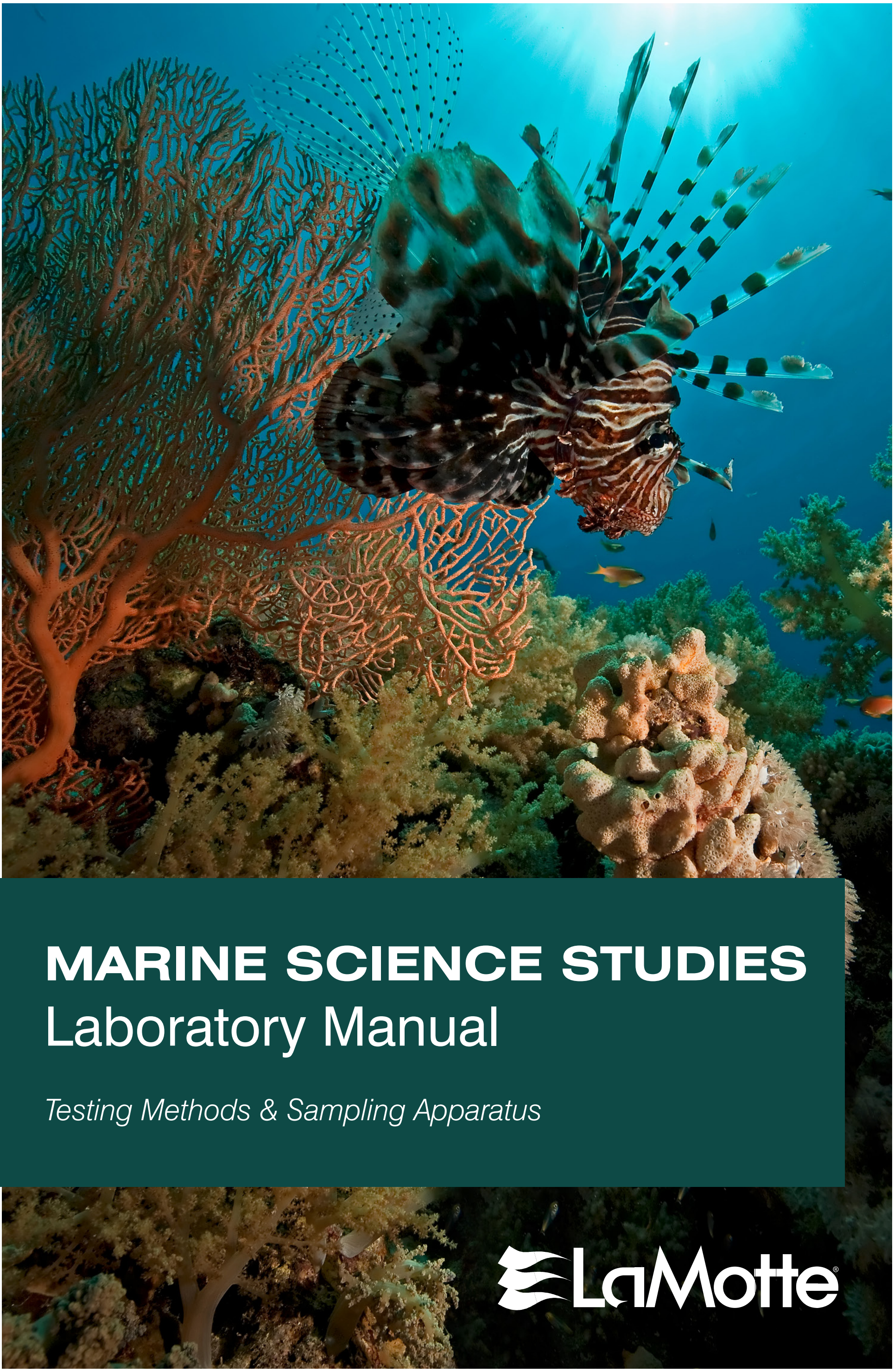


# MARINE SCIENCE STUDIES
## Laboratory Manual

*Testing Methods & Sampling Apparatus*

LaMotte

## INTRODUCTION

From the earliest times, humankind has recognized the differences between fresh water and water from the sea. Contrasts between fresh water supplies (including rain water) and sea water apparent to early humans included sea water's distinctive salty taste,  buoyancy, and the observation of the crusty, white deposits that appeared whenever salt water evaporated. Through the ages, the insatiable curiosity of humankind has been directed toward unfolding the mysteries of the oceans: their composition and their occupants. The tempo of these investigations is at its highest pitch today.

Most peoples of the world are aware that these mammoth bodies of marine water have a known level of frailty. There is an urgent and universal demand for a better understanding of the nature of our oceans, bays, estuaries and the productive coastal marshes. Marine science focuses on an essential segment of our total environment and marine science is an integral part of any scientific inquiry of the planet Earth.

## PURPOSE OF THIS MANUAL

Marine science studies can be grouped into two distinct stages of inquiry, sharing a corridor of overlapping interests. The two stages of oceanographic studies are: (1) the introductory investigations of the marine environment; and (2) the research-oriented, meticulous examination of all facets of the chemical, geological, physical and biological properties of the oceans. This manual has been prepared primarily for those involved in preliminary chemical investigations in oceanography. It is hoped that it will prove to be a handy reference to the more advanced students that are not specialists in chemical oceanography.

The equipment used in chemical investigations may vary from compact field testing outfits for reliable, on-the-spot readings, to elaborate and precise instruments available only to the most well-equipped laboratories afloat or on shore. The testing equipment and sampling apparatus described in this manual are adaptations of professional equipment that has been modified for student use. Valuable data can be obtained through the use of this equipment. It is important to remember, however, that there are some real and logical differences between the capabilities of field test equipment and laboratory instruments. It is also important to understand that the results obtained from any type of testing equipment, whether field or laboratory type, are no better than the sampling technique that is employed to obtain representative samples, nor can it be any more precise than the operator's attention to the details of the analytical procedures.

# COMPOSITION OF SEA WATER

Chemical oceanography is only one of the many scientific disciplines employed in a study of the sea, yet a knowledge of the distribution of certain chemicals in sea water provides important information to oceanographers in both physical studies of water movement and biological studies related to fertility.

Sea water is an aqueous solution of 11 major constituents with a relatively constant composition.

| Major Constituent | Concentration in parts per million (mg/kg of sea water of salinity 35 ppt) |
|---|---|
| Chloride | 19,353 |
| Sodium | 10,760 |
| Sulfate | 2,712 |
| Magnesium | 1,294 |
| Calcium | 413 |
| Potassium | 387 |
| Bicarbonate | 142 |
| Bromide | 67 |
| Strontium | 8 |
| Boron | 4 |
| Fluoride | 1 |

While the above listed components serve to characterize sea water, there are other important elements and compounds that fall under the classification of trace constituents. In this group will be found the various forms of nitrogen and phosphorus, and metals such as iron, copper and manganese. Still other important factors in the chemical examination of sea water are the dissolved gases, oxygen and carbon dioxide, and the pH factor. Because these studies will deal primarily with coastal oceanography and estuarine investigations, greater variations in test results will probably be encountered than would be found in testing offshore ocean water.

