Use of Sugar Flotation and Dye to Sort Benthic Samples

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Study of benthic fauna is an important aspect of many investigations of aquatic environments. Sampling is often restricted because of the time consumed in separating organisms from debris and poor accuracy and precision of many sorting procedures. To be widely applicable, a sorting technique should require little special or elaborate equipment, a minimum of field and laboratory time, and yield reliable results.

Many procedures have been developed to improve the simple method of sieving and hand picking organisms from debris. Flotation techniques have been used with high-density solutions of magnesium sulfate (Ladell, 1936; Beak, 1938), sodium chloride (Lyman, 1943), zinc chloride (Sellmer, 1956), and sugar (Caveness and Jensen, 1955; Anderson, 1959). Carbon tetrachloride flotation has proved useful in some instances (Birkett, 1957; Dillon, 1964; Whitehouse and Lewis, 1966). Successful flotation requires a solution of specific gravity greater than the organisms, but less than the debris. Such a balance is often difficult to obtain and detracts from the general applicability of these techniques in different environments.

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Various dyes have been used to stain organisms in benthic samples (Busbee, 1967; Mason and Yevich, 1967; Korinkova and Sigmund, 1968; Hamilton, 1969). This approach is an improvement over simple hand picking, but organisms must be picked out of debris rather than floating free.

Bayless (1961) constructed an electrical stimulator for recovering benthic organisms from debris. Scarola and Giberson (1967) developed a vacuum device to suck up organisms during hand picking. These techniques worked well under specific applications, but the sample had to be separated fresh (electrical stimulator) or separated a second time into species (vacuum device).

The purposes of this investigation were to compare the effectiveness of the more common techniques used in sorting benthic samples and to recommend a simple procedure useful for routine studies of benthic environments.

METHODS

Field Sampling

Benthic samples were taken with an Ekman dredge (232 sq cm) and sieved through a 0.515 mm mesh wash bucket. The sieved residues were placed in jars and preservative (when used) added to cover the entire sample.

Sample Storage

Seven different treatments were used: (1) jars containing samples to be live picked were kept cool and sorted within a few hours; (2) preservation in 10% formalin; (3) preservation in 70% ethanol; (4) preservation in KAAD, a solution of kerosene, ethanol, acetic acid, and dioxane useful in preserving morphological detail (Peterson, 1962); (5) preservation in rose bengal-formalin solution (0.1 g rose bengal added to 1 liter 10% formalin); (6) preservation in light green SF yellowish-formalin solution (0.1 g light green SF yellowish to 1 liter 10% formalin); (7) preservation in malachite green-formalin solution (0.1 g malachite green added to 1 liter 10% formalin).

Sorting Procedure

Live picking was done by placing sieved residue in a white enamel tray (33 × 55 cm), water added as needed, and organisms were removed with forceps. Sorting was continued until a 2 min examination yielded no further organisms.

Preserved samples were washed thoroughly in a fine mesh dip net to remove fine debris, dye, and preservative, and placed in a white enamel tray (33 × 55 cm). Preliminary trials were made with sugar solutions of various specific gravities to arrive at the most effective concentration. Several liters of sugar solution (sp. gr. 1.125, 440 g sugar added to 1 liter water) were placed in the tray to a depth of 2–4 cm. Most organisms and a small amount of debris floated to the surface. Organisms were removed with forceps until a 2 min examination yielded no further organisms.

Test 1

Twenty-five Ekman dredge grabs were taken over mud-gravel-detritus substrate in Parvin Lake, Colorado, on April 11, 1970. This area of the lake is a plateau at a depth of 4 m. Previous research showed bottom composition to be homogeneous and it was likely that differences in results would be due to treatments rather than to sample bias. Five grabs were randomly selected to be sorted by live picking and 20 to be preserved and stored for several weeks and sorted by sugar flotation. Of these 20 grabs, 5 were randomly selected for formalin preservation, 5 for ethanol preservation, 5 for KAAD preservation, and 5 for rose bengal-formalin preservation. When samples were sorted, the number of larval chironomids, *Lumbriculus* (an aquatic annelid), and sorting time were recorded.

