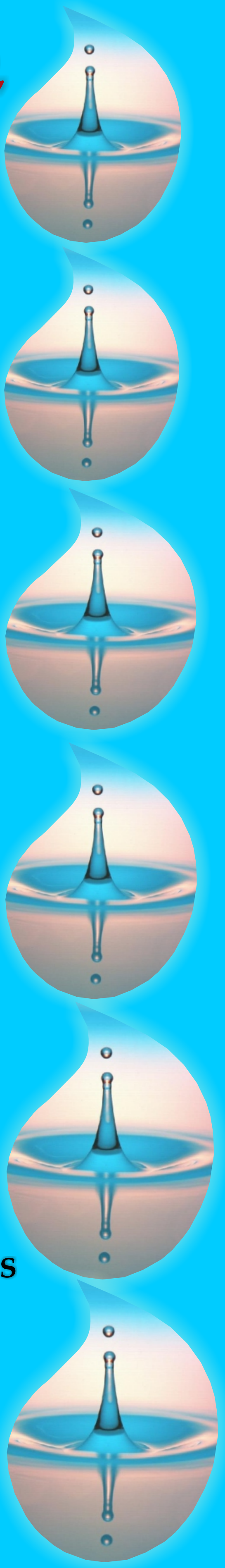
PROCEDURES FOR SEAWATER, SEDIMENT, PLANKTON AND BENTHOS ANALYSIS



CHLOROPHYLL BASED REMOTE SENSING ASSISTED INDIAN FISHERIES FORECASTING SYSTEM

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TRAINING MANUAL

COMPILED BY

BINDU SULOCHANAN, PRATHIBHA ROHIT AND LAVANYA S

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P. B. No. 244, Hoige Bazar, Mangalore, Karnataka - 575 001

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1. Seawater sampling methods

Physical characteristics of seawater viz. temperature, light penetration and pressure are assessed by making *in situ* measurements. For the estimation of most of the physicochemical parameters of seawater, different types of water samplers are used to draw samples from the specific depths. In oceanographic studies seawater samples are drawn from the following internationally defined ‘standard depths’.

Serial number	**Depth (m)**	**Serial number**	**Depth (m)**
1	0 or Surface	18	900
2	10	19	1000
3	20	20	1100
4	30	21	1200
5	50	22	1300
6	75	23	1400
7	100	24	1500
8	125	25	1750
9	150	26	2000
10	200	27	2500
11	250	28	3000
12	300	29	4000
13	400	30	5000
14	500	31	6000
15	600	32	7000
16	700	33	8000
17	800	34	9000

Different types of water samplers are used for the collection of seawater from different depths. Reversible water samplers are operated when use of reversible thermometer is essential. Plastic, PVC and glass samplers are used for collecting samples meant for heavy metal analysis.The following water samplers are commonly used in oceanographic studies.

Meyer's water sampler

It is the simplest sampler for the collection of water samples from any desired depth of shallow systems like the nearshore waters, estuaries and mangroves, where there is no appreciable hydrostatic pressure. Meyer's water sampler consists of an ordinary glass or Perspex bottle of about 1-2 l capacity and is enclosed with a rubber cork. It is weighted below with a lead weight and there are two strong nylon ropes, one tied to the neck of the bottle and other to the cork (fig. 1). While operating, the corked–up (closed) bottle is let down to the desired depth (using the neck-rope) where the stopper is jerked open by a strong pull of the cork rope. The water flows into the bottle. When the bottle is full, which is known by the disappearance of air bubbles, the cork rope is released to keep the cork closed. Afterwards, using the neck rope, the bottle containing the water sample is taken out of the water column. This sampler is suitable up to 20m depth.

Fig. 1 Meyer's water sampler

Friedinger's water sampler

It has a cylindrical portion made of plexiglass or Perspex and is with two hinged covers (fig. 2). During operation, the sampler is sent down in an open state to the desired depth and can be closed by a drop weight messenger (a weight drilled out, so that, it will slide down the wire rope in order to remove or attach it, either hinged or slotted) which falls down inside a sliding rope and closes the covers and makes the bottle water-tight.

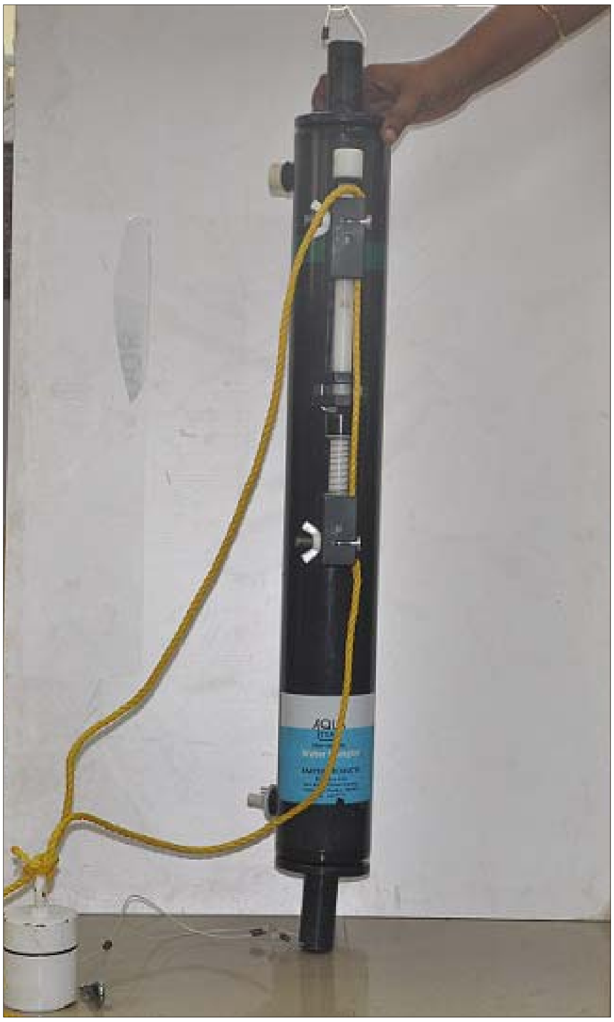


Fig. 2 Friedinger's water sampler

Nansen's reversing water sampler

A Nansen bottle is a device for obtaining samples of seawater at a specific depth (fig. 3). It was designed in 1910 by the early 20th-century explorer and oceanographer Fridjof Nansen and further developed by Shale Niskin. The bottle more precisely a metal or plastic cylinder, is lowered on a cable into the ocean and when it has reached the required depth, a brass weight called a "messenger" is dropped down the cable. When the weight reaches the bottle, the impact tips the bottle upside down and trips a spring-loaded valve at the end, trapping the water sample inside. The bottle and sample are then retrieved by hauling in the cable (fig. 4).

A second messenger can be arranged to be released by the inverting mechanism, and slide down the cable until it reaches another Nansen bottle. By fixing a sequence of bottles and messengers at intervals along the cable, a series of samples at increasing depth can be taken.

The sea temperature at the water sampling depth is recorded by means of a reversing thermometer fixed to the Nansen bottle. This is a mercury thermometer with a constriction in its capillary tube which, when the thermometer is inverted, causes the thread to break and trap the mercury, fixing the temperature reading. Since water pressure at depth will compress the thermometer walls and affect the indicated temperature, the thermometer is protected by a rigid enclosure. A non-protected thermometer is paired with the protected one, and comparison of the two temperature readings allows both temperature and pressure at the sampling point to be determined.

Fig. 3 Nansen sampler

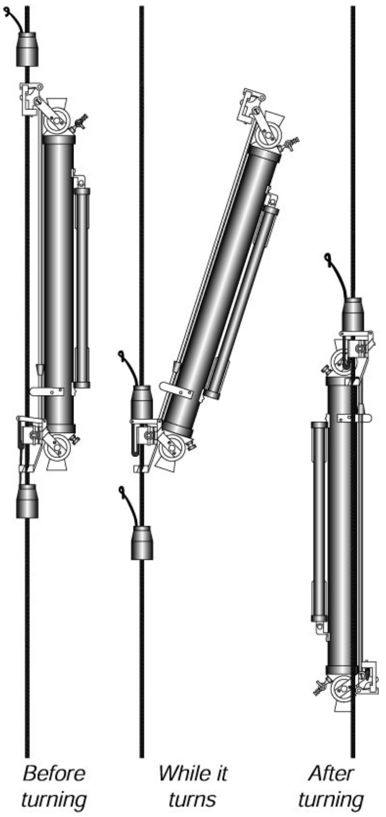


Fig. 4 Nansen sampler in operation

Niskin bottle sampler

The Niskin bottle is a development of the Nansen bottle patented by Shale Niskin in March 1966. Instead of a metal bottle sealed at one end, the 'bottle' is a tube, usually plastic to minimize contamination of the sample, and open to the water at both ends (fig. 5). Each end is equipped with a cap which is either spring-loaded or tensioned by an elastic rope. The action of the messenger weight is to trip both caps shut and seal the tube.

A reversing thermometer may also be carried on a frame fixed to the Niskin bottle. Since there is no rotation of the bottle to fix the temperature measurement, the thermometer has a separate spring-loaded rotating mechanism of its own tripped by the messenger weight.

A modern variation of the Niskin bottle uses actuated valves that may be either preset to trip at a specific depth detected by a pressure switch, or remotely controlled to do so via an electrical signal sent from the surface. This arrangement conveniently allows for a large number of Niskin bottles to be mounted together in a circular frame termed a *rosette* (fig.5a). As many as 36 bottles may be mounted on a single rosette.

Thermistor temperature sensors are more commonly employed on Niskin bottle rosettes due to their higher accuracy compared to mercury thermometers.

Fig. 5 Niskin bottle sampler

Fig 5a. Rosette sampler

2. Handling of seawater samples

Different hydrographic parameters differ in their storage conditions and time limits, it will be useful to subdivide water sample into aliquots which are added in different containers each receiving appropriate storage treatment as given in the table 1.

Table 1 Handling of seawater samples

Parameter	Preservative	Sample Holding Time	Sample Container Size	Type of Container
Alkalinity	4^oC	14 days	100 ml	Plastic or Glass
Ammonia	4^oC, H_2SO_4 to pH < 2	28 days	100 ml	Plastic or Glass
Chloride	None	28 days	50 ml	Plastic or Glass
Chlorophyll a	4^oC	12 hrs	500 ml	Plastic or Glass
Color	4^oC	48 hrs	50 ml	Plastic or Glass
Conductivity	4^oC	28 days	100 ml	Plastic or Glass
Nitrate	4^oC	48 hrs	100 ml	Plastic or Glass
Nitrate-Nitrite	4^oC, H_2SO_4 to pH < 2	28 days	100 ml	Plastic or Glass
Odor	4^oC	24 hrs	200 ml	Glass
Orthophosphate	Filter immediately, 4^oC	48 hrs	50 ml	Plastic or Glass
Particulate Organic matter	4^oC	12 hrs	2 l	Glass
pH	None	Immediately	25 ml	Plastic or Glass
Silica	4^oC	28 days	50 ml	Plastic
TDS	None	7 days	100 ml	Plastic or Glass
Temperature	None	Immediately	1 l	Plastic or Glass

3. Estimation of seawater parameters

Ocean ecosystems are defined by environmental factors and the community of organisms living there. Ocean life is not evenly distributed through time or space due to differences in abiotic factors such as oxygen, salinity, temperature, pH, light, nutrients, pressure, substrate, and circulation. A few regions of the ocean support the most abundant life on earth, while most of the ocean does not support much life.

3.1 Light Penetration

Introduction

Sunlight in the ocean is important for many reasons: It heats sea water, warming the surface layers; it provides energy required by phytoplankton; it is used for navigation by animals near the surface; and reflected subsurface light is used for mapping chlorophyll concentration from space. Light in the ocean travels at a velocity equal to the velocity of light in a vacuum divided by the index of refraction (*n*), which is typically *n* = 1.33. Hence the velocity in water is about 2.25×10^8 m/s. Because light travels slower in water than in air, some light is reflected at the sea surface. For light shining straight down on the sea, the reflectivity is (*n* - 1)2/(*n* + 1)2. For seawater, the reflectivity is 0.02 = 2%. Hence most sunlight reaching the sea surface is transmitted into the sea, little is reflected. This means that sunlight incident on the ocean in the tropics is mostly absorbed below the sea surface. The rate at which sunlight is attenuated determines the depth which is lighted and heated by the sun. Attenuation is due to absorption by pigments and scattering by molecules and particles. Attenuation depends on wavelength. Blue light is absorbed least, red light is absorbed most strongly. Attenuation per unit distance is proportional to the radiance or the irradiance of light:

$$\frac{dI}{dx} = -cI$$

Where x is the distance along beam, c is an attenuation coefficient, and I is irradiance or irradiance.

Radiance is the power per unit area per solid angle. It is useful for describing the energy in a beam of light coming from a particular direction. Sometimes we want to know how much light reaches some depth in the ocean regardless of which direction it is going. In this case we use irradiance, which is the power per unit area of surface.

If the absorption coefficient is constant, the light intensity decreases exponentially with distance.

$$I_2 = I_1 \exp(-cx)$$

where I_1 is the original radiance or irradiance of light, and I_2 is the radiance or irradiance of light after absorption.

In the subtropics and mid-latitudes closer to the coast, sea water contains more phytoplankton than the very clear central-ocean waters. Chlorophyll pigments in phytoplankton absorb light, and the plants themselves scatter light. Together, the processes change the color of the ocean as seen by observer looking downward into the sea. Very productive waters, those with high concentrations of phytoplankton, appear blue-green or green. On clear days the color can be observed from space. This allows ocean-color scanners, such as those on SeaWiFS, to map the distribution of phytoplankton over large areas.

Determination of light extinction coefficient (k)

Light penetration in the sea is measured by using a Secchi disc (fig. 6). A Secchi disc is a circular metal/plate with a diameter of 20 cm. The upper surface is divided into four quadrants and is alternatively painted in black and white. A stable is situated in the centre of the upper surface, and a graduated rope is tied to it. In the centre of the lower surface, a small weight is attached to help in the sinking of the Secchi disc to the depth. The disc is lowered into the water column till the disc disappears on just lifting the disc it will reappear. With the help of the graduated rope, the exact depth of the reappearance of the disc is noted. The values of extinction coefficient of highly transparent deep offshore water and highly turbid coastal waters are calculated using the following formulas:

Calculation of light extinction coefficient (k) in offshore waters

$$K = \frac{1.9}{\text{Secchi disc reading (meters)}}$$

Calculation of light extinction coefficient (k) for shallow coastal waters

$$K = \frac{1.5}{\text{Secchi disc reading (meters)}}$$

It is also possible to calculate the depth of the euphotic zone by using the Secchi disc reading. Since it can be assumed that,

$$K = \frac{1.7}{T}$$ Where T is the Secchi depth.

Based on empirical relationship,

$$0.01 = e^{-K*DE}$$

Where K*DE = 4.6

The depth of the euphotic zone in any coastal water ecosystem is estimated as 2.7 times of the Secchi depth.

Fig. 6 Secchi disc

3.2 Temperature

Ocean temperature is useful for determining the location and variability of ocean currents and eddies, areas of upwelling where nutrients and ocean productivity may be abundant, atmospheric convection, determining heat exchange between the ocean and atmosphere as well and as an indicator of long-term climate change.

Sea Surface Temperature (SST) is a measure of the energy due to the motion of molecules at the top layer of the ocean. Depending on the sensor, space borne measurements give an unprecedented global measurement of sea surface temperatures every few days to a week. Temperatures are measured from approximately 10 µm below the surface (infrared bands) to 1mm (microwave bands) depths using radiometers. Prior to the 1980s, measurements of sea surface temperature were derived from instruments on shorelines, ships and buoys. The first automated method of gathering SST was by measuring water flowing through the input ports of ocean faring ships.

Since the 1980's most of the information about global SST has come from satellite observations. Instruments like the Moderate Resolution Imaging Spectroradiometer on (MODIS) onboard NASA's Terra and Aqua satellites orbit the earth approximately 14 times per day, enabling it to gathering more SST data in 3 months than all other combined SST measurements taken before the advent of satellites.

The motion of electrically charged particles produces electromagnetic radiation of various wavelengths. The electromagnetic spectrum comprises the range of these wavelengths. From longest to shortest the general wavelength categories are radio, microwave, infrared, visible, ultraviolet, x-ray, and gamma ray. The ocean and most other objects emit radiation in the infrared and the microwave wavelengths. The amplitude of these wavelengths vary with the temperature of the ocean and therefore can be used to measure it. Satellite sensors can measure these bands from space. It is important to remember that satellites can only measure temperature at or close to the surface. Other instruments, or models, must be used to determine temperature at depth. Global ocean temperature data below the surface are primarily measured using moorings and drifters. Moorings are good for measuring time series through the depths of the water column and one particular longitudinal/latitudinal location. Deeper ocean temperature data are measured from drifters. There are over 3000 drifters in the ocean today.

Conventional method to measure temperature

Both atmospheric and water temperature of samples collected immediately are measured upon sample collection using mercury thermometer. The atmospheric temperature is measured in a well-ventilated area and in the shade, at 1.2 to 1.5 m above the ground, using a 50^{o}C calibrated (liquid in glass) thermometer. The water temperature is measured by immersing a calibrated thermometer into the water or by measuring the temperature immediately after collection using a calibrated thermometer.

The Mercury-filled thermometer has a precision of ± 0.1^{o}C.

3.3 pH

A pH unit is a measure of acidity ranging from 0-14. The lower the value, the more acidic the environment. When carbon dioxide (CO_2) is absorbed by seawater, chemical reactions occur that reduce seawater pH, carbonate ion concentration, and saturation states of biologically important calcium carbonate minerals. These chemical reactions are termed "ocean acidification". Calcium carbonate minerals are the building blocks for the skeletons and shells of many marine organisms. In areas where most life now congregates in the ocean, the seawater is supersaturated with respect to calcium carbonate minerals. This means there are abundant building blocks for calcifying organisms to build their skeletons and shells.

However, continued ocean acidification is causing many parts of the ocean to become under saturated with these minerals, which is likely to affect the ability of some organisms to produce and maintain their shells.

Principle

pH of a solution is measured with a pH meter (fig.7). pH is the negative log of the hydrogen ion concentration. Hydrogen ion concentration and pH are not the same. The former can be averaged; but pH being a log function should not be averaged. When the electrodes are dipped in two solutions of different pH levels and connected, a potential difference is set up between the two electrodes, which is measured by the potentiometer. This is directly related to the pH of the solution.

Procedure

1. Warm up the instrument for 15-20 min before use.
2. Calibrate the instrument with the standard buffer solutions, (pH 4, 7 or 9). Calibration is done by a buffer solution whose pH is close to that of the sample.
3. Clean the electrode with double distilled water/ deionised water.
4. Immerse the electrode in the unknown sample and stir for 3 min and note the pH.

Fig.7   Bench top pH meter

3.4 Salinity

(Mohr – Knudsen method)

Salinity is defined as the "Total amount of solid materials in grams dissolved in one kilogram of sea water when all the carbonate has been converted to oxide, the bromine and iodine replaced by chlorine and all organic matter completely oxidized."

Salinity is usually estimated either by titrimetric method or using a Salinometer.

Principle

In this method halogen compounds present in seawater (chloride, bromide and iodide) are titrated with silver nitrate using potassium chromate as indicator. The halogen ions (except fluoride) readily react with silver to give insoluble silver halides. In this method silver will react with chromate only after all the halide ions, other than fluoride, are precipitated and as soon as a slight excess of silver ion is present, red silver chromate is formed. A faint red colour of the solution indicates the end point of the titration. Hence, the total quantity of silver required reacting with chloride, bromide and iodide is a true measure of the chlorinity of the sea water.

This method has an accuracy of ± 0.01‰. Cl ± 0.02‰.S.

Reagents required

1. Silver nitrate solution **:** 37.11 g of GR/reagent grade silver nitrate is dissolved in 1 l distilled water
2. Potassium chromate solution: 8g of the salt is dissolved in 100 ml of distilled water.
3. Standard Sea water

Procedure

Pipette out 10 ml of standard sea water into a 250 ml of conical flask. Add 1ml of potassium chromate solution and using a mechanical stirrer, titrate against silver nitrate solution. Titration is preceded till a brownish red colour develops. At this juncture 5 ml of distilled water is added and the precipitate is thoroughly distributed in the flask. Silver nitrate is added drop by drop to stop the titration at the exact end point. Repeat to concordance. Pipette out 10 ml sea water samples into the conical flasks and proceed as above.