## MAJOR FACTORS IN MARINE WATER

### *SALINITY*

Salinity of sea water is classically defined as the weight in grams of solids from 1 kilogram of sea water when all of the carbonate has been converted to oxide, the bromine and iodine replaced by chlorine, all organic matter oxidized and the residue dried at 480°C to constant weight. It is expressed as grams per kilogram or parts per thousand (0/00). This method is only of theoretical interest because it presents too many difficulties for routine use.

Alternative methods are used for determining salinity. Chemical methods measure salinity indirectly through the determination of chlorinity, and direct physical methods measure salinity by density, conductivity and refractivity methods.

Physical methods are used largely because of the speed and the convenience when a large number of samples are to be tested. Of these methods conductivity has been the most widely used; although instruments having the required sensitivity are complicated and expensive. Also, the control and compensation for temperature effects is of utmost importance.

Density can be measured by gravimetric methods with hydrometers that measure different specific gravity ranges. Ordinary stem hydrometers are not sufficiently accurate for anything but approximate work. They are satisfactory, however, for maintenance of a marine aquarium. The following table gives the relationship between salinity and specific gravity of artificial sea water.

### Relationship Between Salinity and Specific Gravity of Artificial Sea Water

| Salinity ppt | Specific Gravity at 20°C (68°F) |
|---|---|
| 0.00 | 1.0000 |
| 2.78 | 1.0020 |
| 5.55 | 1.0041 |
| 8.33 | 1.0061 |
| 11.10 | 1.0082 |
| 13.88 | 1.0102 |
| 16.66 | 1.0123 |
| 19.43 | 1.0144 |
| 22.21 | 1.0164 |
| 24.98 | 1.0185 |
| 27.76 | 1.0206 |
| 30.53 | 1.0227 |
| 33.31 | 1.0248 |
| 36.09 | 1.0269 |
| 38.86 | 1.0290 |
| 41.63 | 1.0311 |
| 44.41 | 1.0332 |
| 47.18 | 1.0353 |
| 49.96 | 1.0374 |
| 52.73 | 1.0396 |

The refractive index of sea water provides another property for measuring its salinity. This is a useful method not only because the refractive index is a function of salinity, but also because the temperature effect, which is small, is not affected appreciably by the salinity of the sample. With precision instruments, an accuracy of 0.05 ppt is obtainable; while with the hand held instruments the accuracy is about 0.5 ppt.

The chemical method for determining chlorinity, from which salinity is calculated, is based on the titration of halide ions in sea water with a standard silver nitrate solution. Silver nitrate ($AgNO_3$) reacts with chloride ($Cl^-$) and bromide ($Br^-$) ions to form insoluble silver chloride and silver bromide. Potassium chromate ($K_2CrO_4$) is used as an internal indicator in the titration, and the endpoint is indicated by the red color of silver chromate ($Ag_2CrO_4$) when a slight excess of silver ions is present after the complete precipitation of the silver halides. In the Knudson method, which is a very precise laboratory procedure, the result is expressed in terms of chlorinity, from which the salinity is calculated by the following equation.

$$\text{Salinity ppt} = 1.805\ Cl^-\ \text{ppt} + 0.030$$

In the Harvey modification of the chlorinity determination the same chemical reactions are involved, but the concentration of the silver nitrate has been adjusted so that it is possible to obtain salinity of sea water on the basis of a single chemical test without further calculations.

## *DISSOLVED OXYGEN*

The dissolved oxygen content of sea water may vary from practically none to concentrations of 10 ppm or more. The "in situ" processes by which the oxygen concentration is altered are principally biological. Oxygen concentrations are high in areas of great photosynthetic activity and low in areas where there is much consumption of oxygen by respiration and by the decomposition of organic materials. Temperature affects the oxygen holding capacity of water. As the temperature increases, the amount of dissolved oxygen decreases. Salinity also affects the solubility of oxygen. As the salinity increases, there is a decrease in dissolved oxygen.

Formula for Determining Oxygen Solubility in Sea Water:

$$S - (C \times F) = \text{Corrected Solubility}$$

S = Solubility in Distilled Water (see table, page 8)

C = Chlorinity reading

F = Factor for Difference per 1.0 ppt Chlorinity (see table, page 8)

Example: Sea water chlorinity of 19 ppt at 20°C

$$9.2 - (19 \times 0.09)$$

$$9.2 - 1.71$$

$$7.49 = \text{Corrected Solubility}$$

# Simplified Table for Calculating the Effect of Chlorinity on Solubility of Oxygen in Sea Water

| Temperature °C | Solubility in Distilled Water mg/L (S) | Difference per ppt Chlorinity (F) |
|---|---|---|
| 0 | 14.6 | 0.17 |
| 1 | 14.2 | 0.16 |
| 2 | 13.8 | 0.15 |
| 3 | 13.5 | 0.15 |
| 4 | 13.1 | 0.14 |
| 5 | 12.8 | 0.14 |
| 6 | 12.5 | 0.14 |
| 7 | 12.2 | 0.13 |
| 8 | 11.9 | 0.13 |
| 9 | 11.6 | 0.12 |
| 10 | 11.3 | 0.12 |
| 11 | 11.1 | 0.11 |
| 12 | 10.8 | 0.11 |
| 13 | 10.6 | 0.11 |
| 14 | 10.4 | 0.10 |
| 15 | 10.2 | 0.10 |
| 16 | 10.0 | 0.10 |
| 17 | 9.7 | 0.10 |
| 18 | 9.5 | 0.09 |
| 19 | 9.4 | 0.09 |
| 20 | 9.2 | 0.09 |
| 21 | 9.0 | 0.09 |
| 22 | 8.8 | 0.09 |
| 23 | 8.7 | 0.08 |
| 24 | 8.5 | 0.08 |
| 25 | 8.4 | 0.08 |
| 26 | 8.2 | 0.08 |
| 27 | 8.1 | 0.08 |
| 28 | 7.9 | 0.08 |
| 29 | 7.8 | 0.08 |
| 30 | 7.6 | 0.08 |

### *Effect of Barometric Pressure on Solubility of Oxygen in Water*

According to Henry's Law, "the weight of a given gas that dissolved in a fixed quantity of a given liquid, at constant temperature, is found to be directly proportional to the partial pressure of the gas above the solution." More simply, as the barometric pressure increases, the solubility of oxygen in water increases. This effect on the solubility of oxygen is determined from the table of solubilities at various temperatures given above (Simplified Table for Calculating The Effect of Chlorinity on Solubility of Oxygen in Sea Water).

Values from the above table are at a total pressure of 760 mm Hg. Under other barometric pressure $P^1$ the solubility $S^1$ is obtained from the equation

$$S^1 = S \quad \frac{P^1}{29.92}$$

While for many analytical procedures instruments have displaced the chemical methods, this is less true for the dissolved oxygen determination where the classical Winkler method modified for marine studies continues to be the method of choice.

