SUBSAMPLER FOR ESTIMATING THE NUMBER AND LENGTH FREQUENCY OF SMALL, PRESERVED NEKTONIC ORGANISMS[1]

When many samples, containing large numbers of organisms, must be processed it is often necessary to take subsamples and assume that they are representative of the total sample. Frequently subsamples are taken in some arbitrary fashion which is described in such terms as "100 fish were randomly selected." However, it is doubtful whether any selection can be adequately random. Therefore, numerous devices have been designed in attempts to secure more representative subsamples and to increase the speed and efficiency of subsampling.

Most subsamplers have been designed for use with plankton, small benthos, and invertebrate drift samples and are generally unsuitable for larger organisms. However, Lewis and Garriott (1971) modified a Folsom plankton splitter for use on meter net samples containing larval fish up to 19 mm long, and Hightower et al. (1976) described a subsampler specifically designed for use with nektonic organisms.

In the present paper I describe the design, operation, and efficiency of a subsampler originally built for research on estuarine nekton (Herke 1971). The subsampler proved to be useful for estimating the number and length frequencies of small nektonic organisms such as the bay anchovy, *Anchoa mitchilli,* tidewater silverside, *Menidia beryllina,* and brown shrimp, *Penaeus aztecus,* as well as young of larger species such as gulf menhaden, *Brevoortia patronus,* and Atlantic croaker, *Micropogon undulatus.* Although different from most subsamplers, the design is fairly similar to that described by Hightower et al. (1976); it bears some similarities to those described by Hewitt and Burrows (1948) for subsampling live hatchery fish, by Cushing (1961) for plankton, and by Södergren (1974) and Hickley (1975) for benthos.

My sampler differs from that of Hightower et al. (1976) in at least four respects: 1) it has fewer moving parts; 2) fewer water jets are required to achieve through mixing of the sample; 3) a central pillar or cylinder prevents organisms from clumping in the center; and 4) the total sample is subdivided by raising vanes through the mixed sample, rather than allowing the sample to settle into baskets. Also, spin-dry weighing is required, but it takes <1 min to complete the subsampling process (after the organisms are placed in the subsampler), rather than several minutes as required for the subsampler described by Hightower et al. I have made no comparative tests between the two subsampler designs, however; individual circumstances may determine which would be most practical in any given situation.

Subsampler Construction

My subsampler can be constructed of various materials, and the same general design can be used for large and small models. A small Plexiglas[2] version (Figure 1) has an outside diameter of 305 mm, and Herke (1971) also illustrated one with a 580-mm outside diameter that utilized part of a 208-l steel drum for the outer cylinder, a 19-l bucket for the inner cylinder, and plywood for the false floor.

The subsampler in Figure 1 was constructed primarily of Plexiglas about 6 mm thick. Plexiglas joints were bonded with solvent (methylene chloride and trichlorethylene). The major parts and their functions are as follows (numbers refer to the parts labeled in Figure 1):

1. Base.
2. Brass hinge for attaching base to edge of table top.
3. Outer cylinder bonded to base; in addition to solvent, a suitable cement may be required to ensure a watertight seal to the base.
4. Central pillar of Plexiglas tube bonded to base at exact center of circle formed by the outer cylinder (3).
5. Rubber stopper in (4) to prevent material from falling inside the piller.
6. Inner cylinder, which slides smoothly up and down over (4).
7. Locking pin for holding (6) in the raised position. Rubber bands around (6) and over a peg through the shaft of (7) hold the pin in place (these are omitted from the diagram to avoid cluttering).
8. Vane bonded to (6); the outer edge almost touches the outer cylinder. In the raised posi-

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[2]Reference to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA.