Calculation

Volume of silver nitrate (ml) required in the titration of the given seawater sample = T_1

Volume of silver nitrate (ml) required in the titration of the same quantity of the standard seawater = T_2

Chlorinity of the seawater sample = Cl_1 ‰

Chlorinity of the standard seawater = Cl_2 ‰

Thus,

$$Cl_1\% = \frac{T_1 \times Cl_2\%}{T_2}$$

(Chlorinity of the standard seawater is 19.38‰)

Salinity of the given seawater sample:

1. For coastal waters: S‰ = 0.03 + (1.805 x Cl_1‰) where the influence of freshwater is very high.
2. For off-shore region: S‰ = 1.80655 Cl‰

Measurement of salinity using conductivity meter

The conductivity (or specific conductance) of an electrolyte solution is a measure of its ability to conduct electricity. The SI unit of conductivity is siemens per meter (S/m). The unit of conductivity is S/m, often also S/cm.

Conductivity measurements are used for applications such as in the production of ultrapure water or determining the salinity of saltwater.

Conductivity is measured by making a measurement of the electrical resistance. The simplest kind of measuring cell used consists of two similar electrodes. An alternating voltage applied to one of the electrodes causes the ions in the solution to migrate towards the electrodes. The more ions in the solution, the greater the current which flows between the electrodes. The instrument (fig. 8) measures the current and uses Ohm’s law to calculate first the conductance of the solution and then – by taking the cell data into account – the conductivity.

Fig. 8 Conductivity meter

3.5 Total Alkalinity

Principle

Total alkalinity is the measure of the capacity of the water to neutralize a strong acid. The alkalinity in the waters is generally imparted by the salts of carbonates, bicarbonates, phosphates, nitrates, borates, silicates etc. together with the hydroxyl ions in free state. However, most of the waters are, rich in carbonates and bicarbonates with little concentration of other alkalinity imparting ions.

Total alkalinity, carbonates and bicarbonates can be estimated by titrating the sample with a strong acid (HCl or H_2SO_4), first to pH 8.3 using phenolphthalein as an indicator and then further to pH between 4.2 and 5.4 with methyl orange or mixed indicator. In first case, the value is called as phenolphthalein alkalinity (PA) and in second case, it is total alkalinity (TA). Values of carbonates, bicarbonates and hydroxyl ions can be computed from these two types of alkalinity.

Reagents

1. Hydrochloric acid, 0.1 N: Dilute 12 N concentrated HCl (sp. Gr. 1.18) to 12 times (8.34→ 1000 ml) to prepare 1 N HCl. Dilute it further to make 0.1 N HCl (100→ 1000 ml). Standardize it against sodium carbonate solution.
2. Methyl orange indicator: Dissolve 0.5 g of methyl orange in 100 ml of distilled water.
3. Phenolphthalein indicator: Dissolve 0.5 g phenolphthalein in 50 ml of 95 % ethanol and add 50 ml of distilled water. Add 0.05 N CO_2 free NaOH solution drop wise, until the solution turns faintly pink.
4. Sodium carbonate, 0.1 N: Dissolve 5.3 g of Na_2CO_3, previously dried at 250 C for about 4 hrs, in distilled water to prepare 1 litre of solution.

Procedure

1. Take 100 ml of sample in an Erienmeyer flask and add 2 drops of phenolphthalein indicator.

2. If the solution remains colourless, PA= O, and total alkalinity is determined as described in step 4.
3. If the colour changes to pink after addition of phenolphthalein, titrate it with 0.1 N HCl until the colour disappears at end point. This is phenolphthalein alkalinity (PA).
4. Now add 2-3 drops of methyl orange to the same sample and continue the titration further, until the yellow colour changes to pink at end point. This is total alkalinity (TA).

Calculation

$$\text{PA as CaCO3, mg/l} = \frac{(\text{A} \times \text{Normality})\text{ of HCL} \times 1000 \times 50}{\text{ml of sample}}$$

$$\text{TA as CaCO3, mg/l} = \frac{(\text{B} \times \text{Normality})\text{ of HCL} \times 1000 \times 50}{\text{ml of sample}}$$

Where, A = ml of HCl used with only phenolphthalein

B = ml of total HCl used with only phenolphthalein and methyl orange.

PA = Phenolphthalein alkalinity

TA = Total alkalinity

Concentration of carbonates, bicarbonates and hydroxyl ions can be determined from the Table 3 using data of PA and TA.

Table 3 Values of hydroxyl ions, carbonates and bicarbonates from the values of phenoplthalein and total alkalinities.

Result of titration	OH alkalinity as $CaCO_3$	CO_3 alkalinity as $CaCO_3$	HCO_3 as $CaCO_3$
P=0	0	0	T
P< ½ T	0	2P	T-2P
P= ½ T	0	2P	0
P>1/2T	2P-T	2(T-P)	0
P=T	T	0	0

Where, P= Phenolphthalein alkalinity

T= Total alkalinity.

3.6 Dissolved Oxygen

(Winkler method)

Principle

This method, popularly known as Winkler method, depends upon the oxidation of manganous dioxide (bivalent manganese) by the oxygen dissolved in the sample resulting in the formation of a tetravalent compound. When the water containing the tetravalent compound is acidified free iodine is liberated from the oxidation of potassium iodide. The free iodine is chemically equivalent to the amount of dissolved oxygen present in the sample and is determined by titration with a standard solution of sodium thiosulphate.

$$MnSO_4 + 2KOH \rightarrow Mn(OH)_2 + K_2SO_4$$

If the precipitate is white there is very little dissolved oxygen in the sample. A brown precipitate indicates that oxygen was dissolved in it and reacted with the manganous hydroxide to form manganic oxide.

$$2Mn(OH)_2 + O_2 \rightarrow 2MnO(OH)_2$$

On addition of acid the precipitate is dissolved forming manganic sulphate.

$$MnO(OH)_2 + 2H_2SO_4 \rightarrow Mn(SO_4)_2 + 3H_2O$$

Due to an immediate reaction between this compound and the potassium iodide added previously, iodine is liberated resulting in the typical iodine colouration of the sample.

$$Mn(SO_4)_2 + 2KI \rightarrow MnSO_4 + K_2SO_4 + I_2$$

The number of molecules of iodine liberated by the reaction is equivalent to the number of molecules of oxygen dissolved in the sample and this can be determined by titrating against standard solution of sodium thiosulphate using starch as indicator.

$$2Na_2S_2O_3 + I_2 \rightarrow Na_2S_4O_6 + 2NaI$$

Reagents

1. Winkler solution A: Dissolve 365 g of manganous sulphate monohydrate, $MnSO_4.H_2O$ in distilled water and make the volume to 1 litre. The salt must be of analytical reagent grade to ensure the absence of ferric iron.
2. Winkler solution B: Dissolve 500 g of sodium hydroxide in 500 ml of distilled water. Dissolve 300 g of potassium iodide in 450 ml of distilled water and mix the two solutions. A great deal of heat will be liberated. Both the sodium hydroxide and potassium iodide used in this method should be of analytical reagent grade.
3. Standard thiosulphate solution (0.01N): Dissolve 2.9 g sodium thiosulphate in 1litre distilled water.
4. Iodate solution (0.0100 N) : Dry a little analytical reagent quality potassium iodate, KIO_3, at 105 ° C for 1 hr. Cool and weigh out exactly 0.3567 g. Dissolve the salt in 200-300 ml of water, warming slightly. Cool, transfer to a 1000- ml measuring flask and make to the mark with distilled water. This solution is stable indefinitely.
5. Starch indicator solution: Prepare a 0.1- 0.2 % solution of soluble starch. A semi preserved solution (stable for several months) may be made as follows:

   Suspend 2 g of soluble starch in 300- 400 ml of water. Add an approximately 20% solution of sodium hydroxide with vigorous stirring until the solution becomes clear (a slight permanent opalescence may remain) and allow the solution to stand for 1- 2 hr. Add concentrated hydrochloric acid until the solution is just acid to litmus paper and then add 2 ml of glacial acetic acid. Finally dilute the solution to 1 litre with distilled water. Discard the solution when the end point colour is no longer a pure blue and takes on a green or brownish tint.

Procedure

1. Remove the stopper from the BOD bottle and add 1.0 ml of manganous sulphate reagent (fig. 9) with an automatic pipette followed at once by 1.0 ml of alkaline iodide solution. Restopper the bottle immediately and mix the contents thoroughly by shaking until the precipitated manganous- manganic hydroxide is evenly dispersed. No air bubbles should be trapped in the bottle.
2. When the precipitate has settled slightly (in 2- 3 min) shake the bottles again. Finally allow the samples to stand until the precipitate has settled at least one third

of the way down the bottle leaving a clear supernatant solution. It is best practice to allow the solution to warm to room temperature at this stage.

3. Add 1.0 ml of concentrated (sp.gr. 1.84) sulphuric acid, restopper the bottle and mix so that all the precipitate dissolves. No air should be trapped in the bottle.
4. Within an hour or so of acidification transfer 50.0 ml of solution (100 ml if desired for oxygen contents less than about 0. 1 mg- at O_2 per litre) into a specially painted conical flask by means of pipette. Titrate at once with standard 0.01 N thiosulphate solutions until a very pale straw colour remains. Add 5 ml of starch indicator and conclude the titration.
5. Alternatively nearly the whole contents of the BOD bottle can be titrated with standard 0.5 N thiosulphate. Pour off 20 ± 0.5 ml into a 25 ml graduated measuring cylinder. Place a small magnetic stirring bar into the bottle and titrate with the stem of the Gilmont micro burette (fig. 9a) well immersed in the bottle. Use 10 ml of starch solution to detect the end point.
6. Subtract any blank correction from the titration to obtain the corrected titration, V ml, and calculate the oxygen content of a sample from the formulae:

$$\text{mg-at } O_2/\text{litre} = \frac{Y}{Y-2} \times \frac{5.00}{X} \times f \times V$$

When an X- ml aliquot is taken from a Y- ml bottle.

In the above expressions *f* is a factor obtained as follows:

Determination of the factor *f*

Fill a 300 ml BOD bottle with seawater, or with distilled water, and add 1.0 ml of concentrated sulphuric acid and 1.0 ml of alkaline iodide solution and mix thoroughly. Finally add 1.0 ml of manganous sulphate solution and mix again.

Withdraw approximately 50-ml aliquots into the titration flasks. Use one or two flasks for blank determinations if necessary , and to the other add 5.00 ml of either 0.100 N or 0.0100 N iodate from a clean 5-ml pipette which should be calibrated as a check on the volume. Allow the iodine liberation to proceed for at least 2 min but not for more than 5 min, during which time the solution should be at a temperature below 25°C and out of direct sunlight. Titrate the iodine with the appropriate thiosulphate solution. If *v* is the titration volume in milliliter then

$$f = \frac{1.00}{v} \quad \text{for the o.5 N thiosulphate}$$

or

$$f = \frac{5.00}{v} \quad \text{for the 0.01 N thiosulphate}$$

The mean value of *f* should be found from at least three and preferably five replicates.

The milliliters of oxygen at NTP present in a liter of water can be calculated from the expression:

Similarly:

$$\text{ml } O_{2\,(NTP)}\ /\text{liter} = 11.20 \ \text{x} \ \text{mg-at } O_2/\ \text{liter}$$

$$\text{mg } O_{2\,(NTP)}\ /\text{liter} = 16.00 \text{ x mg-at } O_2/\text{liter}$$

Determination of Blank

The calibration procedure described in the above section is also used for the blank determination except that no iodate is added. If analytical reagent quality chemicals are used there should be no blue colour with starch. If a slight colouration results a blank correction may be ascertained by titrating with thiosulphate until the solution is colourless. If this blank correction exceeds 0.1 ml, the reagents are suspect and should be prepared afresh. The potassium iodide or manganous reagent is generally the cause of the trouble. If no blue colour is formed on adding starch, check that a blue colour does result when 0.1 ml or less of 0.01 N iodate is added. This guards against the presence of reductants. The blank testing should be undertaken when each new batch of reagents is prepared.

Fig. 9 Fixing DO in the field

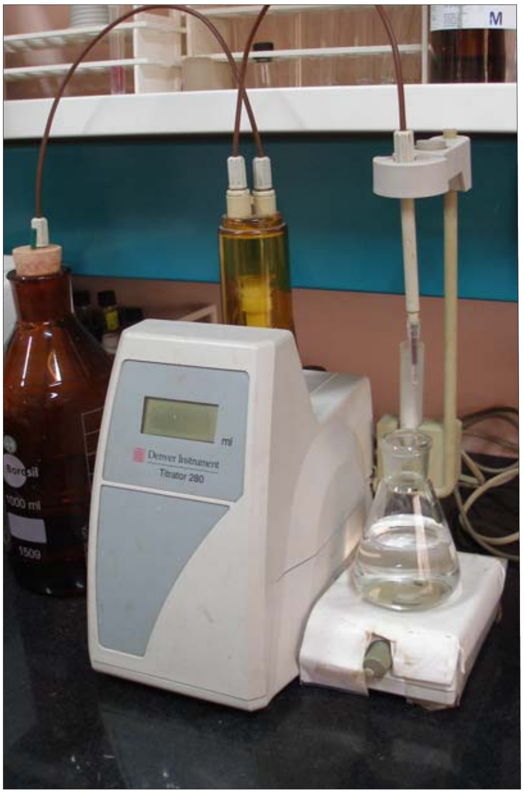


Fig. 9a. Titrator

3.7 Reactive silicate

Introduction

Silicon is present in sea water in the dissolved form mainly as the alkali salts of orthosilicic acid $Si(OH)_4$, is estimated by the method described by Mullin and Riley (1955) and as modified by Strickland and Parsons (1968).

Principle

Determination of silicate in natural waters is based on the principle that yellow silicomolybdic acid is produced when silicomolybdates react with acids. But all forms of silica in solution will not react to give the silicomolybdic complex. Depending on the pH, the silicomolybdate complex exists in two isomeric forms (The alpha and beta silicomolybdic acids). The beta form is very unstable. The alpha form, termed 'reactive silicate' is the most available form, turns into a blue complex on reduction with ascorbic acid, which can be measured photometrically.

Reagents

1. Acid ammonium molybdate: Shake 2 g of ammonium molybdate with approximately 70 ml of water, add 6 ml of concentrated HCl to dissolve the salts completely. Dilute to 100 ml and if necessary filter. Since the reagent takes up silica from glass, it should be stored in polythene bottles.
2. Oxalic acid: Dissolve 10 g of oxalic acid dehydrate in water, dilute to 100 ml and filter.
3. Sulphuric acid: 25 % v/v
4. Metol-Sulphite solution: Dissolve by shaking, 5 g of metol in about 240 ml of water, containing 3 g of anhydrous sodium sulphite and dilute to 250 ml. The solution, after filtration through a Whatman No. 1 filter paper, should be stored in a dark glass bottle.
5. Reducing agents: Mix 100 ml of metol-sulphite solution with 60 ml of 10 % oxalic acid and add, while cooling, 120 ml of 25 % sulphuric acid, dilute to 300 ml. The fresh reducing agent should be prepared fortnightly.
6. Standard silicate solution: 0.960 g of sodium silico fluoride is dissolved in distilled water and make up to 1000 ml. One ml of this solution contains 5µg-at Si.

Treatment of apparatus

Graduated flasks should be allowed to stand overnight with the mixture of concentrated nitric acid and sulphuric acids (1:1) to render them insoluble. After this treatment they should be well washed with tap water and distilled water. The flasks may be drained, but should not be allowed to become completely dry, as this appears to render them more soluble.

Method

Pipette 20 ml of sample (up to 2 µg-at Si) – (If the sample contains more than 2 µg-at Si, take 15 ml of sea water and add about 5 ml of distilled water) – into a 50 ml graduated flask containing 3 ml of the acid molybdate reagent and mix thoroughly. After 10 min add 15 ml reducing agent and make up to 50 ml with distilled water. Allow to stand for 3 hrs. Measure the optical density of the solution at 810 nm in a spectrophotometer. Use a reagent blank and set the instrument at 0.0 absorbance.

Preparation of calibration graph

From the stock solution a series of working standards of known concentrations of silicates are prepared by suitably diluting with distilled water. The diluted working solutions of 2.5, 5.0, 10.0, 25.0, 50.0 and 100.0 µg-at Si/l. are prepared and treated with reagents and OD values measured at 810 nm. Draw a calibration graph.

Calculation

Concentration of reactive silicate in the given sample is obtained from the graph.

Note

1. Glass bottles must be avoided for sampling or storage; plastic containers are suitable. Because of the possible presence of siliceous organisms, storage in the dark is advised, but analysis should in any case not to be delayed for more than 24 hrs. If this were unavoidable, freezing of the samples would probably help to minimize changes.
2. For samples of salinity below 27 %, overnight standing after thawing is essential to allow silicon polymerized by freezing to depolymerize.

3.8 Dissolved Orthophosphate

(Ascorbic acid method)

Introduction

Phosphorous present in seawater in the form of dissolved orthophosphate can be easily determined quantitatively based on the method given by Murphey and Riley (1962).

Principle

Ammonium molybdate and potassium antimony tartrate react in an acid medium with dilute solutions of orthophosphate to form phosphomolybdic acid that is reduced to the intensely coloured molybdenum blue by ascorbic acid. The intensity of the blue colour increases in proportion to the amount of phosphorous present and can be measured photometrically.

Reagents

1. 5-N Sulphuric acid: 70 ml of Conc. H_2SO_4 is diluted to 500 ml with distilled water.
2. Ammonium molybdate: 20 g of ammonium molybdate is dissolved in distilled water and it is made up to 500 ml.
3. Potassium antimony tartrate: Dissolve 1.3715 g $K(SbO)C_4H_4O_6 \cdot \frac{1}{2} H_2O$ in 400 ml distilled water in a 500 ml volumetric flask and dilute to volume. Store in a glass stopper bottle.
4. Ascorbic acid: Dissolve 1.76 g of ascorbic acid in 100 ml distilled water. This solution is stable.
5. Mixed reagent: Mix the above reagents in the following proportions. For 100 ml combined reagent, 50 ml 5N H_2SO_4, 5 ml potassium antimony tartrate solution, 15 ml ammonium molybdate solution and 30 ml ascorbic acid solution. Mix after addition of each reagent. The reagent is stable for 4 hrs.
6. Standard stock phosphate solution**:** Dissolve accurately 0.186 g of anhydrous potassium dihydrogen phosphate (KH_2PO_4) in 1000 ml of distilled water. Store in dark bottle with 1 ml of chloroform. 1 ml of this solution contains 6 µg-at PO_4- P

Procedure

1. To 50 ml of the sample at laboratory temperature add 8 ml of mixed reagent. After 5 min and preferably within the first 30 min measure the extinction of the solution, in a 1 cm cell against water at a wavelength of 885 nm.
2. Warm another portion of the sample to laboratory temperature and measure the extinction to obtain turbidity correction. Correct the measured extinction of the sample by subtracting both the turbidity and reagent blank.

Preparation of calibration graph

Obtain the concentration of PO4- P in the sample from the calibration graph.

Notes

1. Samples are to be collected in polythene bottles and analysis is to be carried out within an hour of collection. If the analysis is delayed the samples must be frozen.
2. All the reagents must reach the room temperature before they are mixed and must be mixed in the order given. If turbidity forms in the mixed reagent shake and let it stand for a few minutes until the turbidity disappears before proceeding.
3. If the samples are high in phosphate, dilute them with distilled water before the reagents are added.

3.9 Reactive Nitrite

There are several methods for the determination of nitrite in water, based on a classical Griess reaction, where nitrous acid is converted to a highly colored "azo" dye. The nitrite in the sea water is allowed to react with sulphanilamide in an acid solution. The resulting diazo compound reacts with N-(1-naphthyl)-ethylenediamine and forms a highly colored azo dye, the extinction of which is measured spectrophotometrically.

