

Microhabitat shifts and substrate selection by the psammophilous predator *Pseudiron centralis* McDunnough (Ephemeroptera: Heptageniidae)

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The predaceous mayfly *Pseudiron centralis* McDunnough exhibited shifts in its association with different types of sandy substrate during larval development: stage I larvae were associated with marginal sands; stage II larvae exhibited no association; and stage III and IV larvae were associated with the substrate of the main-stream channel of the river, where sand is formed into active dunes (shifting sand). Ability to occupy areas of shifting sand is size related, probably because small larvae cannot cope with the physical stresses of these areas. Experiments indicate that stage III larvae avoid gravel, but do not differentiate between sand and silt. Shifting sands are hypothesized to be favourable environments for larger larvae because of greater accessibility of chironomid prey or the absence of potential predators in these areas. Larval spacing behavior is unlikely to affect patterns of larval distribution or density on the riverbed.

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L'éphémère prédateur *Pseudiron centralis* choisit différents types de substrats sablonneux au cours de son développement: les larves de stade I sont associées surtout aux sables riverains; les larves de stade II ne semblent pas avoir d'association particulière avec un type de substrat; et les larves des stades III et IV se tiennent dans le canal central des rivières où les sables s'accumulent en dunes mouvantes (sables mouvants). La capacité des larves de s'associer à des zones de sables mouvants dépend de leur taille, car les petites larves sont probablement incapables de faire face aux stress physiques inhérents à ces zones. Des expériences ont démontré que les larves de stade III évitent la gravier, mais ne font pas de différence entre le sable et le limon. Il semble que les sables mouvants constituent des milieux favorables aux larves plus grosses, peut-être à cause de la disponibilité des chironomides comme proies ou à cause de l'absence de prédateurs possibles dans ces zones. Le comportement d'espacement des larves n'est probablement pas responsable de l'allure de la répartition des larves ou de leur densité dans le lit de la rivière.

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Introduction

Factors influencing microhabitat selection by stream invertebrates have long been of interest to stream ecologists (Hynes 1970). However, most generalizations about the importance of various factors have been applicable only to species occupying gravel and cobble substrates in small headwater streams. A feature of these substrates is that the large size of the dominant substrate particles combines with the small size of most benthic invertebrates to create a rich mozaic of microhabitats on small spatial scales.

In contrast, the beds of most large lowland rivers are dominated by sand and fine gravel forming extensive areas of relatively uniform particle size composition (Leopold *et al.* 1964). The small size of the particles (relative to the size of the organisms) means that microhabitat diversity for nonburrowing benthic invertebrates is greatly reduced on small spatial scales. Thus, patterns of microhabitat association for nonburrowing invertebrates in lowland rivers are more readily observable than those of nonburrowing species in smaller upland streams.

Pseudiron centralis McDunnough is a predatory mayfly found in many larger lowland rivers in North America. It is unique in being the only nonburrowing predator associated with areas of unstable shifting sand in these rivers. It is a specialized predator, feeding almost exclusively on chironomid larvae which it captures while actively foraging over the surface of the sediment (Soluk and Clifford 1984).

Although larvae of *P. centralis* are found in association with shifting sand, they also occur on other types of sandy substrates. This study quantitatively examines patterns of microhabitat association exhibited by *P. centralis* larvae and relates them to patterns of prey and predator abundance in the river bed. Selection for different substrate particle size classes and spacing behaviour were also examined experimentally to assess their potential effects on larval distribution patterns.

Study site

The study area was at the mouth of the Sand River (54°23' N; 111°02' W) in east central Alberta. The main stream channel of the Sand River is dominated by actively moving sand dunes and ripples; marginally there is an area of stable sands; and adjacent to the banks there is a narrow (<1.5 m) band of silt. Gravel bars also occur at irregular intervals in the bed. A more complete description of the study site is provided by Soluk (1983) and Soluk and Clifford (1984).

