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CHEMICAL ANALYSES OF MARINE AND ESTUARINE WATERS USED BY THE GALVESTON BIOLOGICAL LABORATORY

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ABSTRACT

This paper describes the chemical techniques and procedures used by the Biological Laboratory of the U. S. Bureau of Commercial Fisheries, Galveston, Texas, for analyzing samples involved in the chemical and biological survey of the marine and estuarine waters of the Gulf of Mexico and also in the many laboratory and field studies and experiments that have been made pertaining to the red tide investigation.

During the past 12 years the staff of the U. S. Bureau of Commercial Fisheries, Galveston, Texas, has published papers pertaining to the hydrography and chemistry of the Gulf of Mexico and also problems relative to the red tide. Included were data collected from the M/V Pompano from 1949 to 1952 (Graham et al. 1954, and Marvin 1955a), the M/V Alaska from 1951 to 1953 (Collier 1958), and the M/V Kingfish from 1954 to date (Finucane and Dragovich 1959).

The purpose of this paper is to present the chemical methods which were used to obtain the data utilized in these publications. We have endeavored to describe the techniques and procedures in a form usable by chemical aids or technicians with a minimum of supervision.

SAMPLE TREATMENT PRIOR TO ANALYSIS

No attempt was made to stabilize the samples collected from the M/V Pompano. With the exception of salinity and total phosphate analyses, all determinations were performed on board ship immediately after collection.

All samples collected from the M/Vs Kingfish and Alaska that were subject to bacterial and/or chemical deterioration were quick-frozen immediately after collection. Collier and Marvin (1953) showed that significant changes in the phosphate ratio of some waters occurred within 30 minutes after collection, but that the ratio could be effectively stabilized by quick-freezing. May (unpublished manuscript) showed that variations in the carbohydrate-like material in sea water could be minimized by similar treatment. After quick-freezing, samples were stored at below freezing temperatures until delivered to the chemistry laboratory for analysis. Our usual freezing bath consisted of a vat of 38 percent ethylene glycol (commercial antifreeze) held at about -20° C. in a quick-freeze cabinet. Freezing can reduce the air pressure within a sample container to a point where impurities around the cap-glass junction will be drawn into the container. We minimized this effect by sealing the junction with plastic insulation tape (similar to Scotch #33). The seal should not be allowed to come in contact with the freezing solution.

Salinity, copper, and total phosphorus

samples did not require preservation. The latter, however, were "baited" with a pinch of thorium carbonate (Harvey 1948) to prevent the accumulation of phosphorus on the sides of the container. An alternative method that we found satisfactory and have used on occasion consisted of adding the amount of H_2SO_4 called for in the analytical procedure immediately after collection. Sulfide, CO_2, and O_2 samples were analyzed immediately after collection. We found the practice of fixing O_2 samples and titrating them later resulted in an error because the amount of free iodine in the supposedly fixed sample increased with time.

The rate of reactions, which includes the development of color, depends among other things on temperature. For this reason the analyst must be sure that all samples and standards are brought to the same temperature before adding reagents. Standard samples were interspersed among the regular samples rather than being run as a separate group. We have found that this practice reduces operator bias.

SAMPLE STORAGE CONTAINERS

The selection of proper sample containers is of considerable importance. We have found, after considerable experimentation, that pyrex is equal to or superior to soft glass, particularly for the storage of total and inorganic phosphate and nitrate-nitrite samples. During our survey work (M/V Pompano), those samples that were not analyzed on board ship (salinity and total phosphorus) were stored in citrate bottles. During the initial collection programs involving the M/V Alaska, frozen samples were stored in "Kimble" type screw-cap 25 by 200 mm. culture tubes. Salinity and unfrozen total phosphate samples were stored in 4-ounce prescription "Duroglas" bottles. It was during the latter phase of the Alaska program that pyrex was found to be superior to the softer glass, particularly for the analyses mentioned. Subsequent collections (M/V Kingfish) employed pyrex storage containers for all samples except salinities which were stored in 4-ounce prescription-type bottles.

We did not use polyethylene containers because the material acts as an adsorbent, reducing the reliability of an analysis. Further, polyethylene becomes an effective semipermeable membrane under freezing conditions. Samples frozen in this type of container have shown as much as a 2-percent decrease in salinity concentration within a year. Water from the same samples stored at room temperature have shown a salinity increase of over 1 percent during the same period.