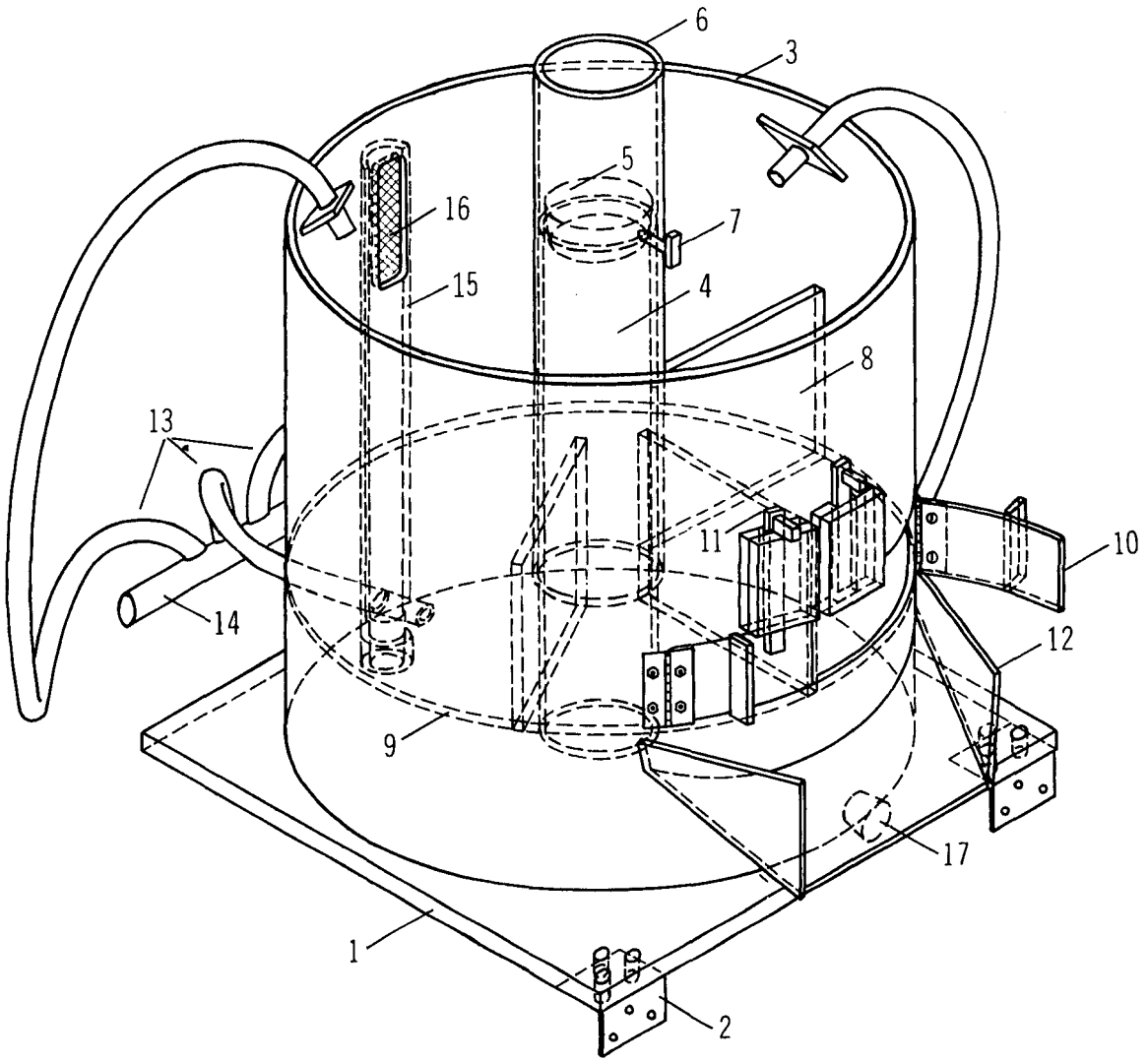

FIGURE 1.—Basic design of the nektonic subsampler; see text for explanation.

tion shown, the three vanes subdivide the sample into portions approximating 0.2, 0.2, and 0.6 of the total.