Methods

Field studies

Data on the distribution and abundance of *P. centralis* larvae were obtained from samples taken along 10 transects established at 5-m intervals along a 50-m reach at the mouth of the Sand River. Three types of sandy substrate were recognized based on visual and tactile criteria: marginal sand (MS), characterized by a firm fine-grained texture underfoot and the presence of some silt on the surface of the sediments; gravelly sand (GS), with a firm coarse-grained texture, no apparent silt, and the presence of gravel on the surface; and shifting sand (SS) with a soft loose texture, no apparent silt, and actively moving sand dunes. Particle size distributions at the surface of these substrates ranged from those of MS areas, dominated by fine sand and

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silt (particles < 0.12 mm), to those of SS areas, dominated by coarser sand (particles 0.25–1.00 mm), and finally to those of GS areas, dominated by coarser particles (2.00–64.00 mm) embedded in a sand matrix.

Samples were obtained from the middle of MS and GS areas along the transects and at intervals of 5, 10, 20, and 30 m from the bank wherever possible in shifting sand areas, using a handled Surber sampler (sampling area 930 cm², mesh size 0.243 mm). Samples were obtained twice a month throughout the open-water season (April to November) in 1980, from April to the end of July in 1981, and on one date in June 1982. A detailed description of the sampling procedure is provided by Soluk and Clifford (1984). Larvae were separated into developmental stages using the wing pad criteria given by Clifford (1970): stage I larvae lack wing pads, stage II larvae have wing pads shorter than the distance between them, stage III larvae have wing pads longer than the distance between them, and stage IV larvae have the black wing pads indicative of the final larval instar.

Prey abundance was determined from cores taken in conjunction with Surber samples collected in May and June 1981. Cores were obtained using a modified version of the core-freezer (Shapiro 1958), which was driven 25 cm into the substrate in the immediate vicinity of where the Surber sampler was to be placed. Soluk (1983) gives a detailed description of the core-freezer and the techniques used to obtain samples with this device. In the laboratory, cores were thawed and chironomid larvae were separated from the sediment by elutriation. Larvae were counted, dried at 60°C for 24 h, and weighed on a microbalance.

Experimental studies

Substrate selection by *P. centralis* larvae was investigated using three different experimental designs. Spacing behavior of these larvae was examined in one experiment. All substrates used in the experiments described below were natural mineral substrates removed from the bed of the Sand River. Before use, substrates were heated to 600°C for 48 h, dry-sieved into appropriate size classes, and washed with distilled water.

Experiment 1

The first experiment for examining substrate selection by larvae of *P. centralis* consisted of a series of pairwise comparisons between seven substrate particle size categories: 0.06–0.12 mm, 0.12–0.25 mm, 0.25–0.50 mm, 0.50–1.00 mm, 1.00–2.00 mm, 2.00–3.36 mm, and 3.36–6.35 mm. Each of the two substrate types to be compared was placed in two trays with dimensions of 16.0 × 5.5 × 1.6 cm, and these trays were then arranged in a 2 × 2 Latin square design. The four trays were then placed in an artificial stream with a width of 11 cm and a water depth of 14 cm. One stage III larva was placed on the substrate in each of the four trays and the stream was then run at a velocity of 12 cm·s⁻¹ (higher velocities caused significant outwash of fine substrates). After 15 min, the number of larvae on each substrate was recorded.

Four replicates were obtained for each pairwise comparison. The total number of individuals found on each particle size category was analyzed for each comparison using a two-tailed binomial test (Zar 1974).

Experiment 2

The second experiment tested for selection among four substrate size categories simultaneously. Material from each category was placed in four Plexiglas trays (6.0 × 6.0 × 2.3 cm), which were randomized within a 4 × 4 arrangement with the constraints that no substrate category occurred more than once in any column or row and all substrate categories contacted all other substrate categories the same number of times. Two runs were conducted, the first using substrate size categories 0.06–0.12, 0.12–0.25, 0.25–0.50, and 0.50–1.00 mm; and the second with categories 0.25–0.50, 0.50–1.00, 1.00–2.00, and 2.00–3.36 mm. One stage III larva was placed on the substrate in each of the 16 trays, and the number of larvae on each of the substrate types was counted after 1 h. Each run consisted of four replicates, all carried out in an air-powered recirculating stream having a width of 30 cm, a depth of 7 cm, and a