Sampling procedure

The 50 ml measuring cylinder and 125 ml Erlenmeyer flasks used for this determination should be rinsed twice with the sample, and drained by inverting and shaking. Then 50 ml of sample is measured into a flask. Samples are stable in subdued light for many hours at room temperature but the analysis should not be delayed for more than about 5-10 hr. If greater delays are unavoidable the samples should be frozen but prolonged storage is not recommended. Withdraw a separate sample of about 30 ml for turbidity measurement for work of the highest precision in inshore areas.

Reagents

a) Sulphanilamide Solution: Dissolve 5 g of sulphanilamide in a mixture of 50 ml of concentrated hydrochloric acid (sp gr 1.18) and about 300 ml of distilled water. Dilute to 500 ml with water. The solution is stable for many months.

b) N-(1-Naphthyl)-Ethylene diamine Dihydrochloride Solution: Dissolve 0.50 g of the dihydrochloride in 500 ml of distilled water. Store the solution in a dark bottle. The solution should be renewed once a month or directly a strong brown coloration

Experimental procedure

i. Add 1.0 ml of sulphanilamide solution from an automatic pipette to each sample (50 ml in a 125-ml flask), mix, and allow the reagent to react for between 2 and 8 min.

ii. Add 1.0 ml of naphthylethylenediamine solution and mix immediately. Between 10 min and 2 hr afterwards measure the extinction of the solution in a 10-cm cell against distilled water. A wavelength of 543 nm should be used. Correct the measured extinction by subtracting both turbidity and reagent blanks. Calculate the nitrite-nitrogen concentration in microgram-atoms of nitrogen per liter (pg-at N/liter) from the expression:

$$\mu\text{g-at N/liter} = \text{corrected extinction} \times F$$

Where F is a factor obtained as described in section Nitrate below.

3.10 Nitrate

(Cadmium Reduction method)

Nitrate is the oxidized state of nitrogen and in oxygenated surface waters; all organic and inorganic nitrogen should be in the form of nitrate. The estimation of Nitrate in seawater is based on a method by Morris and Riley (1963) with some modifications suggested by Grasshoff (1964) and Wood *et al*. (1967).

Outline of the method

The nitrate in sea water is reduced almost quantitatively to nitrite when a sample is run through a column containing cadmium filings loosely coated with metallic copper (fig.10). The nitrite thus produced is determined by diazotizing with sulphanilamide and coupling with N- (1- napthyl) - ethylenediamine to form a highly colored azo dye the extinction of which is measured. A correction may be made for any nitrite initially present in the sample.

Reagents

1. Conc. Ammonium Chloride (NH_4Cl) solution: Dissolve 125 g of analytical reagent quality NH_4Cl in 500 ml of distilled water and store in a glass or plastic bottle.
2. Dilute Ammonium chloride solution: Dilute 50 ml of concentrated ammonium chloride to 2000 ml with distilled water. Store the solution in a glass or plastic bottle.
3. Cadmium- Copper filings (99.9% purity is satisfactory): stir about 100 g of fillings (>0.5mm) at a time with 500 ml of a 2% w/v solution of copper sulphate pentahydrate, ($CuSO_4 . 5H_2O$), until the entire blue color has left the solution and semi-colloidal copper particles begin to enter the supernatant liquid. Fill the column with dilute ammonium chloride solution, or the supernatant liquid from the preparation of cadmium-copper turnings above, and pour in sufficient cadmium-copper mixture to produce a column about 30 cm in length. Add the filings a little at a time, tapping the column hard after each addition to make sure that the fillings are well settled. Wash the column thoroughly with dilute ammonium chloride solution. The flow rate must be such that 100 ml of solution takes between 8 and 12 min to flow completely through the column. If the flow time is less than 8 min,

slow it by restricting the outlet of the siphon or by packing more copper or glass wool at the base of the column.

- When not in use columns must be left with the metal filings completely covered by the dil. NH_4Cl sol.
- When the efficiency of reduction is suspect, columns should be emptied into a beaker and the filings must be washed by stirring vigorously with 300ml of 5% v/v HCl solution, decanting the acid and repeating the procedure once more. Finally wash the metal with 200-300 ml portions of distilled water until the wash is no longer acidic (pH>5) and then decant the liquid to leave metal dry as possible. Retreat the metal with copper sulphate solution as described above.

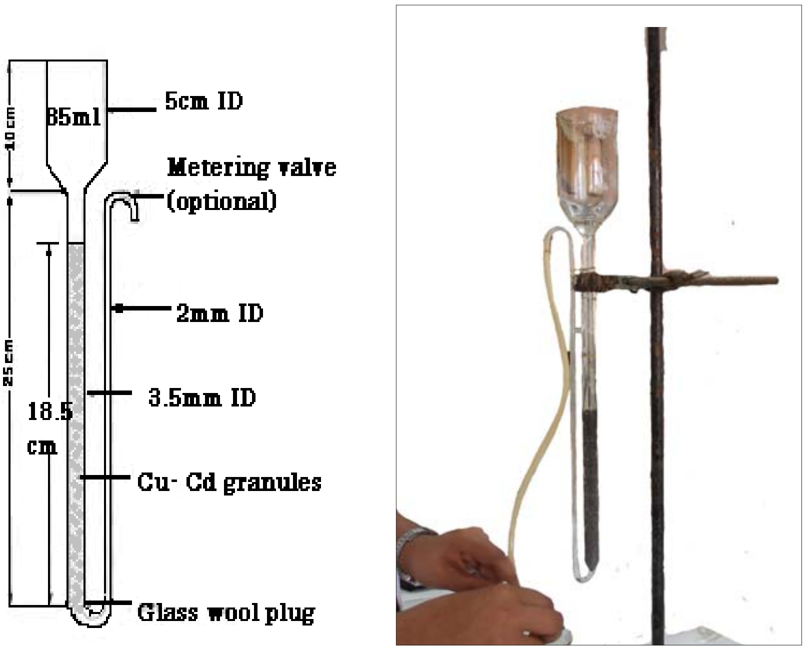


Fig. 10 Nitrate reduction column

4. Sulphanilamide solution: Dissolve 5g of sulphanilamide in a mixture of 50 ml of conc. HCl (sp. gr. 1.18) and about 300 ml of distilled water. The solution is stable for many months.
5. N- (1-naphthyl)- ethylene diamine (NNED) dihydrochloride solution: Dissolve 0.5g of the dihydrochloride in 500 ml of distilled water. Store the solution in a dark bottle. The solution should be renewed once a month or when a strong brown coloration develops.

6. Synthetic seawater: Dissolve 310 g of AR quality sodium chloride ($NaCl$). 100 g AR quality magnesium sulphate ($MgSO_4 . 7H_2O$) and 0.5g sodium bicarbonate ($NaHCO_3 . H_2O$) in 10 lit. of distilled water.

Standard Nitrate solution: Dissolve 1.02 g of AR quality potassium nitrate (KNO_3) in 1000 ml of distilled water. The solution is generally stable in the absence of evaporation. Dilute 2 ml of this solution to 1000 ml with synthetic seawater. This solution be stored in a dark bottle and prepared fresh before use.

Concentration = 20µg – at N/l

Procedure

1. Add 2 ml of conc. ammonium chloride to the sample. Transfer 100 + 2 ml of the sample into an Erlenmeyer flask. Mix the solution and pour about 5 ml on to the top of the column and allow it to pass through.
2. Add the remainder of the sample to the column and place the drained Erlenmeyer flask under the collection tube. Collect about 40 ml and discard. Collect about 50 ml in a graduated cylinder and dispense this into the Erlenmeyer flask, which contained the original sample. Allow the column to drain before adding the next 5 ml sample (as above).
3. To the 50 ml sample add 1 ml of sulphanilamide solution from an automatic pipette. Mix and allow the reagent to react for a period greater than 2 min but do not exceed 8 min. Add 1 ml of napthylethylene diamine (NNED) solution and mix immediately. Between 10 min and 2 hr afterwards, measure the extinction of the solution in a 1cm cuvette against distilled water at a wavelength of 543 nm in a spectrophotometer. Correct the observed extinction by that of the reagent blank.
4. Carry out the procedure given above, using 100 ml of dil. ammonium chloride instead of the seawater sample. Measures the extinction using the same cuvette as is used for the samples and subtract the blank value from the sample values for each column.
5. Also carry out the procedure with 100 ml of the dilute nitrate solution. Measure the extinction for each individual column; then the factor F is

$$F = 20/E_s$$

Where E_s = the extinction of the standard corrected for the blank

Calculation

$$\mu g\text{- at N/l} = (\text{Corrected extinction x F}) - 0.95\ C$$

Where C = the concentration of nitrite in the sample in µg- at N/l

Note

1. Prepare a standard stock nitrite solution by dissolving 0.345g of sodium nitrite in 1000 ml of distilled water (1 ml = 5 µg- at N/l). 1 ml of stock solution is diluted to 100 ml with distilled water. Prepare dilutions of 0.05, 0.2, 0.5, 2.0 and 4.0 µg-at/l. Measure the absorbance at 543 nm and prepare the calibration graph to obtain the nitrite concentration.
2. If the samples are stored they should be frozen at -20°C. In the presence of high concentrations of phytoplankton, samples should be filtered before analysis.
3. Because of the small salt effect, standard nitrate solutions should be made up in synthetic seawater or a low nitrate seawater sample should be spiked with a standard amount of nitrate.
4. Column dimensions can be scaled down proportionally and smaller seawater samples can be used as required.
5. For extinction values of >1.0 or <1.0 use an appropriate cuvette cell length (0.5 or 10 cm respectively) and adjust the factor.
6. In most samples of seawater, the level of nitrite will be insignificant and the correction can be largely ignored. However in some cases, particularly with respect to depth profiles where a nitrate maximum is expected, a correction should be employed. The factor of 0.95 allows for an approximate 5% loss of nitrite on the column compared with the direct determination.
7. For the blank and standard values, the extinctions obtained should be applied to individual cadmium columns and not averaged. Each column may have small consistent differences that are allowed for only if the blank and standards are applied on an individual basis.

3.11 Solids

Solids represent the portion of the water sample that is not lost upon evaporation. Solids include dissolved organic matter, particulate organic matter and dissolved inorganic matter.

3.11.1 Total Dissolved Solids (TDS)

To measure the Total Dissolved Solids (TDS) concentration, a sample is filtered to remove the particulate matter, the filtrate is evaporated, and the residue weighed. The TDS concentration indicates the milligram per liter of dissolved organic and inorganic matter in a sample.

Equipments required

Depending upon the solid analysis conducted, one or more of the following items are required. Glass fiber filtration apparatus, Gelman type A/E glassfiber filters or equivalent. Imhoff cones, 100 ml evaporating dishes, muffle furnace, large desiccators and semi micro analytical balance.

Procedure

i. Prepare glass fiber filters by soaking them in distilled water for 24 hrs and then dry them. Ignite a clean evaporating dish in a muffle furnace at 550^{o}C for 30 min, cool the dish in a desiccator and weigh it.

ii. Position a filter holder, attach the funnel to the holder and attach the apparatus to a vacuum source. Mix the sample and filter 125-150 ml of it through the glass filter.

iii. Measure 100 ml of the filtrate into the evaporating dish with a graduated cylinder. Evaporate the contents of the dish in an oven at 95^{o} C. Increase the oven temperature to 103^{o}C for 1 hr. Cool the dish and residue in a desiccator and weigh.

Calculation

$$\text{TDS (mg/l)} = \frac{(F\text{-}T)1000}{V}$$

Where F = Final weight of evaporating dish and residue in milligrams
T = Tare weight of evaporating dish in milligrams
V = Sample volume in milliliters

3.11.2 Total Suspended Solids (TSS)

The Total Suspended Solids (TSS) can be estimated by weighing the residue retained on the glass fiber filter used in the TDS analysis. The TSS in mg/l is a measure of the particulate matter in suspension.

Procedure

i. Prepare a glass fiber filter by soaking them in distilled water for 24 hrs and then drying. Dry filters in oven at 80-90^{o} C for 24 hrs and tare. Pass 100 ml (or larger) sample through the tared glass fiber filter. Remove the filter with small tongs (do not tough) and dry for 24 hrs at 80-100^{o} C. Cool the filter in a desiccators and weight to five decimal places.

Calculation

$$\text{TSS (mg/l)} = \frac{(F\text{-}T)\ 1000}{V}$$

Where F = Final weight of filter and residue in milligrams
T = Tare weight of filter in milligrams
V = Sample volume in milliliters

The TSS analysis can easily be conducted in conjunction with the TDS analysis. One can simply tare the filter used in the TDS analysis, determine the quantity of the residue resulting from the filtration of the TDS sample and calculate TSS.

3.12 Particulate Organic Carbon

Dissolved and Particulate Organic Carbon (POC) are important components in the carbon cycle and serve as a primary food sources for aquatic food webs. In addition, dissolved organic carbon (DOC) alters aquatic ecosystem chemistry by contributing to acidification in low-alkalinity, weakly buffered, freshwater systems. Furthermore, DOC forms complexes with trace metals, creating water-soluble complexes which can be transported and taken up by organisms. Finally, organic carbon, as well as other dissolved and particulate matter, can affect light penetration in aquatic ecosystems.

Dissolved Organic Carbon (DOC) is defined as the organic matter that is able to pass through a filter (filters generally range in size between 0.7 and 0.22 um). Conversely, POC is that carbon that is too large and is filtered out of a sample. If you have ever seen a body of water that appears straw, tea, or brownish in color, it likely has a high organic carbon load. This color comes from the leaching of humic substances from plant and soil organic matter. This organic matter contributes acids to the stream, resulting in the yellow-brown coloration as well as weathering the soils. Organic carbon can be allochthonous, or sourced from outside the system (e.g. by atmospheric deposition or transported long distances via stream flow) or it can be autochthonous, or sourced from the immediate surroundings of the system (e.g. plant and microbial matter and sediments/soils within the catchment). High amounts of organic matter are common in low oxygen areas, such as wetlands.

Measurement of Particulate Organic Carbon

Equipment required

Muffle furnace, weighing balance of 4-decimal accuracy, Vaccum filtration unit (fig. 11)

Procedure

Particulate organic carbon is measured by determining mass lost upon combustion of a sample. In aqueous samples, this can be done by measuring the dry mass of a filter that had a known amount of water passed through it before and after it is subjected to combustion via heating the filter to 550° C. This method requires that the filter is purged of extraneous POC before filtration (by combusting it at 550° C for 2 hrs), and that the filter and sample are dry (this can be done by putting them in a warm oven) at their pre-combustion weight measurement. The method also requires that the sample has a measurable amount of organic carbon present. POC in soil samples can also be measured by mass loss by measuring the dry weight of a given volume of sample before and after combustion. These methods assume that the mass loss is attributable solely to carbon, rather than any other sample component.

Fig. 11 Vaccum filtration apparatus

3.13 Primary Productivity

The term primary production refers to the rate of formation of organic matter by the phytoplankton. Whereas standing crop refers to the phytoplankton present at any one time in a unit volume of water. In other words, it is the rate at which new organic matter is added to the existing phytoplankton standing crop. It is expressed as grams of carbon per square meter or cubic meter/hr/day/year.

Two sets of bottles are incubated with comparable plankton samples, one exposed to light and the other kept in darkness for a suitable period of time in controlled light and temperature. The changes in dissolved oxygen levels in these bottles are measured and in turn the productivity is expressed in g C fixed/unit volume/hr.

Glassware

a) 125 ml dissolved oxygen bottles 94 numbers clear)
b) 125 ml dissolved oxygen bottles (2 numbers dark)
c) 2 dark cloth bags.

Reagents (preparation procedures as given elsewhere)

a) Winkler A
b) Winkler B
c) Sodium thiosulphate
d) Con. H_2SO_4
e) Starch solution

Procedure

i. Collect water samples in 4 clear BOD bottles and 2 dark BOD bottles as usual. Fix two of the clear bottles immediately with Winkler A and B.
ii. Incubate the other two clear bottles and 2 dark bottles (kept inside in dark cloth bags) for 3 hrs at the place where collection was made. (Alternatively the entire bottle may be incubated in a glass trough with periodic change of water). Or the same came be done *in situ* (fig. 12).
iii. After 3 hrs of incubation fix the clear and dark bottles with equal volumes (1 ml each) of Winkler A and B.
iv. Titrate against sodium thiosulphate and calculate the O_2 in ml/l

Calculation

Let O_2 of light bottle after incubation = x

Let O_2 of dark bottle after incubation = y

Let O_2 of light bottle initially fixed = z

$$\text{Then Gross production} = \frac{(x-y)\times 0.536}{PQ\times t}\ \text{mgC/l/hr}$$

$$\text{And Net Production} = \frac{(x-z)\times 0.536}{PQ\times t}\ \text{mgC/l/hr}$$

Where PQ is the photosynthetic quotient = 1.25

‘t’ is the number of hours of incubation = 3 hrs.

Note: For expressing the productivity in m^3/day, multiply the above by 10000; assuming 10 hrs being the sunshine hours affecting photosynthesis in a day.

Fig. 12 *In situ* measurement of primary productivity

3.14 Chlorophyll

(*Spectrophotometric method)*

Introduction

The chlorophyll pigments a, b and c are seen in the marine phytoplankton. Of these chlorophyll a is important as it represents the biomass of phytoplankton in the sea. Moreover, chlorophyll a measurement is required to calculate assimilation number, i.e. the physiological index of the photosynthetic efficiency of phytoplankton**.**

Principle

Chlorophyll bearing organisms present in a known volume of water sample is filtered and dissolved in solvent (acetone 90% v/v). The pigment content dissolved in unit volume of acetone is measured spectrophotometrically. Since on an average, primary production in the ocean bears a fairly constant relation to the chlorophyll content, measurement of these pigments is also used as an index of productivity.

Requirements

Glass filter paper (GF/C), vacuum filtering unit, measuring jar, centrifuge and centrifuge tubes with caps

Sampling procedure and sample storage

Generally 500 ml to 5 l sample is filtered through a small piece of clean 0.3 mm mesh nylon netting to remove the larger zooplankton. For open sea sampling, filtration of 500 ml through a 0.15 mm mesh net will suffice. Two or three drops (0.1 – 0.2 ml) of magnesium carbonate ($MgCO_3$) suspension is added. The sample may be stored in a cool dark place for a maximum of about 8 hr. It is desirable, that samples may be filtered through a membrane filter at the time of collection.

Reagents

1. Use analar grade acetone and add a little pinch of granular anhydrous sodium carbonate and shake well. Decant and use the solution.
2. Magnesium Carbonate Suspension: Add approximately 1 g of finely powdered magnesium carbonate (light weight of Levis grade) of analytical reagent quality to

100 ml of distilled water in a stoppered Erlenmeyer flask. Shake vigorously to suspend the powder immediately before use.

Procedure

1. Water samples collected for chlorophyll pigments must be passed through a coarse filter 0.2 mm mesh to remove zooplankton. Thoroughly mix the sample and known volume (500 ml) of the sample was filtered through a 47 mm dia Millipore AA filter or 4.5 cm Whatman GF/C glass filter paper.

Note

1. If $MgCO_3$ has not been added previously, introduce about 1 ml of magnesium carbonate suspension to the last few hundred milliliters of sample being filtered. The magnesium carbonate is added at this stage to ensure that the phytoplankton chlorophyll is prevented from becoming acid with the resulting decomposition to give phaeophytin pigments.

2. Millipore filters have the advantage that they dissolve in acetone completely, give no complications at the centrifugation stage, and require no particular precautions during filtration. However, unless great care is taken, undesirably high blanks will occur when using Millipore filters, making the determination of small concentrations of carotenoids difficult. On the other hand use Glass filters results in practically no blank. In this case the glass filter used has to be shaken vigorously for a longer time till it completely dissolves. During this time the cells are ground due to the physical shaking and it results in better extraction of cell pigments.
3. Drain the filter thoroughly under suction before removing it from the filtration equipment and if a Millipore filter is used trim away the peripheral excess of the unstained membrane with clean scissors.

