SEARS FOUNDATION FOR MARINE RESEARCH
BINGHAM OCEANOGRAPHIC LABORATORY, YALE UNIVERSITY

JOURNAL OF
MARINE RESEARCH

VOLUME IV 1941 NUMBER 3

APPARATUS FOR COLLECTING WATER SAMPLES FROM DIFFERENT DEPTHS FOR BACTERIOLOGICAL ANALYSIS[1]

By

CLAUDE E. ZOBELL

Scripps Institution of Oceanography[2]
University of California
La Jolla, California

Prerequisite to quantitative studies on the occurrence and importance of bacteria in the sea or other natural waters is a satisfactory device for collecting water samples for bacteriological analysis from any desired depth. Such a device must be easily sterilized, susceptible to aseptic manipulation under the most rigorous field conditions, possible to operate at high hydrostatic pressures and it must be made of biologically inert materials. It is desirable that the bacteriological water sampler can be used on the standard hydrographic or sounding wire in tandem or multiple units to provide for the simultaneous collection of samples from different depths and in conjunction with Nansen, Ekman, or Allen bottles, reversing thermometers or other hydrographic apparatus on the same wire. Sturdiness of construction, convenience of operation and economy of manufacture are also important features.

A survey of the literature reveals that more than a hundred bacteriological water samplers have been described introducing new

[1] Assistance in the preparation of these materials was furnished by the personnel of Work Projects Administration Official Project No. 65-1-07-2317.

[2] Contribution new series No. 150.

principles or modifications, but they all have limitations which restrict their usefulness. Unfortunately the data obtained from the bacteriological analysis of water collected with many samplers are open to criticism or are of only historical significance because the containers could not be sterilized, they were subjected to possibilities of extraneous contamination or they were made of metals which exert a bactericidal effect. The majority of the samplers which have been described are suitable only for collecting surface samples or samples from shallow depths. Others are unreliable, excessively expensive, intricately complicated or otherwise unsatisfactory. The apparatus which is described below has given good results under various conditions, it is simple to construct and it is positive in operation at any depth.

HISTORICAL

Besides the numerous methods which have been described for collecting water samples from the surface, three different types of samplers have been used: (1) weighted glass bottle from which the stopper is removed by a string, spring or messenger at the desired depth, (2) cylinder to which water is admitted by opening valves or by withdrawing a piston and (3) partially evacuated glass receptacle to which water is admitted by breaking a capillary glass tube.

Johnston (1892), Heydenreich (1899), Abbott (1921), Whipple (1927), Zillig (1929), Eyre (1930) and others have described ingenious devices for removing the stopper from a sterile bottle after it has been lowered into the water. However, it is obvious that the operation of such samplers is restricted to relatively shallow depths because the hydrostatic pressure at greater depths prevents the removal of stoppers from empty bottles. (The hydrostatic pressure of water increases approximately one atmosphere, or 15 lbs. per square inch, for each ten meters of depth.) In fact, unless provisions are made to prevent their descent, rubber stoppers will be pushed into the bottle at 50 to 100 meters and eschewing this, the empty bottles will be crushed at depths of a few hundred meters.

Otto and Neumann (1904) collected samples in a metal cylinder the ends of which could be closed with rubber gaskets at the desired depth. A nickel-plated cylinder with cocks operated by a messenger was used by Bertel (1911). However, it is objectionable to send such a cylinder down open because it might become contaminated with extraneous material while descending through the water and it is virtually impossible to perfect leak-proof valves or cocks which operate with sufficient ease to permit a pressure-resistant cylinder to be sent down empty. Matthews (1913) sought to obviate

this difficulty by filling a glass-lined cylinder with 95 per cent alcohol which provided for the sterility of the apparatus as well as for the equalization of pressure. One messenger was dropped to open the ends of the cylinder thus permitting the disinfectant to diffuse out, after which a second messenger closed the valves to entrap a water sample from the desired depth. Drew (1914) used this apparatus with expressed confidence to depths of 800 meters. Others have used it with phenol solution or other disinfectants. Besides the expense and inconvenience of operating this apparatus there is always a possibility of the disinfectant diffusing out prematurely through a faulty valve, or all of the disinfectant may not be washed out during the time the valves are opened and closed.

Young, Finn and Bedford (1931) fitted a phosphor-bronze cylinder with a brass piston which is withdrawn by a messenger-activated mechanism thereby aspirating a water sample. In order to eliminate the bactericidal effect of metals, Renn (unpublished) used a large ground-glass cylinder fitted with a solid ground-glass piston or plunger. It is doubtful, though, if such cylinder and piston arrangements can be made so that they are absolutely leak proof at great depths and it is especially difficult to exclude water from the orifice until the messenger is dropped. Unless it is closed right to the end, the orifice may admit a small amount of surface water which will be aspirated when the piston is withdrawn. This objection applies to Reyniers' (1932) glass cylinder which is ingeniously opened and closed by flexible rubber tubing.