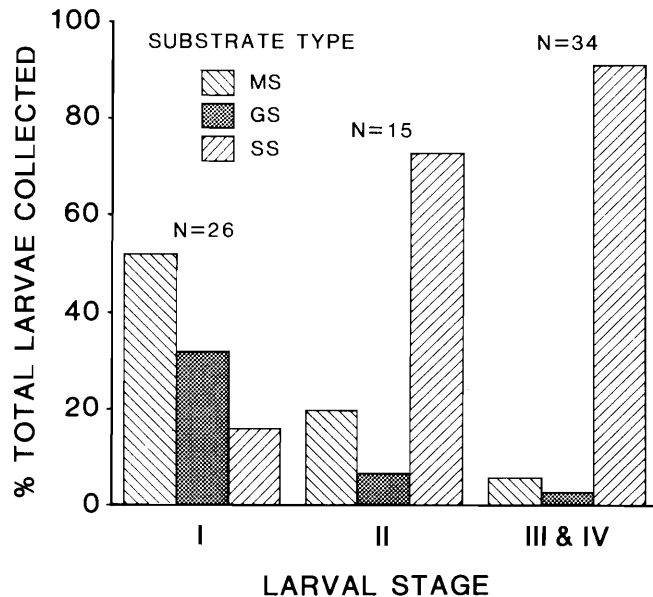


FIG. 1. Proportion of *P. centralis* larvae of different developmental stages obtained from shifting sand (SS), marginal sand (MS), and gravelly sand (GS) areas of the Sand River (N = sample size for a particular stage). Stage III and IV larvae are pooled into one category.

mean velocity of 12 cm·s⁻¹.

Experiment 3

A third experiment was conducted to determine whether *P. centralis* larvae discriminate between fine sand (particle size 0.06–0.12 mm) and silt (particle size < 0.06 mm). Four trays (11.5 × 11.5 × 3.0 cm), two containing fine sand and two containing silt, were arranged in a 2 × 2 Latin square. Two stage III larvae were placed on the substrate in each tray. After 1 h, the number of larvae on each substrate type was recorded. There were four replicates. This experiment was run in the absence of current, since even the lowest detectable water velocities caused some outwash of silt.

Experiment 4

Spacing behavior of *P. centralis* larvae was examined experimentally to determine: (i) whether field densities and the random dispersion pattern exhibited by larvae (Soluk and Clifford 1984) might be due in part to interactions between individuals; and (ii) whether interaction between individuals may have affected the results of the substrate selection experiments.

Four trays (6.0 × 6.0 × 2.3 cm) containing sand (particle size 0.25–1.00 mm) were placed side by side in an air-powered flow tank with a velocity of 16 cm·s⁻¹ and a depth of 7 cm. Eight stage III larvae were introduced upstream of the substrate trays. After 1 h, the number in each tray was recorded. Another four larvae were then introduced and the number in each tray was recorded again after 1 h. This process was repeated until 28 larvae had been introduced into the flow tank.

Results

Larval distribution and abundance

Larvae show definite shifts in their association with the three types of sandy substrates during development (Fig. 1). Contingency table analysis (Zar 1974) was used to determine whether particular larval stages exhibited associations with particular types of sandy substrate (Table 1). When significant associations were indicated, subdivision of the contingency table (Zar 1974) was used to assign particular associations. Data for each stage were assembled only from dates on which the stage in question was present; stages III and IV were considered together. Analysis indicated that stage I larvae were

TABLE 1. Number of samples obtained from the three categories of sandy substrate (SS, shifting sand, MS, marginal sand, and GS, gravelly sand) in which *P. centralis* larvae of particular stages were present or absent

	Substrate category			
	SS	MS	GS	Total
Stage I larvae				
Absent	71	34	31	136
Present	4	11	6	21
Total	75	45	37	157
Stage II larvae				
Absent	66	33	27	126
Present	10	3	1	14
Total	76	36	28	140
Stages III and IV larvae				
Absent	48	26	24	98
Present	24	2	1	27
Total	72	28	25	125

associated with marginal sand ($p < 0.05$) and stage III and IV larvae were associated with shifting sand areas ($p < 0.001$). The low number of stage II larvae necessitated a pooling of the marginal sand and gravelly sand categories; these larvae exhibited no significant differentiation between this pooled category and shifting sand.

