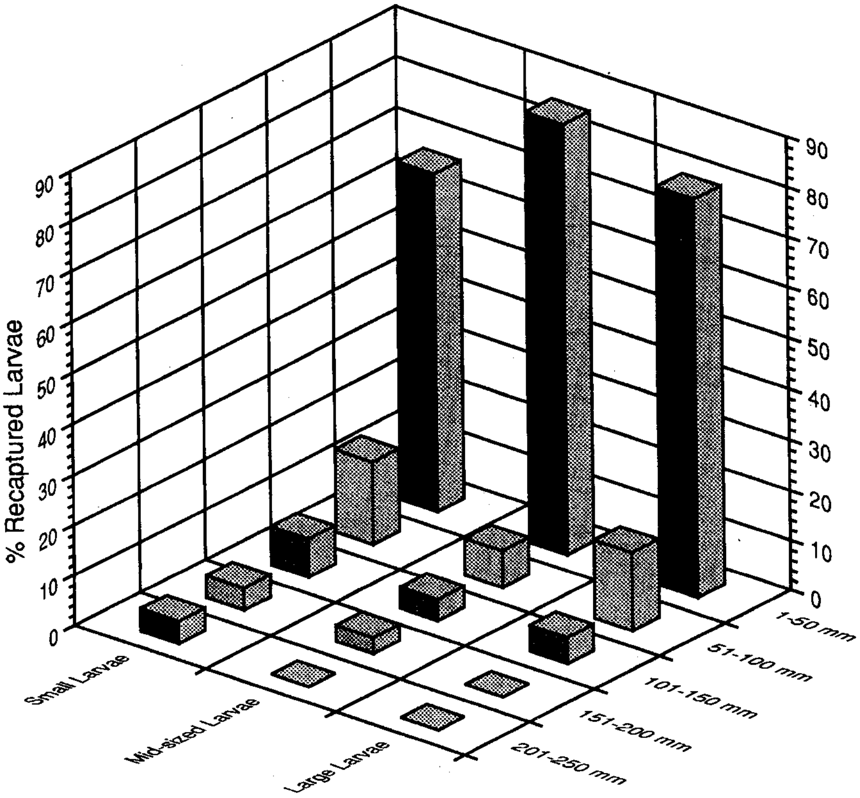

Fig. 4. Experimental vertical distribution of *A. verticis* larvae within medium gravel.