Russell (1892) applied the unpublished evacuated glass-bulb capillary-tube idea of Massea by fitting a small flask with a glass tube the end of which was sealed hermetically. Provisions were made for breaking the end off the glass tube with a messenger thus permitting the ingress of water. Modifications of this capillary tube idea have been described, in most cases independently, by Praum (1901), Miquel and Cambier (1902), Portier and Richard (1906), Druse (1908), Parsons (1911) and Issatschenko (1914). In its most practical form Wilson (1920) used a large test tube fitted by means of a rubber stopper with a capillary glass tube the end of which is sheared off by a lever activated by a messenger. Gee (1932) attached it to an Ekman bottle, provided a long capillary tube to retard the sudden inrush of water when the tip was broken at great depths and used a thick-walled test tube with the neck constricted to prevent the descent of the rubber stopper into the test tube with increasing pressure. ZoBell and Feltham (1934) bent the glass inlet tube in such a way that it was broken at a point remote from pos-

sibilities of contamination by the messenger and simplified the breaking mechanism.

BACTERICIDAL EFFECT OF METALS

Alloys of copper, lead, nickel, silver, tin, zinc or other metals lend themselves readily to the manufacture of water sampling apparatus but it has been recognized for many years that all except the noblest metals have an oligodynamic effect (Raadsveld, 1934). After finding a reduction of 97 to 100 per cent in the bacterial population in 100 ml. quantities of sea water exposed to 2 square inches of bronze, nickel, brass and other alloys, Drew (1914) concluded that platinum is the only metal suitable for the interior of bacteriological water sampling apparatus. According to Bedford (1931) the bactericidal effect of the bronze and brass sampling bottle of Young et al. (1931) was inappreciable in sixty minutes but noticeable in ninety minutes.

Observations made at the S. I. O. during the last decade indicate that the less noble metals have a pronounced effect on the survival and activity of bacteria in samples of sea water. Under certain conditions most of the bacteria in sea water are killed within a few minutes when in contact with bright brass, bronze or other alloys containing copper, nickel, tin or zinc. Sea water itself stored in brass receptacles for a few hours becomes bacteriostatic for some bacteria although it enhances the growth of other bacteria. It is beyond the scope of this paper to consider the factors which influence the oligodynamic effect of heavy metals on the activity of bacteria in sea water but a few experiments will be summarized to show that metal containers for bacteriological water samples should be shunned.

Investigators on several oceanographic expeditions have analyzed bacteriologically water samples collected with Nansen, Ekman or other metal bottles. As a rule they observe that in spite of the fact that the bottles were not sterilized, few or no bacteria could be demonstrated in the water. Such observations have been interpreted as indicating that there are few or no bacteria in the sea. That such an interpretation is not always valid is proved by the data recorded in Table I which gives a protocol of a series of experiments in which sea water was stored in Nansen bottles.

Thoroughly cleaned Nansen bottles were filled with raw sea water. After different periods of storage at 22° C. samples were withdrawn for bacteriological analysis. Control water samples were stored in glass bottles for the same time. The bacterial population was

determined by plating procedures using nutrient sea water agar (ZoBell, 1941). It will be observed from Table I that whereas the

TABLE I—Number of Bacteria per Cubic Centimeter of Sea Water After Different Periods of Storage at 22° C. in Glass Bottles and in Brass Nansen Bottles

Period of Storage	Experiment 1		Experiment 2		Experiment 3		Experiment 4	
	Glass bottle	Nansen bottle	Glass bottle	Nansen bottle	Glass bottle	Nansen bottle	Glass bottle	Nansen bottle
0	94	—	108	—	143	—	294	—
1 hour	86	30	92	25	137	80	253	86
2 hours	72	21	102	18	89	26	222	19
3 hours	90	15	110	10	118	25	266	4
5 hours	52	0	76	3	226	1	207	0
24 hours	446	6	539	0	590	1	890	2

number of bacteria in the control sea water in glass containers decreased slightly with storage and then started to increase, the bacteria rapidly disappeared from the sea water which was stored in Nansen bottles for a few hours.