The relative proportions of the various substrate types in the study reach (approximately 70% SS, 15% MS, and 15% GS) did not change substantially during the larval period in any year of our study.

Prey abundance, in terms of both biomass and number of chironomid larvae, was similar in the three types of sandy substrate (Fig. 2). Analysis of variance of $\log(n + 1)$ -transformed data indicated no significant differences ($p > 0.05$) in abundance of chironomids on the substrate types for any of the dates on which samples were taken.

Experimental studies

Experiment 1

Substrates with particle sizes in the range from 0.063 to 2.00 mm appeared to be the only ones actively chosen by stage III larvae of *P. centralis* (Table 2). When larvae were given the choice between the two coarsest particle size classes (2.00–3.36 and 3.36–6.35 mm), 10 of the 16 larvae left the substrate and drifted in the water column after spending only a short time on the substrate. In contrast, the highest number of larvae drifting in all other comparisons was two.

Although discrimination was exhibited between substrates of nonadjacent size classes, no significant discrimination was exhibited between adjacent classes. This suggests that if the experiment had compared only adjacent classes then no significant selection would have been observed. Larvae did not remain in the tray in which they were initially placed, but moved actively between the substrate trays especially during the first 5 min after the current was turned on; thus it is unlikely that placement of larvae tended to bias against selection in this experiment.

Experiment 2

The first run of this experiment examined substrate selection

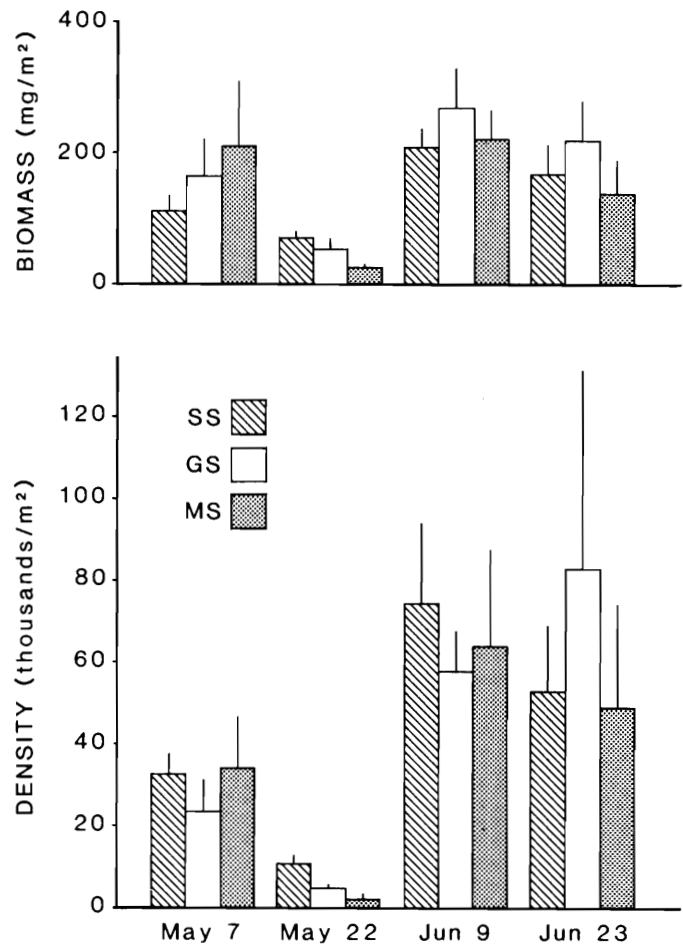


FIG. 2. Abundance of chironomid larvae (+SE) in terms of biomass (upper figure) and density (lower figure) in shifting sand (SS), gravelly sand (GS), and marginal sand (MS) areas of the Sand River.

for the four categories from 0.06 to 1.00 mm (Table 3). The χ^2 value was 15.19, indicating significant differences ($p < 0.005$) among the four categories. Subdivision of the χ^2 analysis (Zar 1974) indicated that the two smallest size categories (0.06–0.12 and 0.12–0.25 mm) were selected significantly more often ($p < 0.05$) than the larger size categories (0.25–0.50 and 0.50–1.00 mm). In the second run, the χ^2 value was 38.96, indicating highly significant differences among the categories ($p < 0.001$). Subdivision of the analysis further indicated that particle sizes from 0.25 to 1.00 mm were selected significantly more often than particle sizes from 1.00 to 3.36 mm ($p < 0.05$).

