A Field Method to Quantitatively Sample Sand Invertebrates

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ABSTRACT. — The use of CO_2 flotation was found to be 89-95.6% effective in separating invertebrates from sand samples. It can be used under field conditions to obtain samples of living material and to make quantitative estimates of sand populations. For quantitative work, two fresh CO_2 rinses are necessary. The paper also gives preliminary estimates of populations of Chironomidae in the clean sand of the Blackwater River, Florida.

Many streams in northern Florida, particularly the acid streams, have a unique benthos associated with sand substrates. One of these is the Blackwater River, an undisturbed, protected, sand-bottom river in the Blackwater River State Forest, Okaloosa and Santa Rosa Counties, Florida. Many studies on sand substrates have been restricted by a lack of precise methods for separating the fauna from the sand, but there are two studies that provide quantitative estimates of the Blackwater River sand fauna (Bass & Hitt 1977, Tsui & Hubbard 1979).

Separating the fauna from the sand either with sieves or by hand has presented problems. Invertebrates, particularly small ones, are probably missed in both processes, and hand-sorting is too tedious and time-consuming for general use. Other separation methods include flotation (soap, oil, sugar, CO_2), elutriation, and centrifugation (Edmondson & Winberg 1971). Centrifugation requires that samples be sorted with a centrifuge in the laboratory, limiting its use in the field. We tried the other methods at least once under field conditions in an effort to find a method that separated live invertebrates from sand with relative ease and accuracy. Flotation with CO_2 offered the greatest promise and this paper gives results of experiments designed to find the most efficient way of using CO_2 to float live invertebrates from the clean sand.

METHODS

Studies were made in the clean sand of the Blackwater River, Okaloosa Co., Florida, which was described by Beck (1973), Peters and Jones (1973), Bass and Hitt (1977), and Peters and Peters (1977). Sand analysis showed that 75-90% of sand by weight was in the size categories from 0.25-1.00 mm diameter (∅1,2) with less than 0.5% very fine sand and silt ($\leq$0.125 mm diameter). Changes in the relative proportions of ∅1 and ∅2 occurred with changes in current. Experiments were conducted on 26-IV, 2-V, and 9-V-1981 in water 36 to 68 cm deep with a current of 45 to 60 cm/sec and temperatures of 21.2 to 23.5°C.

Circular samplers of three standard lengths (5 cm, 10 cm, 15 cm) and various widths retrieved approximately 100 cm^3 of sand per sample. A corer of PVC pipe was used to sample depths greater than 15 cm. Since compactness of the sand varied and some of each sample was lost during retrieval, actual sand volume was measured for each sample so that the number of animals per unit volume of sand could be determined with precision.

Each sample was placed in a 0.35 litre jar. The jar was filled with water saturated with CO_2, corked, agitated 10-30 sec, and the elutriate decanted over filter paper. At first, we used bottles of club soda as a source of CO_2, but later changed to a soda water bottle with CO_2 gas canisters. For experiments, the filter paper, the sample, and the sand were stained with a solution of 1% rose bengal and preserved in 10% formalin. Formalin was used to keep oligochaetes intact until counted and this was fairly successful. We then counted all specimens floated by the CO_2 and all specimens left in the sand under a microscope. Each taxon in a sample was treated as an individual trial. Only trials containing more than 10 specimens were included in Table 3.

Results

Experiments with a single CO_2 rinse collected only 59.8 ± 20.8% of invertebrates present (13 trials, range 26.7-94.0%), a result too low and too variable for quantitative work. Results for two samples with four rinses, recharging the CO_2 before the first and third rinse, are given in Table 1 (data pooled for all groups except Chironomidae). Most animals were collected only after the freshly charged first and third rinses, suggesting that a fresh CO_2 charge was necessary. Table 2 gives total data for a single core using three fresh CO_2 rinses. Although Chironomidae were underrepresented, it was these data that influenced the choice of two CO_2 rinses since a third rinse only contributed about 5% to the total numbers. Subsequent samples used two fresh CO_2 rinses. Two CO_2 rinses, both freshly charged, will give overall estimates of better than 90% of populations present (Table 3). In the Chironomidae, an average 95.6% of the population was estimated (range 89.3-100%).

	N	Number of rinses 1	2	3	4
Chironomidae	67	65.7	74.6	95.5	97.0
	27	70.4	77.8	88.9	92.6
Other benthos	230	56.5	65.2	90.9	91.3

TABLE 1. Percentage of invertebrates collected after four consecutive rinses with CO_2 recharged before first and third rinse.

Depth into sand	Volume (cm^3)	Taxon	Number/rinse 1	2	3	N in sand	Total
0.0–4.7 cm	60	CH	2	2	0	0	4
		EN	9	6	1	1	17
		NE	3	2	0	0	5
		CO	0	1	0	0	1
4.7–10.1 cm	70	CH	1	3	0	0	4
		EN	34	17	3	4	58
		NE	6	1	1	0	8
		CO	11	0	2	1	14
10.1–15.7 cm	72	EN	15	20	2	1	38
		NE	1	1	0	0	2
		CO	4	10	1	0	15
15.7–20.7 cm	64	EN	30	4	1	0	35
		NE	4	0	0	0	4
		CO	78	1	4	0	83
	266		198	68	15	7	288

TABLE 2. Numbers of invertebrates collected in a single core (2V-1981, water depth of 55 cm) treated with three rinses. CO_2 was recharged before each rinse. Abbreviations: CH, Chironomidae; EN, Enchytraeidae; NE, Nematoda; CO, Copepoda.