Samples should be analyzed for dissolved oxygen as soon as possible after they have been collected and in no case should the analysis be started later than one hour after the sample has been collected.

## *pH*

The term pH is used to define the degree of acidity or alkalinity of a solution. Technically pH is defined as the negative logarithm of the hydrogen ion concentration or: $pH = \log 1/[H^+]$; $pH = -\log[H^+]$. The value of pH 7.0 represents neutrality or a solution where the hydrogen ($H^+$) and hydroxyl ($OH^-$) ions are present in equivalent quantities. Below pH 7.0 the $H^+$ ions predominate and the solution is acid. Above pH 7.0 there is an excess of $OH^-$ ions and the solution is alkaline. The two instrumental methods for determining pH are colorimetric and electrometric. Colorimetric indicator methods have the advantage of simplicity and low cost; however the electrometric method is taken as the standard for pH measurements. Where a sufficient difference exists between the two methods a correction factor can be applied to the colorimetric determination in order to obtain exact agreement. An example of this will be shown later.

The pH of ocean water is relatively constant, generally falling within the range of 7.8 - 8.3 with an average value of 8.1. This constancy of pH is attributed principally to the buffer system of dissolved carbon dioxide, carbonate and bicarbonate ions. Some pH variations are due to temperature shifts and biological activity. In small bodies of water where stratification may develop during the summer growing season, there may be a marked difference in the pH of the surface and bottom waters. Photosynthetic uptake of $CO_2$ will raise the pH at the surface and in the oxygen deficient water of the bottom layer the pH will be lowered. The pH change associated with production or loss of carbon dioxide provides useful information on circulation. Diurnal changes as large as 1.5 pH unit have been reported for shallow waters, and heavy rainfall may cause a considerable change in pH. Brackish waters, estuarine, harbor waters and areas of local industrial contamination show considerable differences from the pH of ocean water.

The electrometric pH value is the recognized standard for sea water. Small differences exist between the electrometric and colorimetric or indicator methods depending on the salinity of the sea water. The following table shows the correction factors to be applied to the colorimetric method in order to obtain values corresponding to the electrometric values when Cresol Red is the indicator. Some test kits for salt water include this correction factor.

| Salinity 0/00 (parts per thousand) | Colorimetric pH Correction |
|---|---|
| 5 | –0.11 |
| 6 | –0.13 |
| 7 | –0.14 |
| 8 | –0.15 |
| 9 | –0.16 |
| 10 | –0.17 |
| 11 | –0.18 |
| 12 | –0.19 |
| 13 | –0.20 |
| 14-15 | –0.21 |
| 16-17 | –0.22 |
| 18-19 | –0.23 |
| 20-22 | –0.24 |
| 23-26 | –0.25 |
| 27-31 | –0.26 |
| 32-35 | –0.27 |

**Example:** Sea water with a colorimetric pH of 8.30 and salinity of 33 ppt

| | |
|---|---|
| Colorimetric pH | 8.30 |
| pH Correction | –0.27 |
| Corrected pH | 8.03 |

The test for pH should be made as soon as possible after the sample has been collected. pH tests can be made with a pH meter or with colorimetric test kits.

## *CALCIUM AND MAGNESIUM*

Calcium and Magnesium make a major contribution to sea water salinity. In normal sea water with salinity of 35 ppt, the calcium content is 0.413 grams per kilogram and the magnesium content 1.294 grams per kilogram, with only minor variations from these values. The cations $Ca^{++}$ and $Mg^{++}$ are present largely as compounds of carbon dioxide.

Calcium and Magnesium in sea water can be determined by both gravimetric and volumetric methods. Since the determination of calcium and magnesium by a gravimetric method is a somewhat involved laboratory procedure, the volumetric method using complex formation with aminopoly-carboxylic acids such as EDTA and suitable endpoint indicators is now widely used. By this latter method, it is now possible to make on-the-spot tests for calcium and magnesium. The calcium and magnesium tests should be of special value in testing estuarine waters since it seems to have been well established that, other factors being constant, the more calcium and magnesium in water, the greater the productivity.

Waters containing small concentrations of the alkaline earths, calcium and magnesium, are normally called soft, and those containing large concentrations are called hard, with the results of analytical tests being expressed in terms of calcium carbonate.

## *ALKALINITY AND FREE CARBON DIOXIDE (CARBONIC ACID)*

Sea water owes its alkaline properties to the presence of bicarbonate, carbonate, borate, phosphate, silicate and fluoride ions. In sea water the alkalinity is the sum of the analytical concentrations of these ions, but for practical purposes, in terms of contribution to alkalinity, the carbonate system is the governing factor. The combined carbonate and bicarbonate concentrations of sea water are of the order of 2.4 meq/L. The alkalinity balance is instrumental in maintaining the relatively constant pH of sea water. If the alkalinity is low, the water will be poorly buffered and thus subject to greater changes in pH. This may be experienced in testing estuarine waters. A number of methods have been proposed for accurately determining the alkalinity of sea water, one of these being the procedure described by Strickland and Parsons[1]. This method involves treating the sample with an excess of acid and then determining the pH of the solution with a pH meter at an accuracy of 0.01 pH unit. With the prepared tables and the above pH measurement, an alkalinity reading can be obtained. Alkalinity may also be determined by direct titration with standard acid to pH values between 4.5 - 5.0. This is the method described in Standard Methods and APHA, and is the procedure employed in the portable field test kits.

Because only 1% or less of the total carbon dioxide content of sea water is in the form of carbonic acid (free carbon dioxide), this value is usually calculated from previously determined factors which include carbonate alkalinity, salinity, temperature, pH, and temperature at which the pH was measured. In some cases, however, as with estuarine waters where the pH is markedly lower than that of sea water, a direct chemical determination can be performed. This involves titration with a standard base solution to a phenolphthalein endpoint.

[1] *J.D.H., Strickland, and T.K. Parsons, A Practical Manual of Sea Water Analysis. Fish. Res. Bd. Canada. Bull. No. 167. (Ottawa, Ontario, Canada: Queens Printer, 1968)*

Analytical results may be expressed in terms of parts per million (ppm), milligrams per liter (mg/L) or milliequivalents per liter (meq/L). The units of ppm and mg/L are often used interchangeably. One milliequivalent of calcium carbonate per liter equals 50.04 milligrams per liter, therefore

$$\text{meq/L } CaCO_3 = \frac{\text{mg/L}}{50.04}$$

## MICRO CONSTITUENTS OF MARINE WATER

It was previously stated that sea water, for the most part, is an aqueous solution of 11 major constituents with a relatively constant composition. If carbon, hydrogen and oxygen are added to these, we have a total of 14 elements in concentrations equal to or greater than one part per million. The remaining elements in sea water which are present in fractional parts per million concentration or parts per billion are those which are generally involved in the most important chemical and biochemical reactions of sea water and shall be discussed in this section. The inorganic constituents of sea water most intimately involved in the life process are water, oxygen, carbonate, silicate, phosphate, ammonia, nitrite, nitrate and certain trace elements such as iron, copper and manganese.