Sample evaporation can virtually be eliminated by sealing containers with poly-seal screw caps. Salinity samples, for example, have remained constant for as long as a year and a half when sealed in this manner, and we strongly recommend their use

ANALYTICAL METHODS

The colorimetric procedures that follow refer to the Fisher A. C. electrophotometer, the instrument used for the determination of the majority of samples collected from the M/V Kingfish and also the M/V Alaska. The colorimetric analyses of samples collected from the M/V Pompano, however, were carried out visually with Nessler tubes.

Standard Samples

With a few exceptions, the methods described below require the use of laboratory-prepared standard samples. Initially we employed distilled water in the preparation of these. However, it became evident that the percent recovery depended, to some extent, on the type of water. For this reason we endeavored, when possible, to employ used portions of the samples for the preparation of standards. If a sufficient volume cannot be obtained for this purpose, it is recommended that the percent recovery be determined by "spiking" a few samples with the standard solution.

Standard samples were prepared from compounds of known purity and formula weight that contained the element, nutrient, etc., under consideration. The compounds selected were readily soluble but were not hygroscopic or deliquescent to the extent that they absorbed large amounts of atmospheric moisture. The compounds used were either bone dry or of a known moisture content. This enabled all weights to be reduced to a bone-dry basis. Final amounts used in standard samples were obtained by dilution of the relatively concentrated stock solution.

Two compounds were usually employed in the preparation of each series of standard samples. We discouraged the practice of using stock solution more than once. The practice of starting each series of standard samples from the "dry" compounds rather than from preserved stock solutions, and also of using two different compounds to a series of standard samples, enabled the analyst to detect systematic errors that could reduce the accuracy of his data.

To correct for glassware and reagent contamination, blanks consisting of "pure" water were treated in the same manner as unknown samples. Any color that developed was due to impurities which were corrected by subtracting the blank sample readings from those of the regular samples. Systematic errors can be introduced by using a low quality distilled water in preparing blank samples. We have found that a satisfactory grade can be prepared by distilling tap water two times in a borosilicate glass still. The arrangement has been described by Marvin and Lansford (1958).

Phosphate

We used the method of Robinson and Thompson (1948a) for the determination of inorganic phosphate, and that of Harvey (1948) for the determination of total phosphate. To make total and inorganic analyses on aliquots of the same sample, we used Harvey's method for both analyses, but, in this case, did not digest the inorganic samples.

The mechanics of the phosphate determinations are as follows:

Inorganic Only

1. Remove frozen samples from cold storage and thaw by placing in front of an electric fan. Do not thaw more than 50 samples at a time.

2. While samples are thawing, set up enough 50-milliliter pyrex Erlenmeyer flasks to take care of duplicate determinations from each vial.

3. With a 2-milliliter automatic pipette, add 0.25 ml. of molybdate reagent to each flask.

4. When the samples have thawed and warmed to room temperature, shake them vigorously. Clean each cap-glass junction with a jet of distilled water, remove caps, and dispense 25-milliliter portion into the sample flasks with a large bore 25-milliliter graduated pipette.

5. Add 1 drop of stannous chloride reagent to the first sample flask and then swirl the flask thoroughly. Thirty seconds later, add a drop to the second flask and swirl. Continue at 30-second intervals until all flasks have been treated.

6. Determine the density of the blue color that will develop if there is any phosphate present with a Fisher a.c. electrophotometer equipped with a 650 A filter and a 23-milliliter cell. Color-density determinations are made 30 minutes after the addition of the stannous chloride reagent. If analyst cannot check a sample every 30 seconds, increase the time interval in item 5 as necessary.

7. Convert the density units (-log T) into concentration units (µg at PO_4-P/l) by dividing by the slope $\frac{\text{-log T}}{\text{µg at } PO_4\text{-P/l}}$ obtained by running known standards along with regular samples.

Reagents:

a. Molybdate reagent. Add 1 part of a 10-percent ammonium molybdate solution to 3 parts of 18N H_2SO_4.

b. Stannous chloride reagent. Dissolve 1 gm. of stannous chloride in 5 ml. of 12N HCl (if necessary, heat gently until acid solution clears) and dilute to 100 ml. with triple-distilled water.

Total and Inorganic

1. Remove about 50 frozen samples at a time from cold storage and thaw by placing in front of an electric fan.

2. While samples are thawing, set up two sets of 50-milliliter pyrex Erlenmeyer flasks. One set will be used for inorganic and the other for organic analyses.

3. With a 2-milliliter automatic pipette, add 0.38 ml. of 18N H_2SO_4 to each flask. Cover immediately with 30- or 50-milliliter beakers.

4. When the samples have thawed to room temperature, shake thoroughly and clean the cap-glass junction with a jet of distilled water. Remove cap, and with a large bore 25-milliliter graduated pipette, dispense 25-milliliter portions into the appropriate flask.