4. Place the filter in a 15 ml stoppered centrifuge tube. If a Millipore filter was used add approximately 8 ml of 90% acetone, stopper the tube, and dissolve the filter by shaking the tube vigorously. If a glass filter paper was used add approximately 10 ml 90% acetone, stopper the tube, and disperse and disintegrate the paper by shaking the tube vigorously. Allow the pigments to be extracted by placing the tube in a refrigerator in complete darkness for about 20 hrs (It is good practice to shake the tubes vigorously once more after they have been 1 or 2 hr in the refrigerator).

5. Remove the tubes from the refrigerator and let them warm up in the dark nearly to room temperature. Add 90% acetone to make the extracts upto 10 ml and those from glass filters to exactly 12 ml.

6. The extract is centrifuged (4000 rpm) for 10 min. Decant the clear supernatant into a 10 mm path length cuvette and measure the extinction at the following wavelengths (750, 665, 645 and 630 nm) against a cell containing 90% acetone. The amount of pigments in the sample is calculated using the formula of Strickland and Parsons (1972).

$$\text{C (chlorophyll a)} = 11.6\ E_{665} - 1.31\ E_{645} - 0.14\ E_{630}$$

$$\text{C (chlorophyll b)} = 20.7\ E_{645} - 4.34\ E_{665} - 4.42\ E_{630}$$

$$\text{C (chlorophyll c)} = 55\ E_{630} - 4.64\ E_{665} - 16.3\ E_{645}$$

Where

- E stands for the absorbance at different wavelengths obtained above and corrected by the 750 nm reading.
- Chlorophyll a, b and c are the amounts of chlorophyll

7. Calculate the concentration of pigments in sea water from the equation:

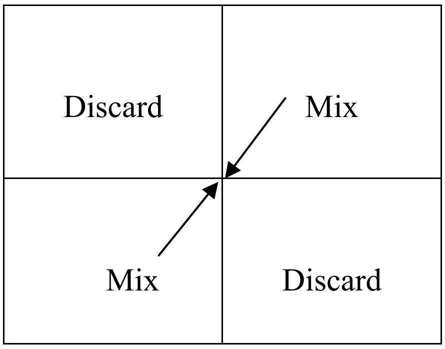
$$\text{mg pigment}/\text{m3} = \frac{C \times 10}{V}$$

Where C is a value obtained from the Strickland and Parsons equations and
V is the volume of seawater filtered in liters.

4. Sediment

Sediment sampling

Sediment sample is collected from the sea / river using a grab. Sample should be representative of the area sampled. For this, collect samples from at least 4 sites in an area. Pool these samples in wide basin. The pooled sediment is mixed thoroughly. Then quartering is done, removing the opposite quarters as shown below,



Quartering is done till sizeable quantity i.e., around 500 g sediment is obtained. Keep in heavy-duty plastic bags.

Sediment processing

Do not store the sediment in wet condition for more than 1 day. Wet samples should be kept under refrigeration if it is stored for more than 1 day. Air dry the sediment in shade in well ventilated places. To reduce the drying time, oven drying can be done at 50-60°C (Temperature should not be increased above 60°C, since it will cause loss of nutrients). Then pulverize the sediment gently by breaking clods using mortar and pestle. (Do not over grind the sediment so as to break the sand particles, which will cause errors in textural analysis). Then sediment samples can be stored in well labeled, capped plastic bottles or heavy-duty plastic bags for future analysis. Store in a dry place.

4.1Organic Carbon

***(Walkley and Black's titration method*)**

Principle

The soil is digested with potassium dichromate solution and sulphuric acid making use of the heat of dilution of sulphuric acid. The excess of potassium dichromate not reduced by the organic matter of the soil, is determined by titration with standard ferrous sulphate or ferrous ammonium sulphate solution.

Reagents

1) Potassium dichromate 1N: Dissolve 49 g of potassium dichromate in water and make it to 1 liter.
2) Sulphuric acid + silver sulphate solution: Dissolve 25 g of silver sulphate in 1 liter of conc. sulphuric acid.
3) Phosphoric acid (85%)
4) Diphenylamine indicator solution: Dissolve 0.5 g of diphenylamine in a mixture of 100 ml sulphuric acid and 20 ml water and store in a colored bottle.
5) Ferrous ammonium sulphate (N/2): Dissolve 196 g of A.R grade $FeSO_4 (NH_4)_2SO_4 . 6H_2O$ in water. Add 20 ml sulphuric acid and dilute to 1 liter.

Procedure

Take 0.5 – 2 g of soil (0.5 mm sieved) in 500 ml conical flask. Add 10ml of 1N potassium dichromate and 20 ml conc. H_2SO_4. Shake well for a minute or two and allow it to stand for about 30 min. Add 200 ml water, 10 ml phosphoric acid and 1 ml diphenylamine indicator solution. A deep violet color will appear. Titrate with N/2 ferrous ammonium sulphate solution, till the violet color changes to blue and finally to green. In the same way carry out a blank determination also and calculate the results as follows.

Calculation

Weight of soil taken = W g

Volume of 0.5 N ferrous ammonium
sulphate required for reducing 10 ml
$K_2Cr_2O_7$ solution (Blank Reading) = X ml

Volume of 0.5 N ferrous ammonium
Sulphate required for reducing the
excess of dichromate (experimental reading) = Y ml

Difference = (X – Y) ml

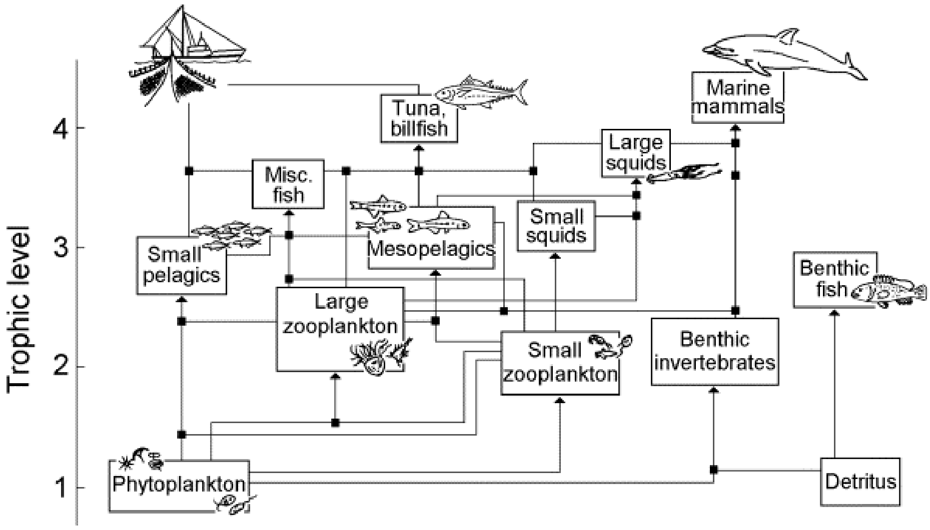
1 ml of 1N $K_2Cr_2O_7$ = 0.003 g carbon

$$\text{\% of carbon in soil} = \frac{(x\text{-}y) \times N \times 0.003 \times 100}{W}$$

Where N = normality of ferrous ammonium sulphate

5. Plankton

Plankton are diverse group of organisms that live in the surface or water column of the water. They are not capable of swimming on their own and drift along with the current. They include diverse organisms from algae, bacteria to protozoans, crustaceans, molluscs and representatives from almost all phyla. The name plankton is derived from the Greek adjective planktos, meaning errant, and by extension "wanderer" or drifter. It was coined by Victor Hensen. While some forms are capable of independent movement and can swim hundreds of meters vertically in a single day (a behavior called diel vertical migration), their horizontal position is primarily determined by the surrounding currents. They are the major source of food for many large aquatic organisms, such as fish and whales. (fig.13). The existence and importance of nano and even smaller plankton was only discovered during the 1980s, but they are thought to make up the largest proportion of all plankton in number and diversity. Though many planktonic species are microscopic in size, plankton includes organisms covering a wide range of sizes, including large organisms such as jellyfish. Aside from representing the bottom few levels of a food chain that supports commercially important fisheries, plankton ecosystems play a role in the biogeochemical cycles of many important chemical elements including the ocean's carbon cycle. Photosynthesis by the phytoplankton accounts for up to half of global primary production. They also provide the primary food source for the zooplankton, and together form the base of the oceanic food chain. Larger and larger zooplankton, fish, and mammals depend on these plankton for their survival. Primarily by grazing on phytoplankton, zooplankton provides carbon to the planktic foodweb, either respiring it to provide metabolic energy, or upon death as biomass or detritus.



An example of a simplified food web, which defines the various elements of such webs ('functional groups'), the flow between them, and so-called 'trophic levels', which indicate the position of each functional group within the web.
From "Fishing down marine food webs' as an integrative concept" by Daniel Pauly (University of British Columbia, Canada),

Organic material tends to be denser than seawater and as a result it sinks into open ocean ecosystems away from the coastlines, transporting carbon along with it. This process is known as the biological pump and it is one reason that oceans constitute the largest carbon sink on earth.

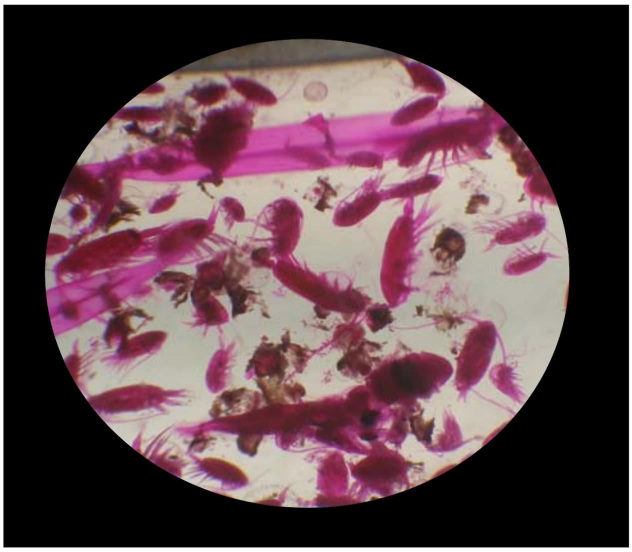


Fig. 13 A group of plankton viewed under microscope

The growth of phytoplankton populations is dependent on light levels and nutrient availability. Environmental variability at multiple scales influences the nutrient and light available for phytoplankton, and as these organisms form the base of the marine food web, this variability in phytoplankton growth influences higher trophic levels. For example, at interannual scales phytoplankton levels temporarily plummet during *El nino* periods, influencing populations of zooplankton, fishes, sea birds, and marine mammals. Changes in the vertical stratification of the water column, the rate of temperature-dependent biological reactions, and the atmospheric supply of nutrients have important impacts on future phytoplankton productivity.

Plankton are primarily divided into broad functional (or trophic level) groups:

- **Phytoplankton** (from Greek *phyton*, or plant), autotrophic, prokaryotic or eukaryotic algae that live near the water surface where there is sufficient light to support photosynthesis. The important groups are (Appendix I & II) the diatoms, cyanobacteria, dinoflagellates and coccolithophores.
- **Zooplankton** (from Greek *zoon*, or animal), small protozoans or metazoans (e.g. crustaceans and other animals) that feed on other plankton. Some of the eggs (Appendix I, II & III) and larvae of larger animals, such as fish, crustaceans and annelids are included under this head.
- **Bacterioplankton**, bacteria and archaeas (single celled microorganisms with no cell nucleus) which play an important role in remineralising organic material down the water column (note that the prokaryotic phytoplankton are also bacterioplankton).

This divides the plankton community into producer, consumer and recycler groups. However, determining the trophic level of some plankton is not straightforward. For example, although most dinoflagellates are either photosynthetic producers or heterotrophic consumers, many species are mixotrophic depending upon circumstances. Plankton are also often described in terms of size. Usually the following divisions are used.

Group	Size range (ESD)	Examples
Megaplankton	>20mm	Metazoans; eg. Jellyfish, ctenophores, salps and pyrosomes (pelagic Tunicata); Cephalopoda; Amphipoda
Macroplankton	2 – 20mm	Metazoans; eg. Pteropods, Chaetognaths, Euphausiacea(krill),Medusae, salps. Doliolids and pyrosomes, Cephalopoda, Amphipoda
Mesoplankton	0.2 – 2 mm	Metazoans; eg. Copepods, medusa, cladocera, Ostracoda, chaetognaths, pteropods, tunicates, Heteropoda
Microplankton	20 -200 µm	Large eukaryotic protists; most phytoplankton, Protozoa (Foraminifera); ciliates, Rotifera, juvenile metazoans – Crustacea (copepod nauplii)
Nanoplankton	2-20µm	Small eukaryotic protists, Small Diatoms, Small Flagellates, pyrrophyta, Chlorophyta, Chrysophyta, Xanthophyta
Picoplankton	0.2-2µm	Small eukarytotic protists; bacteria, Chrysophyta
Femtoplankton	<0.2µm	Marine viruses

However, some of these terms may be used with very different boundaries, especially on the larger end of the scale.

Collection of samples

Zooplankton

The standard plankton net (fig. 13a) is the most commonly used equipment for collection of plankton (IOE- Standard net has 1 m^2 mouth and mesh size of 300µm). It comprises essentially of a conical bag of bolting silk (or similar material) mounted on a ring or hoop, to which are attached three thin rope bridles spliced to a small ring, by means of which the net can be shackled to a towing rope or warp . A weight is attached to the warp to facilitate sinking of the net to the required depth and to keep horizontal positioning of the mouth. Horizontal or oblique hauling is commonly employed though, vertical hauling can also be made as required, with suitable change in the attachment of weight (fig.13b).

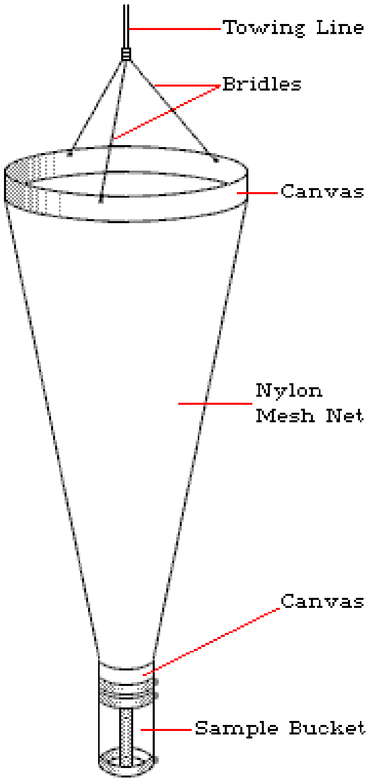


Fig 13a. Standard Plankton net

For qualitative sampling, the standard net is dragged at 1- 1.5 knots for 10 min. For quantitative assessment, it is important know the volume of water filtered through the net for estimating the quantity of plankton in unit volume. For this a calibrated flow meter has to be attached at the mouth, a little behind the front of the net, positioned exactly at the middle (fig 13c). If one digit reading of the flow meter is equal to d digit of the water flow, then the volume of water filtered is arrived as follows:

$$\text{Volume } (m^3) \text{ of water filtered, } V = \pi r^2 d \times f$$

Where, r is the radius in meters of the net opening and *f* is the flow meter reading (initial reading minus final reading).

Fig. 13b Zooplankton collection

Fig. 13c Flow meter

Preservation of sample

Examination of live plankton has many advantages, but the facilities available may not permit long storage of live specimen. Therefore the sample must be fixed using formaldehyde; sufficient to bring the concentration to about 4 % in the final preserved fluid (add 20 ml of 40 % formaldehyde to 200 ml seawater containing plankton). Use screw capped jars, preferably plastic, for storage of the preserved plankton with label carrying relevant information.

Measurement and counting

Larger organisms (macro plankton) which are usually present in small numbers can be identified and enumerated examining with the naked eye. Members of the micro plankton can be identified and enumerated only under a dissection microscope. As they are found in large numbers, the sample can be thoroughly mixed and small aliquot portion can be taken for examination and counting. Devices such as *Folsom Separator/Splitter* (fig.14) can be conveniently used for accurately making aliquots of sample and a *Bogorov Counting Tray* for accurately counting organisms. The enumerated organisms have to be recorded in systematic order. Individual items that are difficult to identify have to be kept in separate specimen tubes, properly labeled for future identification by experts. Organisms of special interest also can be separated for measurement, identification and enumeration. While feeding the data on a spreadsheet, enter the sample/ stations along the rows and parameters including the number of different species along the columns. This would help easy statistical analysis of the data.

The volume of plankton can be determined by the displacement method. Extremely large specimens such as jelly fish have to be separated before taking measurements in order to avoid unusual values. First the total volume (ml) of the concentrated plankton plus preserving fluid (v_1) is measured. The final representation of the plankton volume will be as:

$$\text{Plankton volume (ml/ m}^{-3}\text{)} = \frac{w1 - w2}{V}$$

Where, V is the volume (m^3) of water filtered through the net arrived at from the formula given earlier.

For estimating the dry weight biomass, the filtered plankton has to be dried at 50°C in a desiccator inside an oven and then weighed rapidly. Alternatively divide the fresh sample, before addition of formalin into two equal portions and preserve one portion for subsequent sorting and counting. The other portion has to be washed, filtered, dried and weighed.

Phytoplankton

Net sampling for phytoplankton has many draw backs. The nanno plankton, which consists predominantly of small flagellates, can only be obtained quantitatively from bottle samples. Though the quantity of sample has to be decided depending upon the density of plankton, concentration methods such as sedimentation technique (Utermöhl technique) can be conveniently followed for samples from coastal waters.

To one litre of seawater sample, add 3 ml of Lugol's Iodine and allow it to stand for 24 hours in a measuring cylinder. After all the particulate matter had settled down to the bottom, siphon off the supernatant water taking care that the settled sediment is left undisturbed. The one litre sample may thus be reduced to about 60 ml. Use only clear glass bottles to store this reduced samples (plastic bottles will take up iodine from the solution).

Fig. 14. Folsom splitter

Counting with Rafter Cell

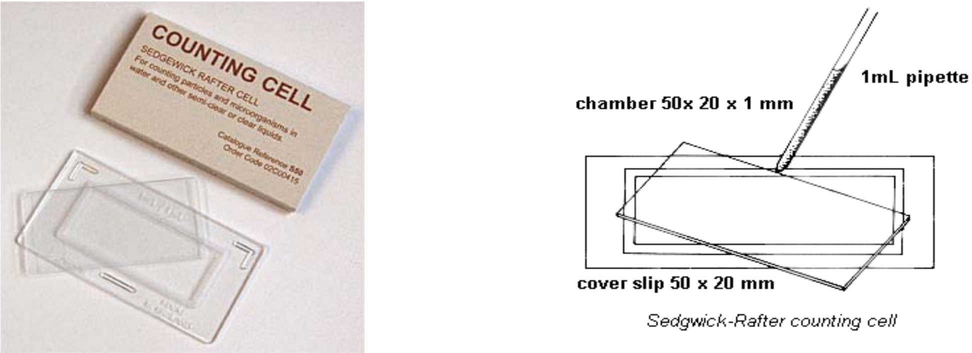


Fig 14a. Sedgwick- Rafter counting cell

Shake the settled sample thoroughly and count 1 ml aliquot of the sample using a Sedgwick- Rafter counting chamber (fig.14a) under binocular microscope (fig 18b) Qualitative and quantitative enumeration has to be made by counting replicate aliquots and the average taken for estimating the cell count of the algae in one litre. In the case of chain forming species, the number of chains has to be counted. The cell count of different species (n_i) per litre can be arrived at by the following formula:

Number of cells per litre of ith species, $n_i = \bar{x}_i \times \frac{v}{V}$

Where, $\bar{x}_i$ is the average count of ith species, ‘***V***’ the volume (l) of sample and ‘*v*’ the volume (ml) to which the sample was reduced

The total plankton cell count (*N*) per litre can be arrived at by

$$N = \sum_{i=1}^{S} n_i$$

Counting with Haemocytometer

Alternatively, when the plankton cells are numerous and less than 30 µ in size, the cell counting can be done using a haemocytometer (fig. 15). The haemocytometer with an improved Neubauer ruling has to be rinsed clean and dried. The face of the counting chamber is composed of two surfaces with grids, separated by canals. The cover slip has to be placed on the support bars along the canals. A drop of homogeneously mixed algal suspension (of the 60 ml reduced sample detailed above) is delivered from a Pasteur pipette by touching the pipette tip to edge of the cover slip, where it hangs over the V-shaped loading port. Both the chambers must be loaded to seat the cover slip properly. If the algal suspension overflows either side, the chamber must be cleaned and refilled.