The combined results of experiments 1 and 2 indicate that stage III *P. centralis* larvae select fine to medium sands (0.06–1.00 mm) most often, selecting coarse sand (1.00–2.00 mm) less often, and generally avoiding substrates with particle sizes greater than 2.00 mm.

Experiment 3

Results of the four replicates of this experiment were summed to yield counts of 17 and 14 individuals in the fine sand and silt categories respectively. χ^2 analysis of this data indicated no significant discrimination between the two substrates ($\chi^2 = 0.32$, $p > 0.50$).

Experiment 4

If *P. centralis* larvae exhibit active spacing behavior then, as numbers increase for a limited amount of optimal substrate, the

TABLE 2. Number of *P. centralis* larvae on each substrate in pairwise comparisons (expressed as row versus column) of seven different particle size categories: I (0.06–0.12 mm), II (0.12–0.25 mm), III (0.25–0.50 mm), IV (0.50–1.00 mm), V (1.00–2.00 mm), VI (2.00–3.36 mm), VII (3.36–6.35 mm)

Particle size category	I	II	III	IV	V	VI	VII
I	—	7	9	12	9	15*	16*
II	9	—	10	13*	12	16*	16*
III	7	6	—	9	14*	14*	16*
IV	4	3*	7	—	10	15*	16*
V	7	4	1*	6	—	11	15*
VI	1*	0*	2*	1*	3	—	5
VII	0*	0*	0*	0*	1*	1	—

*Significantly different pairs (binomial test, $p < 0.05$).

TABLE 3. Total number of larvae on each substrate type in each run of experiment 2

Particle size (mm)	Total no. of larvae on each category	
	First run	Second run
0.06–0.12	25	—
0.12–0.25	14	—
0.25–0.50	8	23
0.50–1.00	7	29
1.00–2.00	—	6
2.00–3.36	—	0

mean density on that substrate should approach an asymptote with extra individuals being displaced either into the drift or to less optimal substrates. There was no apparent tendency toward an asymptote, except at the highest larval density used in the experiment, at which there was almost no unoccupied space left in the substrate trays (Fig. 3). Calculation of an index of dispersion (Southwood 1978) indicated that larvae were dispersed randomly among substrate trays for all densities used ($0.25 < p < 0.90$).

Discussion

Larval habitat associations

Shifts in microhabitat utilization during development have been reported for several species of aquatic insects (e.g., Hildrew *et al.* 1981; Gotceitas and Clifford 1983). These shifts generally seem to consist of a broadening of microhabitat utilization by larger developmental stages (small larvae restricted to nearshore areas, large larvae found in a variety of microhabitats in the main stream), and have been variously attributed to food availability or avoidance of predation.

The shift in habitat association by *P. centralis* larvae is especially dramatic since larger larvae (stage III and IV) show a high degree of specificity for a habitat type generally considered to be extremely unfavourable for benthic macroinvertebrates. The costs of surviving on the surface in areas of actively shifting unstable sands are probably high (maintaining position in the face of high current speeds and loose substrates, resisting the abrasive forces of sand grains) and the absence of other nonburrowing macroinvertebrates attests to harshness of this habitat. We suggest that the ability to cope with the rigors of shifting sand areas is size related; thus stage I larvae were