Only when collected from appreciable depths is there a likelihood of the water being in the Nansen bottle for an hour or longer. However, further experiments summarized in Table II indicate that many

TABLE II—Number of Viable Bacteria in Sea Water After Different Periods of Storage in Different Receptacles at 22° C., the Number Being Expressed as the Per Cent of the Original Number Present

Time in minutes	Nansen bottle No. 3	Nansen bottle No. 13	Nansen bottle No. 18	Nansen bottle No. 19	Glass bottle control
0	100	100	100	100	100
5	89	94	85	69	103
10	77	82	74	62	95
30	71	78	56	40	96
60	42	70	51	36	92

bacteria are killed or rendered incapable of multiplication in Nansen bottles within a few minutes. The average results of from three to six different experiments with four different Nansen bottles are given. Some of the Nansen bottles had a greater bacteriostatic effect than others, probably due to differences in the metallic surfaces which were exposed to the water samples. It is generally recognized that brightly polished metallic surfaces are more bacteriostatic than those which are coated with oxides or other film-forming substances.

GLASS BOTTLES

After trying different sizes and kinds of flasks, test tubes and bottles it was found that citrate of magnesia (CM) bottles with a one-hole rubber stopper substituted for the patented stopper, or ordinary pop or beer bottles are the best for collecting water samples. They are economical, rugged in construction, of sufficient volume and they stand erect on their flat bottoms without the need of special racks. Moreover, unlike the test tubes used by Wilson (1920), Gee (1932) and ZoBell and Feltham (1934) there is less tendency for the stoppers to be pushed in by the hydrostatic pressure. This can be prevented completely by inserting a piece of 8 mm. glass tubing of such a length that its ends rest upon the bottom of the bottle and the lower end of the stopper.

The use of the specially bent capillary inlet tubes as devised by various workers are somewhat difficult to construct and they are easily broken during sterilization, transportation or handling. The use of a piece of pressure rubber tubing, as used by Schach (1938), provides for flexibility and greatly simplifies the construction of the piece of glass that is broken at the time a sample is collected. Combining the most desirable features of the samplers of Schach (1938) and ZoBell and Feltham (1934), we have a device which is simple to construct and positive in action.

By means of a No. 1 one-hole rubber stopper a CM bottle is fitted with a four inch piece of 5 mm. glass tubing bent at right angles one inch from the end. The longer piece of the resulting L goes through the stopper into the bottle and the shorter arm is fitted with a four inch piece of ⅛ inch heavy-wall rubber pressure tubing. This part of the apparatus can be used over and over again almost indefinitely. The piece of projecting rubber tubing is fitted with a four inch length of 4 mm. glass tubing hermetically sealed at the outer end in a flame. The assembled apparatus is sterilized in the autoclave with the stopper resting loosely on the neck of the bottle held in place by the extruding glass tube. As soon as the bottles can be removed from the autoclave, preferably while they are still full of steam, the stoppers are firmly seated exercising aseptic precautions. When cool the bottles will be from 50 to 90 per cent evacuated.

To collect a sample of water the bottle is clamped into the brass carrier illustrated in Figure 37. The rubber tubing is bent around at an angle of 180° and held in this position by the spring clamp T. C. which is operated with the thumb. When lowered into the water to the desired depth the messenger is dropped. The glass tubing