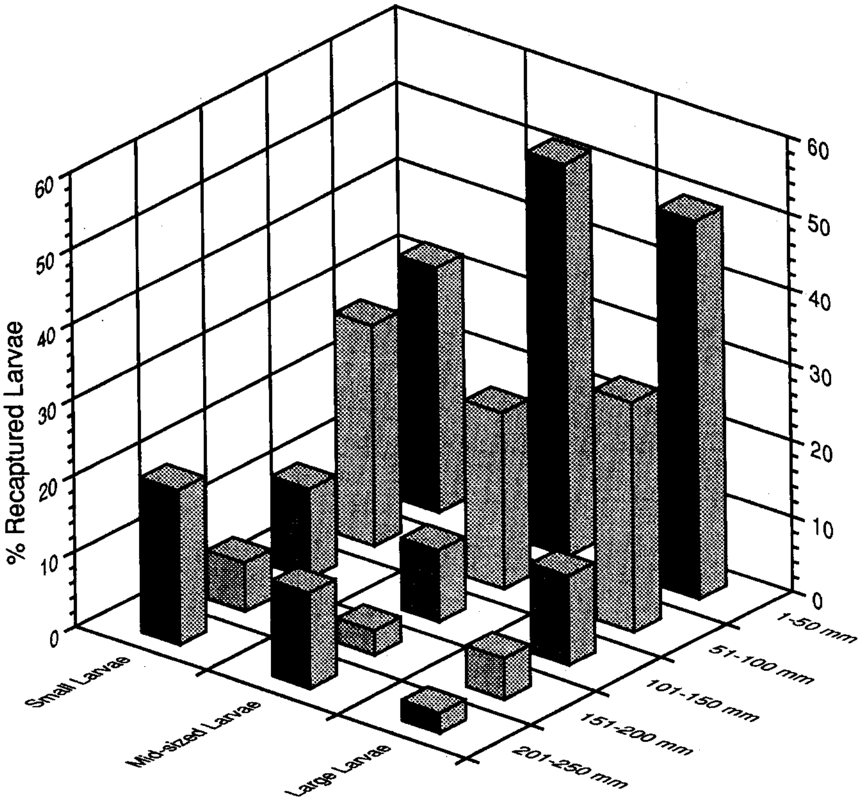

Fig. 5. Experimental vertical distribution of *A. verticis* larvae within coarse gravel.

Results

Field investigation

Our field observations and general sampling data indicated that *A. verticis* larvae live interstitially among coarse particulate substrate to depths of at least 0.4 m. This macrohabitat is describable in general terms as somewhat erosional (moderately fast current) with substrate often composed of a mix of coarse sand, gravel, pebble, and cobble-sized rocks. Larvae were often found at the interface of a stone and finer substrate, such as sand/gravel, usually using the stone for purchase (Fig. 1). Larvae were most abundant in this hyporheic biotope in the interstices of coarse gravel and small pebbles, sometimes mixed with some coarse sand deposits. Only rarely were larvae found in slower flowing, depositional areas with typically larger percentages of sand and silt. Larvae were not taken from swift current/cobble habitats that lacked sand and gravel.

Quantitative sampling data indicated that the overall abundance of larvae was nearly the same between coarse-gravel and small-pebble substrates (Table 1). There were differences, however, based on the distribution of the different size classes of larvae represented (Table 1). Small larvae preferred coarse gravel over small pebbles and large larvae preferred small pebbles over coarse gravel, while mid-sized larvae were quite evenly distributed between the two substrate types.

Lab experiments

In our substrate selection experiment, the different size classes of *A. verticis* larvae clearly showed a preference for certain substrate particle sizes.

Table 1. Number of *A. verticis* larvae collected from fifteen 0.037 m^3 samples (for a total of 0.555 m^3) each of coarse gravel (Phi = −3: 8–16 mm; QD = 0.5) and small pebble (Phi = −4: 16–32 mm; QD = 0.5) substrates in the Tippecanoe River, Indiana.

		Coarse gravel	Small pebble	p
Small	tot.	135	39	
larvae	$\bar{x}$/samp.	9.0	2.6	0.048
	sd	6.633	3.647	
Mid-sized	tot.	60	69	
larvae	$\bar{x}$/samp.	4.0	4.6	0.384
	sd	2.345	3.715	
Large	tot.	18	87	
larvae	$\bar{x}$/samp.	1.2	5.8	0.030
	sd	2.168	4.147	
All	tot.	213	195	
larvae	$\bar{x}$/samp.	14.2	13.0	0.414
	sd	7.294	9.513	

Among the 125 larvae of each size class used for this experiment, 98 (78.4%) of the small larvae were recaptured alive (8.8% died, 5.6% were lost), 116 (92.8%) of the mid-sized larvae were recaptured alive (1.6% died, 5.6% were lost), and 118 (94.4%) of the large larvae were recaptured alive (3.2% died, 1.6% were lost, 0.8% emerged).

When presented with different substrate types ranging from fine gravel to large pebbles, 155, or 46.7% of all larvae recaptured alive were taken from coarse gravel (Table 2). Small and mid-sized larvae were most abundant (57.1% and 55.2%, respectively) in coarse gravel, while large larvae were most abundant (60.2%) among small pebbles (Fig. 3). Large majorities of mid-sized larvae (93.1%) and large larvae (89.8%) were found in coarse gravel and small pebbles, but small larvae were more evenly distributed in the substrates provided. Abundance of the three size classes differed most in small pebbles (Fig. 3).

In our vertical distribution experiment, the different size classes of *A. verticis* larvae selected certain depths of penetration. Less than 1.0% of the larvae remained at the surface of substrates. Among the 100 total larvae used from each size class and each substrate type, 91 and 93 small larvae were recaptured alive from medium and coarse gravel, respectively. Likewise, 96 and 95 mid-sized larvae, and 78 and 76 large larvae were recaptured alive. The distinctly fewer large larvae retrieved was due to numerous emergences during the experiment.