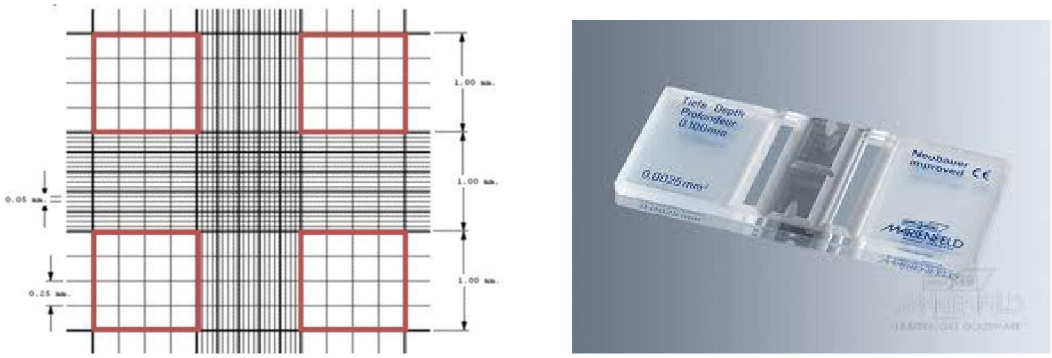


Fig. 15. Haemocytometer

Each half of the haemocytometer surface contains nine large grids. Only those algal cells, which fall within the four large corner grids (numbered 1 to 4) are to be counted. Each larger corner grid is further divided into 16 small quarters. Moving systematically back and forth across the squares, a minimum of 200 algal cells may be counted in as many grids as necessary. Cells falling on the border are counted if at least half the cell is within the square, taking necessary caution not to count same cell twice. The number of algal cells per ml can be arrived at by dividing the number of cells counted by the larger corner grid area covered for counting, multiplied by 10,000. For example, if one and a half large corner grids (or 24 small squares) were covered to count 300 cells, the cell density is equal to 2.0×10^6. In general the ultimate cell count per litre can be worked out by the formula:

$$\text{Number of cells per litre} = \frac{n}{g} \times 10000 \times \frac{v}{V}$$

Where, n is the number of algal cells counted, g the number of corner grids covered, ‘V’ the volume of sample and ‘v’ the volume to which the sample is reduced after settlement.

6. Benthos (Macrobenthos)

The bottom fauna or benthos forms an important link in the food web of the sea. Bottom dwelling fishes and crustaceans feed mainly on the benthic organisms and hence the abundance of benthic fauna is a major factor determining the demersal fishery potential of a given region of the sea. In recent years, long- term work on marine benthic communities has been gaining importance in pollution studies and assessment of ecosystem health. Therefore, study of the qualitative and quantitative aspects of benthos is very important in oceanography and fisheries research.

The macrofauna and microfauna (meiofauna or interstitialfauna) are mainly distinguished by their relative size. It is generally accepted that those animals retained in 0.5- 2.0 mm mesh sieve (fig. 16) are macrofauna and those which pass through this sieves but are retained by a sieve of about 60µm mesh are termed meiofauna.

Fig 16. Sieves of different mesh size

Sampling

A Petersen grab or Van Veen grab can be conveniently used for collecting benthos samples from near shore waters. The area of the grab, which is the denominator while working out the density and biomass per square meter, has to be noted for the instrument. While taking the grab sample onboard, a check on the quantity of the sediment must be made because improper performance of grab may result in reduced quantity of sediment and erroneous values of population and biomass. The sediment temperature has to be noted immediately after the grab is brought down to the check. A sub-sample of the sediment has to be taken if sediment characteristics have to be analyzed.

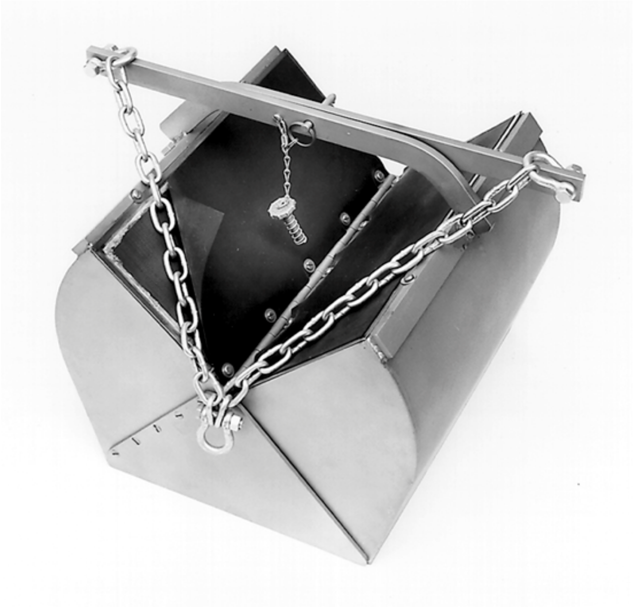


Fig.17. Van Veen Grab

Fig 17a. Petersen grab

Processing sample onboard

Transfer the sediment sample without spilling into a tub or bucket. Wash the sediment through a sieve with a copious flow of water, taking care not to spill over or clog the mesh. A series of sieves can be used if the size composition of the fauna is of interest. Three sieves of mesh sizes 2000µm (BBM-8), 1000µm (BSS-16) and 500 µm (BSS- 30) in that order will give an idea about the size structure of the fauna. If information on size is not important, a single sieve of 500 µm can be used for segregating the macro fauna.

The benthic organisms retained in each sieve have to be collected in separate bottles and preserved with 4 % formalin onboard (collect the sample in approximately 100 ml sea water and add 10 ml of 40% formalin). A few drops of Rose Bengal stain (1:500) added to this sample will facilitate sorting, as the organisms will take on a deep purple colour. Use of a soft brush for transferring the organisms is desirable.

Processing in the laboratory

Larger animals can be picked up, enumerated and weighed separately before the sorting and enumeration of smaller ones. The smaller animals have to be sorted and enumerated under a dissection microscope using fine brushes, needle and forceps. Care has to be taken not to exert pressure on any delicate worms resulting in breakage and counting of the two pieces as separate animals. The displacement volume and wet weight (after properly blotting the moisture with filter paper) of animals sorted out can be taken before identification and enumeration.

Before estimation of dry weight, shells and other hard material should be removed. The organisms have to be dried at about 105˚C and weighed without much delay. Being a destructive process, it is not always practicable to estimate the dry weight of all samples. A convenient method would be to work a formula for conversion of wet weight (or volume) into dry weight. This can be achieved by taking dry weight of a few numbers of samples whose wet weight (volume) are known, and by working out a conversion factor based on the relationship.

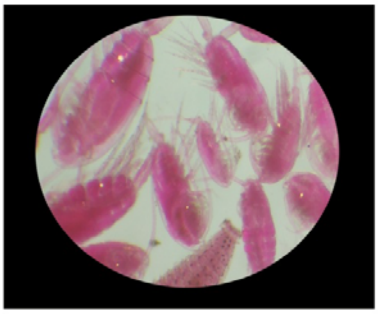
The sorted animals have to be preserved in alcohol in glass specimen tubes. Plastic tubes may crack or leak and cause drying up of specimens. Formalin should never be used for preserving or fixing sponges and ctenophores, as rapid disintegration or total destruction of the animals may occur. Though formalin is a better fixative than alcohol in microscopy, other fixatives/preservatives such as FAA or Bouin’s fluid are preferable. A more convenient preservative is alcohol- glycerin, in which glycerine will prevent total desiccation as well as act as a clearing agent.

Fig 18a. Dissection microscope

b. Binocular microscope

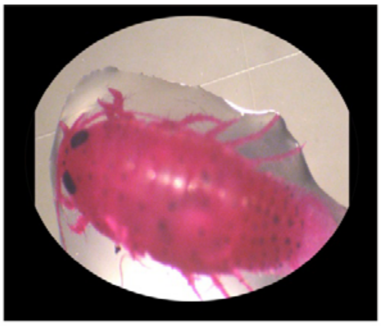
Appendix I

Photograph of common phytoplankton, zooplankton and benthos (genus/groups) observed off Mangalore.



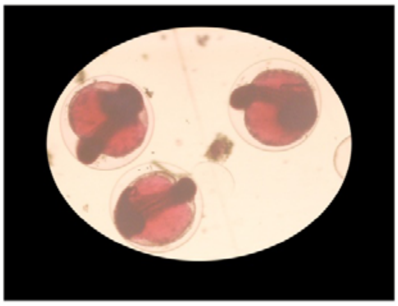
Copepods

Amphipods

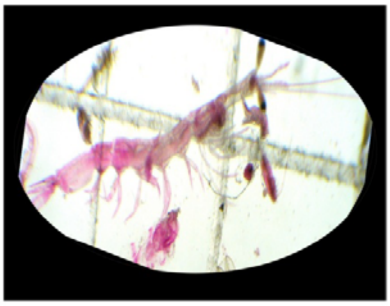


Isopod

Fish larvae

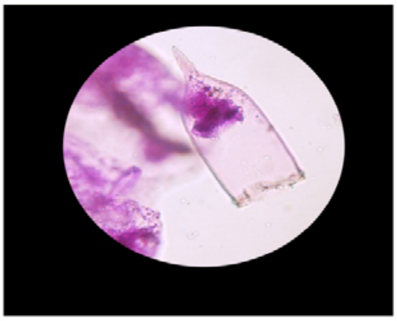


Fish eggs



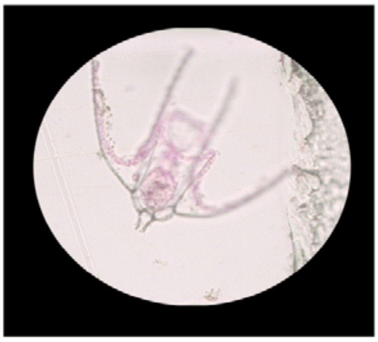
Lucifer spp.

Oikopleura spp.

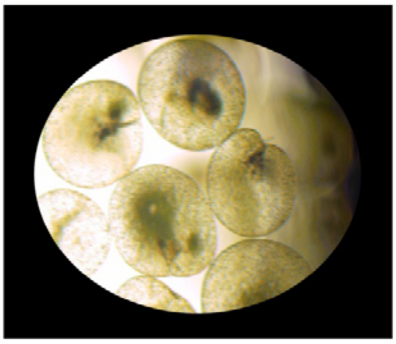


Tintinnid spp.

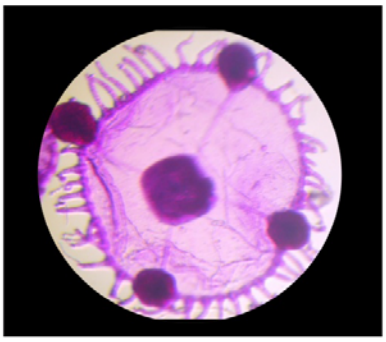
Polychaete larvae



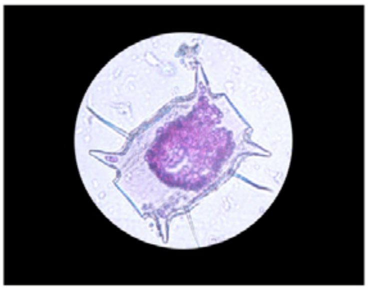
Echinoderm larvae

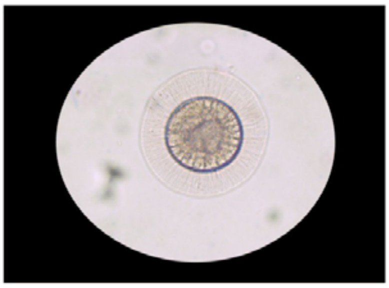


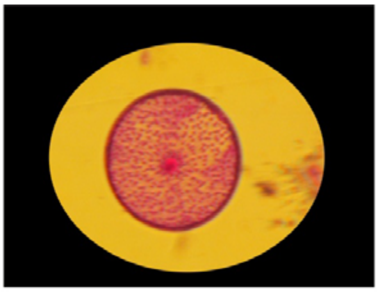
Noctiluca spp.

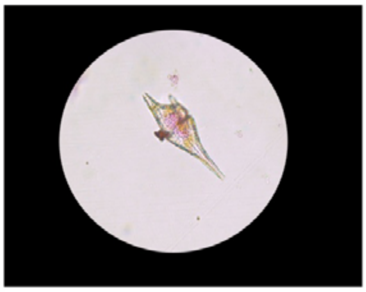


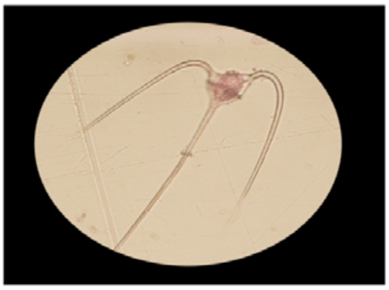
Medusae

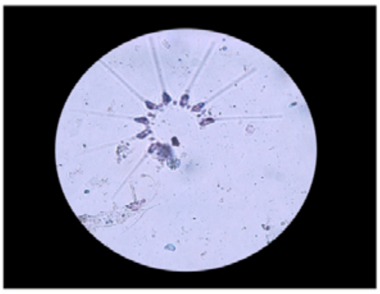

Biddulphia spp.


Planktoniella sol

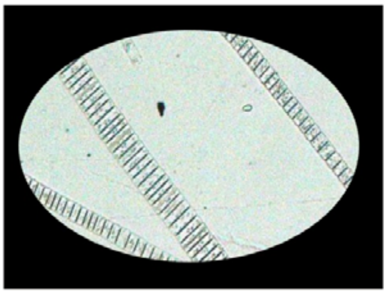

Coscinodiscus spp.

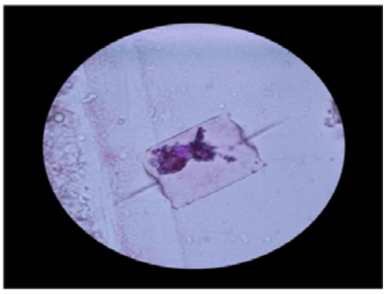

Ceratium spp.

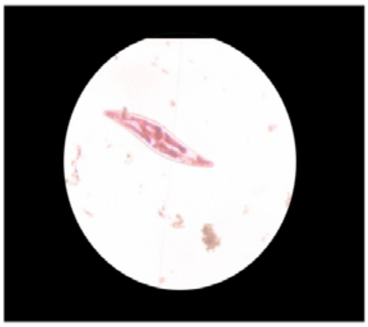

Ceratium spp.

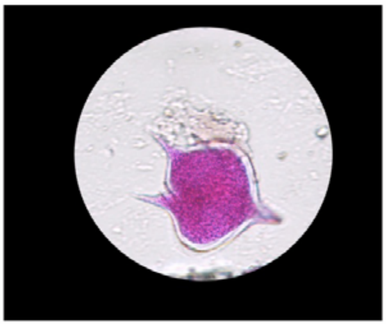

Asterionella spp.

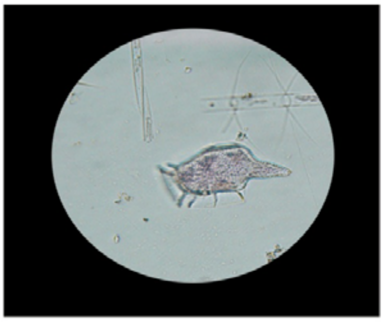

Chaetoceros spp.


Fragillaria spp.

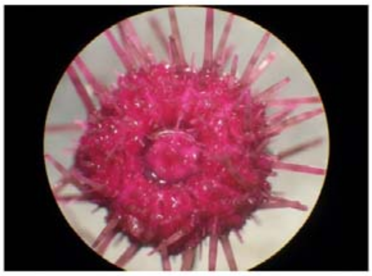

Ditylum spp.

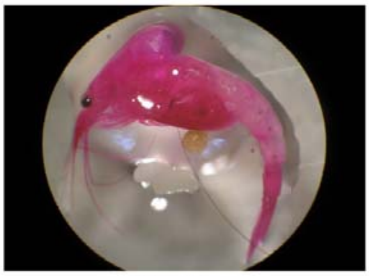

Pleurosigma spp.


Protoperidinium spp.


Dinophysis spp.

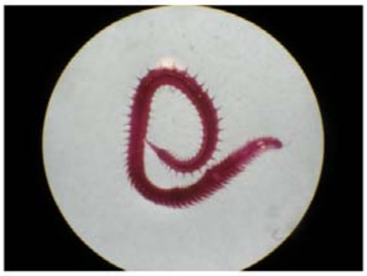

Cumacea

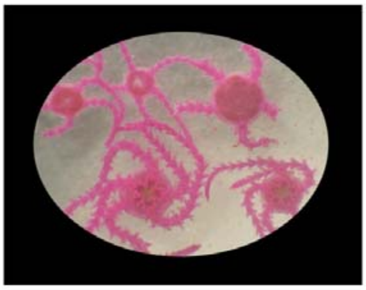

Sea urchin


Mysidiacea

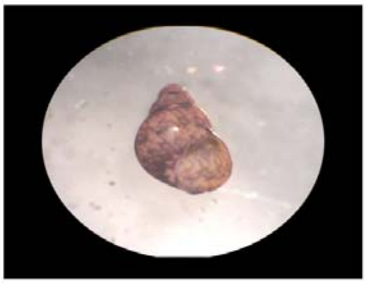

Decapod larvae

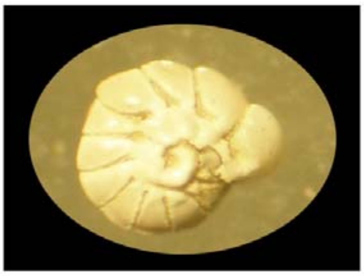

Brachyura (crabs)