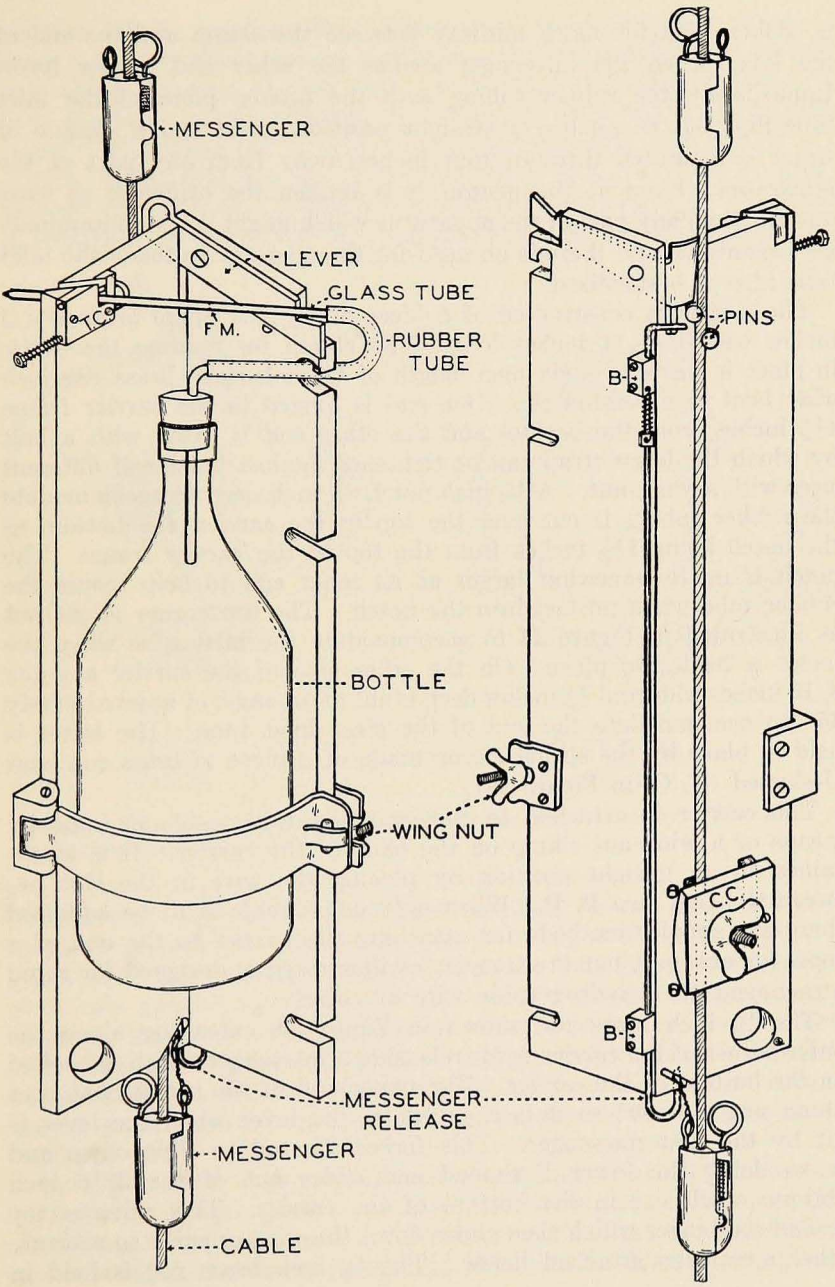

Figure 37. Front and back view of the J-Z bacteriological sampler.

is broken at a file mark midway between the clamp and the end of the lever when the messenger strikes the other end of the lever. Immediately the rubber tubing with the broken piece of the inlet tube flips out to assume a straight position again and a sample of water is aspirated three or four inches away from any part of the apparatus. Even at the instant it is broken the orifice is at least an inch from any part of the apparatus which might carry contaminating organisms, and there is no need for the operator to touch the inlet tube after it is sterilized.

The carrier is constructed of a piece of ¼ inch angle brass 2 × 2 inches wide and 11 inches long. The clamp for holding the bottle in place is made of a six inch length of ⅛ inch strap brass one inch wide bent in a semi-circle. One end is hinged to the carrier frame 4½ inches from the bottom and the other end is fitted with a bolt by which the brass strap can be tightened against bottles of different sizes with a wing-nut. A ⅛ inch notch ½ inch deep to accommodate the rubber tubing is cut over the top of the carrier, the bottom of the notch being 1½ inches from the top of the carrier frame. The notch is made somewhat larger at its inner end to help secure the rubber tube when pushed into the notch. The top corner is cut out as illustrated in Figure 38 to accommodate the inlet tube when the latter is bent into place. On the other side of the carrier a notch 3/16 inches wide and ¾ inches deep is cut at an angle of approximately 45° to accommodate the end of the glass inlet tube. The latter is held in place by the spring clamp made of a piece of brass rod bent U-shaped (T. C. in Figure 38).

The carrier is attached to the sounding wire, cable or rope by means of a wing-nut clamp on the back of the carrier. It is maintained in an upright position by placing the wire in the slot between the two pins P. P. When only one sample is to be collected there are simpler methods for attaching the carrier to the end of a rope, for example, but the sampler as illustrated is designed for rapid attachment to a hydrographic wire or cable.

The ⅛ inch brass rod shown in Figure 38 extending along the outer corner of the carrier is for releasing a messenger which is hooked on the bottom of the carrier. The upper end of the rod is bent over at an angle of 90° so it is engaged by the lever when the lever is hit by the first messenger. This forces the rod to slide down and in so doing the lower U-shaped end slides out of the 3/16 inch oblique notch cut in the bottom of the carrier. This releases the second messenger which then slides down the wire or cable to activate other apparatus attached below. The ⅛ inch brass rod is held in

position by the bearings BB. These are constructed from pieces of brass ¼ inches thick and ⅝ inches square. After drilling a ⅛ inch hole along the edge of them they are fastened in place with two ⅛ inch machine screws although they can be soldered to the carrier frame. The brass rod is held in position by means of a spring.

This device with the attached glass bottle has proved to be satisfactory for collecting water samples when used singly or on a line in combination with other apparatus in lakes as well as in the ocean.

