

Name _____

ALGAE AND DIATOM LAB

Collection techniques / Marine Macroalgae

<http://www.nmnh.si.edu/botany/projects/algae/Alg-CoPr.htm#preservation>

Important note: There are numerous laws that pertain to the collection of specimens. Collecting may not be allowed, or permits may be required for many locations, both local and foreign. It is your responsibility to be aware of and adhere to these rules. Museums now routinely require copies of collecting permits or documentation of adherence to regulations for the accession of specimens.

The objective of taxonomic collections is to represent the natural population in size and form. Therefore, it is important to collect a representative sample of specimens (seasonally), being careful to collect the entire plant (including the holdfast) as well as representative plants from various habitats. Noting information about the habitat for the label may be as important as the specimen itself.

The majority of benthic macroalgal specimens collected for the U.S. National Herbarium have been gathered by hand in the intertidal zone, or subtidally by the use of **SCUBA**. Specimens may also be collected from ships by mechanical means such as bottom grabs, dredges and nets, or by submersibles fitted with claws, grabs or suction devices.

Further information about equipment and techniques may be found in the "References" section of this discussion. A good overview is given in:

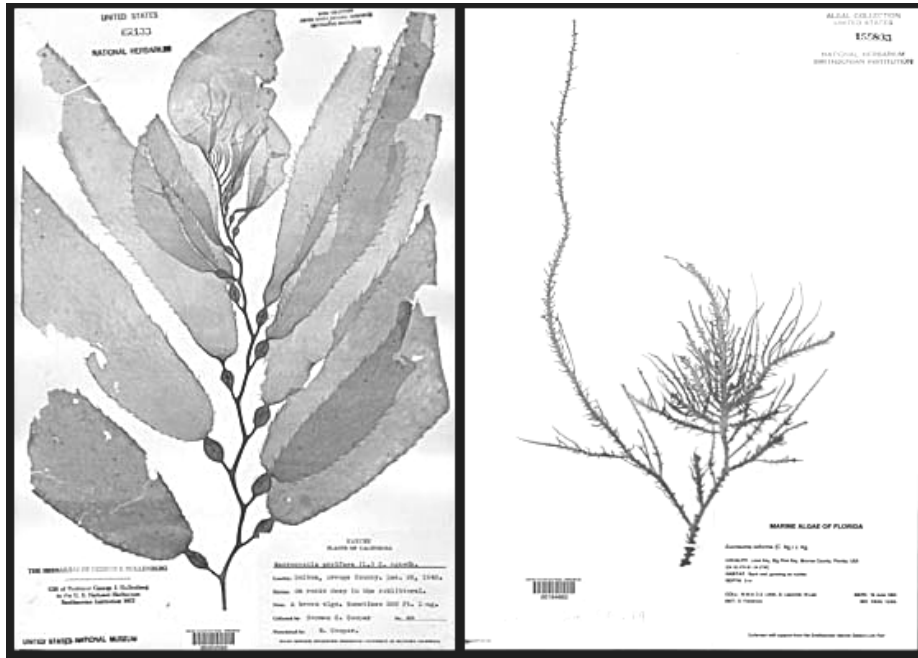
- Tsuda, Roy T. and Isabella A. Abbott. 1985. *Collection, handling, preservation and logistics*, pp. 67-68. In: Littler, M.M. and Littler, D.S. (eds.), *Ecological Field Methods: Macroalgae. Handbook of Phycological Methods*. Cambridge Univ. Press, New York, 617 pp.

~ PRESERVATION OF MARINE ALGAE ~

Classical taxonomists generally agree that macroalgae should first be preserved in a Formalin solution and then pressed dry, with a portion preserved in a liquid medium as described below. Note that different species may require slightly different handling and/or preservative techniques. The accompanying reference list should aid in determining other procedures which may be required for some species.

DRYING SPECIMENS

ALGAE LAB



It is important that dry specimens be prepared carefully so that important morphological characters are displayed as fully and completely as possible. Portions of the specimens (fruiting structures or thallus sections) may be removed and placed in a vial of preservative for microscopic observation, which often is essential for identification. The following procedure, with a bit of practice, should produce good quality dried specimens.

1. Fixing the specimen:

The color of most specimens is best preserved by "fixing" the specimens in 3-5% buffered Formalin seawater away from direct sunlight (overnight fixation is adequate but algae may remain for longer periods of time without damage in this preservative if kept away from light, which causes bleaching). Deterioration may commence upon collection, so it is advantageous to have Formalin handy immediately following collection.

The staff of the U.S. National Herbarium prefers to sort the collection by taxa, while still in the field. The specimens are then preserved in separate plastic whirl-pac bags into which waterproof labels have been inserted. All of the specimens from a station are placed in a large labeled plastic bag which is then placed in a light-proof shipping container (liqua-pac).

2. Preparing the specimens:

- Fleshy specimens.

Having been properly fixed, fleshy specimens should be rinsed free of any sand or debris. (Tap water may be used for this.) Remove any artifacts (shells, animals) which are not part of the specimen -- although records of their presence should be made for ecological reference. If the holdfast is too thick and resistant to be pressed, either split it to remove portions, thereby facilitating pressing, or remove the holdfast and dry it separately (properly tagged for reference to the original specimen).