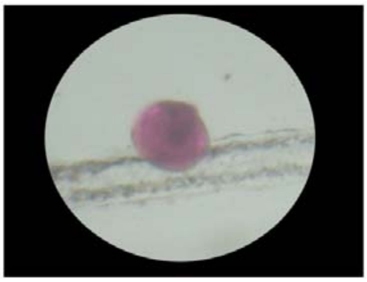

Polychaetes


Brittle star


Star fish


Gastropod


Foraminifera

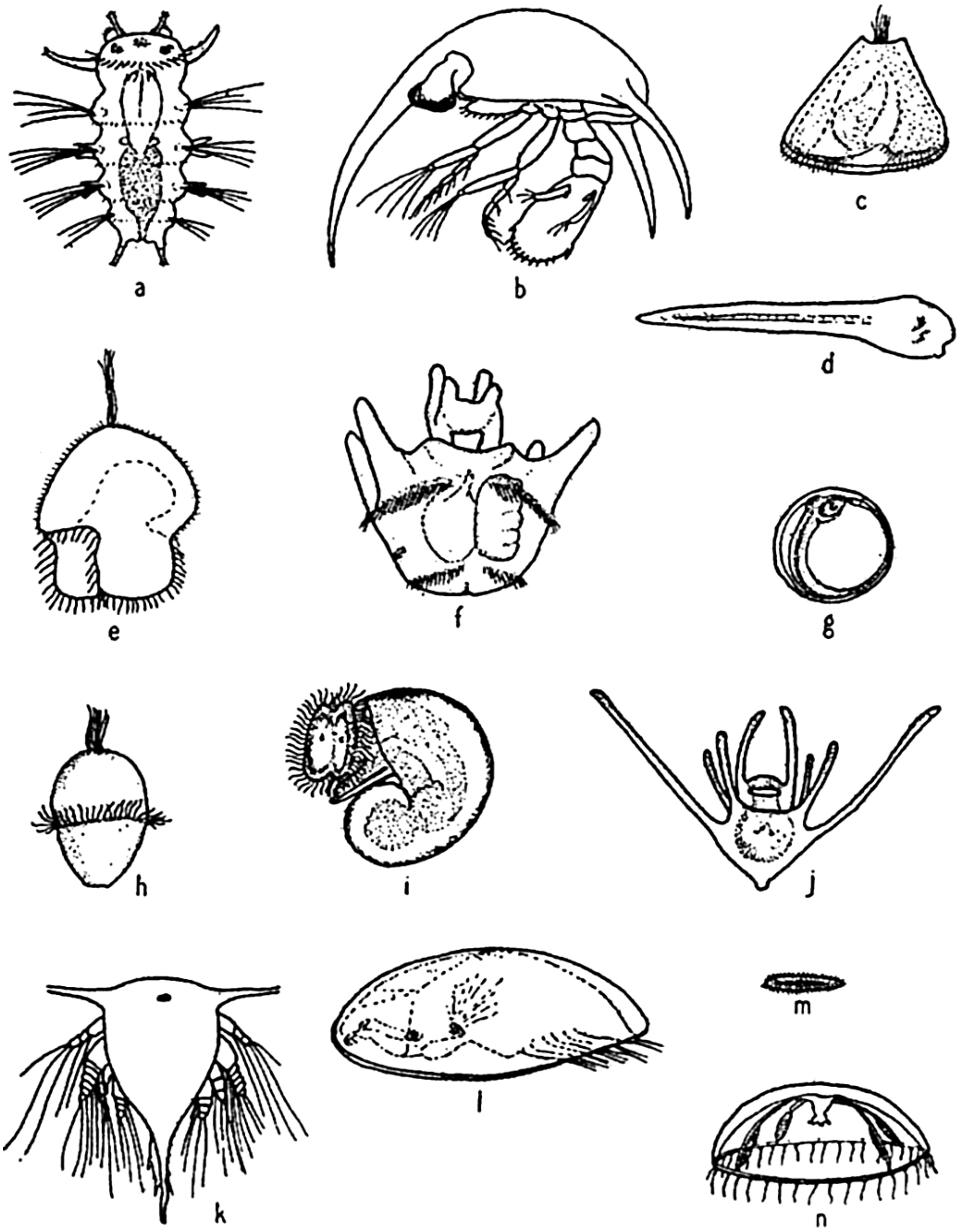

Bivalves


Hydrozoan

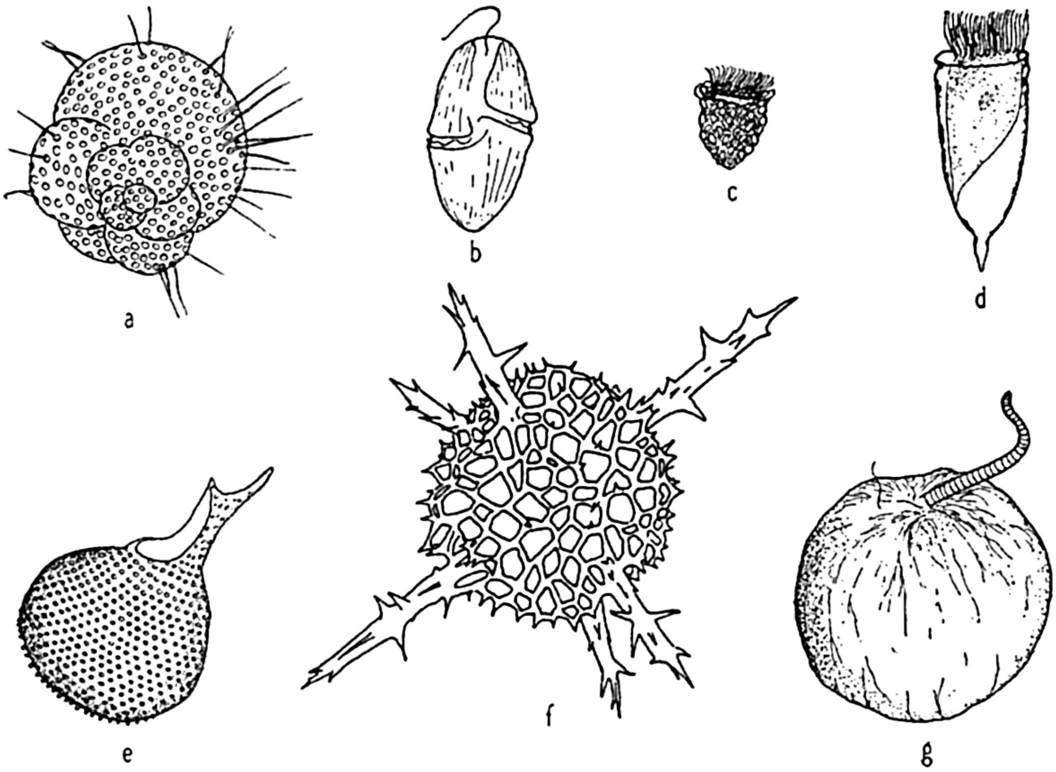
Appendix II

Line diagrams of common phytoplankton, zooplankton and benthos observed off Mangalore

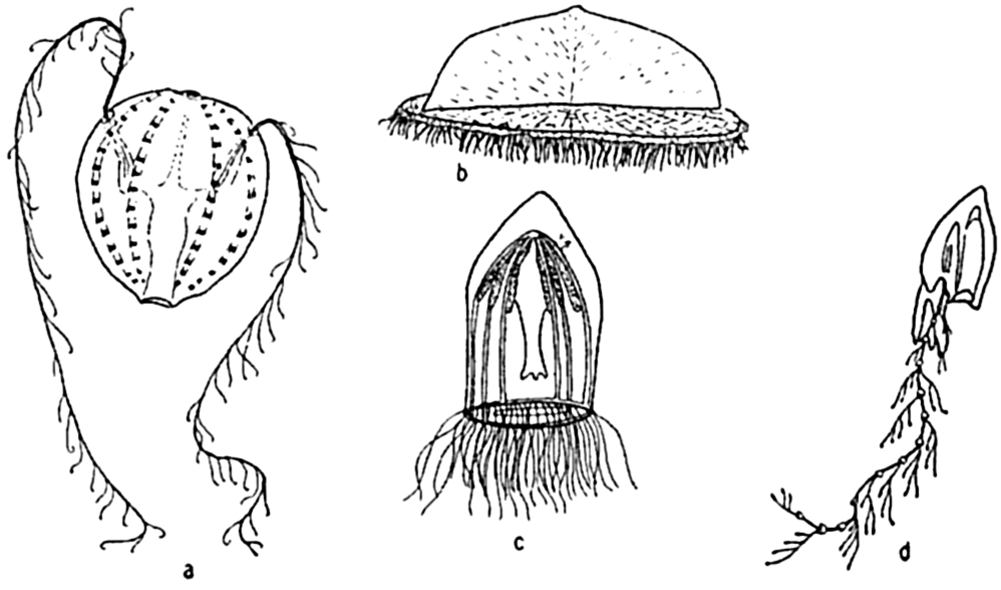
Line drawings of common zooplankton



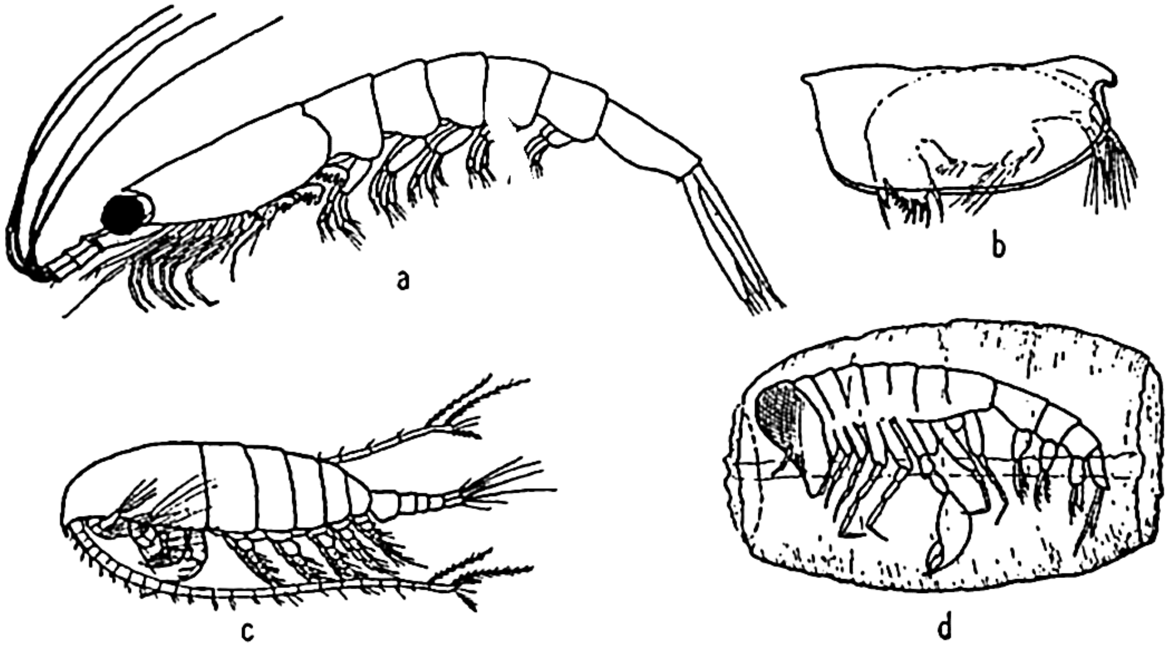
Characteristic larvae of the meroplankton: (a) polychaetate larva of the annelid *Platynereis agassizi* (b) zoea of sand crab, *Emerita analoga* (c) cyphonautes larva of bryozoa (d) tadpole larva of sessile tunicate (e) pilidium larva of nemertean worm (f) advanced pluteus larva of sea urchin (g) fish egg with embryo (h) trochophore larva of scaleworm (i) veliger larva of snail (j) pluteus larva of brittle star (k) nauplius larva of barnacle (l) cypris larva of barnacle (m) planula larva of coelenterate (n) medusa of hydroid**.**



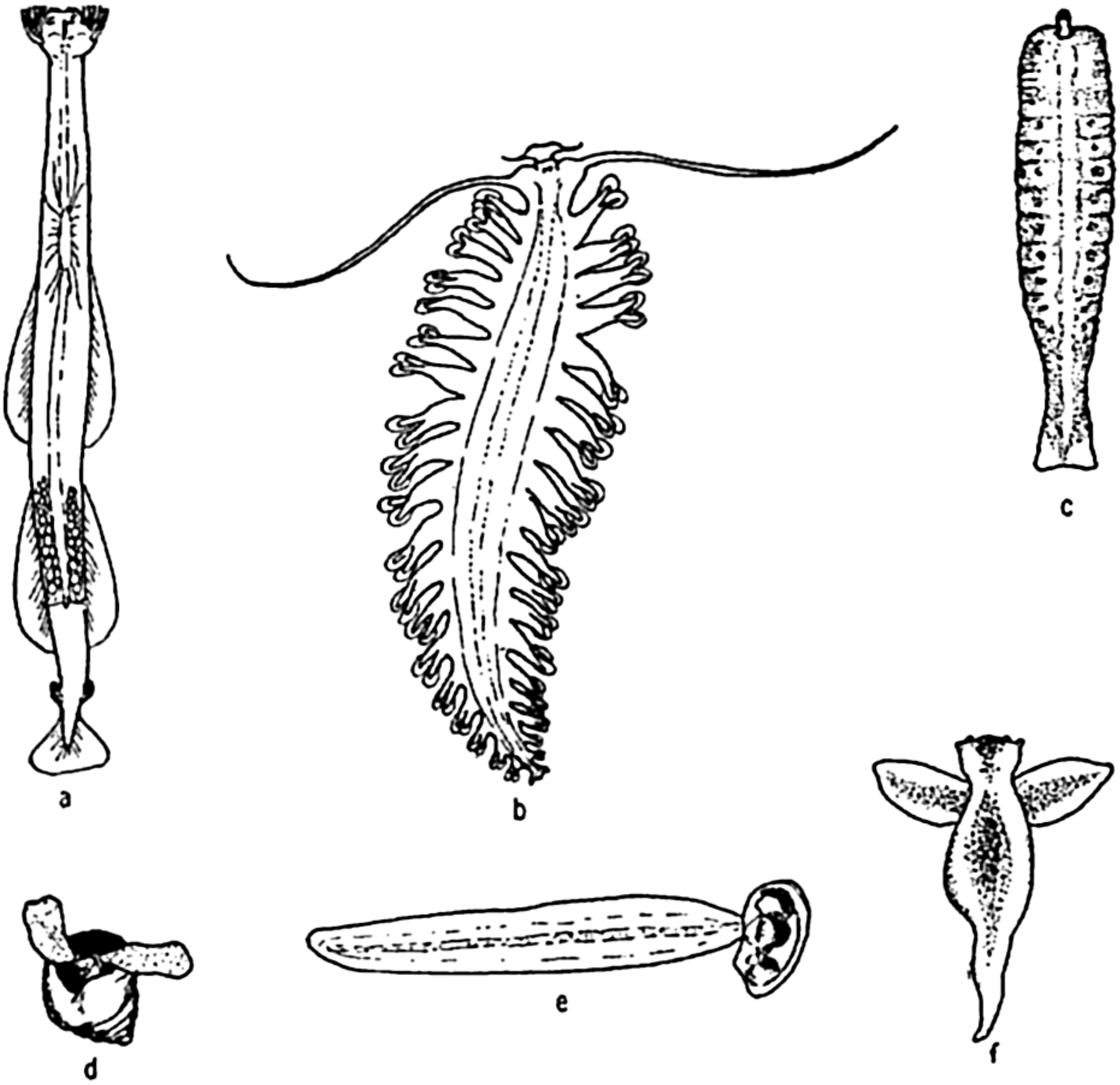
Characteristic holoplankton protozoa (a) foraminifera (*Globigerina)* (b) dinoflagellate (*Gymnodinium*) (c) tintinnid (*Stenosomella)* (d) tintinnid (*Favella)* (e) radiolarian (*Protocystis*) (f) radiolarian (g) dinoflagellate (*Noctiluca*).



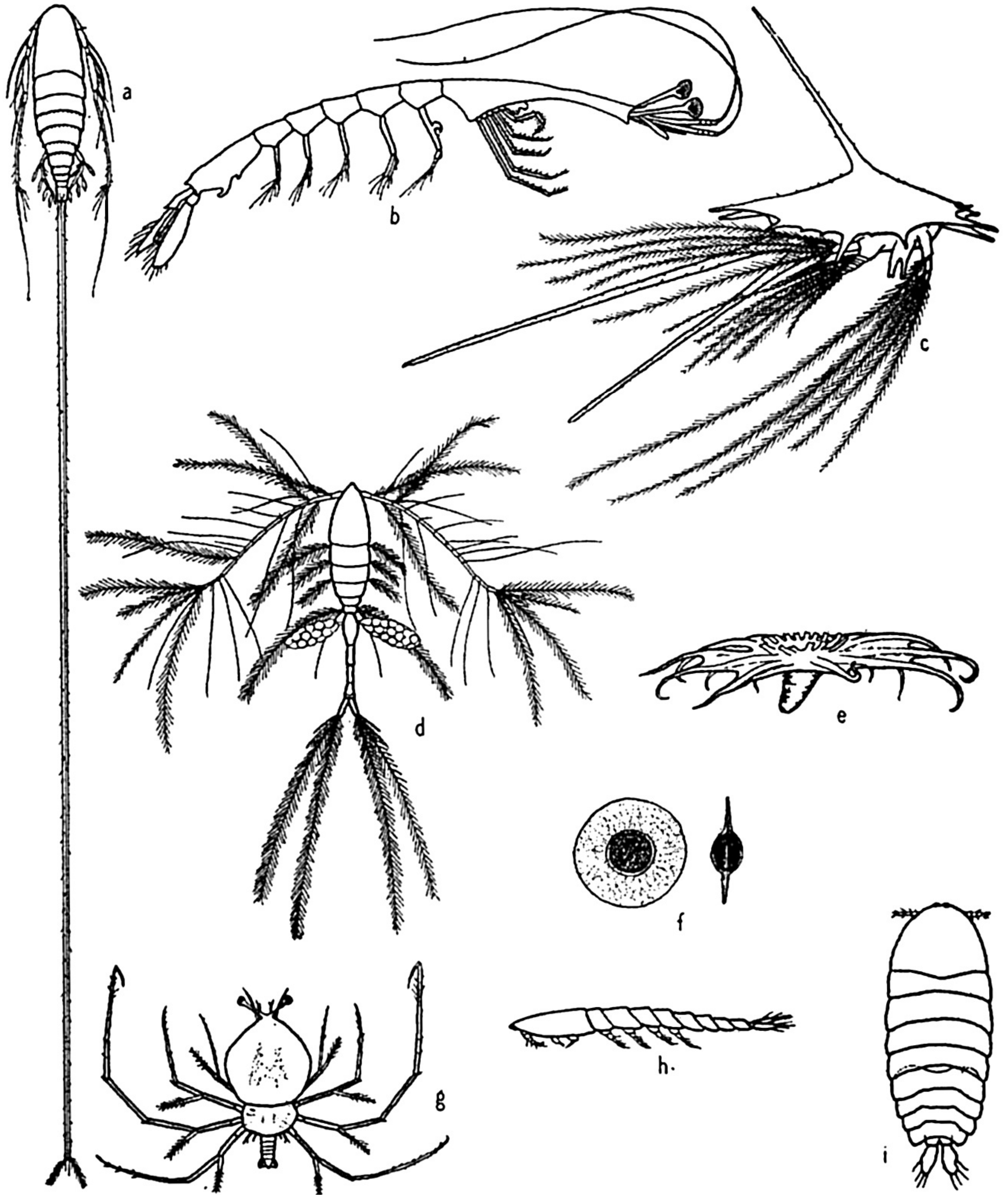
Characteristic holoplankton coelenterates and ctenophores (a) comb-jelly (*Pleurobrachia)* (b) siphonophore (*Velella*) (c) jellyfish (*Aglantha*) (d) siphonophore (*Diphyes*).



Characteristic holoplankton crustaceans: (a) euphausiid (*Euphausia)* (b) ostracod (*Conchoecia*) (c) copepod (*Calanus)* (d) amphipod (*Phronemia*) in empty mantle of the pelagic tunicate *Salpa*.

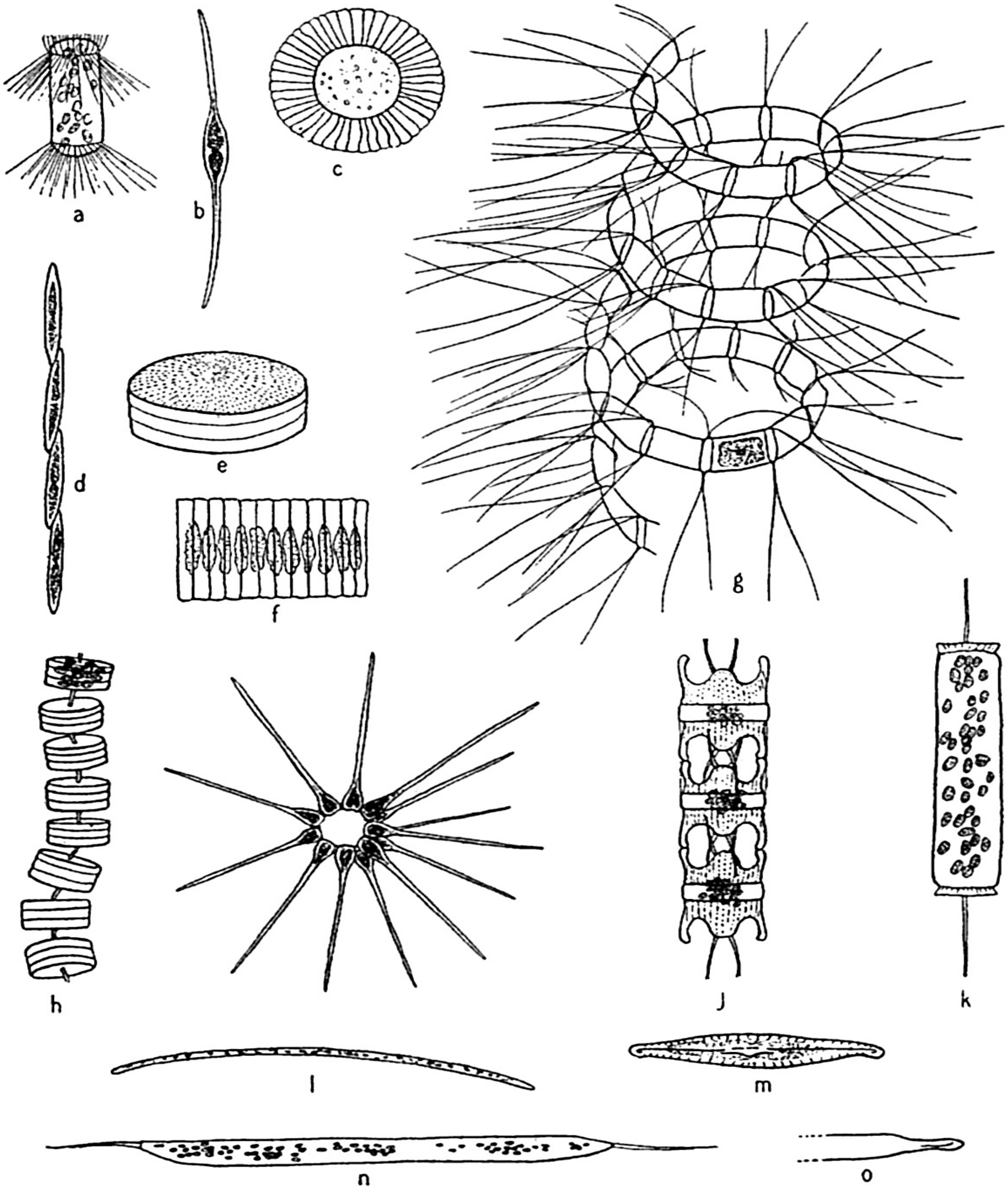


Characteristic holoplankton, miscellaneous: (a) arrow worm (*Sagitta*) (b) annelid (*Tomopteris*) (c) nemertean (*Nectonemertes*) (*d*) pteropod mollusc (*Limacina*) (*e*) tunicate (*Oikopleura*) (*f*) pteropod mollusc (*Clione*).