In medium gravel (Table 3, Fig. 4), the vast majority of larvae were recaptured from 1–50 mm depth (91.9% of all larvae were taken from 1–50 mm depth in fine gravel). Only small larvae were found below 200 mm, while mid-sized and large larvae were not found below 200 mm and 150 mm, respectively, in medium gravel. Of the larvae recaptured from medium gravel, 17.6% of small, 60.0% of mid-sized, and 67.8% of large larvae were found at the various depths along the interface of the gravel and side of the aquarium.

In coarse gravel (Table 3, Fig. 5), only 44.0% of all larvae were recaptured from the 1–50 mm

Table 2. Number of *A. verticis* larvae recaptured alive from five experimental substrate types (5 repetitions) (see also Fig. 3).

		Fine gravel	Medium gravel	Coarse gravel	Small pebble	Large pebble
Small larvae	tot.	10	17	56	13	2
	$\bar{x}$/samp.	2.0	3.4	11.2	2.6	0.4
	sd	1.871	2.408	3.271	1.673	0.548
Mid-sized larvae	tot.	1	7	64	44	0
	$\bar{x}$/samp.	0.2	1.4	12.8	8.8	0.0
	sd	0.447	1.949	5.630	3.962	0.000
Large larvae	tot.	3	6	35	71	3
	$\bar{x}$/samp.	0.6	1.2	7.0	14.2	0.6
	sd	0.548	0.837	4.743	5.718	0.894

Table 3. Numbers of *A. verticis* larvae recaptured from different depths of medium and coarse gravel (5 repetitions each) (see also Figs 4, 5)

		Depth from substrate surface				
		1–50 mm	51–100 mm	101–150 mm	151–200 mm	201–250 mm
			Medium gravel			
Small larvae	tot.	61	15	7	4	4
	x̄/samp.	12.2	3.0	1.4	0.8	0.8
	sd	1.483	0.707	1.140	0.837	0.837
Mid-sized larvae	tot.	81	7	4	3	0
	x̄/samp.	16.2	1.4	0.8	0.6	0.0
	sd	1.924	0.894	1.304	0.548	0.000
Large larvae	tot.	61	12	4	0	0
	x̄/samp.	12.2	2.4	0.8	0.0	0.0
	sd	3.033	2.302	1.095	0.000	0.000
			Coarse gravel			
Small larvae	tot.	30	27	11	6	19
	x̄/samp.	6.0	5.4	2.2	1.2	3.8
	sd	3.000	2.966	1.789	0.837	1.789
Mid-sized larvae	tot.	49	22	9	3	12
	x̄/samp.	9.8	4.4	1.8	0.6	2.4
	sd	2.864	1.949	1.304	0.548	1.140
Large larvae	tot.	38	23	9	4	2
	x̄/samp.	7.6	4.6	1.8	0.8	0.4
	sd	2.702	3.362	1.789	0.447	0.894

depth increment, and most were found between 51–250 mm. Small larvae were relatively evenly distributed among the depth increments compared to mid-sized and large larvae. Some 61.3% of small, 74.7% of mid-sized, and 80.3% of large larvae were found from 1–100 mm. Only 8.6% of small, 21.1% of mid-sized, and 32.9% of large larvae were found at the gravel/aquarium interface.

In additional experiments on vertical distribution, the data for fine gravel and small pebble showed similar tendency to those of medium gravel and coarse gravel, respectively.