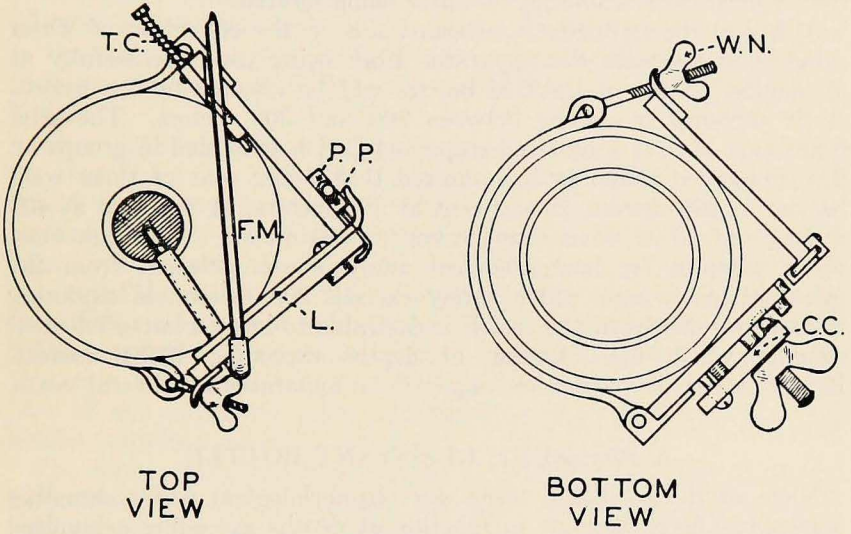

Figure 38. Top and bottom view of the J-Z bacteriological sampler with a citrate of magnesia bottle and its connections in place.

As many as eight of them fastened seriatum on the same line have made it possible to obtain samples from eight different depths in one cast. The repeated failure to recover test pigmented bacteria purposely applied to all parts of the carrier, the cable and the messenger proves that uncontaminated water samples can be collected under the most rigorous conditions. After a few words of instruction, uninitiated deck-hands and technicians alike can collect uncontaminated water samples with the apparatus suitable for bacteriological analysis.

In order to get a sample of water near the surface it is necessary to have the bottle partly evacuated. However, at depths exceeding 10 meters the bottle rapidly fills with water even if the bottle is not evacuated, the time required for the bottle to fill decreasing with

depth due to the increasing hydrostatic pressure. Once equilibrium has been established, which requires from two to ten minutes after the inlet tube is broken, there is no possibility of water from higher levels entering the bottle as it is pulled toward the surface because the pressure within the bottle is greater than that in the water at higher levels. Actually there is a slight tendency for water or air to be forced out of the bottle as it is raised. Sometimes when bottles fitted with small capillary tubes are hauled rapidly to the surface they appear with a fine jet of water being ejected.

Although the hydrostatic pressure aids in the collection of water samples, it prevents the apparatus from being used successfully at all depths. Some of the CM bottles will be crushed by the hydrostatic pressure at depths between 200 and 300 meters. The trial immersion of over a hundred stoppered CM bottles tied in groups on the submerged sounding line showed that 7 per cent of them were broken at 200 meters, 29 per cent at 300 meters, 81 per cent at 400 meters and all of them were broken at 600 meters. Although most water samples for bacteriological analysis are collected from the principal biotic zone which rarely exceeds 200 meters, in exploring the abyssal depths of the sea, it is desirable to have a bacteriological sampler which will function at depths exceeding 10,000 meters. Bacteriologists have been seeking such an apparatus for several years.

A PRESSURE RESISTANT BOTTLE

None of the so-called "deep sea" bacteriological water sampling bottles has been designed to function at depths exceeding a hundred meters or so, with the possible exception of those filled with a disinfectant (Matthews, 1913, Drew, 1914).

As mentioned above, valves and pistons on metal bottles are not leak-proof as far as bacteria are concerned. Evacuated glass bulbs which are large enough to collect a satisfactory sample are broken by the high hydrostatic pressure encountered at greater depths. The best high pressure bottle we could find without going to the expense of having special ones fabricated was a 125 ml. thick-walled flask with a round bottom supplied by the Corning Glass Company. It was cracked at a depth of 1200 meters.