5. Upon completion of the dispensing operation remove the total phosphate set and place flasks in digester. Adjust controls to digest for either 3 hours at a steam pressure of 85 ± 5 pounds per square inch or 5 hours at a steam pressure of 30 ± 5 pounds per square inch,

6. With a 2-milliliter automatic pipette, add 0.75 ml. of the molybdate reagent to each inorganic sample. Shake immediately after the addition.

7. Add 1 drop of stannous chloride reagent to the first sample flask and then swirl the flask thoroughly. Thirty seconds later, add a drop to the second flask and swirl. Continue at 30-second intervals until all flasks have been treated.

8. Determine the density of the blue color that will develop if there is any phosphate present, with a Fisher a.c. electrophotometer equipped with a 650 A filter and a 23-milliliter cell. Color-density determinations are made 30 minutes after the addition of the stannous chloride reagent. If analyst cannot check a sample every 30 seconds, increase the time interval in item 5 as necessary.

9. Convert the recorded density units (-log T) to concentration units (µg at PO_4-P/l) by dividing by the slope $\frac{-\log T}{\mu g \text{ at } PO_4\text{-}P/l}$ obtained by running known standards along with the unknown samples.

10. After the total phosphate samples have been digested for the required time, remove them from autoclave. Adjust all volumes to 25 ml. with triple-distilled water and treat exactly as indicated for the inorganic set, starting with item 6.

Reagents:

a. Molybdate reagent. Dissolve 3.3 gm. ammonium molybdate in 200-milliliter triple-distilled water to which has been added 3 ml. of 36N H_2SO_4.

b. Stannous chloride reagent. The same as that listed under Inorganic Only.

Total Only

1. If samples are frozen, thaw by placing in front of an electric fan.

2. While samples are thawing, set up enough 50-milliliter pyrex Erlenmeyer flasks for duplicate analysis.

3. To each flask add 0.38 ml. of 18N H_2SO_4, 1/ and with a large bore 25-milliliter graduated pipette, a 25-milliliter portion of the sample. Before removing cap, clean the cap-glass junction with a jet of distilled water.

4. Place samples in the autoclave and digest for a period of 3 hours at a steam pressure of 85 ± 5 pounds per square inch.

5. Remove samples and cool to room temperature. Adjust volumes at 25 ml. by adding triple-distilled water. Add 0.75 ml. of molybdate reagent to each flask (see reagents under Total and Inorganic) and swirl.

6. Add 1 drop of stannous chloride reagent to the first sample flask and then swirl the flask thoroughly. Thirty seconds later, add a drop to the second flask and swirl. Continue at 30-second intervals until all flasks have been treated.

7. Determine the density of the blue color that will develop if there is any phosphate present with a Fisher a. c. electrophotometer equipped with a 650 A filter and a 23-milliliter cell. Color-density determinations are made 30 minutes after the addition of the stannous chloride reagent. If analyst cannot check a sample every 30 seconds, increase the time interval in item 5 as necessary.

8. Convert from density to concentration units by comparing with standard samples that were run with the regular samples.

1/ Omit if acid was added immediately after sample collection.

Reagents:

The same as those listed under Total Only.

Nitrate-Nitrite

We used the method of Zwicker and Robinson (1944) as modified by Marvin (1955 b) for the determination of nitrate-nitrite.

The steps that were involved in the determination are as follows:

1. Remove frozen samples from cold storage and thaw by placing in front of an electric fan. Thaw 24 samples at a time.

2. While samples are thawing, set up the required number of 10-milliliter pyrex test tubes to take care of duplicate analyses from each vial. Clean the cap-glass junction of each sample bottle before opening.

3. If the range of nitrate concentration is unknown, proceed as follows[2/]:

a. With a 1-milliliter pipette, transfer 1 ml. of sample into its designated test tube.

b. With a 2-milliliter automatic pipette, add 2 ml. of distilled water to each of the 10-milliliter tubes.

c. Using a 5-milliliter pipette, add 3 ml. of strychnidine reagent to each tube. To prevent boiling during this addition, tilt the tube about 45° and allow the reagent to run down the inside so that it forms a layer under the water sample. Perform this step carefully to avoid unnecessary mixing of reagent and sample.

d. With another 10-milliliter pyrex tube, mix by gently pouring the contents of each tube into the mixing tube and back again only once. Use least 15 mixing tubes in rotation, allowing each one to drain after use.

e. Store samples in darkness for 3 to 5 hours, then check color density with a Fisher a. c. electrophotometer equipped 3-milliliter micro cells and a 525 B filter.

f. Check the results against standard samples run with the unknown samples.