9. False floor consisting of three sections bonded to the inside of the outer cylinder (3) at a height so that the upper surface is exactly even with the upper edges of the vanes when (6) is lowered to the base. The vanes move up and down through the slits left between the sections of the false floor. Enough space must be left between the inner edges of the false floor and the inner cylinder (6) and its attached vanes to allow the inner cylinder and vanes to move freely up and down. Conversely, the clearance must be small enough to prevent organisms from falling into the space below the false floor. Omitted from the diagram are braces extending from the base to near the inner edges of the two smaller sections of false floor.
10. Hinged door for removing subsampled organisms.
11. Latch holding the other door closed.
12. One side of a spout into which the water and organisms pour when doors are opened; the organisms are collected in a sieve below the spout.
13. Rubber tubes to carry water; the middle one enters the cylinder (3) beneath the false floor (9).

14. Copper tube with outlets for each rubber tube (13). Water to operate the subsampler comes through a large-diameter garden hose and pistol-type hose nozzle (not shown) attached to this tube.
15. Overflow tube attached to the outside of the cylinder (3). The cut edges of a longitudinal section of Plexiglas tubing are bonded to the cylinder from the overflow intake to the bottom of the base (1). Below the base the tube is not sectioned (i.e., left intact) so a drain hose can be attached to it.
16. Aluminum window screen covering overflow intake; bottom of intake opening is level with the top of the vanes (8) when they are raised.
17. Rubber stopper in drain hole below spout.

Also omitted from the diagram are "stops" on the bottom edges of the vanes (which prevent the vanes from pulling through the false floor) and spongy, foam gaskets attached to the doors with rubber cement.

Subsampling Procedure

In subsampling, one pushes the inner cylinder (6) with the attached vanes down until it rests on the base; in this position the tops of the vanes are even with the top of the false floor so that the vanes and floor form a single flat surface. The entire sample is then placed on the false floor. The hose nozzle trigger is squeezed fully open, squirting water rapidly through the rubber tubing. (Normally, the space below the false floor is still filled with water from previous use.) Some of the water rises through the three vane slits in the false floor, thereby inhibiting downward passage of the smaller specimens; most of the water squirts out of the upper tubes, causing the water above the false floor to swirl rapidly. Turbulence thoroughly mixes the sample as both sample and water revolve. When the water almost reaches the bottom of the overflow intake, the inner cylinder (6) and attached vanes are quickly raised as far as possible so that the locking pin (7) slides farther through its hole in (6) and over the top edge of (4); simultaneously, the hose nozzle trigger is released. The sample has now been divided into three parts equal to about 0.2, 0.2, and 0.6 of the whole.

The entire subsampler is next tilted on its hinges (2) in preparation for emptying. If a 0.2 subsample is desired, only one door is opened and the contents of that compartment flow through the spout (12) into a sieve. (To avoid bias, the user should always open the same door first. Occasionally fish balance on top of the vanes; the user can avoid personal bias by always pushing the fish so it falls headfirst.) Opening both doors produces a 0.4 subsample and the remainder of the material in the subsampler constitutes a 0.6 subsample. The 0.6 subsample is removed by first taking out the 0.4 subsample and then lowering the vanes as far as they will go. The 0.6 subsample may then be washed into a sieve below the spout. (When removing any subsample, it is easier to wash the organisms out of the subsampler than to pick or push them out.) A wide variety of subsample ratios can be obtained by sequentially subsampling subsamples (e.g., $0.8 \times 0.2 \times 0.2 = 0.032$).

Small organisms do occasionally fall through the vane slits into the space between the base and the false floor. Such losses are normally insignificant compared with the total number being subsampled, but they are noticeable through the Plexiglas. These organisms may be recovered by washing them out through the drain hole plugged by the rubber stopper (17).

No special leveling of the subsampler is required for proper operation; it may be mounted on any reasonably level surface such as a table top or laboratory bench.