While unquestionably glass bottles holding 100 ml. or more could be fabricated to tolerate any pressure in the sea, the cost is excessive and, moreover, it is doubtful if evacuated bottles should be used at great depths for collecting water samples for bacteriological analysis. When the capillary inlet tube on such a bottle is broken at a depth of 1000 meters, for example, the bacteria aspirated with the

water sample are subjected to an instantaneous pressure change of approximately 100 atmospheres, and correspondingly greater pressure changes at greater depths. The recovery of viable bacteria by Certes (1884), Carey and Waksman (1934) and others from depths near 5000 meters proves that neither the pressure encountered (approximately 500 atmospheres) nor its gradual release, effected by bringing the samples to the surface, killed all of the bacteria but it is indeterminate if some failed to survive this change. The work of Larsen et al. (1918) indicates that while certain bacteria tolerate pressures of 6000 atmospheres (about six times as high as any encountered in the sea), they are injured by the sudden release of pressures of 50 atmospheres or less depending upon the gas tension and other factors.

After experimenting with many types of devices to obviate the pressure factor for collecting samples at great depths, we have perfected the collapsible rubber bottle idea suggested to us by Dr. Austin Phelps of the Hopkins Marine Station. In its simplest form heavy rubber pear-shaped aspirator bulbs holding around 100 ml. are used (Figure 39). A 5 inch length of ⅛ inch heavy rubber pressure tubing is cemented in the 6 mm. opening of the rubber bulb with rubber cement. The rubber tubing is fitted with a 4 inch length of 4 mm. glass tubing with the outer end sealed in a flame, this part of the apparatus being the same as that used with the CM bottles described above.

The rubber bulbs can be sterilized in the autoclave after which the inlet tubes with the sealed ends are fitted aseptically with the bulb collapsed or depressed. At sea they can be sterilized by boiling, or in an emergency they can be sterilized by filling with 70 per cent alcohol or other disinfectant and then rinsed with sterile water after squeezing out the disinfectant, exercising aseptic technique. In practice we prepare and sterilize in convenient receptacles or paper-wrapped packets several hundred of the glass inlet tubes preparatory to embarking on a cruise. The rubber bulbs are sterilized by boiling or in a pressure cooker as needed, the bulbs being fitted with the glass inlet tubes while the former are still quite warm to lessen the likelihood of contamination and to get a better evacuation of the bulbs.

These rubber bulbs function in precisely the same way as the similarly fitted CM bottles. When the capillary inlet tube is broken by the lever activated by the messenger, the rubber bulb aspirates a water sample as it assumes its normal shape. It cannot be broken by the pressure regardless of whether it is collapsed or inflated

with air because as it is compressed by the increasing hydrostatic pressure, the pressure will always be nearly the same inside and outside the bulb due to the flexibility of the rubber. Consequently there is very little pressure change as the water sample is aspirated.

The rubber bulbs are secured to the same carrier as is used for the CM bottles by means of an auxiliary collar made of a 1¼ inch length of brass pipe 1⅛ inches in inside diameter. A hole is drilled ⅜ of an inch from the edge of the collar and threaded to take a ¼ inch machine screw 1¼ inches long having a knurled or wing-nut head. Two nuts are placed on the screw as illustrated in Figure 39 to keep the collar and attached rubber bulb in the proper position in the carrier. The collar is slipped over the neck of the sterilized pear-shaped bulb which is ready for use. Then the bulb is placed in position on the carrier and fastened there by slipping the screw projecting from the brass collar into a ¼ inch slot which is cut ¾ inches deep 4 inches from the top of the carrier. It is secured by tightening the machine screw and in so doing the end of the screw presses into the neck of the rubber bulb for about ⅛ inch thereby preventing the bulb from dropping out under any conditions. The collars are made detachable to facilitate handling the water samples collected in the rubber bulbs as well as to facilitate the sterilization of the latter and their assembly.

The trial immersion of the assembled rubber bulbs to depths exceeding 4000 meters without breaking the inlet tube proved that they were leak-proof. When the inlet tube was broken by the messenger-activated mechanism, the bulbs came up filled with water. Although we have not had an opportunity to send them down to the greatest depth in the sea, there seems to be no reason why they will not be just as efficient at 10,000 meters as at 4000 meters.

With the brass collar removed the rubber bulbs stand in an upright position on their flat bottoms. The sample can be transferred to a sterile glass container by squeezing the bulb, or aliquot portions can be removed as needed. The water samples should be stored in the refrigerator until they can be analyzed, although to insure reliable results the water should be analyzed for its bacterial population as soon as possible to minimize the changes which accompany the storage of water samples (ZoBell and Feltham, 1934, ZoBell and Anderson, 1938).