Strychnidine reagent:

Dissolve 0.3 millimole of strychnidine in a liter of 36N Du Pont c. p. H_2SO_4.

Nitrite

For the determination of nitrite in sea water, we used the method of Robinson and Thompson (1948b).

Steps involved in the determination are:

1. Remove frozen samples from cold storage and thaw by placing in front of an electric fan. Thaw 24 samples at a time.

2. While samples are thawing, set up the number of 60-milliliter Erlenmeyer flasks required to make duplicate analyses of each sample.

3. When samples have thawed to room temperature, clean the cap-glass junction with a jet of distilled water and remove the cap.

4. Add 25 ml. of sample to each flask with a large bore 24-milliliter graduated pipette.

5. Prepare working reagent by mixing together equal volumes of the alpha-naphthylamine and sulfanilic acid reagents.

6. Add 1 ml. of the working reagent to each sample and swirl flask gently after each addition.

7. After 1/2 hour determine the color density with a Fisher a. c. electrophotometer, using a 525 B filter. If the salinity of the sample is zero or close to zero, allow 2 to 3 hours for color development.

2/ If samples are known to be low in nitrate concentration (below 8 µg at /l), proceed as in item No. 3, but omit step "a" and add 3 ml. of sample in step "b" rather than 2 ml. of distilled water.

8. Compare to desired units by comparing sample results with those obtained by concurrently analyzing a set of standards.

Reagents:

a. Alpha-naphthylamine. Boil 0.2 gm. of alpha-naphthylamine in 40-milliliter distilled water. Separate the clear portion from the dark-colored precipitate and then add the clear portion to 15 ml. of glacial acetic acid. Add 285 ml. of distilled water and stir.

b. Sulfanilic acid. Add 1 gm. of sulfanilic acid to 30 ml. of a 50-50 mixture of glacial acetic acid and distilled water. Stir thoroughly, gradually adding more water until 270 ml. in all have been added. During this addition, gently warm until solution is complete.

Salinity

We used the Knudsen (1901) method of salinity determination incorporating a slight modification. If coastal and river waters (which require an accuracy of only 0.2 to 0.3 parts per thousand) were being analyzed, the Knudsen burette was replaced with a 50-milliliter self-leveling burette. For the greater accuracy required for off-shore samples, the end point was determined by an electrometric rather than a colorimetric method, and the entire operation conducted in a constant temperature room. All final determinations requiring an accuracy of at least 0.1 °/₀₀ were based on the average of duplicate titrations. Also, the calibration for off-shore samples was achieved by titrating a Copenhagen standard sea water sample every sixth time. We recommend that the effects of systematic errors be minimized by making each titration with a separate set of equipment (including reagent and standard), and, if possible, by different analysts. If only one set of equipment and one analyst are available, make individual titrations on separate days using two sets of reagents and standards. For greatest accuracy, use Copenhagen standard sea water for standardization. Store all samples at the same temperature as the reagents and apparatus for a period of 8 hours before analysis.

The step-by-step procedure used for the analysis of coastal and river waters is as follows:

1. Using a 15-milliliter Knudsen pipette, draw out a 15-milliliter portion of sample and dispense into a 100-milliliter beaker.

2. Add 1 ml. of 8 percent solution of K_2CrO_4 in water to the beaker.

3. Place beaker on the magnetic stirring assembly.

4. Stir at a slow rate of speed while simultaneously adding the silver nitrate reagent from a self-leveling burette.

5. As the end point approaches, increase the stirring speed.

6. Record the end-point reading in the record book.

7. Maintain a record of the strength of the silver nitrate by checking with a standard sea water sample that has been standardized against the Copenhagen standard water.

8. Convert burette readings to salinity units (parts per thousand) by means of the Knudsen tables or a suitable alignment chart (Marvin 1957).

Silver nitrate reagent:

Dissolved 705 gm. of $AgNO_3$ in 19 liters of triple-distilled water and then adjust the solution so that 38.75 ml. of the solution will be required to titrate 15 ml. of standard sea water (cl = 19.375 °/₀₀).

Copper

We have used the method of Hoste, Eeckhout, and Gillis (1953) and also that of Chow and Thompson (1952) for the determination of copper. The former is preferred because the reagent used is more selective for copper at the pH range encountered in river and coastal waters. In addition, the copper complex extract of the first method has less tendency to cloud in such waters.

The procedure used in carrying out the preferred method follows:

1. Filter all samples through pyrex wool. Do not use paper filters.

2. Pour duplicate 100-milliliter portions of each sample into 250-milliliter bottles and add approximately 0.1 gm. of hydroxylamine hydrochloride with a small spatula.