Discussion

The subsampler is useful for estimating both total numbers in a sample and the total length-frequency distribution. If the total sample is not first separated by species, one should at least make a thorough scan of the sample, before subsampling, to remove any unusually large or odd specimens. As stated by Hightower et al. (1976), these can later be added to the total estimate, which is derived by extrapolating the subsample results. However, subsampling can give erratic results for inconspicuous species present in small numbers. Therefore, I think it usually is best to first separate the total sample into individual species, and subsample only the abundant ones. For each of these species, a subsample is first taken, and its weight and that of the remainder are obtained by the spin-dry method described by Herke (1973). (In contrast to plankton, preserved fishes and many crustaceans can be easily and precisely weighed without damage by using the spin-dry method.) All organisms in the subsample are then

counted and the number in the total sample is estimated on the basis of the weights of the subsample and total sample. Since the estimate is based on weight rather than volume, the three vanes need not divide the subsampler into exactly 0.2, 0.2, and 0.6 segments.

If a length-frequency estimate is desired, the subsample can be further subsampled. Since the number in the first subsample is now known, any desired number for the length-frequency subsample can be closely approximated by selecting the proper sequence of subsamples. For instance, suppose the first subsample contains 3,371 anchovies and a length frequency is desired from approximately 100 fish; $3{,}371 \times 0.2 \times 0.4 \times 0.4 = 108$. Therefore, subsamples taken in this sequence should produce the desired number for measuring.

The consistency with which the desired number is obtained may be judged (Table 1) by comparing the "theoretical" and "actual" numbers obtained in 20 successive trials. The two subsamplers used in these trials had a tendency to slightly exceed the desired number; one or both of the smaller compartments in each subsampler probably contained a bit more than 0.2 of the whole. However, the increased subsample size actually improves the probability of obtaining an accurate length-frequency estimate. Also, with use, one soon learns whether the tendency is to obtain more or fewer than the theoretical number and can select the subsampling sequence accordingly.

How well the length-frequency estimates derived from subsampling groups of anchovies and menhaden represented the true length frequencies of the groups was examined by using the Kolmogorov-Smirnov one-sample, two-tailed test, which is a test of goodness of fit. The test involves comparing the observed cumulative frequency distribution from a subsample with the cumulative frequency distribution of the total sample. It is sensitive to any kind of difference between the two distributions—differences in location (central tendency), in dispersion, in skewness, etc. According to Siegel (1956) the Kolmogorov-Smirnov test is definitely more powerful than the chi-square test when samples are small, and may be more powerful in all cases.

The cumulative length-frequency distribution for only one subsample was significantly different ($\alpha = 0.05$) from its corresponding total sample (Table 1). In the other 19 tests, the probability was greater than 0.20 that a divergence of the observed magnitude would occur if the observations were really a random subsample from the total sample (0.20 is the highest probability listed in Siegel's table).

TABLE 1.—Results of 20 tests to determine the correspondence between: 1) the theoretical and actual number of bay anchovies or gulf menhaden in the subsample, and 2) the cumulative length frequency distribution of fish in the subsample and in the corresponding total sample. Subsamples were returned to the total sample after each trial. The cumulative distribution shown in italics (in the same row with the number in the total sample) was the true distribution obtained by measuring every fish in the sample.

Number in total sample	Subsample sequence	Final subsample no.		Standard length in millimeters[1]									
		Theoretical	Actual	15	20	25	30	35	40	45	50	55	60
3,371					*0.539*	*0.772*	*0.821*	*0.861*	*0.914*	*0.957*	*0.984*	*0.995*	*1.000*
anchovies	(0.2) (0.4) (0.4)	108	133		.481	.797	.835	.880	.947	.970	.977	.992	1.000
			124		.524	.758	.838	.863	.911	.960	.976	1.000	
			134		[2].418	.739	.791	.858	.932	.962	1.000		
			141		.489	.709	.773	.822	.894	.950	.979	.993	1.000
			146		.479	.740	.781	.856	.925	.938	.986	1.000	
1,505				*0.361*	*.553*	*.643*	*.774*	*.846*	*.908*	*.964*	*.991*	*.998*	*.999*
anchovies	(0.2) (0.2)	60	49	.347	.551	.571	.673	.734	.795	.917	.958	.999	
			59	.322	.576	.661	.729	.814	.848	.933	.967	1.001	
			77	.338	.520	.559	.676	.793	.832	.949	.988	1.001	
			70	.357	.528	.628	.728	.799	.885	.956	.985	.999	
			71	.389	.570	.598	.667	.778	.875	.958	1.000		
Same	(0.4) (0.2)	120	128	.328	.586	.672	.766	.836	.914	.992	1.000		
1,505			125	.272	.544	.600	.776	.864	.920	.976	1.000		
anchovies			133	.353	.556	.654	.789	.842	.880	.940	.985	.993	1.001
			134	.306	.507	.589	.768	.843	.903	.970	1.000		
			152	.283	.526	.598	.710	.780	.881	.934	.987	1.000	
1,221								*.020*	*.273*	*.756*	*.980*	*.998*	*1.000*
menhaden	(0.4) (0.2)	98	90					.000	.278	.656	1.000		
			116					.026	.198	.733	1.000		
			115					.017	.252	.765	.991	1.000	
			128					.031	.242	.664	.969	.984	1.000
			113					.027	.345	.796	1.000		