Lab observations

Videomacroscopic observations in the laboratory confirmed that all size classes of larvae can burrow and maintain themselves in medium and coarse gravels or mixed substrate including these gravels (Fig. 1). Larvae also lived interstitially among pebble substrates but because of the smallness of our observational cells, detailed interstitial behavior among pebbles could not be recorded. Larvae clearly demonstrated positive geotaxis upon being placed on penetrable substrate. During burrowing and once settled, the slightly upcurved mandibular tusks (Fig. 6) were used to excavate substrate particles from the region immediately anterior to the head. This was accomplished mainly by repeated lifting of the head. Gill pulsation created a microcurrent anterior to posterior over the body within the interstices occupied. Defined burrows or tubes were never formed by larvae, and footholds on larger available substrate, *i.e.*, stones in mixed substrate,

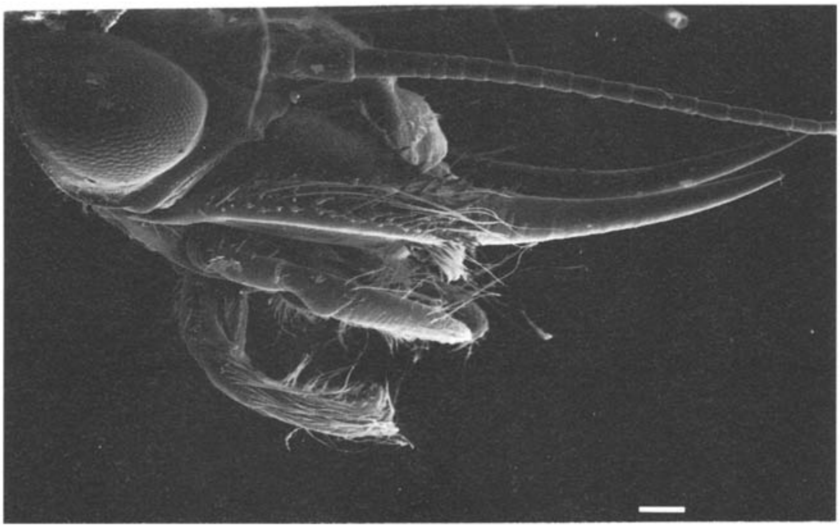

Fig. 6. A. verticis larva, head, lateral, bar = 0.1 mm.

particularly in crevices, were commonly maintained.

Discussion

Previous reports of the general habitat of *Anthopotamus* larvae as erosional, relatively shallow reaches of medium sized to large rivers (Argo, 1927; Needham *et al.*, 1935; McCafferty, 1975; Bartholomae & Meier, 1977; Munn & King, 1987b) agree with our macrohabitat data. These same studies, however, either reported *Anthopotamus* as surface benthos or did not clearly address the larval microhabitat. Certain studies (Morgan, 1913; Needham, 1920) had indicated that *Anthopotamus* larvae inhabited silted depositional areas of streams similar to those habitats of the common burrowing mayflies of the genus *Hexagenia* (family Ephemeridae), but these reports were evidently due to the incorrect association of the larval stage with adults of genera of other families (McCafferty, 1975). In this study and in over 20 years of general sampling throughout the range of *Anthopotamus*, we have not encountered these mayflies in fine sand or silt bottomed depositional zones. We also have not found them in homogeneous cobble or bedrock, erosional habitats. We instead have found them in gravel or pebble beds or in mixed substrates, sometimes strewn with large cobble or bolders, in erosional zones.

Anthopotamus larvae in both our field and lab investigations showed definite preferences for coarse-gravel and small-pebble substrates (Tables 1, 2, Fig. 3). Data strongly suggest that there is an increase in degree and depth of burrowing as particle size increases from medium to coarse gravel and as larval size decreases (Table 3, Figs 4, 5). In addition, the fact that more larvae, especially older larvae, were found from the interface of the aquarium and medium gravel than from a similar interface with coarse gravel, suggests to us that medium gravel or finer substrates are not optimal microhabitats for them without there being an adjacent larger surfaced object, such as an embedded stone, available for purchase. This may also explain why larvae do not burrow in homogeneous coarse sand or finer substrates. In coarse gravel, on the other hand, larvae were not only able to burrow but were able to maintain themselves in the gravel interstices. The fact that *Anthopotamus* larvae have been reported only on stones in other published studies is possibly because the stones were embedded in finer, mixed substrates, or because homogeneous or heterogeneous substrates consisting of medium or coarse gravel, or small pebbles simply had not been sampled adequately.