Since the rubber is not entirely inert the samples should not be left in the rubber bulb any longer than is necessary. There are certain bacteria found in the sea which slowly attack rubber as well as the synthetic products, neoprene and isoprene, as manifested by

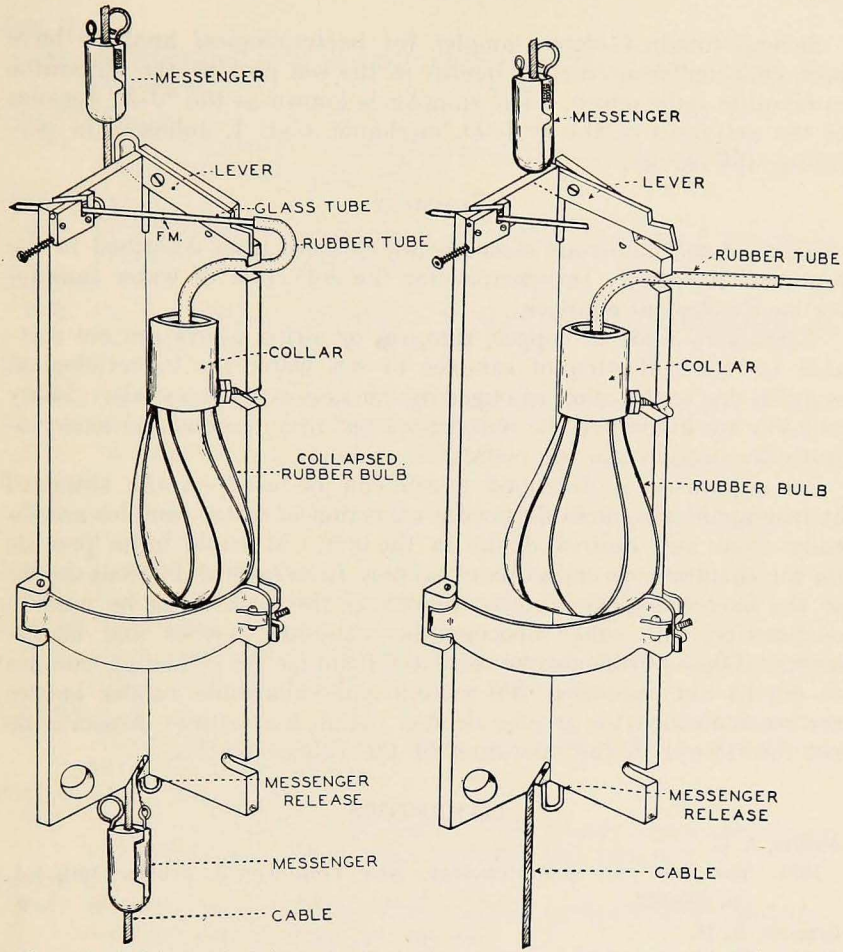

Figure 39. J-Z bacteriological sampler fitted with a pressure-resistant collapsible rubber bottle before and after collecting a sample of water. TC, thumb clamp, PP, pins for guiding cable; FM, file mark on glass inlet tube; L, lever which breaks tube; WN, wing nut on bottle clamp; CC, cable clamp for securing apparatus to cable.

oxygen consumption and the increasing bacterial population after several days. However, there is no evidence that the rubber is attacked for several days, so this possible source of error can be discounted provided the water samples are not left in the rubber bulbs for more than a few hours. The bacterial population and other properties of water samples of known bacterial content stored in rubber bulbs for 24 hours were not unlike those of water stored in glass receptacles under comparable conditions.

Several hundred water samples for bacteriological analysis have been collected from various depths in the sea proving the apparatus to be quite satisfactory. The sampler is known as the "J-Z" because of the activities of the S. I. O. mechanic, Carl I. Johnson, in perfecting the carrier.

Summary

None of the numerous samplers which have been described in the literature is entirely satisfactory for the collection of water samples for bacteriological analysis.

Containers made of copper, zinc, tin or nickel alloys are not suitable for the collection of samples of sea water for bacteriological analysis due to the inimical oligodynamic action of the metals. Many bacteria are killed and the sea water itself may be rendered bacteriostatic by exposure to the metals.

An apparatus is described which can be used on the standard hydrographic wire or cable for the collection of water samples aseptically from any desired depth in the sea. Multiple units provide for the simultaneous collection of samples from several different depths or the bacteriological sampler, known as the "J-Z", can be used in conjunction with other hydrographic sampling bottles and instruments. Glass bottles can be used to advantage for collecting samples at depths not exceeding 200 meters and collapsible rubber bottles are recommended for greater depths. High hydrostatic pressures do not interfere with the operation of the rubber bottles.

LITERATURE

Abbott, A. C.
1921. The Principles of Bacteriology, New York, Lea & Febiger, 10th Ed., pp. 650–652.

Bedford, R. H.
1931. The bactericidal effect of the "Prince Rupert" sea water sampling bottle. Contr. Can. Biol. and Fish., 6: 423–426.