[1]Measured in 5-mm increments; i.e., 15 = 15.0–19.9, 20 = 20.0–24.9, etc.
[2]The probability of a divergence this large in a random subsample from the total sample was between 0.05 and 0.01. The probability for the 19 other subsamples was >0.20.

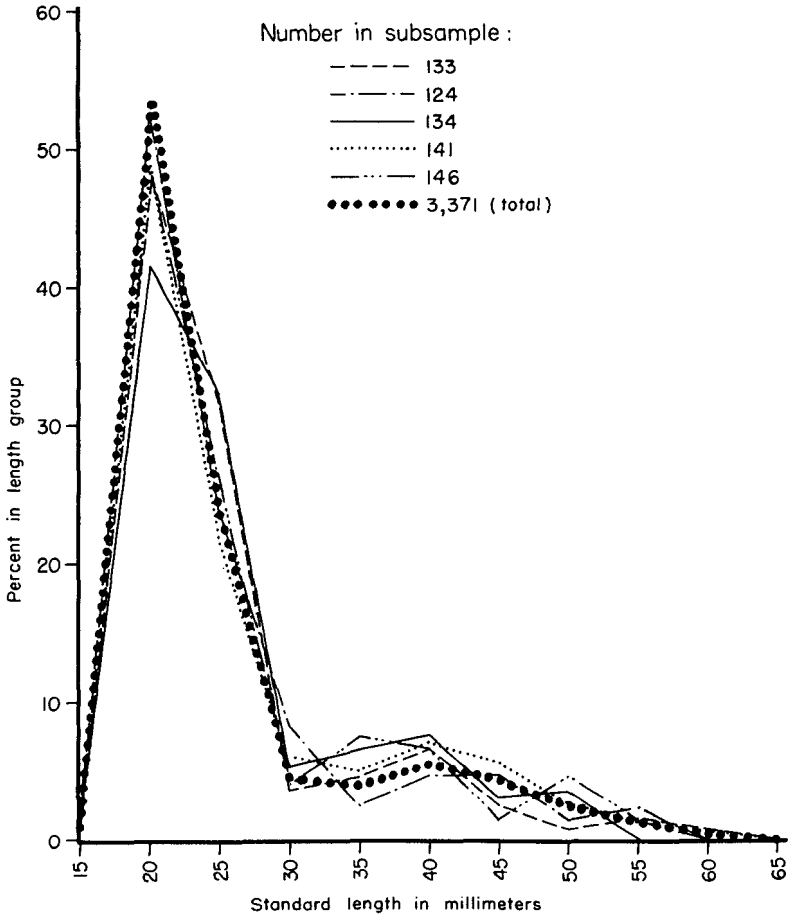

FIGURE 2.—Length-frequency distribution of a total sample of 3,371 bay anchovies, and of each of five subsamples taken from the total. (From Herke 1971.)

It is difficult to visualize, from inspection of the cumulative length-frequency distributions, how well the percentage of fish in each subsample length group represents the percentage in the corresponding length group in the total sample. Therefore, this comparison is shown graphically (Figure 2) for the first five subsamples listed in Table 1.

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