Our data show that *A. verticis* larvae are part of the endemic, or permanent, hyporheos of habitable streams within their geographic range. Two factors probably explain why their true biotope was not recognized in the past. First, many common methods of sampling macrobenthos in streams, such as kicking or disturbing substrate upstream of a catchnet or screen, would have yielded some larvae of *Anthopotamus* since many are located in shallow substrate depths, as our data have shown. Similarly, picking the undersides of stones could also yield some of the larvae because the interface of stones and gravel can be occupied by larvae in mixed substrate. These two methods are in fact the means by which we commonly had collected *Anthopotamus* prior to discovering that larvae are often present deep within homogeneous gravel. Such qualitative collecting would not necessarily ever have led to the conclusion that *Anthopotamus* larvae were hyporheic.

The second possible factor contributing to inconsistencies in the literature regarding *Anthopotamus* ecology has to do with morphology. The general facies of potamanthid larvae had not been associated with burrowing and did not exactly fit either of the two basic body types that are generally associated with burrowing [long, slender, and flexible or small, blunt, and hard (Williams & Hynes, 1974)]. Potamanthid larvae were assumed to have traits more typical of sprawlers or clingers [depressed with laterally oriented legs (Stuart, 1958; Ambühl, 1959; Edmunds *et al.*, 1976)].

Bartholomae & Meier (1977) did not observe the burrowing habit or interstitial habitat of the congener *A. myops* in the Huron River, Michigan. They later concluded that larvae migrated between the undersides and upper surfaces of stones as a response to diel periodicity (Meier & Bartholomae, 1980). We have not observed such diel cyclic movement in *A. verticis*. More recently, Munn & King (1987b), in a laboratory and field study of *A. myops* from the Chippewa River, Michigan, did find that larvae were located interstitially between 'stones and bottom sand'.

With regard to particle size limitations that our data have suggested for *Anthopotamus* larvae, certain sized interstices or crevices within the substrate typical of their preferred microhabitat may be necessary. Minimum sized interstices may be required for optimizing *Anthopotamus* filter-feeding activities (McCafferty & Bae, 1992) and/or for allowing adequate space for natural current flow or flow generated by gill ventilation and presumably needed for respiratory and osmoregulatory purposes. Perhaps a critical ecological factor limiting these mayflies is available DO, such as was suggested for hyporheic lotic benthos (Williams & Hynes, 1974) and for interstitial lotic meiofauna in general by Pennak (1988). Eriksen (1964) showed that the burrowing mayfly *Ephemera simulans* (Ephemeridae) usually selected fine gravel for burrowing in laboratory tests. He also showed that although the mayfly could burrow in a variety of substrate sizes, the relatively low DO of finer sediments in field situations limited the mayfly to substrates with larger interstices (Eriksen, 1968).

McCafferty (1975) first discovered young larvae of *Anthopotamus* within substrate (at approximately 0.5 m depth) while searching for burrowing mayflies of the genus *Ephoron* on the Tippecanoe River. Our vertical distribution data indicated a tendency for smaller larvae to burrow deeper than larger larvae, and particularly in finer substrates (Table 3, Figs 4, 5). We suspect that one of the reasons for this is because of an optimal body size/substrate size relationship allowing interstices for functions suggested above. On the other hand, it may simply reflect a possible tendency for young instars to be found deeper in substrates (Coleman & Hynes, 1970), although Bishop (1973) found no such deep substrate nursery apparent from his depth data in a Malaysian stream. Tolkamp & Both (1978) found the burrowing mayfly *Ephemera danica* in sand and gravel as young larvae, but preferring gravel as larger larvae.

Because of the nature of substrates in erosional zones where *Anthopotamus* larvae occur, and because these mayflies do not form discrete burrows [as do, e.g., *Hexagenia* and *Pentagenia* larvae in silt and clay (Keltner & McCafferty, 1986)], we consider *Anthopotamus* larvae to be only crude burrowers. There is a fundamental difference evident among burrowing mayflies. Those such as *Anthopotamus* actively burrow but dwell in interstices or available crevices, and although capable of excavating moveable substrates, they are limited in their ability to manufacture spaces. By comparison, more advanced burrowers construct, and dwell within, walled burrows in finer or more compacted substrate. Intermediate gradations of burrowing ability are also found among the ephemeroid mayflies (Bae & McCafferty, 1993).