Test 2

Twenty-four Ekman dredge grabs were taken from a mud-detritus bottom at 10 m depth in Parvin Lake on June 7, 1970, which was shown to be fairly homogeneous. Six grabs were preserved in formalin, 6 in rose bengal-formalin, 6 in light green-formalin, and 6 in malachite green-formalin. All 24 samples were stored for several weeks and then sorted by sugar flotation. The number of *Chaoborus* (phantom midge), *Lumbriculus*, and sorting time were recorded.

TABLE 1.—*Estimated number of larval chironomids and* Lumbriculus *per square meter and sorting time as determined by live picking and flotation with various preservatives (Test 1)*

		Flotation			
	Live picking	Formalin	Ethanol	KAAD	Rose bengal-formalin
		Chironomids			
Mean	2755	3024	2540	1529	2117
95% conf. int.	1257–4253	2425–3623	1913–3167	916–2142	1408–2826
		F = 3.41*			
		Lumbriculus			
Mean	3829	2754	2255	2047	7707
95% conf. int.	2800–4858	1747–3761	1125–3385	814–3281	5827–9586
		F = 24.97**			
		Sorting time			
Mean	43	37	36	36	33
95% conf. int.	29–56	29–44	22–49	28–44	28–37
		F = 0.98 ns			

*—ANOVA significant at $\alpha = .05$.
**—ANOVA significant at $\alpha = .01$.
ns—ANOVA not significant at $\alpha = .05$.

RESULTS AND DISCUSSION

Live picking is usually thought to be the most desirable method of separating organisms from debris (American Public Health Association, 1965). The results of test 1 (Table 1) show this is a good method, but not the most efficient for recovering small or transparent invertebrates. For estimating the number of larval chironomids (relatively easy animals to recover in these samples), live picking had the widest confidence interval (1257–4253). This variability is due in part to the activity of organisms, which depends on how soon they were sorted after collection. This source of variation is not present in preserved samples.

For estimating the number of *Lumbriculus* (difficult animals to recover in these samples), rose bengal-formalin was clearly the most efficient with live picking second (Table 1). These worms are small and remain floating only a few minutes in sugar solution and are thus easily missed unless moving or brightly stained.

Sorting time (Table 1) in test 1 showed no significant difference even though rose bengal-formalin treatment yielded many more organisms.

Since the flotation method with dye (rose bengal) appeared to be superior to live picking and flotation with other preservatives based on test 1, a second test was made using dyes suggested by Busbee (1967) for comparison to rose bengal. For recovery of *Chaoborus*, which are easy organisms to recover by any flotation method, no significant difference between treatments was shown (Table 2).

For estimating *Lumbriculus*, rose bengal-formalin was superior to the other treatments (Table 2). Although light green-formalin and malachite green-formalin were superior to formalin, the variability in dying effectiveness was great. Some organisms were dyed dark green and blended with the plant material, while others were unaffected.

Sorting time among the four treatments in test 2 was significantly different (Table 2). Light green-formalin and malachite green-formalin hastened recovery of *Chaoborus* and the larger *Lumbriculus* compared to formalin (hence the shorter sorting times), but these

TABLE 2.—*Estimated number of* Chaoborus *and* Lumbriculus *per square meter and sorting time as determined by flotation with various preservatives and dyes (Test 2)*

	Formalin	Rose bengal-formalin	Light green-formalin	Malachite-formalin
		Chaoborus		
Mean	934	517	1200	1279
95% conf. int.	244–1625	309–725	154–2246	799–1759
		F = 1.69 ns		
		Lumbriculus		
Mean	3256	9803	3392	2623
95% conf. int.	462–6049	5677–13929	–36–6819	1203–4043
		F = 7.79**		
		Sorting time		
Mean	40	32	23	18
95% conf. int.	25–55	20–45	12–34	12–24
		F = 4.80*		

*—ANOVA significant at $\alpha = .05$.
**—ANOVA significant at $\alpha = .01$.
ns—ANOVA not significant at $\alpha = .05$.

three methods were ineffective in recovering the smaller, more difficult, *Lumbriculus*.

CONCLUSION

A solution of rose bengal and formalin is a good all around preservative for benthic samples. Sieved benthic samples can be preserved in this solution for long periods and then sorted by sugar flotation to obtain excellent results.

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