A Note on Bacteriological Sampling in Seawater[1]

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ABSTRACT

In a comparative study, two existing bacteriological samplers and a newly designed device were tested for obtaining uncontaminated seawater samples. *Serratia marinorubra* was used as a tracer organism. It was possible to eliminate contamination to a large extent.

Introduction. During recent studies on growth characteristics of heterotrophic marine bacteria from various oceanic environments, satisfactory deep-sea sampling presented a considerable problem. Although a few microorganisms of external origin may be tolerable when certain metabolic types are to be isolated from a particular environment, it is essential to avoid unidentifiable contaminants or contaminants that displace slow-growing indigenous bacteria of low substrate specificity during the isolation procedure. Therefore, a special sampling device has been constructed and tested, employing the pigmented bacterium *Serratia marinorubra* as a tracer organism.

With the bacteriological samplers commonly used, contamination of the sample results from the breaking of a glass capillary or the cutting of a rubber intake tube by an unsterile object. Furthermore, the sample is taken near the sampling gear, and, even if autoclaved prior to sampling, the sampler can be heavily contaminated by microorganisms released with lubricating materials from the hydrowire and dispersed on the sea surface.

The Sampler. In order to avoid these difficulties, the intake of our sampler (Fig. 1) consists of a 15-cm long silicone rubber tube covered with a cellophane dialysis bag and attached to a 50-ml syringe (Fig. 2). This unit is sterilized as a whole. When the sampler is triggered by a messenger, the intake tube

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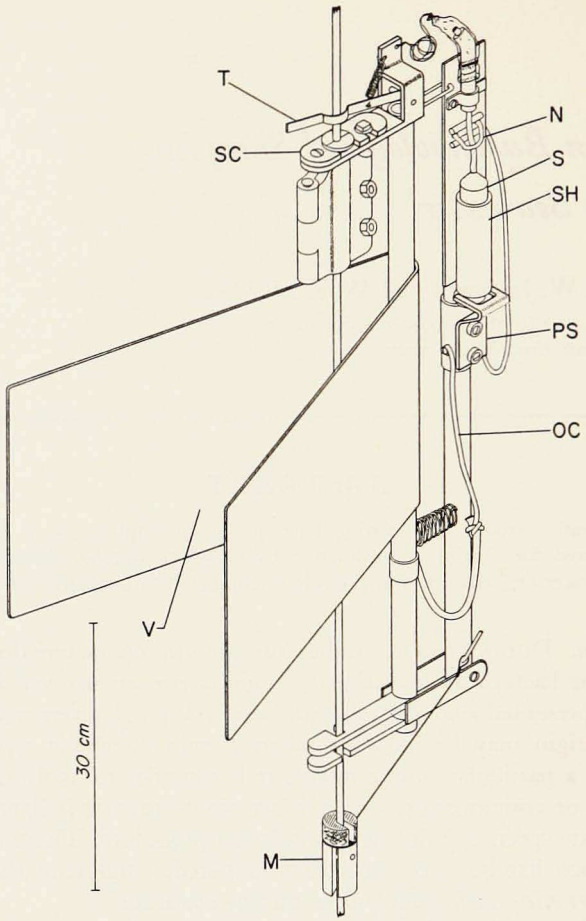

Figure 1. Sampler attached to hydrowire. (T) trigger, (SC) swivel clamp, (N) closing noose, (S) syringe, (SH) syringe holder, (PS) plunger slide and plunger-holding clamp, (OC) operating cable, (M) second messenger, (V) vane.

remains covered while the syringe swings away from the hydrowire. At a distance of about 75 cm, the dialysis bag starts to roll back, progressively exposing new sterile surface until the end of the intake tube is reached and sampling begins, as illustrated in Fig. 3. The sampler rotates freely on a swivel and is headed into the current by means of a vane.

Immediately after the syringe is autoclaved, about half of the water-filled open cylinder is sealed with a hot 1:1 mixture of paraffin-paraffin oil (Fig. 2). For work at sea, a number of syringes can be preautoclaved and kept in aluminum foil. In the closed condition, the water-filled syringe and intake tube are free of air, which makes the sampler suitable for collecting anaerobic micro-

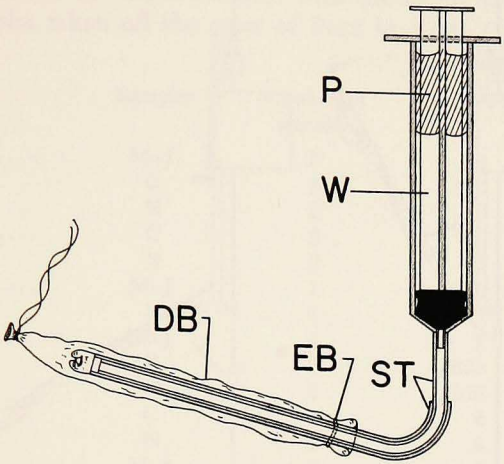

Figure 2. Syringe assembled for sterilization. (DB) dialysis bag filled with water, (EB) elastic band, (ST) silicone rubber tubing, (W) water-filled space behind plunger, (P) soft paraffin-oil mixture (added after autoclaving).

organisms. The flexible dialysis bag accommodates volume changes under pressure equilibration. The volume of the sample may be too small for some types of microbiological studies, but a larger sampler operating on the same principle is conceivable.