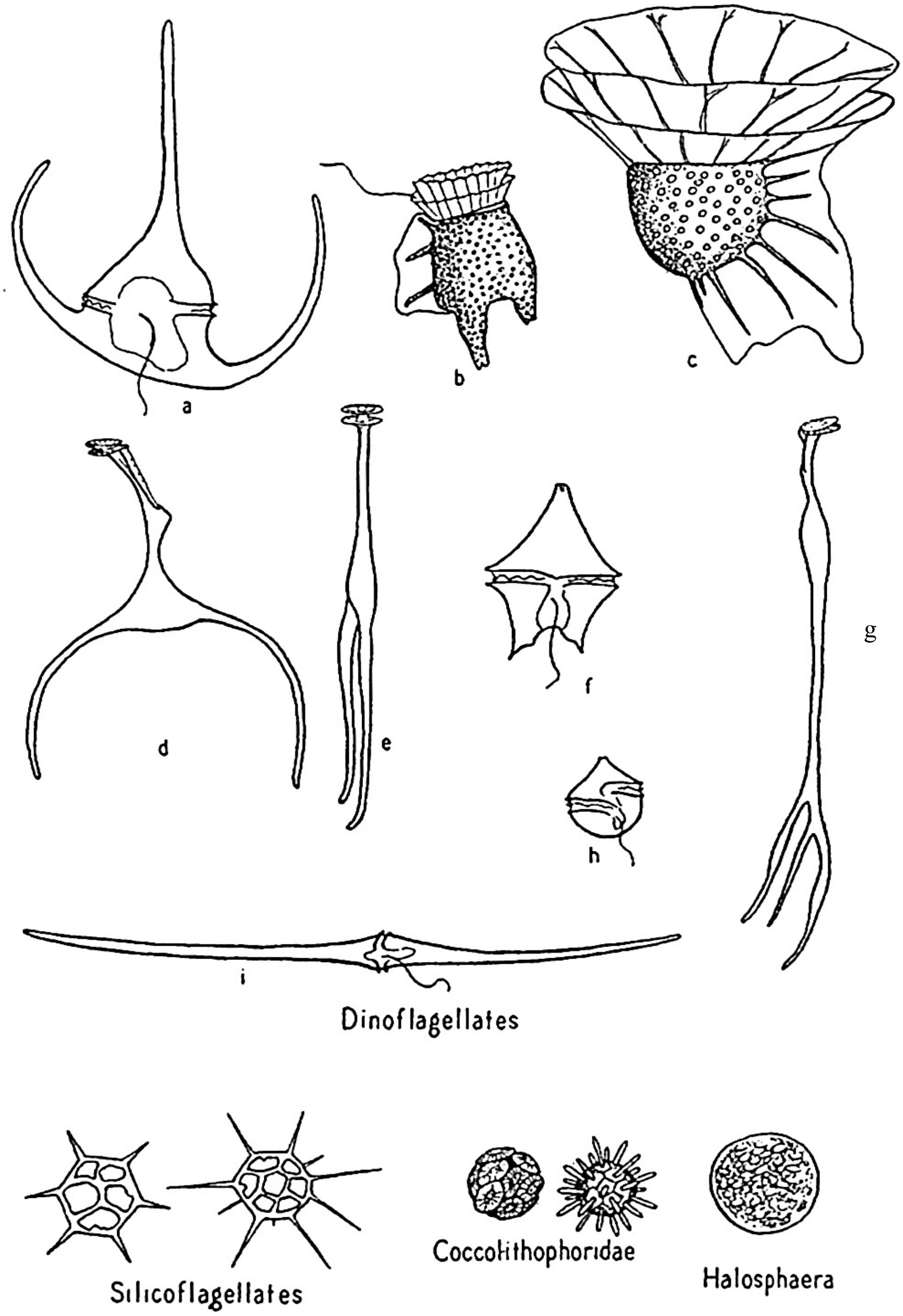


(a) copepod (*Aegisthus*) (b) decapod (*Lucifer)* (c) barnacle nauplius (d) copepod (*Oithona)* (*e*) holothurian (*Pelagiothuria*) *(f*) pelagic egg of copepod (*Tortanus)* (*g*) phyllosome larva of lobster (*h, i*) copepod (*Sapphirina*), side and dorsal views.

Line drawings of common phytoplankton



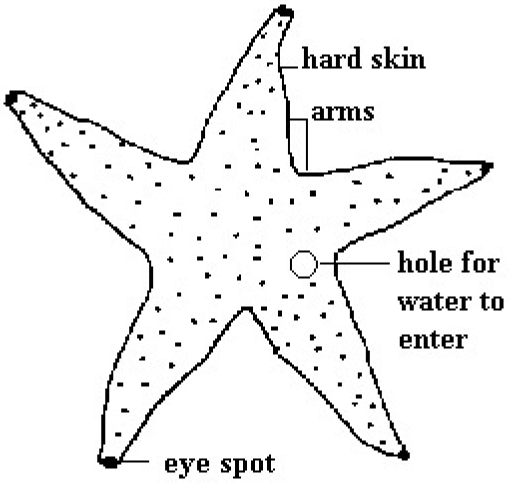
Characteristic types of diatoms. (a) Corethron (b) *Nitzschia clostrium* (c) *Planktoniella* (e) *Coscinodiscus* (f) *Fragilaria* (g) *Chaetoceros* (h)*Thalassiosira* (i) *Asterionella* (j) *Biddulphia* (k) *Ditylum* (l) *Thalassiothrix* (m) *Navicula* (n,o) *Rhizosolenia semispina*, summer and winter forms**.**



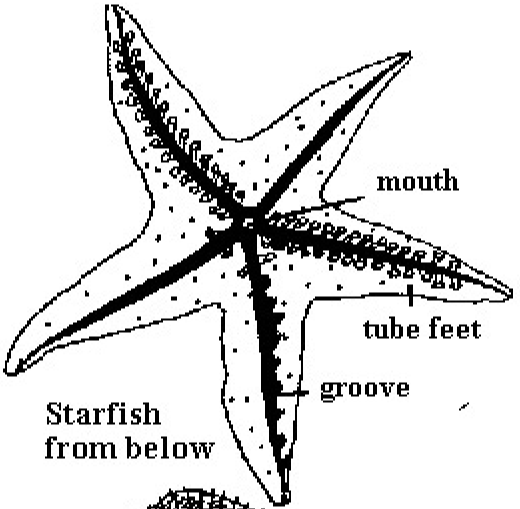
Dinoflagellates and other phytoplankton organisms. (a) *Ceratium tripos* (b) *Dinophysis* (c) *Ornithocercus* (d &e)*Triposolenia*, front and side view(f) *Peridinium* (g) *Amphisolenia* (h) *Goniaulax* (i) *Ceratium fusus*.

Line drawings of common benthic organisms

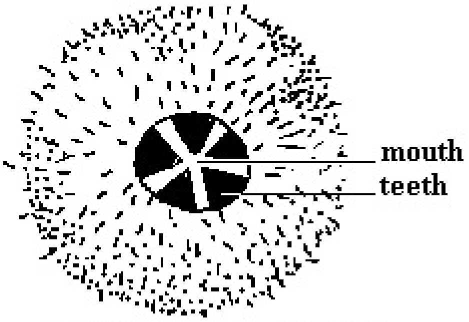
Common echinoderms



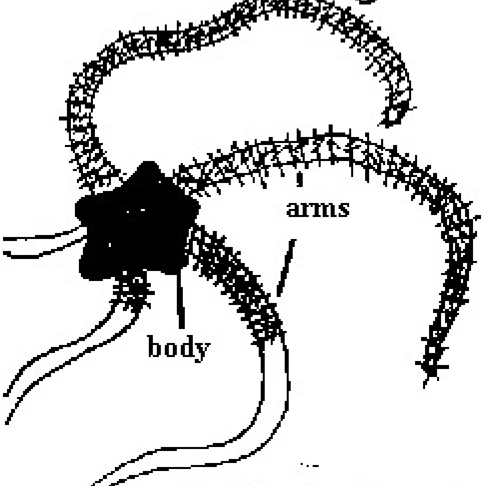
Starfish from above



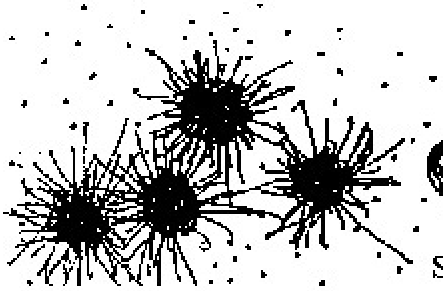
Starfish from below



Sea urchin from below

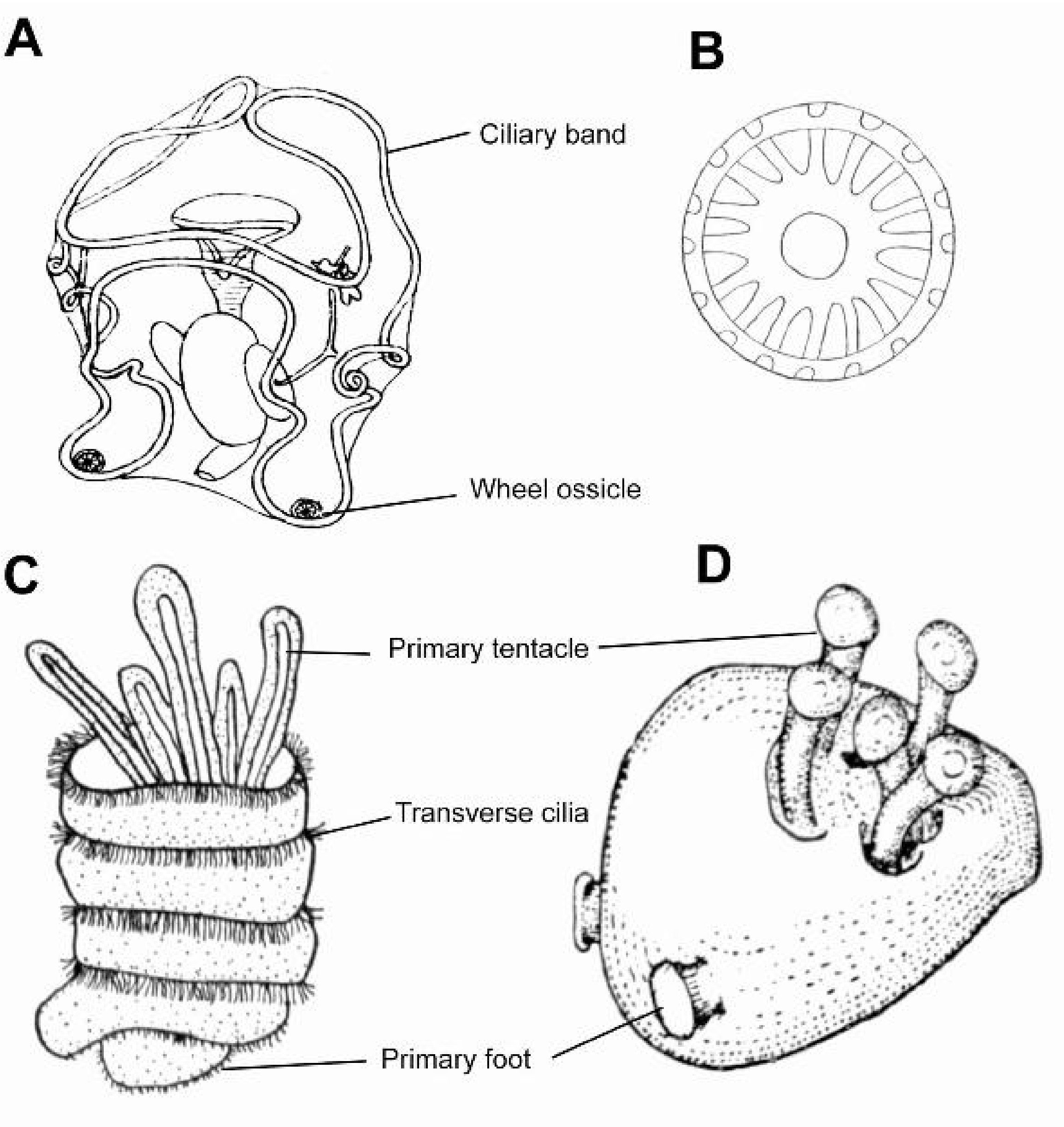


Brittle star from above



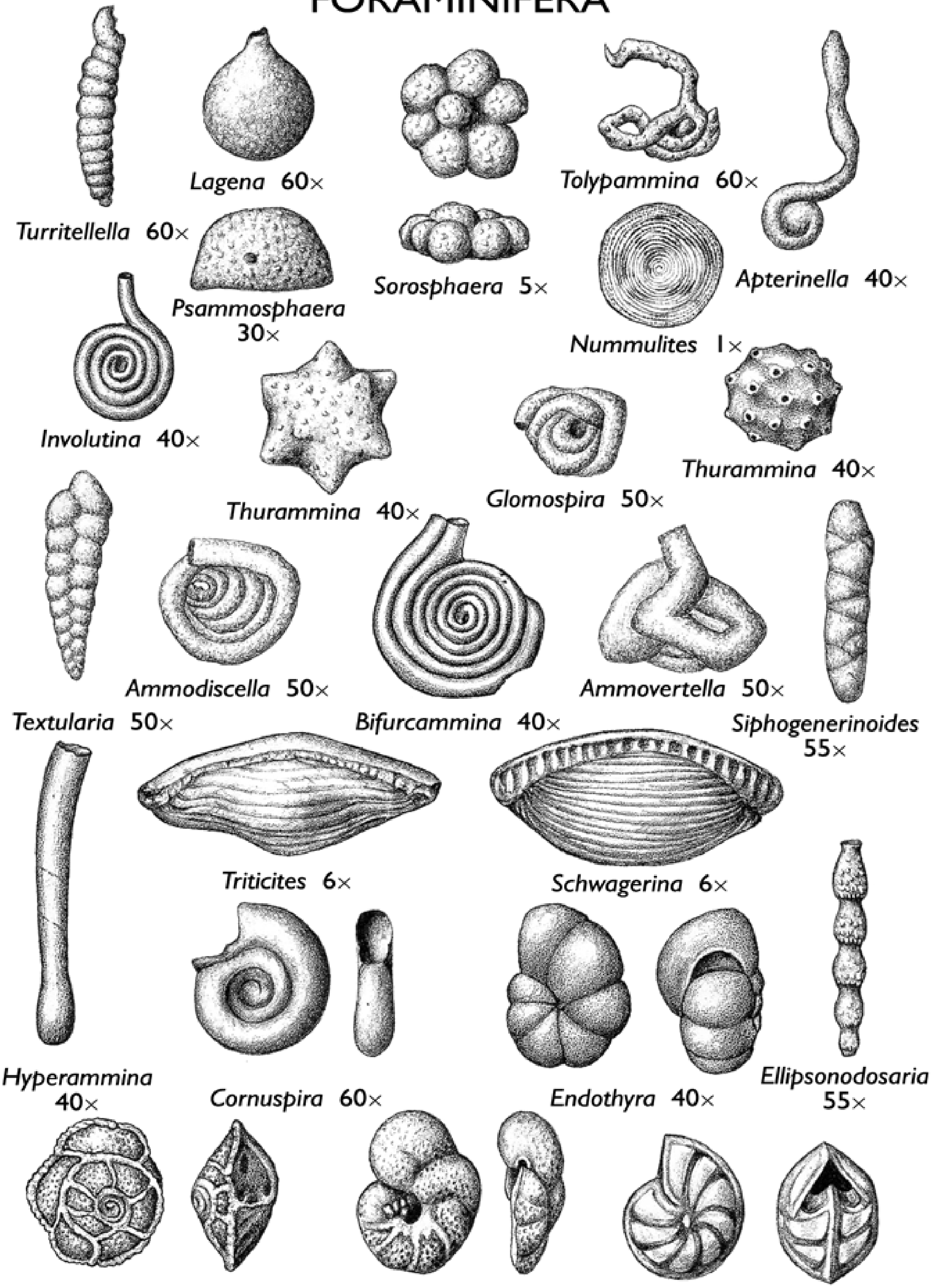
Sea urchins from above

Sea cucumber, trepang, bech-de-mer

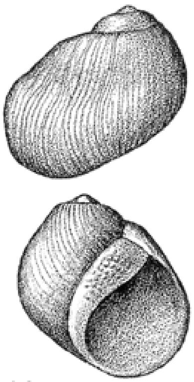


Holothurian auricularia and pentactula larvae. (A) Auricularia (B) Wheel ossicle (C) Early pentactula (D) Late pentactula.