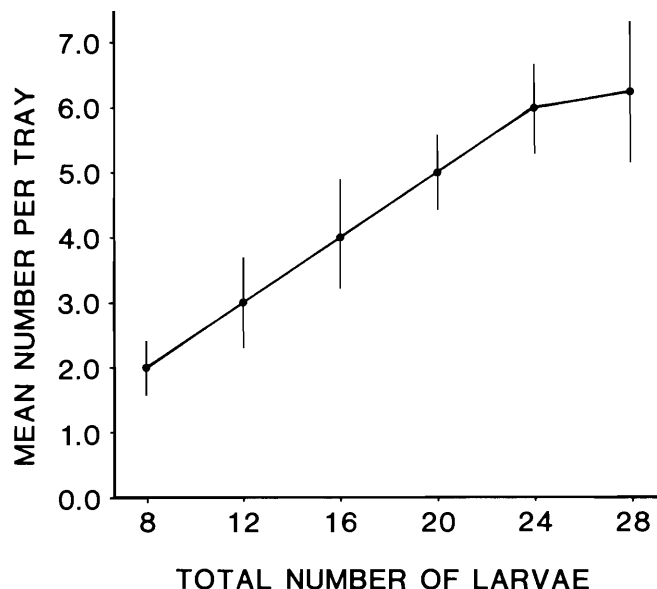


FIG. 3. Mean number (\pm SE) of *P. centralis* larvae in each of four trays, as a function of larval density in the experimental tank.

largely confined to marginal areas, and stage II larvae were transitional in their association between marginal areas and shifting sand areas. This does not necessarily imply active selection of marginal areas by small larvae, but such a restriction may occur by passive processes such as lateral transport of dislodged or drifting animals (Ciborowski 1983).

Stage III and IV larvae appeared to have no problem maintaining themselves in shifting sand areas and were found almost exclusively in these areas of the river bed. Results of the substrate selection experiments provide a possible explanation for the absence of larvae from gravelly sand areas, since they indicated that larvae avoid gravel. However, there is no indication as to why stage III and IV larvae were absent from marginal sand areas. Larvae were maintained at low current velocities for weeks in the laboratory at temperatures similar to those measured in the field; thus selection for higher current velocities to satisfy immediate physiological needs is unlikely.

Although prey abundance was not significantly higher in shifting sand areas than in the other areas examined, relative accessibility of chironomid larvae may be higher in shifting sand. The observed preference of *P. centralis* larvae for the eroding upstream face of sand dunes (Soluk and Clifford 1984) suggests that movement of the sand may expose chironomid larvae. Since *P. centralis* larvae are only able to make shallow excavations in the sand (3 or 4 mm, using a unique behavioral mechanism; Soluk 1983), they probably exploit prey items exposed largely by the action of the current and the instability of the substrate in shifting sand areas. A complicating factor is that changes also occur in the composition of the chironomid communities associated with the substrate types. Shifting sands are dominated almost exclusively by chironomid species with larvae that live in the interstices between sand grains (Soluk 1983), whereas marginal sand and gravelly sand areas support large numbers of tube-dwelling and burrowing forms which may be less susceptible to capture by *P. centralis* larvae.

Pseudiron centralis larvae are the only nonburrowing macroinvertebrates that occupy shifting sand areas in the Sand River. Possibly by occupying this area, they avoid interactions with the relatively diverse epibenthic fauna of gravelly sand and

marginal sand areas. Specifically, such areas in the Sand River support large numbers of larvae of the family Gomphidae (Odonata). Tsui and Hubbard (1979) suggest that these predaceous dragonfly larvae restrict larvae of the mayfly *Dolania americana* (a burrowing predator) to shifting sand areas through competition for food resources. For *P. centralis* larvae, predation by odonates is probably more important as a determinant of distribution than competition for food resources. This is suggested by the rapid decline in the number of stage III larvae in laboratory streams containing gomphid larvae (D. A. Soluk, personal observation). Possibly an active foraging strategy, such as that of *P. centralis* larvae, is incompatible with the presence of a significant number of "sit-and-wait" predators, such as gomphid larvae.

Larval spacing behavior

Observations suggest that individual *P. centralis* larvae will not tolerate physical contact with other *P. centralis* larvae. Such contact elicits a response whereby one or both individuals will rapidly swim or crawl away. The spacing experiment indicated that larvae seem to tolerate a mean density of about six individuals in a 36-cm² area. This density could easily have been accommodated in the substrates used in the substrate selection experiments, and thus it is unlikely that spacing of individuals had an appreciable effect on these experiments. Further evidence of the lack of significant spacing or aggregation behavior is the random assortment of *P. centralis* larvae between substrate trays at all densities in the spacing experiment. Larvae also exhibit a random dispersion pattern on shifting sand areas in the river bed (Soluk and Clifford 1984).