### *PHOSPHORUS*

Because of its importance as a nutrient, the phosphorus content of sea water has been a subject of great interest, and phosphorus has been described as the master element in the marine ecosystem. The average phosphorus content of sea water has been estimated at 70-75 µg/l (parts per billion); although in the uppermost layer it is usually much less, since it is in this layer that photosynthesis takes place. The photosynthetic process uses phosphorus and releases oxygen, thus these two constituents are inversely related. Phosphorus is a primary nutrient for phytoplankton and phytoplankton provides the food for zooplankton. Thus, as the population in each step grows, the concentration of its predecessor diminishes.

Phosphorus is present in dissolved, inorganic, organic, absorbed and insoluble forms. Filtration through a membrane filter of 0.5µm pore size separates the soluble and insoluble forms and permits their determination. Usually, soluble ortho-phosphate is the predominating form, but the partitioning of the phosphorus content of sea water is seasonally dependent. In addition, the phosphorus cycle in sea water is not strictly a closed one, since there is some exchange with deposits on the ocean floor.

### *INORGANIC NITROGEN*

Although nitrogen may occur in any one of nine states of oxidation (-3 to +5), ammonia, nitrite, and nitrate present the three most important forms of inorganic nitrogen found in sea water. The highly reduced forms of nitrogen in ammonia and organic nitrogen compounds are end products of nitrogen assimilation by marine plants or bacteria. The more highly oxidized forms of nitrogen are nitrite (+3) and nitrate (+5). Over extended periods of time, the amount of inorganic nitrogen which is oxidized equals or exceeds the reduced forms. All marine algae capable of using inorganic nitrogen can use ammonia, and most of the common forms attain comparable rates of growth with ammonia, nitrite or nitrate.

The main fluctuations in dissolved inorganic nitrogen in surface waters of coastal areas are mostly seasonal. Nitrate is often depleted to trace amounts in summer, and during winter months high nitrate concentrations may result with rather uniform vertical distribution.

### *SILICON*

The silicon content of sea water shows a wider range of variations than that of almost any other element. It is affected by chemical and biological events and concentrations may vary from undetectable amounts to concentrations as high as 2 to 4 ppm in deep ocean waters. Silicon dioxide is an essential part of the structure of silicoflagellates and diatoms. Vigorous growth of these plants may quickly exhaust the immediately available supply of dissolved silica.

In sea water silica is found in solution as silicate and silicic acid and in suspension in the form of clay particles and combined in organisms. Seasonal variations in silicate in sea water have been found to resemble those of phosphate, a winter maximum being followed by lower concentrations in the spring corresponding to the increased biological activity. Coastal waters will have quite high silicon concentrations since they are affected by land drainage.

Because of the very low concentration of silica involved in this test it is important to compensate for the effect of any silica that might be contained in the reagents by running a blank test along with the water sample.

### *IRON, COPPER, MANGANESE*

Iron, copper and manganese are important trace elements in the process of photosynthesis and are essential in the enzyme reactions which take place. A shortage of iron, which has been found under some marine conditions, will give rise to cells with lowered photosynthetic potential. There is no definite information as to the iron concentration requirements. As for copper and manganese, known shortages of these elements have not been demonstrated.

The average concentration of manganese in sea water is under 2 micrograms per liter (2 ppb) which is too low for any method which oxidizes this element to the permanganate form for color comparisons. Even the most sensitive method known, which is based on the manganese-catalyzed oxidation of the leuco-base of malachite green, is used close to its limit of detection and requires from 4 to 5 hours under controlled conditions of temperature and salinity for full color development.

## COLOR

Color in sea water is caused by dissolved and suspended materials, due principally to humic acids and to particles ranging in size from colloids to particulate matter. The color of an untreated sample would be considered as “apparent” color while the “true” color would be that of a filtered or centrifuged sample from which the particulate matter has been removed. Field practice often consists of measuring the “apparent” color without previous treatment of the sample.

In coastal waters color is due to the comparatively large amounts of humic materials from land drainage where colloid carrying fresh waters mix with saline sea water.

Humic acids have an effect on the biological processes which take place in sea water. One effect seems to be an increase in the availability of certain nutrients and a decrease in the toxicity of other elements due to a chelation process. Another is the removal of growth inhibiting substances excreted by the plants themselves

A numerical value for the color of water is obtained by viewing the water sample by transmitted light and matching the color with cobalt-platinum color standards of assigned color values or units. This is an APHA method.

## VISIBILITY

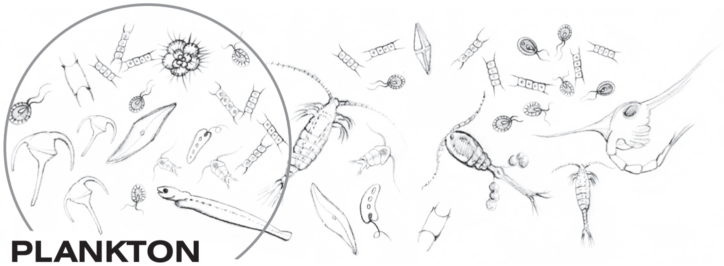
The Secchi Disk provides a convenient method for measuring light penetration below the water surface. By using a Secchi Disk, one can determine the transparency or limit of visibility of the water and estimates of light transmission can be derived. The limit of visibility is approximately the region of transmission of 5% sunlight (Reid 1961). Once the limit of visibility is established calculations can be made to determine the lower limits of the euphotic zone which is usually three times the Secchi Disk depth (Welch 1948).

High concentrations of dissolved substances such as fats, proteins, and carbohydrates, and particulate organic matter such as phytoplankton, zooplankton, detritus and colloidal substances which are found in the highly productive coastal waters, cause most of the light to be absorbed within the first few meters of water. On the other hand, some of the clearest ocean water with low concentrations of dissolved substances and particulate organic matter may have a Secchi Disk reading of 40 meters or more.

It must be remembered that all the light received at the water surface does not penetrate or enter the water. A large portion of the solar radiation is reflected. This depends upon the angle of the incidence of the light rays striking the water. Also, it is important to consider the types or kinds of light penetrating the water surface. In natural waters blue light or light with short wavelengths generally penetrate farther than light possessing longer wavelengths, such as red or orange light. When considering productivity, the penetration of biologically active wavelengths such as red and blue, which are used in photosynthesis, must be measured.