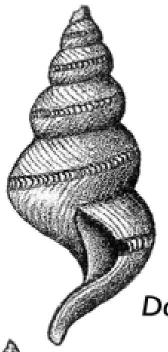
FORAMINIFERA



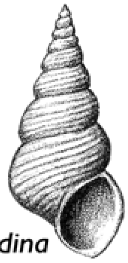
GASTROPODS



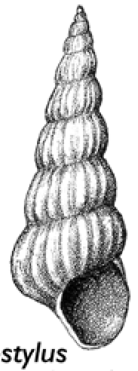
Naticopsis (Jedria) 1×



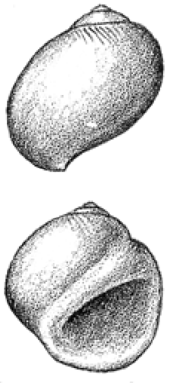
Hormotoma 1⅓×



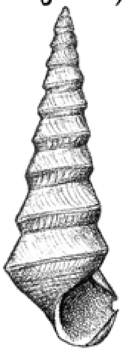
Donaldina 5×



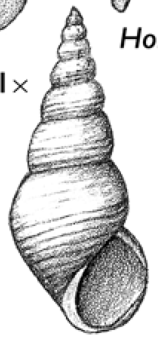
Palaeostylus (Pseudozygopleura) 1⅓×



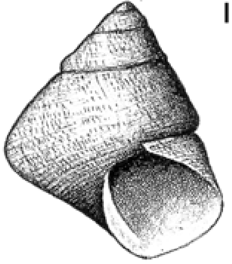
Naticopsis 1½×



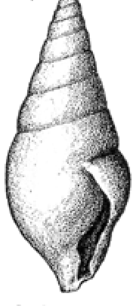
Goniasma 1⅓×



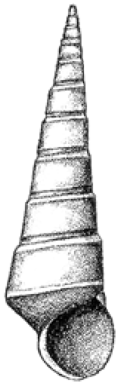
Aclisina 3×



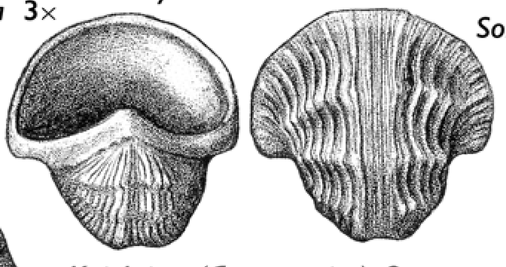
Cyclonema 1⅓×



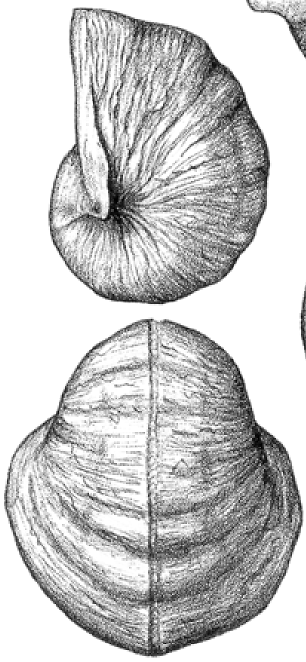
Soleniscus 1×



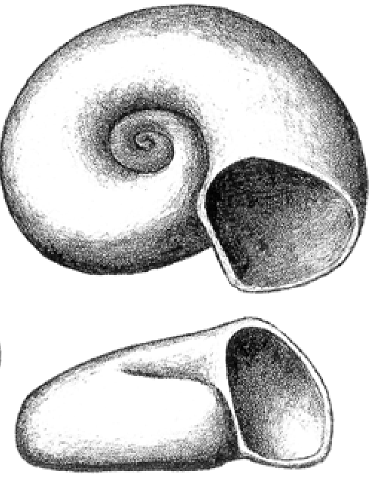
Orthonema 1⅓×



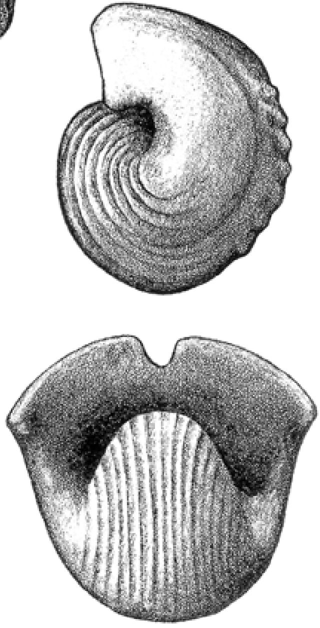
Knightites (Cymatospira) 2×



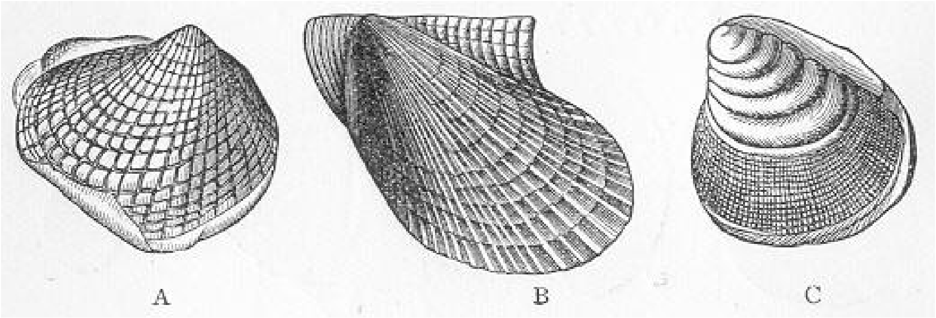
Bellerophon ⅔×



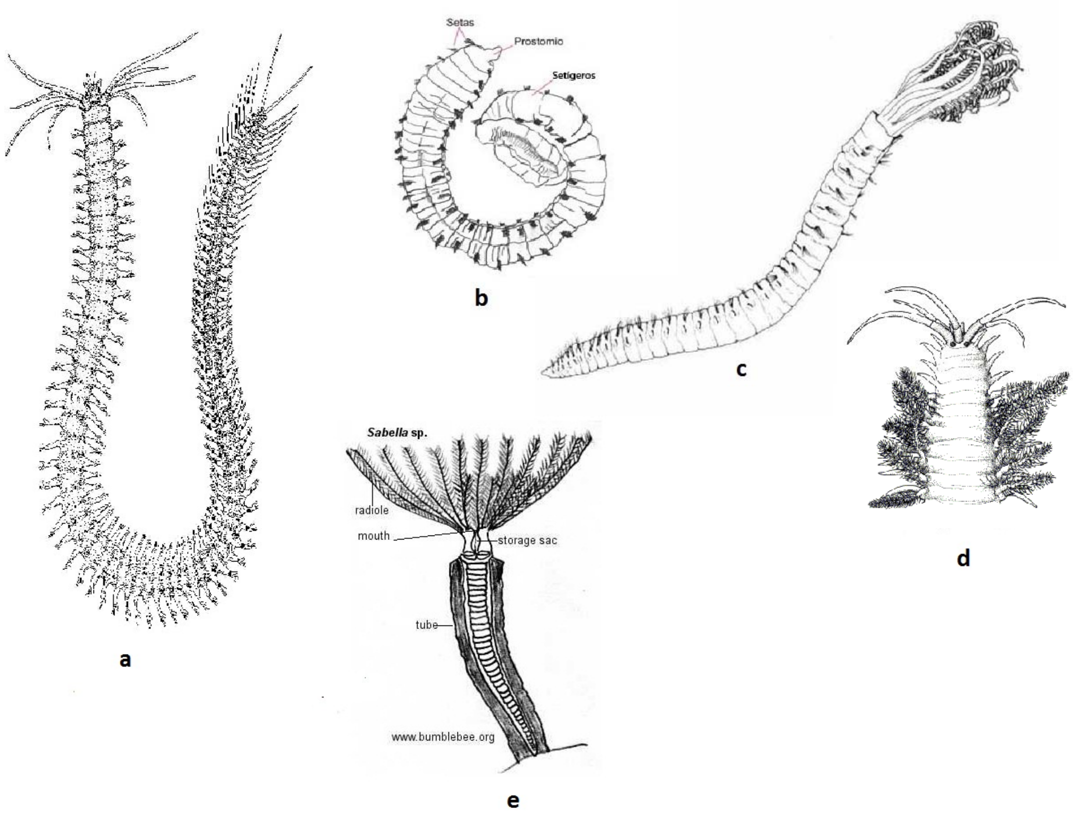
Maclurites ⅔×



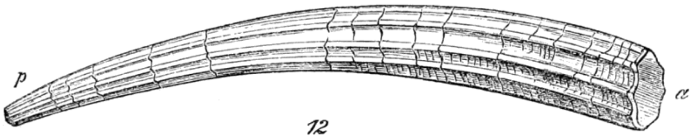
Nodocarinatus 1×



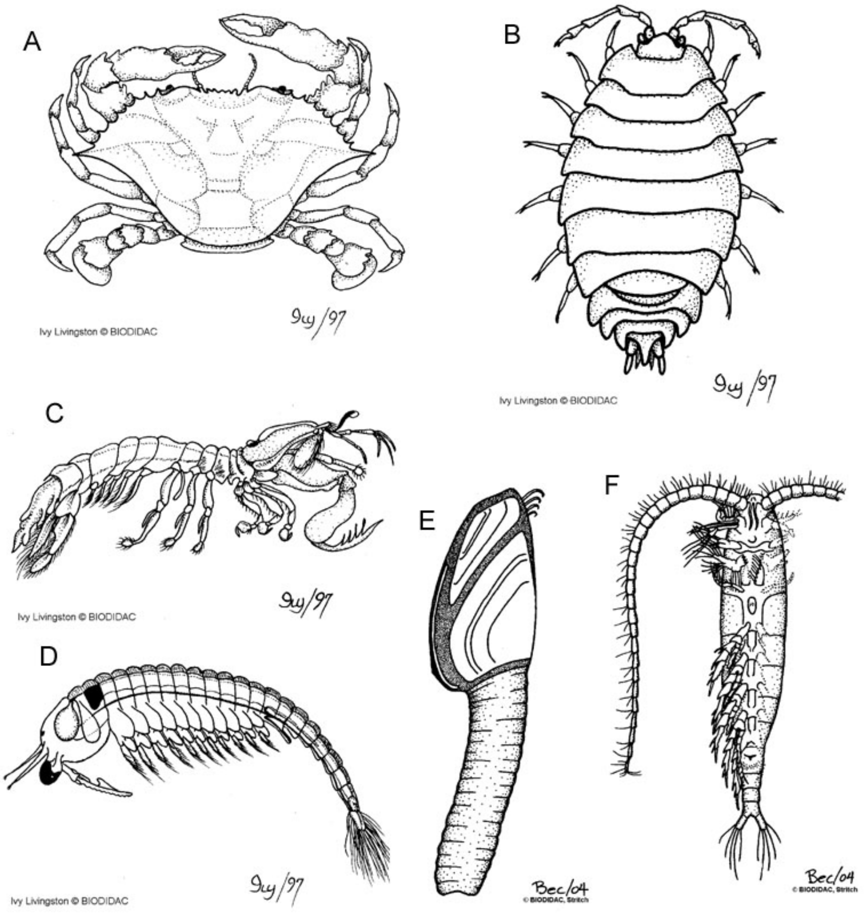
Upper Silurian Bivalves. *A, Cardiola interrupta; B, Pterinea subfalcata; C, Cardiola fibrosa,*



Commonly seen Polychaetes belonging to the families: a. Nereididae b. Capitellidae d. Onuphidae e. Sabelleridae



Dentalium elephantinum

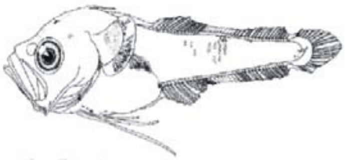


Examples of crustacean diversity. A. Brachyura (crabs) B. Isopoda (slaters and sea lice) C. Stomatopoda (mantis shrimps) D. Branchiopoda (brine shrimp, sea monkeys) E. Cirripedia (barnacles) F. Copepoda (copepods).

Appendix – III

Line diagram of common fish larvae

www.**sea**fdec.or.th/dmdocuments/TDTRB81_**EarlyStages**.pdf



c 6.5 mm SL (type 1)

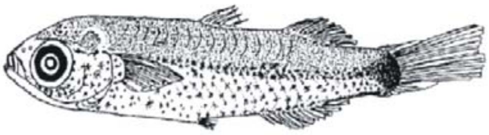
Bregmaceros spp.(Type 1)

f 8.5 mm SL (type 2)

Bregmaceros spp. (Type 2)

b 7.5 mm TL

Exocoetidae sp./spp.

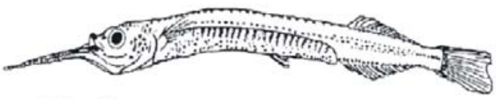


d 15.4 mm TL

Mugilidae sp./spp.

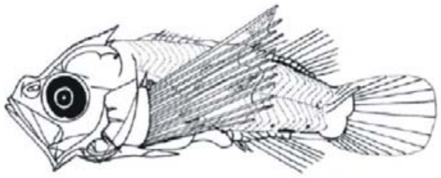
c 20.1 mm TL

Stolephorus sp.



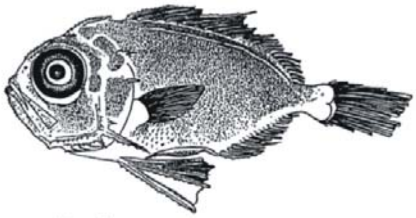
b 19.6 mm TL

Hemiramphidae sp./spp.



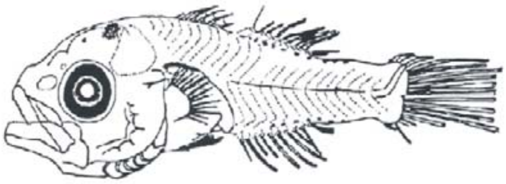
c 6.71 mm TL (*Minous* sp.)

Scorpaenidae spp.



c 14.4 mm TL

Priacanthus sp.



c 6.2 mm TL (Type 3)

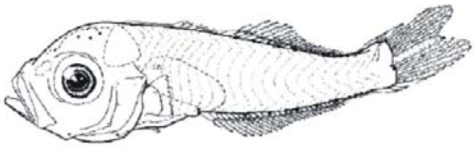
Apogonidae spp.

a 6.60 mm SL

Sillago sp.

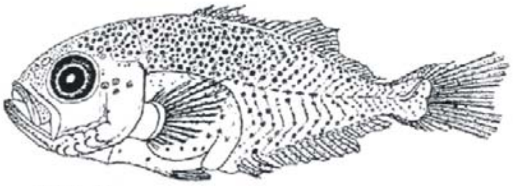
c 11.00 mm TL

Rachycentron canadum



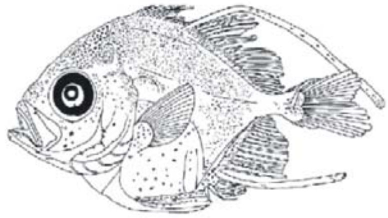
a 6.0 mm SL

Lactarius lactarius



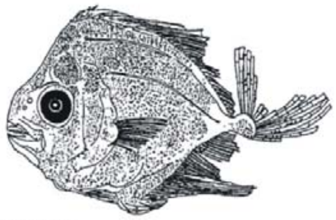
c 8.3 mm TL

Atule mate



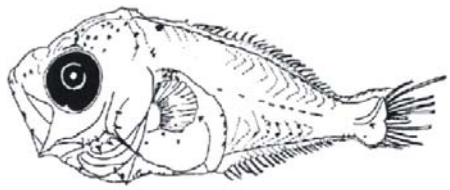
a 11.0 mm TL

Alectis ciliaris



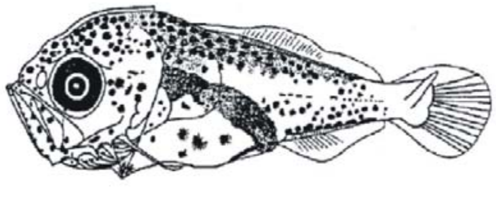
b 15.2 mm TL

Parastomateus niger



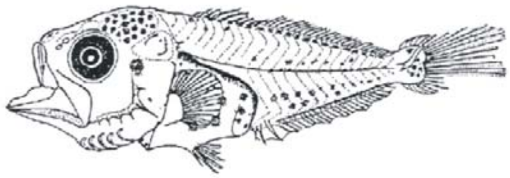
c 5.8 mm TL

Selaroides leptolepis



a 5.76 mm TL

Elagatis bipinnulata

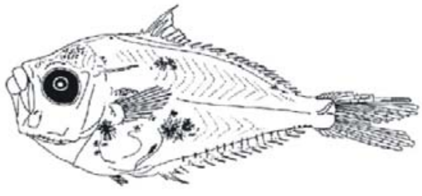


c 9.3 mm TL

Selar crumenophthalmus

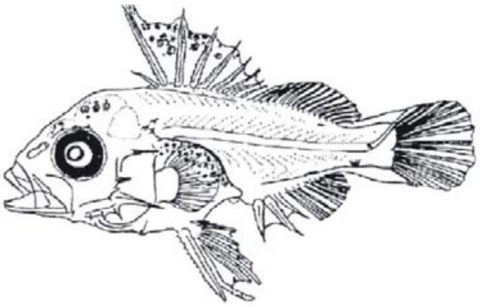
c 10.73 mm TL

Naucrates ductor

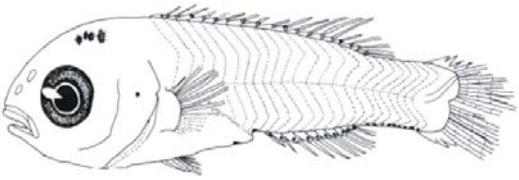


d 13.1 mm TL

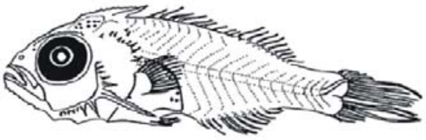
Leiognathidae spp.



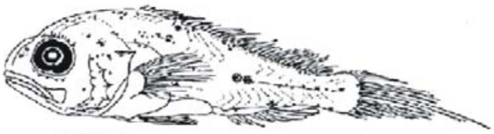
Lutjanidae spp.



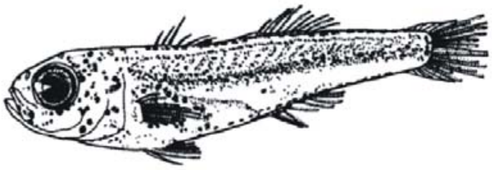
Nemipteridae spp.



Lethrinidae spp.

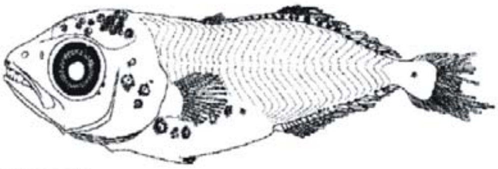


Sciaenidae spp.

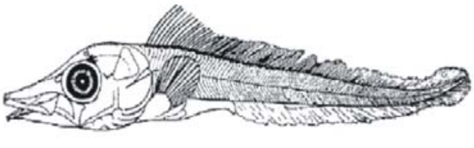


Mullidae sp.

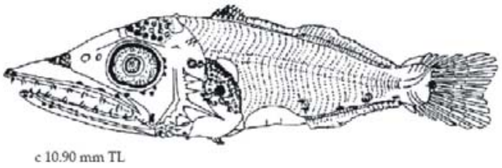
Sphyraena spp.



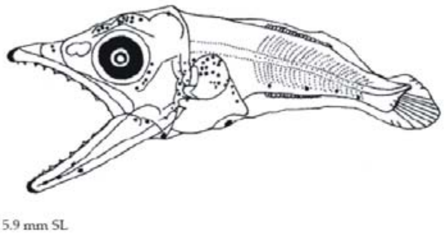
Rastrelliger spp.



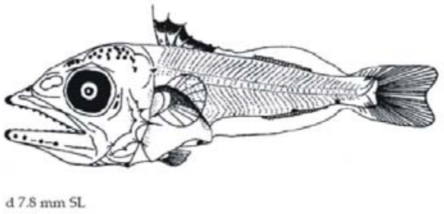
Trichiurus leptulus



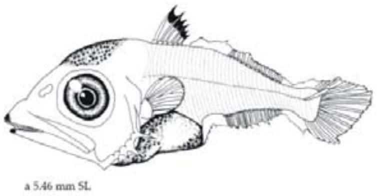
Scomberomorus commerson



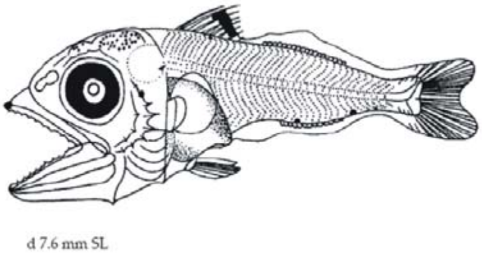
Scomberomorus guttatus



Euthynnus affinis

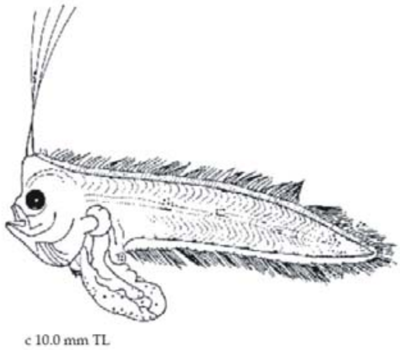


Thunnus albacares

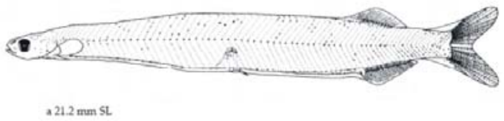


Thunnus tonggol

Pampus sp.



Cynoglossus sp.



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