Densities of *P. centralis* larvae in the Sand River were less than 10 larvae·m⁻² (Soluk and Clifford 1984). The density tolerated by larvae in the spacing experiment was equivalent to 1600 larvae·m⁻²; it thus seems unlikely that spacing is an important determinant of field densities, even given that actively foraging individuals would probably tolerate only a much lower density.

Although stage III *P. centralis* larvae do show a degree of discrimination in their association with various size categories of substrates, they are less selective than was expected based upon the distribution of larger larvae in the river bed. This suggests that substrate size may be an important factor in determining broadscale distribution (sandy substrates versus gravel substrates), but other factors determine the final microhabitat specificity of larvae.

Shifting sand areas are considered harsh habitats because of the continuous motion of the sand, and because these areas support few species. However, on a longer time scale, shifting sand areas are less subject to the effects of fluctuations in discharge than other types of substrates in rivers (Barton 1980) and as such present a relatively uniform environment that can be exploited if an organism can cope with substrate instability,

high current speeds, and the abrasive forces of moving sand grains. *Pseudiron centralis* larvae not only cope with these stresses, but are probably able to use them to their own advantage. Further studies on this unusual animal and its habitat should lead to insights into possible combinations of factors that control habitat and microhabitat selection in nonriffle invertebrates associated with larger streams.

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- BARTON, D. E. 1980. Benthic macroinvertebrate communities of the Athabasca River near Ft. Mackay, Alberta. *Hydrobiologia*, **74**: 151–160.
- CIBOROWSKI, J. J. H. 1983. Downstream and lateral transport of nymphs of two mayfly species (Ephemeroptera). *Can. J. Fish. Aquat. Sci.* **40**: 2025–2029.
- CLIFFORD, H. F. 1970. Variability of linear measurements throughout the life cycle of the mayfly *Leptophlebia cupida* (Say) (Ephemeroptera: Leptophlebiidae). *Pan-Pac. Entomol.* **46**(2): 98–106.
- GOTCEITAS, V., and H. F. CLIFFORD. 1983. The life history of *Dicosmoecus atripes* (Hagen) (Limnephilidae: Trichoptera) in a Rocky Mountain stream of Alberta, Canada. *Can. J. Zool.* **61**: 586–596.
- HILDREW, A. G., C. R. TOWNSEND, and J. HENDERSON. 1981. Interactions between larval size, microdistribution and substrate in the stoneflies of an iron rich stream. *Oikos*, **35**: 387–396.
- HYNES, H. B. N. 1970. The ecology of running waters. University of Toronto Press, Toronto.
- LEOPOLD, L. B., M. G. WOLMAN, and J. P. MILLER. 1964. Fluvial processes in geomorphology. Freeman, San Francisco.
- SHAPIRO, J. 1958. The core freezer: a new sampler for lake sediments. *Ecology*, **39**: 758.
- SOLUK, D. A. 1983. Ecology of shifting sand areas in rivers. M.Sc. thesis, University of Alberta, Edmonton.
- SOLUK, D. A., and H. F. CLIFFORD. 1984. Life history and abundance of the predaceous psammophilous mayfly *Pseudiron centralis* McDunnough (Ephemeroptera: Heptageniidae). *Can. J. Zool.* **62**: 1534–1539.
- SOUTHWOOD, T. R. E. 1978. Ecological methods: with particular reference to the study of insect populations. 2nd revised ed. Chapman and Hall, London.
- TSUI, P. T. P., and M. D. HUBBARD. 1979. Feeding habits of the predaceous nymphs of *Dolania americana* in northwestern Florida (Ephemeroptera: Behningiidae). *Hydrobiologia*, **67**(2): 119–123.
- ZAR, J. H. 1974. Biostatistical analysis. Prentice-Hall, Englewood Cliffs, NJ.