Because *A. verticis* larvae are similar to their congeners in morphological detail, and because those congeners are known from similar macrohabitats, conclusions about microdistribution and burrowing behavior are most likely applicable to other *Anthopotamus* species. Recent ecological observations on other Potamanthidae, including *Potamanthus formosus* (as *P. kamonis*) in Japan (Watanabe, 1988), *P. yooni* in Korea (Bae, unpublished), *P. luteus* in France and Algeria (A. G. B. Thomas, personal communication), *P. sabahensis* in northern Borneo (Bae *et al.*, 1990), and *Rhoenanthus coreanus* from Korea (Bae, unpublished), all indicate or suggest a hyporheic habitat essentially similar to that which we have reported for *Anthopotamus*. The other species and genera cited above represent all major phyletic lineages of Potamanthidae (Bae & McCafferty, 1991) and may indicate a general familial habitat.

Acknowledgements

Lynda Corkum provided constructive comments for experimental design. Dave McShaffrey assisted with videomacroscopy and scanning work, Dan Bloodgood, Steve Yanoviak, and Carlos Lugo-Ortiz assisted with field work and experimental apparatus, and Arwin Provonsha provided Fig. 1. The SEM was made available by the Electron Microscope Center in Agriculture at Purdue University with support from NSF Grant PCM-8400133. This article is published as Purdue Experiment Station Journal No. 12842.

References

Allan, J. D., 1975. The distributional ecology and diversity of benthic insects in Cement Creek, Colorado. Ecol. 56: 1040–1053.

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

Argo, V. N., 1927. The North American species of the genus *Potamanthus*, with a description of a new species. J. N. Y. Ent. Soc. 35: 319–329.

Bae, Y. J. & W. P. McCafferty, 1991. Phylogenetic systematics of the Potamanthidae (Ephemeroptera). Trans. Am. Ent. Soc. 117: 1–143.

Bae, Y. J. & W. P. McCafferty, 1993. Ephemeroptera tusks and their evolution. In L. Corkum & J. Ciborowski (eds), Proceedings of the seventh international conference on Ephemeroptera. Sandhill Crane Press, Gainesville, Florida, in press.

Bae, Y. J., W. P. McCafferty & G. F. Edmunds, Jr., 1990. *Stygifloris*, a new genus of mayflies (Ephemeroptera: Potamanthidae) from southeast Asia. Ann. Ent. Soc. Am. 83: 887–891.

Bartholomae, P. G. & P. G. Meier, 1977. Notes on the life history of *Potamanthus myops* in southeastern Michigan (Ephemeroptera: Potamanthidae). Great Lakes Ent. 10: 227–232.

Berner, L., 1959. A tabular summary of the biology of North American mayfly nymphs (Ephemeroptera). Bull. Florida St. Mus., Biol. Sci. 4: 1–58.

Bishop, J. E., 1973. Observations on the vertical distribution of the benthos in a Malaysian stream. Freshwat. Biol. 3: 147–156.

Brusven, M. A. & K. V. Prather, 1974. Influence of stream sediments on distribution of macrobenthos. J. Ent. Soc. British Columbia 71: 25–32.

Coleman, M. J. & H. B. N. Hynes, 1970. The vertical distribution of the invertebrate fauna in the bed of a stream. Limnol. Oceanogr. 15: 31–40.

Craig, D. A., 1990. Behavioural hydrodynamics of *Cloeon dipterum* larvae (Ephemeroptera: Baetidae). J. N. Am. Benthol. Soc. 9: 346–357.

Crisp, C. B. & N. H. Crisp, 1974. Substrate preference of benthic macroinvertebrates in Silver Creek, Madison County, Kentucky. Trans. Kentucky Acad. Sci. 35: 61–66.