Tests and Experiments. A wild strain of *Serratia marinorubra* (obtained from A. F. Carlucci) was grown in 0.5% yeast-extract seawater (75%)-agar, harvested, and emulsified in a few milliliters of motor oil. There was no perceptible growth inhibition of the organisms when the emulsion was plated after 1 to 7 days of incubation. Prior to sampling at sea, the hydrowire or the sampling gear was artificially contaminated with this emulsion at several places. Samples were obtained at different depths and plated on the same medium as that described above. Following an incubation period of 48 hours at 28°C, the strain of *S. marinorubra* produced deep-red colonies. No such colonies were observed on blanks. Tables I and II show the numbers of red colonies compared with the numbers of unpigmented colonies. The counts are given as means of ten plates. For all samples, the error ranged between 4.5 and 9.4% (standard deviation of means/means).

Experiment I. Our sampler (M–J) was fastened to the hydrowire between two Cobet samplers.[2] The samplers were placed 5 m apart. The Cobet sampler resembles the J–Z sampler (ZoBell 1959) but omits the lever device for breaking the glass capillary. The messenger hits the capillary directly.

2. Hydro Products Division, San Diego, California.

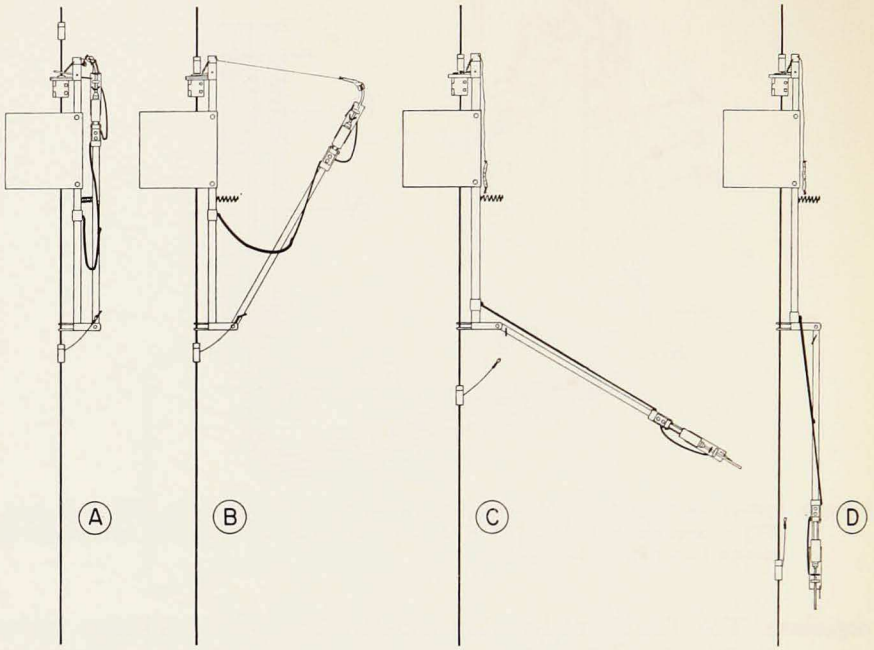

Figure 3. Operating sequence in sampling. A. Sampler at depth; messenger about to strike trigger. B. Moveable arm of sampler released by trigger. Movement initiated by spring and completed by force of gravity. Dialysis bag everted and stripped away from intake tube. Syringe about to fill when operating cable slack has been taken up. C. Syringe taking in sample. With continuing motion, the noose will be drawn tight to close the intake tube. Second messenger is released. D. Sampling completed, intake tube closed. Elastic portion of operating cable extended to permit sampler to hang vertically while being raised to the surface. Friction where noose passes through support bracket keeps the intake tube closed even if the operating cable should be slackened.

Smears of the tracer emulsion were applied to the lead weight at the end of the hydrowire and to the outside of the dialysis bag of the M–J sampler. The results are shown in Table I.

Table I. Comparison of results obtained with the M–J and Cobet samplers. Samples taken three miles (4.8 km) north of Vineyard Haven in March 1966.

Depth (m)	Sampler	Colony count/ml	
		S. marinorubra	Unpigmented colonies
15.....	C	890	4
20.....	M–J	0 (1)	25
25.....	C	1800	0

Experiment II. M–J, Cobet, and Niskin (Niskin 1962) samplers were tested for contaminants in depths to 3000 m. In all cases the tracer emulsion was applied to the end of the hydrowire, thus contaminating the seawater shortly before the samplers were lowered. The results are shown in Table II.

Table II. Comparison of results obtained with the M–J, Cobet, and Niskin samplers. Samples taken off the coast of Peru in April 1966.

Depth of sampling (m)	Sampler	Number of samples	Colony count/ml	
			S. marinorubra	Unpigment. colonies
10	M–J	2	0	95
	C	2	22	54
	N	2	0	135
25	C	3	60	14
	N	2	8	28
50	M–J	1	0	157
	N	2	55	61
100	M–J	4	2	33
	C	3	1600	0
	N	2	250	11
500	C	2	6	1
	N	2	3	17
1000	M–J	3	0	12
	C	2	84	0
	N	2	12	2
2000	M–J	2	0	4
	N	1	120	0
3000	M–J	2	0	7
	C	1	210	0
	N	1	27	2

Discussion. Obviously, the relative number of unpigmented colonies is strongly affected by the number of colonies of *S. marinorubra.* At a high-colony density of the tracer organism on the plates, no growth of other marine bacteria was found.

The newly designed sampler consistently yielded samples that included few or none of the tracer organism. A rinsing effect on the tracer organism is clearly indicated. In some cases, however, it led to the absence of tracer organisms from samples taken with the Cobet and the Niskin sampler. The comparatively high degree of contamination with use of the Cobet sampler may be explained by the fact that the head of the messenger must be heavily contaminated as it slides down the hydrowire before breaking the glass capillary. The great reduction in contaminants with the M–J sampler made it a satisfactory tool for the purpose of our study.

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