Although the Secchi Disk provides a convenient method for measuring the limit of visibility, the observer must list the conditions under which the Secchi Disk readings are made. Welch (1948) lists the following conditions for Secchi Disk measurements:

**Clear sky; sun directly overhead, shaded, protected side of boat under a sun shade under minimal waves or ripples. Any necessary departure from the conditions should be specifically stated in the records.**



## MARINE PLANKTON

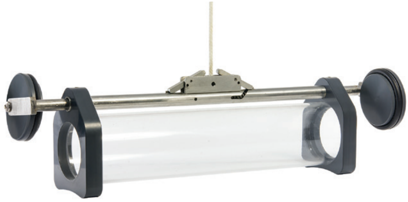
A marine science study would not be complete unless an important part of the aquatic community is discussed: the marine plankton. Plankton are microscopic plants and animals that are found either suspended or weakly-swimming in the sea. The plankton may be very primitive, one-celled organisms or they may be composed of many cells to form complex cellular systems. Marine plankton constitute the greatest source of organic matter in the sea and for this reason nearly all other marine life is dependent upon them as food.

Plankton are subdivided into two basic forms; phytoplankton and zooplankton. Phytoplankton refers to plant forms such as the bacteria, fungi, and algae that are found floating in the sea. Most of the phytoplankton have the same cellular properties as terrestrial plants, but they have no vascular tissue and show no cellular organization to form leaves, stems, or roots. Since most of the phytoplankton in the sea possess chlorophyll, they use the sun's energy and convert this into chemical energy through a series of complex chemical reactions. Representing the first trophic level in a complex food chain, the phytoplankton are the primary producers of food in the oceans. Phytoplankton should be distinguished from other aquatic plant life which inhabits the sea, for there are many types of algae that are found attached to the bottom by means of a holdfast or some other anchoring structure. Attached plants are part of the benthos and include such plants as the seaweeds and kelps.

Zooplankton refers to the free floating or weakly-swimming, usually microscopic, animals in the sea. Although the zooplankton encompasses a vast array of animal life in the sea that remains permanently floating, zooplankton also includes the development states of animal life such as the small larvae of fish, oysters, and crabs. As consumers, zooplankton graze upon phytoplankton and are completely dependent upon them as a food source.

There are numerous ways marine plankton may be collected from the sea. Probably the device that it used most often is the plankton net. This device consists of a fine-meshed bolting cloth made of nylon or other fabric which has very small pores. It is sewn to form a cone-shaped net. A rigid ring and towing bridle at the opening of the plankton net keep the net open as it is being pulled through the water. At the narrow end of the net is a conical graduated tube to collect the plankton. In most cases the pore size of the net is small enough to retain the plankton and concentrate them into the conical collecting tube at the end of the net while the water is filtered through the net.

Some modifications and improvements of the plankton net have been made to increase its usefulness. Although the plankton net was primarily designed as a qualitative sampler, rather successful attempts to adapt the plankton net for quantitative sampling have been achieved. A quantitative sample is made by first determining the amount of water strained through the net. By either using a flow meter which is mounted in the mouth of the plankton net or calculating the volume of water filtered through the plankton net by a formula, an estimate of the number of organisms per unit volume of water can be derived. In order to take samples at different depths, it is possible to attach weights to the net to collect samples from predetermined depths.



Plankton samples may also be taken by other mechanical devices. The Van Dorn and Kemmerer samplers are quantitative samplers and are used to collect plankton samples at a designated depth. These devices consist of a hollow cylinder with a volume capacity of 1 liter or greater, two chamber covers, a measured and calibrated rope or cable, messenger, and trigger mechanism. The sampler is cocked above the water surface and allows the tube to descend to the desired depth. When the sampler reaches the desired depth, a weighted messenger is sent down the rope to trigger the closing of the chamber covers to prevent the collected sample from mixing with unsampled water from intermediate regions during ascent to the surface.

After the plankton sample has been collected by using any one of the sampling apparatus above, it is best to keep a record of how the sample was collected, time, date, temperature, name of collector, exact locations, and any other relevant data for future correlations.

## EXAMINATION OF PLANKTON

Since most of the plankton is too small to be observed with the naked eye, a suitable microscope must be used. Usually if an aliquot (a small portion) of the sample is taken and examined immediately, valuable observations of the behavior and motility of the organisms are obtained. Identifying plankton in its natural state is easier than identifying plankton that has been preserved for a period of time. Populations of plankton tend to change with time because the zooplankton are constantly depleting the phytoplankton populations. Also, if the sample is kept too long without any preservative measure, pigmented planktonic forms fade with time and make identification more difficult.

When observing the plankton under the microscope for preliminary investigations, a petri dish, depression plate, or deep wall slide should be used. For more exacting work after the initial observations have been made, a small sample is transferred to a regular slide with an appropriate coverslip to observe cellular characteristics under a higher magnification of the microscope.

The reporting of the number and kinds of plankton you find in the sample is left to the individual study objectives. A survey of the types of plankton can be reported and a list of important observations can be compiled. If a quantitative examination is desired, some means of calibrating the sample volume you are observing under the microscope are required.

Counting plankton per unit volume of sample is accomplished by a number of scientific devices that are adapted to the microscope. Ocular micrometers, haemocytometers, Sedwich-Rafter counting cells, or Whipple micrometers, serve as excellent instruments to make quantitative plankton studies.

### *PLANKTON SAMPLE PRESERVATION*

If an immediate analysis of the plankton sample cannot be conducted, preservation of the sample is necessary. Instant refrigeration and storage in the dark keeps the activity of the organisms to a minimum. Using this method, the sample can be kept a day or two without drastic changes. Preservation can be maintained for longer periods of time, weeks or months, with a formalin solution (37-42% aqueous solution of formaldehyde), by adding 40 mL of solution to a liter of sample. A few drops of chloroform, when added to a plankton sample, cause an instant kill; but chloroform has a tendency to distort cellular structure and may leach pigments from the cells of the plankton if stored for a period of time. There is no absolutely sound preservative measure that can be used for all plankton. One of the above or other recommended procedures should be selected with care to meet the individual needs of the investigator.

## TEMPERATURE

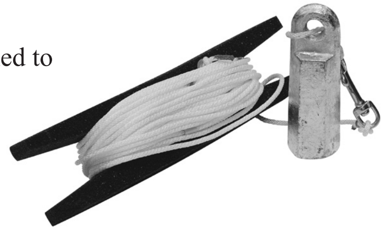
Although temperature may be one of the easiest measurements to perform, it is probably one of the most important parameters to be considered in marine science studies. Many biological, physical, and chemical principles are temperature dependent. Among the most common principles which are temperature dependent are the solubility of compounds in sea water, distribution and abundance of organisms living in the sea, rates of chemical reactions, density, inversions and mixing, and current movements.

Providing the experimenter has a good quality thermometer, the surface temperature of a body of water can be measured with ease. A mercury thermometer represents just one way temperature is measured. Depending upon the type of temperature measurement one desires, a number of adaptations to the mercury thermometer have increased its usefulness to include taking temperatures at various depths. An example is the reversing thermometer. Electronic temperature devices have become quite popular, since a constant recording of temperature provides a more accurate monitoring of temperature with time and depth.