Bertel, R.
1911. Ein einfacher Apparat zur Wasserentnahme aus beliebigen Meerestiefen für bakteriologische Untersuchungen. Biol. Centralbl., 31: 58–61.

Drew, G. H.
1914. On the precipitation of calcium carbonate in the sea by marine bacteria and on the action of denitrifying bacteria in tropical and temperate seas. Carnegie Inst. Wash., 5: 7–45.

Eyre, J. W. H.
1930. Bacteriological Technique. Wm. Wood & Co., New York, 3rd Ed., pp. 493–495.

Gee, H.
1932. Bacteriological water sampler. Bull. Scripps Inst. Oceanog., Tech. Ser., 3: 191–200.

Heydenreich, L.
1899. Einige Neuerungen in der bakteriologischen Technik. Zeitschr. f. wissensch. Mikroskopie, 16: 144–179.

Issatchenko, B. L.
1914. Investigations on the bacteria of the glacial arctic Ocean. Monograph, Petrograd, 300 pp. (In Russian.)

Johnston, W.
1892. On the collection of samples of water for bacteriological analysis. Canadian Rec. of Sci., 5: 19–28.

Kruse, F.
1908. Beitrage zur Hygiene des Wassers. Zeitschr. f. Hyg., 59: 6–94.

Larsen, W. P., Hartzell, T. B. and Diehl, H. S.
1918. The effect of high pressures on bacteria. Jour. Infect. Dis., 22: 271–279.

Matthews, D. J.
1913. A deep-sea bacteriological water-bottle. Jour. Marine Biol. Assn. U. K., 9: 525–29.

Miquel, P., and Cambier, R.
1902. Traite de Bacteriologie pure et appliquée. Paris. 372 pp.

Otto, M. and Newmann, R. O.
1904. Ueber einige bacteriologische Wasseruntersuchungen im Atlantischen Ozean. Centralbl. f. Bakt., II Abt., 13: 481–489.

Parsons, P. B.
1911. Apparat zur Entnahme von Wasser aus grösserer Tiefe. Centralbl. f. Bakt., Abt. II, 32: 197–207.

Portier, P. and Richard, J.
1906. Sur une methode de prelevement de l'eau de mer destinee aux études bacteriologiques. Compt. rend. Acad. Sci., 142: 1109–1111.

Praum, L.
1901. Einfacher Apparat zur Entnahme von Wasserproben aus grösseren Tiefen. Centralbl. f. Bakt., I Abt., 29: 994–996.

Raadsveld, C. W.
1934. The oligodynamic effect of metals and metal salts. Chemisch Weekblad, 31: 497–504.

Reyniers, J. A.
1932. Apparatus for taking water samples from different levels. Science, 75: 83–84.

Russell, H. L.
1892. Bacterial investigations of the sea and its floor. Botanical Gazette, 17: 312–321.

Schach, H.
1938. Ein Apparat zur Entnahme von Meerwasserproben aus der Tiefe für bakteriologische Untersuchungen. Jour. du Conseil, 13: 349–356.

WHIPPLE, G. C.
1927. The Microscopy of Drinking Water. John Wiley & Sons, New York, Ed. 4, 585 pp.

WILSON, F. C.
1920. Description of an apparatus for obtaining samples of water at different depths for bacteriological analysis. Jour. Bact., 5: 103–108.

YOUNG, O. C., FINN, D. B., BEDFORD, R. H.
1931. A deep sea bacteriological water bottle. Contrib. Canadian Biol. & Fish., 6: 417–422.

ZILLIG, A. M.
1929. Bacteriological studies in Lake Erie. Bull. Buffalo Soc. Nat. Sci., 14: 51–55.

ZOBELL, C. E.
1941. Studies on marine bacteria. I. The cultural requirements of heterotrophic aerobes. Jour. Mar. Res., 4: 42–76.

ZOBELL, C. E. AND ANDERSON, D. Q.
1936. Observations on the multiplication of bacteria in different volumes of stored sea water and the influence of oxygen tension and solid surfaces. Biol. Bull., 71: 324–342.

ZOBELL, C. E. AND FELTHAM, C. B.
1934. Preliminary studies on the distribution and characteristics of marine bacteria. Bull. Scripps Inst. Oceanogr., Tech. Ser., 3: 279–296.

ACKNOWLEDGEMENT

Ideas from several of my associates at the Scripps Institution of Oceanography, the Limnological Laboratory of the University of Wisconsin, Hopkins Marine Station, and the Woods Hole Oceanographic Institution have contributed to the perfection of the bacteriological sampler. This help together with the material assistance rendered by Dr. Sydney C. Rittenberg and Catharine B. Feltham is gratefully acknowledged.