Taxon	Trials	N collected	N present	Average %/trial
Chironomidae	6	424	436	95.6 ± 4.0
Enchytraeidae	10	910	1016	90.7 ± 3.6
Nematoda	6	363	374	95.2 ± 5.5
Copepoda	3	104	112	89.0 ± 9.1
	25	1801	1938	92.7 ± 5.3

TABLE 3. Percentages of invertebrates collected after two rinses with CO_2 recharged before each rinse.

Few of the invertebrates living in the sand can be identified with any degree of certainty. Some of the Copepoda belong to the harpacticoid genus *Parastenocaris* (based on Pennak 1978). Although they have been sent to specialists, the Nematoda are not yet identified. A single species of Enchytraeidae, *Barbidrilus paucisetus* Loden & Locy, represents the oligochaetes in clean sand. The Chironomidae, identified from larvae or pupae, belong to the Orthocladiinae (*Lopescladius* Oliveira, *Rheosmittia* Brundin, *Corynoneura* Winnertz, *Thienemanniella* Kieffer) and the *Harnischia* group of Chironomini [*Robackia claviger* (Townes), "*Cryptochironomus*" sp. Pagast, *Demicryptochironomus* Lenz]. Additional genera and species of Chironomidae are likely to be found in sand samples from other dates and habitats.

Discussion

The CO_2 flotation method was efficient in clean sand substrates and apparently anesthetized and bubbled out invertebrates present in the sand; individuals returned directly to fresh water remained alive. It was also effective in fine gravel/sand (unpublished data). The method was unsuccessful in silt habitats because silt was bubbled off with the fauna.

We sampled Chironomidae in different sand habitats of the Blackwater and our results using CO_2 flotation can be compared with those of two published reports. For comparison, we have converted all results to numbers/m^2 to a depth of 10 cm into the sand. Bass and Hitt (1977)

Body length	1,2-III-1981* N	%	9,12-V-1981** N	%
≥6 mm	3	.4	5	.3
<6mm >1 mm	321	37.5	201	12.7
≤1 mm	532	62.1	1372	87.0

* 28 samples totaling 1998 cm^3 sand.
** 26 samples totaling 2315 cm^3 sand.

TABLE 4. Size groupings of Chironomidae collected from sand habitats in the Blackwater River, Florida.

surveyed the fauna of the Blackwater using an Ekman dredge and sieves. Their samples taken "near-shore" and "mid-river" averaged 31 (2-II-1977) and 113 (9-V-1977) chironomids/m^2. Tsui and Hubbard (1979) hand-sorted litre samples of sand which had been taken from random depths and preserved in formalin stained with rose bengal. These samples averaged 6,500-9,000 chironomids/m^2 (II, III, IV-1974). Our averages were 53,744 (1,2-III-1981) and 75,723 (9,12-V-1981). These averages were calculated from totals for three 10 cm samples each at depths of 38 to 45 cm (current 44 to 45 cm/sec) and 60 to 72 cm (current 60 to 62 cm/sec). Mean numbers/cm^3 for the two habitats were respectively: March, .846 ± .102 and .153 ± .058; May, 1.044 ± .118 and .469 ± .211.

The differences between our data and those of Bass and Hitt (1977) demonstrate that dredge and sieve methods underestimate populations of sand chironomids. Sieves cannot adequately separate sand particles from midges when both are of similar size, and Flannagan (1970) showed that the Ekman dredge is not reliable in sand. Only when all chironomids less than 6 mm in body length (Table 4) are excluded from our data do the CO_2 flotation estimates compare with the dredge and sieve estimates.

Table 4 gives totals for all Chironomidae collected from samples 2 cm to 27 cm into the sand. These data give a rough estimate of the size distribution of chironomids: most were larvae of 1 mm body length or less (Table 4). Quantitative estimates based only on the percentage of larvae greater than 1 mm body length would approximate the figures given by Tsui and Hubbard (1979). However, the samples of Tsui and Hubbard were taken in a different year when the river was 15 to 58 cm deeper, so differences in estimates of chironomid populations may represent annual population fluctuations or a broadened dispersal. The increased substrate available with a wider stream bed compensates for an apparent reduction in species density, as was shown for mayflies by Lehmkuhl and Anderson (1972).

We do suspect that hand-sorting large samples of sand overlooks many chironomids less than 1 mm in body length. In using hand-sorting to evaluate invertebrates left in the sand (Tables 1-3), we used small samples sorted with care under a microscope, and it seems that the small midges were the first to be floated off by CO_2; however, some may have been missed since every sand grain was not individually examined. If so, overall efficiency may be somewhat less than calculated in Table 3. Whether or not hand-sorting is as accurate as CO_2 flotation, the use of CO_2 has other advantages: sorting time is reduced; small larvae are easily retrieved; and live material is collected.

Acknowledgments

We thank Jarl K. Hiltunen, Great Lakes Fishery Laboratory, Ann Arbor, for identification of oligochaetes, and William L. Peters, A. Elizabeth Gordon, and Michael D. Hubbard, Florida A&M University, for critical review of the manuscript. We also thank the Florida Game and Freshwater Fish Commission, the Florida Division of Forestry, and Robert Carr, State Fish Hatchery, for help and support. This research was supported by a research program (FLAX 79009) of CSRS, USDA.

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