3. Add 8 ml. of biquinoline reagent to each sample and shake for 10 minutes on the mechanical shaker.

4. Remove from shaker and add enough distilled water to fill the bottles. The alcohol reagent will layer in the neck where it can be easily removed.

5. After at least 10 minutes, remove 3 ml. of the alcohol reagent with a 5-milliliter automatic pipette and dispense into Fisher electrophotometer micro cells.

6. Check the color density through a 525 B filter and convert to concentration units by comparing with standard samples that were analyzed with the regular samples.

Biquinoline reagent:

Dissolve 0.2 gm. of 2, 2'-biquinoline in 1 liter of isoamyl alcohol as follows: add all the biquinoline to 100 ml. of alcohol; heat until solution is complete; and then add the remaining alcohol.

Sulfide

The method we used was similar to that of Pomeroy (1941). This laboratory has found no suitable means of stabilizing the sulfide content of a sample and for this reason all analyses are carried out immediately after collection. Aeration will decrease the sulfide content of a sample. Therefore, we treat all samples and standards so as to minimize exposure to air. Standards were made from dilutions of $Na_2S \cdot 9H_2O$. A moderately concentrated solution was iodimetrically standardized and then diluted to final working standards immediately before use.

The determination is carried out as follows:

1. With a 50-milliliter graduated cylinder, measure out 50-milliliter portions of the sample into 200-millimeter culture tubes.

2. With a 5-milliliter automatic pipette, add 3 ml. of amine-sulfuric acid test solution. Then with a 22-milliliter automatic pipette, add 0.65 ml. of ferric chloride reagent.

3. Mix by closing vials with thumb and inverting once or twice.

4. Allow vials to stand for 10 minutes. Then, to each, add 11 ml. of ammonium phosphate reagent and again mix.

5. Allow vials to stand for an additional 5 minutes and check the color density with a Fisher electrophotometer using 23-milliliter cells and a 650 A filter.

6. Convert from density to concentration units by comparing with standard samples analyzed with unknown samples.

Reagents:

a. Stock amine-sulfuric solution. Distill para-amino-dimethyl-aniline in an all-glass apparatus in which the air has been replaced with nitrogen. Mix 50 ml. of concentrated H_2SO_4 with 30 ml. of water and cool. Add this to 20 gm. of the purified amine, stirring until solution is complete. Make up to 100 ml. with water. This stock will discolor somewhat on standing, but its usefulness will remain unimpaired.

b. Amine-sulfuric acid test solution. Dilute 10 ml. of the stock amine-sulfuric acid solution with 990 ml. of 18N H_2SO_4.

c. Ferric chloride solution. Dissolve 100 gm. of $FeCl_3 \cdot 6H_2O$ in enough water to make 100 ml. of solution.

d. Ammonium phosphate solution. Dissolve 400 gm. of diammonium phosphate in enough water (805 ml.) to make 1 liter of solution.

Oxygen

We used Winkler's (1888) method, slightly modified, (Dean and Hawley 1947;

Krogh 1935; Yoder and Dresher 1934; Pomeroy and Kirschman 1945) for this determination. The oxygen samples taken from the M/V Pompano were collected in the conventional 300-milliliter B. O. D. bottles. Using 50-milliliter burettes, 100-milliliter aliquots were titrated.

Since then many analyses have had to be made on samples of limited volume. For this reason a semimicro method is now employed. Samples are collected in 60-milliliter pyrex reagent bottles. Since their volumes vary considerably, each one has to be calibrated and the appropriate volume correction included in the final calculations.

The semimicro determination is carried out as follows:

1. Very carefully displace the bottom layer of the sample with 0.6 ml. of NaOH-NaI reagent. Make this addition with a 2-milliliter automatic pipette.

2. In the same manner add 0.6 ml. of the $MnCl_2$ reagent.

3. Displace the portion of sample in the bottle neck by carefully replacing the glass stopper. If air should be inadvertently trapped in the bottle, discard sample.

4. Shake sample, being careful not to introduce air.

5. After 5 minutes add 2.5 ml. of 12N HCl with an automatic pipette.

6. Replace stopper displacing excess sample and shake vigorously.

7. Draw out a 10-milliliter portion with a 10-milliliter volumetric pipette and add to a 50-milliliter Erlenmeyer flask.

8. Using a 5-milliliter micro burette, titrate the 10-milliliter portion with approximately 0.003N sodium thiosulfate. As the end point is approached, add 3 drops of starch solution. The titration is complete when the blue coloration disappears.