The temperatures of surface and subsurface waters are usually different. With an increase in depth, the water generally becomes colder. This results in thermal stratification of deep water and can lead to density differences. Although temperature stratifications are measured regularly, the ocean water seldom varies more than 25°C, and for this reason the oceans act as a thermostat for keeping the earth's climate within certain limits.

The change of temperature with depth is more characteristic in a particular layer of water known as the thermocline. Within the thermocline the temperature changes are more extreme than layers above or below the thermocline. Below the thermocline in the deepest regions of the ocean, the temperature of the water is usually found to be between 0°C and 4°C.

## DEPTH



A sounding lead, a lead weight that is attached to a graduated line, is used to determine the depths of bodies of water. Precise determinations of depth are not practical when bottom sediments are soft, since the depth at which the weight sinks into the bottom ooze cannot be measured accurately. Most soundings are read to the nearest foot or 0.3 meters in estuarine waters, whereas in oceanic regions the fathom is the standardized unit of measurement. Exact locations of all soundings are required for future reference.

## SAMPLE COLLECTION FOR CHEMICAL ANALYSIS

When collecting water samples for analysis, enough of the sample should be collected to perform the necessary tests. For certain determinations larger sample may be required, especially if the sample has to be concentrated or extracted. Generally, a greater volume of a collected sample is more representative of an area than a small sample. The possibility of loss due to evaporation or contamination of the sample is less with a greater sample volume.

In general, the best analytical results are obtained when the time interval between taking a sample and conducting an analysis is held to the minimum. For certain chemical and physical constituents of sea water, immediate analysis is required. This is especially true for pH, dissolved gases, alkalinity, and temperature. Due to the inherent reduction and oxidation reactions caused by microbial growth, the chemical composition of a sample can change in a short period of time.

It is imperative that the sample to be analyzed is truly representative of the existing conditions under which the sample was taken. For this reason, it is important to handle the sample in such a way as to prevent deterioration or contamination before the analysis is performed. Chemical data for an area is greatly enhanced when a series of tests are conducted on samples that have been collected from sites over a period of time. This permits the investigator to chart these changes and to further investigate any abnormal variance in the chemical balance of the water. Sufficient information is gathered to insure the duplication of results by another investigator. Factors such as the name of the collector, date, how the sample was collected, exact location, temperature, depth, atmospheric pressure and any other relevant data are helpful in evaluating the test results.

The selection of a storage container requires much consideration. The choice is usually limited to glass (preferable borosilicate) or plastic. Glass containers with a suitable plastic cap or stopper are used for salinity and trace constituents, but undesirable for silicate analysis. All glassware should be treated with dilute hydrochloric acid and rinsed several times with good quality distilled water to remove contaminants attached to the glass. Among the advantages of using glass containers is its ability to prevent water loss due to evaporation.

Plastic storage containers should be treated with dilute hydrochloric acid to remove ions left by the industrial process of making plastic containers, and then rinsed with distilled water.

## SAMPLE PRESERVATION

If an immediate analysis of the sample cannot be conducted, it is essential that the sample be preserved. Remember that there is no one preservative for all chemical constituents in sea water. There are a few recommended procedures which preserve the sample, but no adequate substitution can be made for conducting an immediate analysis.

Use an acid or germicide to prolong storage and to protect the sample from being changed by microbial growth. Some specific procedures are recommended for the following sea water constituents.

1. Samples for dissolved oxygen are removed immediately and treated with Winkler reagents and stored under acidic conditions.
2. Analysis of some micro constituents; nitrogen, nitrate, nitrite, ammonia, and silica, should be analyzed within an hour. If this is not possible the sample can be kept by freezing (0°C) for a few hours. Samples requiring a longer storage period (a few months) should be quick-frozen at -20°C and stored at this temperature.
3. Filtered phosphate samples can be kept for several weeks by adding chloroform and storing the sample in the dark.

## SAMPLING APPARATUS

Different types of sampling apparatus are required to collect sea water samples for chemical analysis. The type of sampling apparatus to be used is determined by what kind of sample is needed. Surface sampling only requires some means of gathering sea water in a suitable containers such as a bottle or bucket, while sub-surface sampling requires more sophisticated equipment to sample at known depths and to bring the sample to the surface in an unmodified condition.

## *WATER SAMPLING BOTTLE*

VanDorn, Kemmerer, and Nansen samplers are also used extensively in collecting water samples for chemical analysis. With the exception of differences in the tripping mechanisms, all three samplers operate in more or less the same fashion. The sampler is lowered on a graduated rope to the desired depth, and a messenger is sent down the rope triggering the closing of two fitted plungers which seal the sampler. Providing the sampler is completely sealed during ascent to the surface, the sample will contain a representative water sample from the desired depth.

## *BOTTOM SAMPLING DREDGE*

The Peterson dredge provides a convenient method for collecting bottom sediments. It is used for collecting sediments which may contain mud, sand, ooze, and gravel; however, it is not designed to collect samples from rock bottoms. By using a bottom sampling dredge a number of different analyses can be made. Since the bottom sediments represent a good area to find macro invertebrates and benthic algae, one can easily study quantitatively and qualitatively the communities of organisms living in the bottom habitat. If a physical examination of the bottom is conducted, one may only be interested in the structural characteristics of the bottom and possibly relate this to the biological and chemical analysis. All of these different studies are made possible through the use of a bottom sampling dredge.

Whenever samples of the bottom are taken, it is imperative that every effort be made to insure that the sample is truly representative of the whole area. Much of this depends upon the judgment of the individual taking the sample and their individual study objectives. Ordinarily, as many samples as practical should be taken since one sample has no value statistically. The time interval between sampling and analysis is not critical for a mechanical or physical analysis but prompt analysis isrecommended for both biological and chemical examinations.

## *CORE SAMPLING*

Vertical core samplers are used to sample bottom sediments and soil in depth. Basically, there are three types varying only in the way the samples are retrieved. The most commonly used vertical core sampler for bottom sediments in preliminary marine science studies is the hand operated model, though the explosion driven and self-surfacing models are used to some extent for special sampling in deep water or atypical sediment.

In most cases the length of the core sample will vary with the texture of the soil and the nature of the bottom sediments. Consideration must be made to the kind of analysis to be conducted. Most core sampling devices are designed to sample a small diameter of soil and bottom sediments in depth. It is conceivable that, unless the benthic fauna and flora are known beforehand, representative samples of the organisms in the soil or sediments cannot be obtained unless a large number of samples are taken.

Much of the bottom sampling for biological analyses rely primarily upon dredges, larger coring devices, or bottom scoops, which sample more surface area.

Primarily the vertical coring device is designed to study the physical nature of various soils and bottom sediments, but with the proper precautions the cores can be analyzed chemically to determine the presence or absence of biologically important compounds.