Cummins, K. W., G. F. Edmunds, Jr. & R. W. Merritt, 1984. Summary of ecological and distributional data for Ephemeroptera (mayflies). In R. W. Merritt & K. W. Cummins (eds), An introduction to the aquatic insects of North America. 2nd edn. Kendall/Hunt, Dubuque, Iowa: 122–125.

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

DeMarch, B. G. E., 1976. Spatial and temporal patterns in macrobenthic stream diversity. J. Fish. Res. Bd Can. 33: 1261–1270.

Edmunds, G. F., Jr., S. L. Jensen & L. Berner, 1976. The mayflies of North and Central America. Univ. Minnesota, Minneapolis, Minnesota.

Eriksen, C. H., 1964. The influence of respiration and substrate upon the distribution of burrowing mayfly naiads. Verh. int. Ver. Limnol. 15: 903–911.

Eriksen, C. H., 1968. Ecological significance of respiration and substrate for burrowing Ephemeroptera. Can. J. Zool. 46: 93–103.

Erman, D. C. & N. A. Erman, 1984. The response of stream macroinvertebrates to substrate size and heterogeneity. Hydrobiologia 108: 75–82.

Fuller, R. L. & P. E. Rand, 1990. Influence of substrate type on vulnerability of prey to predacious aquatic insects. J. N. Am. Benthol. Soc. 9: 1–8.

Hawkins, C. P., 1984. Substrate associations and longitudinal distributions in species of Ephemerellidae (Ephemeroptera: Insecta) from western Oregon. Freshwat. Invert. Biol. 3: 181–188.

Hynes, H. B. N., 1970. The ecology of running waters. Liverpool Univ., Liverpool, U. K.

Ide, F. P., 1935. Life history notes on *Ephoron*, *Potamanthus*, *Leptophlebia*, and *Blasturus* with descriptions (Ephemeroptera). Can. Ent. 67: 113–125.

Keltner, J. & W. P. McCafferty, 1986. Functional morphology of burrowing in the mayflies *Hexagenia limbata* and *Pentagenia vittigera*. Zool. J. Linn. Soc. 87: 139–162.

Lamberti, G. A. & V. H. Resh, 1979. Substrate relationships, spatial distribution patterns, and sampling variability in a stream caddisfly population. Envir. Ent. 8: 561–567.

McCafferty, W. P., 1975. The burrowing mayflies (Ephemeroptera: Ephemeroidea) of the United States. Trans. am. Ent. Soc. 101: 447–504.

McCafferty, W. P., 1981. Aquatic entomology. Jones & Bartlett, Boston, Massachusetts.

McCafferty, W. P., 1991. Toward a phylogenetic classification of the Ephemeroptera: a commentary on systematics. Ann. Ent. Soc. Am. 84: 343–360.

McCafferty, W. P. & Y. J. Bae, 1992. Filter-feeding habits of the larvae of *Anthopotamus* (Ephemeroptera: Potamanthidae). Ann. Limnol. 28: 27–34.

McClelland, W. T. & M. A. Brusven, 1980. Effects of sedimentation on the behavior and distribution of riffle insects in a laboratory stream. Aquat. Insects 2: 161–169.

McShaffrey, D. & W. P. McCafferty, 1986. Feeding behavior of *Stenacron interpunctatum* (Ephemeroptera: Heptageniidae). J. N. Am. Benthol. Soc. 5: 200–210.

McShaffrey, D. & W. P. McCafferty, 1987. The behavior and form of *Psephenus herricki* (DeKay) (Coleoptera: Psephenidae) in relation to water flow. Freshwat. Biol. 18: 319–324.

McShaffrey, D. & W. P. McCafferty, 1988. Feeding behavior of *Rhithrogena pellucida* (Ephemeroptera: Heptageniidae). J. N. Am. Benthol. Soc. 7: 87–99.

McShaffrey, D. & W. P. McCafferty, 1990. Feeding behavior and related functional morphology of the mayfly *Ephemerella needhami* (Ephemeroptera: Ephemerellidae). J. Insect Behav. 3: 673–688.