9. Convert the burette readings to oxygen concentration units (ml/l or ppm) with the aid of the oxygen conversion chart. Do not forget to correct for sample bottle volume.

Reagents:

a. NaOH-NaI reagent. Dissolve 900 gm. of NaI and 400 gm. of NaOH in 550 ml. of water.

b. $MnCl_2$ reagent. Add 40 gm. of $MnCl_2$ to 10 ml. of 6N HCl and dilute to 100 ml. with distilled water. Stir until solution is complete.

c. 0.003N sodium thiosulfate reagent. This reagent is standardized with potassium biniodate or recrystallized potassium dichromate.

d. Starch reagent. Prepared by dissolving 1 gm. of starch in a saturated NaCl solution.

Total Carbon Dioxide

For the determination of total CO_2 we used the Van Slyke volumetric blood gas apparatus and followed the method of Greenberg, Moberg, and Allen (1932). The technique is as follows:

1. Rinse the apparatus' reaction chamber with a small portion of the sample. Discard the washings and eliminate all air from the apparatus by completely filling with Hg.

2. Introduce 1 ml. of Hg into the cup by opening the connecting valve between the cup and reaction chamber. Remove with filter paper any water entering the cup with the Hg.

3. Add exactly 10 ml. of sample to reaction chamber. For measuring and introducing sample, use a 10-milliliter pipette, accurately calibrated to deliver between marks, and having a stopcock seal between either the upper or lower end. Place a rubber tip on the end of the pipette to prevent air from entering. Remove any portion of the sample on top of the Hg seal with a piece of filter paper.

4. Run 1 ml. of CO_2-free 0.2N lactic acid[3/] into the cup on top of the mercury.

3/ The CO_2 is removed in the Van Slyke apparatus after which the solution is kept under oil.

If air bubbles are noticed in the capillary part of the cup, remove them by means of a wire. Introduce the Hg and acid into the reaction chamber by carefully opening the connecting cock and lowering the leveling bulb. Reseal the cup capillary with a few drops of Hg.

5. Lower leveling bulb until mercury is at the 50 ml. mark and then shake sample for 3 minutes.

6. Allow the Hg level to rise until the liquid is exactly on the 2 ml. mark. Record manometer reading.

7. Add 0.2 ml. of 5N NaOH to the cup over the Hg seal. Making sure there are no bubles in the capillary, introduce the NaOH into the reaction chamber. Reseal cup with a few drops of Hg.

8. Lower the Hg level to about 25 ml. and shake the sample for 1 minute. After shaking, place the leveling bulb in exactly the same position as in item 7 and allow liquid to again ascend to the 2 ml. mark.

9. Record the manometer reading.

10. From the manometer readings and the ambient temperature, compute the quantity of CO_2 in the sample analyzed from the following equation (Van Slyke and Neill 1924):

$$V^{\circ}_{,760} = \left(P \frac{ai}{760\ (1+0.00384t)}\right)\left(1 + \frac{S}{A-S}\, a'\right)$$

where $V^{\circ}_{,760}$ = volume of CO_2 measured under standard conditions;

P = partial pressure of CO_2--i.e., difference in manometer readings;

a = volume of the gas at which P is measured;

i = reabsorption coefficient of the gas in the liquid in the reaction chamber;

t = temperature at which the manometer readings are made;

S = collective volume of sample and reagents;

A = capacity of the extraction chamber;

a' = the distribution coefficient of CO_2 of sea water are supplied in table 1.

Table 1.--Factors for calculating CO_2 content of sea water.
(When A = 50 ml., S = 11 ml., a = 2 ml., and sample = 10 ml.)

Temp. °C	a'	Factors for CO_2 per liter of sea water		
		Ml.	Mg.	Millimoles
15	0.946	0.3209	0.6341	0.01442
16	0.922	0.3176	0.6279	0.01427
17	0.901	0.3151	0.6228	0.01416
18	0.8815	0.3125	0.6178	0.01404
19	0.864	0.3103	0.6134	0.01394
20	0.848	0.3079	0.6086	0.01383
21	0.829	0.3057	0.6042	0.01373
22	0.812	0.3033	0.5995	0.01362
23	0.794	0.3011	0.5950	0.01352
24	0.772	0.2984	0.5899	0.01341
25	0.756	0.2962	0.5854	0.01331
26	0.737	0.2940	0.5810	0.01321
27	0.720	0.2918	0.5765	0.01311
28	0.701	0.2896	0.5723	0.01301
29	0.683	0.2873	0.5676	0.01290
30	0.665	0.2854	0.5641	0.01282

11. Clean the reaction chamber between each determination as follows:

a. Draw 6 ml. of 1N lactic acid into the chamber. Lower the Hg to about 25 ml. and shake for a minute.

b. Discard the lactic acid by displacing with Hg and then repeat the entire cycle using distilled water in place of the acid.