# USE OF CHEMICAL TEST EQUIPMENT

## SAFETY DATA SHEETS (SDS)

*WARNING: Reagents marked with an * are considered to be potential health hazards. To view or print a Safety Data Sheet (SDS) for these reagents go to www.lamotte.com. Search for the four digit reagent code number listed on the reagent label, in the contents list or in the test procedures. Omit any letter that follows or precedes the four digit code number. For example, if the code is 4450WT-H, search 4450. To obtain a printed copy, contact LaMotte by email, phone or fax.

Emergency information for all LaMotte reagents is available from Chem-Tel (US, 1-800-255-3924) (International, call collect, 813-248-0585).

To order individual reagents or test kit components, use the specified code number.

## USE PROPER ANALYTICAL TECHNIQUE

1. Hold dropper bottles and pipets vertically to dispense uniform drops.
2. Thoroughly clean and rinse test tubes after each test.
3. Wipe test tubes dry before placing them in a comparator or meter.
4. Measure reagents and samples carefully.
5. Follow instructions for waiting and mixing times.

## SAFETY

1. See SDS information above.
2. Store reagents and equipment in a cool, dry place. Protect from freezing. Avoid prolonged exposure to direct sunlight or extreme temperatures.
3. Read instructions and reagent labels before using reagents or apparatus.
4. Keep all reagents and equipment out of the reach of small children.
5. Children should use test kits with adult supervision.
6. Avoid contact between reagents and skin, eyes, nose and mouth.
7. Wear protective eyewear and gloves when handling reagents.
8. Wipe up spills as soon as they occur.
9. Cap reagents after use. Do not interchange caps between reagents.
10. As a general rule, replace reagents annually.

# CONVERSION FACTORS

To convert the units in Column A to the units in Column C, multiply the unit in Column A by the factor in Column B. To convert the units in Column C to the units in Column A, divide the units in Column C by the factor in Column B.

| Column A | Column B | Column C |
|---|---|---|
| Acres | 43560 | square feet |
| Acres | 4,047 | square meters |
| Centigrade | (C° x 9/5) + 32 | Fahrenheit |
| Centimeters | 3.281 x 10-2 | feet |
| Centimeters | 0.3937 | inches |
| Centimeters | 10-5 | kilometers |
| Centimeters | 0.01 | meters |
| Centimeters | 10.0 | millimeters |
| Centimeters/sec. | 0.03281 | feet/sec |
| Cubic centimeters | 0.06102 | cubic inches |
| Cubic centimeters | 0.001 | liters |
| Cubic centimeters | 2.113 x 10-3 | pints (U.S. liq.) |
| Decigrams | 0.1 | grams |
| Deciliters | 0.1 | liters |
| Drams | 1.7718 | grams |
| Drams | 27.3437 | grains |
| Drams | 0.0625 | ounces |
| Fahrenheit | 5/9 F°– 32 | centigrade |
| Fathom | 1.828804 | meter |
| Fathoms | 6.0 | feet |
| Feet | 30.48 | centimeters |
| Feet | 0.3048 | meters |
| Feet | 1.645 x 10-4 | miles (naut.) |
| Feet | 1.894 x 10-4 | miles (stat.) |
| Feet | 304.8 | millimeters |
| Gallons | 3,785.0 | cu cm |
| Gallons | 0.1337 | cu feet |
| Gallons | 231.0 | cu inches |
| Gallons | 3.785 x 10-3 | cu meters |
| Gallons | 3.785 | liters |
| Grains/U.S. gal. | 17.118 | parts/million |

## Conversion Factors (cont.)

| Column A | Column B | Column C |
|---|---|---|
| Grains/U.S. gal. | 142.86 | pounds/million gal. |
| Grams | 0.001 | kilograms |
| Grams | 1,000 | milligrams |
| Grams | 0.03527 | ounces (avdp) |
| Grams | 2.205 x 10-3 | pounds |
| Grams/liter | 58.417 | grains/gal |
| Grams/liter | 8.345 | pounds/1,000 gal. |
| Grams/liter | 1,000.0 | parts/million |
| Inches | 2.540 | centimeters |
| Inches | 2.540 x 10-2m | meters |
| Inches | 25.40 | millimeters |
| Kilograms | 1,000.00 | grams |
| Kilograms | 2.205 | pounds |
| Knots | 6,080 | feet/hr |
| Knots | 1,8532 | kilometers/hr |
| Knots | 1.0 | nautical miles/hr |
| Knots | 1.689 | feet/sec |
| League | 3.0 | miles (approx.) |
| Liters | 1,000.0 | cu cm |
| Liters | 0.03531 | cu feet |
| Liters | 0.2642 | gallons (U.S. liq.) |
| Liters | 2.113 | pints (U.S. liq.) |
| Meters | 100.0 | centimeters |
| Meters | 3.281 | feet |
| Meters | 39.37 | inches |
| Meters | 5.396 x 10-4 | miles (naut.) |
| Miles (naut.) | 6,080.27 | feet |
| Miles (naut.) | 1.853 | kilometers |
| Miles (naut.) | 1.1516 | miles (statute) |
| Milligrams | 0.001 | grams |
| Milligrams/liter | 1.0 | parts/million |
| Millimeters | 3.281 x 10-3 | feet |
| Ounces | 28.349527 | grams |

## Conversion Factors (cont.)

| Column A | Column B | Column C |
|---|---|---|
| Ounces | 0.0625 | pounds |
| Ounces (fluid) | 0.02957 | liters |
| Parts/million | 0.0584 | grains/U.S. ga |
| Parts/million | 0.07016 | grains/Imp. gal. |
| Parts/million | 8.345 | pounds/million gal. |
| Pints (liq.) | 473.2 | cu cm |
| Pints (liq.) | 0.4732 | liters |
| Pounds | 0.4536 | kilograms |
| Pounds of water | 0.01602 | cu feet |
| Pounds of water | 27.68 | cu inches |
| Pounds of water | 0.1198 | gallons |
| Pounds/sq ft | 4.882 | kg/sq meter |
| Quarts (liq.) | 946.4 | cu cm |
| Quarts (liq.) | 0.03342 | cu feet |
| Quarts (liq.) | 9.464 x 10-4 | cu meters |
| Quarts (liq.) | 57.75 | cu inches |
| Square centimeters | 1.076 x 10-3 | sq feet |
| Square centimeters | 0.1550 | sq inches |
| Square centimeters | 0.001 | sq meters |
| Square kilometers | 247.1-4 | acres |
| Square kilometers | 106 | sq meters |
| Square meters | 2.471 x 10 | acres |
| Square meters | 104 | sq cm |
| Square meters | 10.76 | sq feet |
| Square millimeters | 0.01 | sq cm |
| Square millimeters | 1.550 x 10-3 | sq inches |
| Temperature (F°) | 5/9 F°– 32 | temperature (C°) |
| Yards | 91.44 | centimeters |
| Yards | 9.144 x 10-4 | kilometer |
| Yards | 0.9144-4 | meters |
| Yards | 4.934 x 10-4 | miles (naut.) |
| Yards | 914.4 | millimeters |

# GLOSSARY OF MARINE SCIENCE CHEMISTRY TERMS

**Alkalinity:** Capacity of a water to accept protons; usually refers to bicarbonate, carbonate and hydroxide components of natural water; expressed as milliequivalents per liter.