McShaffrey, D. & W. P. McCafferty, 1991. Videomacroscopy for the study of Ephemeroptera and other aquatic macroinvertebrates. In J. Alba-Tercedor & A. Sanchez-Ortega (eds), Overview and strategies of Ephemeroptera and Plecoptera. Sandhill Crane, Gainesville, Florida: 15–24.

Meier, P. G. & P. G. Bartholomae, 1980. Diel periodicity in the feeding activity of *Potamanthus myops* (Ephemeroptera). Arch. Hydrobiol. 88: 1–8.

Minshall, G. W., 1984. Aquatic insect-substratum relationships. In V. H. Resh & D. M. Rosenberg (eds), The ecology of aquatic insects. Praeger, N.Y.: 358–400.

Morgan, A. H., 1913. A contribution to the biology of mayflies. Ann. Ent. Soc. Am. 6: 371–426.

Munn, M. D. & R. H. King, 1987a. Life history of *Potamanthus myops* (Walsh) (Ephemeroptera: Potamanthidae) in a central Michigan stream. Am. Midl. Nat. 117: 119–125.

Munn, M. D. & R. H. King, 1987b. Ecology of *Potamanthus myops* (Walsh) (Ephemeroptera: Potamanthidae) in a Michigan stream (USA). Hydrobiologia 146: 71–75.

Nachtigall, W., 1974. Locomotion: mechanics and hydrodynamics of swimming in aquatic insects. In M. Rockstein (ed.), The physiology of Insecta. Vol. 3, 2nd edn., Academic Press, N.Y.: 381–432.

Needham, J. G., 1920. Burrowing mayflies of our large lakes and streams. Bull. Bur. Fish. 36: 266–304.

Needham, J. G., J. R. Traver & Y. C. Hsu, 1935. The biology of mayflies. Comstock, Ithaca, N.Y.

Osborne, L. L. & E. E. Herricks, 1987. Microhabitat characteristics of *Hydropsyche* (Trichoptera: Hydropsychidae) and the importance of body size. J. N. Am. Benthol. Soc. 6: 115–124.

Pennak, R. W., 1988. Chapter 4. Ecology of freshwater meiofauna. In R. P. Higgins & H. Thiel (eds), Introduction to the study of meiofauna. Smithson. Inst., Washington, D. C.: 39–60.

Reice, S. R., 1980. The role of substratum in benthic macroinvertebrate microdistribution and litter decomposition in a woodland stream. Ecol. 61: 580–590.

Statzner, B. & T. F. Holm, 1982. Morphological adaptations of benthic invertebrates to stream flow – an old question studied by means of a new technique (Laser Doppler Anemometry). Oecol. 53: 290–292.

Stuart, A. M., 1958. The efficiency of adaptive structures in the nymph of *Rhithrogena semicolorata* (Curtis) (Ephemeroptera). J. exp. Biol. 35: 27–38.

Tolkamp, H. H. & J. C. Both, 1978. Organism-substrate relationship in a small Dutch lowland stream. Preliminary results. Verh. int. Ver. Limnol. 20: 1509–1515.

Ward, J. V., 1975. Bottom fauna-substrate relationships in a northern Colorado trout stream: 1945 and 1974. Ecol. 56: 1429–1434.

Watanabe, N. C., 1988. Life history of *Potamanthodes kamo-*

nis in a stream of central Japan (Ephemeroptera: Potamanthidae). Verh. int. Ver. Limnol. 23: 2118–2125.

Wentworth, C. K., 1922. A scale of grade and class terms for clastic sediments. J. Geol. 30: 377–392.

Williams, D. D., 1978. Substrate size selection by stream invertebrates and the influence of sand. Limnol. Oceanogr. 23: 1030–1033.

Williams, D. D., 1980. Some relationships between stream benthos and substrate heterogeneity. Limnol. Oceanogr. 25: 166–172.

Williams, D. D. & H. B. N. Hynes, 1974. The occurrence of benthos deep in the substratum of a stream. Freshwat. Biol. 4: 233–256.

Wright, L. L. & J. S. Mattice, 1981. Substrate selection as a factor in *Hexagenia* distribution. Aquat. Insects 3: 13–24.