Ammonia

We have had most success determining NH_3 by Nesslerization after the addition of Rochelle salt to prevent subsequent precipitation (Wattenberg 1931). Treadwell's Nessler reagent has been shown by Wirth and Robinson (1933) to be the most satisfactory of several reagents investigated, since it does not have a completely insensitive region at very low NH_3 concentrations.

The determination is carried out as follows:

1. Remove frozen samples from cold storage and thaw by placing in front of an electric fan. Do not thaw more than 20 samples at a time.

2. After a sample has warmed to room temperature, clean the cap-glass junction with a jet of distilled water, shake vigorously, remove cap and with a 50-milliliter graduated cylinder, measure out 50 ml. of the sample. Discard remainder of sample in the collection vial and pour the measured portion back into the empty vial.

3. To each of the 50-milliliter samples add 2 ml. of 30-percent solution of potassium sodium tartrate.

4. Shake all samples thoroughly.

5. Add 1.6 ml. of 10N NaOH to samples and immediately shake. Quickly add 1 ml. of Nessler reagent and shake again.

6. Exactly 2 minutes after the addition of the sodium hydroxide, read color density in a Fisher electrophotometer using 425 B filter.

7. Convert from density to concentration units by comparing values with those of standards which were analyzed with the regular samples.

Nessler reagent: Prepare as follows:

a. Dissolve 115 gm. HgI and 80 gm. of KI in enough water to make 500 ml.

b. Add 500 ml. of 6N NaOH and mix thoroughly.

c. Let stand overnight in a 1-liter cylinder.

d. Decant clear solution into reagent bottle. Discard settlings.

Chlorophyll

Chlorophyll was determined by the method of Richards with Thompson (1952) as modified by Cretiz and Richards (1955). The steps involved are as follows:

1. Add a teaspoon of magnesium carbonate to each 2-liter sample received.

2. Store in refrigerator until following day.

3. Filter duplicate 1-liter samples using the millipore apparatus.

4. When filtration is complete, carefully cut away the excess filter paper and place the pad containing the chlorophyll sample in a screw-top culture tube.

5. Add 5-milliliter 90-percent redistilled acetone to each culture tube.

6. Triturate samples and allow to stand in the dark for 18 to 24 hours.

7. If samples cannot be read within this time limit, do not add acetone, but place tubes and samples in a vacuum desiccator. When samples can be analyzed, add acetone and proceed as in steps 6 and 7.

8. Again triturate samples in tubes, and centrifuge for 3 minutes at 2,000 rpm.

9. Read in the Beckman DU spectrophotometer in 1-centimeter Corex cells against a blank of 90-percent redistilled acetone at the following wave lengths--480, 510, 630, 645, and 665 mu.

10. Make calculations according to

the following equations:

Chlorophyll types:

$C_a = 15.6D_{665} - 2.0D_{645} - 0.8D_{630}$ (in mg/l of extract)

$C_b = 25.4D_{645} - 4.4D_{660} - 10.3D_{630}$ (in mg/l)

$C_c = 10.9D_{630} - 12.5D_{660} - 28.7D_{665}$ (in MSPU/L) 4/

where C_a = concentration of chlorophyll a; C_b = concentration of chlorophyll b; C_c = concentration of chlorophyll c.

Carotenoid types:

$D_{res,\ 510} = D_{510} - 0.0026C_a - 0.0035C_b - 0.0021C_c$

$D_{res,\ 480} = D_{480} - 0.0019C_a - 0.0136C_b - 0.0054C_c$

$C_{astacin} = 2(4.45D_{res,\ 510} - D_{res,\ 480})$ (in MSPU/L)

$C_{non\text{-}astacin} = 7.6(D_{res,\ 480} - 1.49D_{res,\ 510})$ (in MSPU/L)

In all above equations D refers to the optical density reading at the given wave length.

"Carbohydrates"

We used the method of Zein-Eldin and May (1958) based upon the method of J. G. Erdman and A. B. Little (unpublished manuscript) for determining the arabinose equivalent of "carbohydrate-like" material in sea water.

The determination is carried out as follows: (Rinse all glassware used in preparing and transferring the reagent in c. p. H_2SO_4.)

1. Thaw frozen samples with the aid of a fan. Clean cap-glass junctions with a jet of distilled water before removing caps.