**Low Range Comparator:** A device used for color comparison purposes; provides readings of faint colors by looking down through the axis of the sample rather than across the axis.

**Benthos:** Pertains to that portion of the marine environment inhabited by marine organisms which live permanently in or on the bottom; all submarine bottom terrain regardless of depth.

**Chlorinity :** The total amount in grams of chloride, iodide, and bromide contained in one kilogram of sea water, assuming that the iodide and bromide have been replaced by the chloride.

**Chlorosity:** The weight of chloride and bromide in a liter of sea water at 20°C, the bromide being replaced by an equal amount of chloride; expressed in grams per liter of sea water.

**Color-Apparent:** Term which is used to designate color of an original sample of water without filtration or centrifugation of suspended or colloidal substances.

**Color-True:** Term which is used to designate color from which turbidity has been removed.

**Colorimetric:** A name given to a chemical test method whereby the result of the test is determined by the intensity or the shade of the color produced.

**Comparator:** A device for holding color standards and a test sample in adjacent positions so that the colors may be easily compared, one with the other.

**Conductivity:** Specific conductance - A measure of a water's capacity to convey an electric current; related to the total concentration of the ionized substances in water; expressed in micromhos per centimeter.

**Density (Sea Water):** The weight of a given volume of sea water at a specified temperature compared with the weight of the same volume at a temperature of 4°C.

**Diatoms:** Microscopic, unicellular, marine or fresh water algae having siliceous cell walls; a major component of plankton.

**Direct Reading Titrator:** A simple device for dispensing precise micro amounts of titrating reagent. Test results are then read directly from the scale on the barrel of the Titrator.

**Electromagnetic:** Pertaining to a device consisting of an iron or steel core which is magnetized by electric current passing through a surrounding coil.

**Euphotic Zone:** A zone or region in bodies of water into which sufficient light penetrates to permit photosynthesis.

**“Fixed” (as in D.O.):** Refers to rendering a test solution unreactive until a time when the test can be repeated.

**Food Chain:** Refers to the consumption of energy and matter through a complex system of trophic levels. Within this system herbivores graze upon the plants, carnivores prey upon the herbivores, and larger carnivores prey upon smaller ones.

**Germicide:** A substance which is used for the purpose of disinfection and controlling disease organisms.

**Grams Per Kilogram:** Weight of a solute in grams per kilogram of solution. See Chlorinity.

**Gravimetric:** Denoting a method of analyzing compounds by finding the weights of their elements.

**Hydrometer:** A sealed cylinder with weighted bulb and graduated stem for determining the specific gravity of liquids by reading the level of the liquid on the emerging stem

**Indicator:** A chemical reagent added to a test sample to bring about a color reaction.

**Macro Constituents:** The major constituents of sea water which contribute significantly to the salinity; sometimes called the conservative elements.

**Macroinvertebrates:** Macroscopic animals without a backbone; invertebrate animals which can be seen without magnification.

**Messenger:** A weight which is sent down a rope or cable to trigger the closing of some sampling apparatus in the water.

**Micro Constituents:** Chemical elements found in sea water which exist in micro molar and sub-micro molar concentrations. May be involved in some of the most important inorganic and biochemical reactions of the marine environment.

**Milliequivalent (meq):** One one-thousandth of an equivalent. An equivalent is the weight in grams of a substance which combines with or displaces one gram of hydrogen. A milliequivalent is obtained by dividing the formula weight by the valence.

**Milligram (mg):** One one-thousandth of a gram.

**Mineral:** A class of substances occurring in nature, usually comprising inorganic substances of definite crystal structure. Sometimes taken to include aggregations of these substances and certain natural products of organic origins.

**Oceanography:** The application of the sciences to the phenomena of the oceans. It includes a study of the physical, chemical, and biological features of those phenomena.

**Organic:** Pertaining to a class of chemical compounds which formerly comprised only those existing in or derived from living organisms, but now includes these and all other compounds containing the element carbon.

**Oxidation:** To convert an element into its oxide; to take away hydrogen by the action of oxygen; to add oxygen or any non-metal; to remove electrons or to increase the valence of an element.

**Parts per Million (ppm):** A unit of measurement used in chemical testing which indicates the parts per weight in relation to one million parts of solution. One part per million is equivalent to 8.3 pounds of material in a million gallons of water. Equivalent to milligrams per liter.

**Parts per Thousand (0/00) (ppt):** A unit of measurement used in marine sciences to indicate parts by weight in relation to one thousand parts of solution

**pH:** This is a scale based on the hydrogen ion concentration by which water and other substances are measured to determine if they are acid, neutral, or alkaline. The midpoint of the scale is pH 7.0. Readings from 0.0 to 7.0 are acid and the lower the pH value, the more strongly acid the material. Readings 7.0 to 14.0 are alkaline and the higher the reading, the more strongly alkaline the material.

**Photometer:** An instrument for measuring the intensity of light or the relative illuminating power of different lights.

**Photosynthesis:** The process by which green plants convert solar energy (light) into chemical energy. The green pigment chlorophyll is responsible for absorbing light and through a series of chemical reactions converting this to useful chemical energy and food.

**Phytoplankton:** Microscopic aquatic plants which are usually suspended or weakly swimming in surface waters.

**Plankton:** Minute aquatic plants and animals which are found suspended or weakly swimming in surface waters.

**Productivity:** Refers to the total amount of organic matter resulting from photosynthesis after subtracting losses due to respiration and other processes.

**Reduction:** To convert an element into a reduced state by the addition of hydrogen; to add electrons or to decrease the valence of an element.

**Refractive Index:** Ability of a substance to cause a change in the direction of a light ray in passing obliquely from one medium into another which causes the light path to change.

**Respiration:** The process by which oxygen and carbohydrates are assimilated into a living system and the oxidation products (carbon dioxide and water) are given off.

**Salinity:** The total amount of solid matter in grams contained in one kilogram of sea water when all the carbonate has been converted to oxide, all bromide and iodide replaced by chloride and all organic matter oxidized; a measure of the quantity of dissolved salts in sea water, expressed as parts per thousand (0/00).

**Silico-Flagellates:** Flagellated or mobile organisms which have some parts of their bodily structure impregnated with silicon or silicon compounds.

**Specific Gravity:** The ratio of the density of a given substance to that of distilled water at 4°C and at a pressure of one atmosphere. In oceanography the specific gravity is considered numerically equal to density.

**Spectrophotometer:** An instrument for measuring spectral composition of transmitted and scattered light of solutions in terms of optical density or percent transmission.

**Thermal Stratification:** Distinct layering or divisions of bodies of water due to temperature differences.

**Thermocline:** The layer or region in which maximum temperature change occurs in deep bodies of water.

**Zooplankton:** Minute animals which are found suspended or weakly swimming in surface waters; includes developmental stages such as larva and eggs.

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