2. Pipette 3-milliliter samples in duplicate into 50-milliliter Erlenmeyer flasks using 30-milliliter beakers as covers.

3. Add reagent to groups of 12 samples (6 duplicates) as follows:

a. At time 0, add 5 ml. of reagent to sample I from the first automatic acid burette.

b. At time 30 seconds, add 5 ml. of reagent to sample II from second automatic acid burette.

c. At 60 seconds, add an additional 22 ml. to sample I from the first burette (27 ml. total reagent added).

d. At 90 seconds, add an additional 22 ml. of reagent to sample II from the second burette.

e. Pump up both burettes and continue until acid has been added to all 12 samples.

4. Place the samples in a water bath at 70° C. for 30 minutes.

5. Place the samples in the refrigerator and cool to room temperature.

6. Read in the Fisher electrophotometer using the G. A. B. 5/ 560 mu interference filter in the D position or, if unavailable, a 525 B filter in the B position. Read against distilled water.

7. Treat triple-distilled water blanks and arabinose standards of 1, 2, 3, 5, and 10 mg. per liter similarly.

N-ethyl carbazole reagent:

Crude N-ethyl carbazole is recrystallized from an ethanol solution and precipitated with triple-distilled water until pure white, needle-shaped,

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4/ MSPU/L = one-thousandth of a specified pigment unit (SPU).

5/ Obtained from Photovolt Corporation, New York.

crystals are obtained. Dissolve 1 gm. of the crystals in 1 liter of Du Pont c. p. H_2SO_4, and transfer to amber-glass automatic acid burettes to protect it from the light.

"Protein"
(Tyrosine equivalent)

We used the Z. P. Zein-Eldin and B. Z. May (unpublished manuscript) modification of the P. J. Wangersky method (Collier et al. 1958) for the determination of protein (tyrosine equivalent).

The determination is carried out as follows:

1. Thaw frozen samples. When sample has attained room temperature, clean cap-glass junction with a jet of distilled water.

2. Pipette duplicate 200-milliliter samples into 50-milliliter glass reagent bottles that have ground-glass stoppers.

3. Add 20 ml. of 0.75N NaOH from an automatic burette.

4. Autoclave at 80 lbs. for 2-3 hours.

5. Centrifuge until clear.

6. Read on the Beckman DU spectrophotometer, using the optical density scale. Set wave length at 330 mμ and use the ultraviolet light source. Using the 5-centimeter silica cells, read against distilled water.

7. Treat blanks and samples of tyrosine (1, 3, 5, mg. per liter) similarly. Prepare blanks from Brujewicz' artificial sea water (Sverdrup, Johnson, and Fleming 1942):

NaCl	- 26.518 gm.
$MgCl_2$	- 2.447 gm.
$MgSO_4$	- 3.305 gm.
$CaCl_2$	- 1.141 gm.
KCl	- 0.725 gm.
$NaHCO_3$	- 0.202 gm.
NaBr	- 0.083 gm.
Triple-distilled water	- to 1,000 gm.

WASHING PROCEDURES FOR ALL ANALYTICAL GLASSWARE

We recommend the following procedure for use in routine analyses:

Glassware for all determinations except nitrite, copper, carbohydrate, protein, and salinity should be treated as follows:

Immediately following use, rinse all glassware and fill with distilled water until it is to be washed. Wash with 10-minute immersion in dichromate-sulfuric acid cleaning solution. Then rinse successively with tap and single-distilled water, followed by three rinses with triple-distilled water. If not used immediately, rerinse with triple-distilled water just prior to use.

Nitrate-nitrite tubes--rinse these in distilled water and then place in technical grade H_2SO_4. Prior to use, rinse the tubes with triple-distilled water as mentioned in the general procedure.

Salinity bottles--pour out any remaining sample. Rinse bottles with distilled water, and then fill with about 50 ml. of distilled water.

Copper bottles--rinse these in distilled water and dip in a 5-percent hydrofluoric acid solution for cleaning. The rinsing procedure is the same as that given above.

Carbohydrate flasks--do not use tap water. After use, rinse the flasks with distilled water and immerse overnight in Du Pont c. p. H_2SO_4. Before reusing, rinse once in single-distilled and three times in triple-distilled water and allow to dry. If clean flasks are not used within a week, reimmerse them in acid before use.

Protein bottles--place these directly into a hot detergent solution (Alconox) for at least 24 hours. Rinse these bottles, dip in a 5-percent hydrofluoric acid solution and rerinse as above.

Before use, leach all new glassware at least 12 hours with 10-percent H_2SO_4 in an autoclave at 30 - 40 pounds steam pressure.

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