- Calcareous specimens.

ALGAE LAB



After first fixing the specimens in 3-5 % Formalin, select portions to be liquid preserved in a vial. Soak for several days in a solution of about 40% glycerin in 3% buffered Formalin seawater. Dry and place in small boxes without pressing or, where appropriate, glue carefully to herbarium paper.

- Additional information may be found at: An Introduction to Nongeniculate Coralline Algae. This server is maintained by Derek Keats at the University of the Western Cape, South Africa.

NOTE: Some researchers prefer that glycerin not be used, as it may harm the reproductive structures inside the conceptacles.

3. Pressing the specimen:



Prior to pressing the specimen, a number or note should be written on the lower right hand corner of the herbarium sheet. This will aid in identifying specimens and allow for affixing the proper herbarium label after pressing. Also keep in mind the layout of the herbarium sheet when placing specimens on the sheet with regard to the label location (lower right corner) and herbarium stamp (usually upper right corner).

- The larger coarser forms may be placed directly on herbarium paper. However, delicate forms must be placed on the paper while it is submerged in a tray or pan of adequate size containing water (it may be tap water if specimens have been fixed), which will allow for spreading the specimen.



- Place specimen (in water) over paper, and using forceps, a pointed instrument, or a small, soft paint brush for more delicate forms, pull out and separate branches, and spread the specimen to reveal branching patterns and small structures. If necessary, trim away (and note that this was done) an appropriate amount of the specimen where excess material obscures structures.
- Remove the paper very carefully from the tray, at an angle so that water flows will tend to spread the branches as the paper is being lifted. A squirt bottle of water may be used at this point to further spread branches. Place the paper containing the specimen(s) on a blotter. Then place a piece of muslin, cheesecloth, a cloth "diaper" or crumpled waxed paper over the specimen, and add another blotter on top.
- Place the blotter and specimen sandwich between standard plant press ventilators (corrugated cardboard or

ALGAE LAB

aluminum).



- Continue adding specimens to the plant press so that each specimen is covered with non-sticking material and between a layer of blotters, enclosed by a layer of ventilators. Finally, firmly tighten the straps of the plant press.
- Moderately warm artificial heat should be applied from below the press as it is placed on its side so that warm dry air passes upward through the corrugations of the ventilators. Alternately, the plant press may be placed in a fume hood so that the sash closes on the press, allowing air to be drawn through the corrugations.

Heaters may be devised which include a rack to hold the press and a heating source, such as light bulbs, a heating coil or a hot plate. Note that warm air is all that is required. Too much heat will cause the algae to darken and become brittle. Check specimens at least every 24 hours removing any that are dry; wet blotters and "diapers" should be changed and the straps of the plant press retightened. If a heat source is not available, blotters and diapers must be removed daily and replaced with dry ones. Drying may require many days by this method, so a heat source should be used if at all possible.

- Remove press from heat source and allow to cool with the straps tightened before opening. Specimens may curl if exposed to a humid atmosphere before cooling, in which case they must be wetted and re-dried.

4. Gluing the dried specimen:

Many specimens will remain attached to the herbarium sheet following drying due to the presence in the algal walls and intercellular spaces of colloidal "glues". The coarser, non-gelatinous forms (e.g. some Phaeophyta) may not remain attached after drying and may require "glue".

Any good clear-drying glue may be adequate, such as white glue or a white PVA resin. However due to problems with white glue becoming soft / sticky again (under humid conditions), the U.S. National Herbarium prefers to use "tin" paste applied in spots, to the underside of the specimen. Gummed linen herbarium tapes may also be used to "strap" the specimen(s) to the sheet.

5. Applying specimen label:

Using good quality (100% rag acid-free) herbarium label paper, complete the label and affix by means of a clear-drying cement (tin paste), to the lower right-hand corner of the sheet. **AVOID** gummed labels on poor quality paper.

The completed herbarium sheet should include a label, and may also have museum and barcode numbers, as well as annotation notes written directly on the sheet or on separate labels.

ALGAE LAB

AIM: To make yourself familiar with specimens of algae. As with all other groups of organisms, certain species of algae are found in certain habitats. The purpose of this material is to demonstrate that you

ALGAE LAB

can identify some of the more common species and learn where some of these species are commonly found.

MATERIALS: Microscopes, algae samples, herbarium samples

PROCEDURE: Examine specimens of the different groups of algae noting the distinguishing characteristics of each.

DIVISION CHLOROPHYTA

The Chlorophyta are the green algae. They are an ancient group, possibly extending back to the origin of photosynthetic, cellular plants. Complex green plants are considered to have arisen from green algae.

Distinguishing Characteristics:

1. Pigments---chlorophyll a and auxiliary pigments, chlorophyll b and carotinoids (yellow and orange pigments).
2. Food reserve--true starch.
3. Cell wall--cellulose..(exceptions)
4. Flagellation--when present, always two to four; always anterior.



I. Examine the various specimens on demonstration. Describe one macroscopic and one microscopic sample on your paper. Use the following terms to help with the description:

Branching, cell shape, color, chloroplasts..(shape, size, location), filaments, presence of

any reproductive structures, any outer sheath present and anything else you can describe it with!

DIVISION PHAEOPHYTA

Most brown algae (Phaeophyta) grow in the intertidal zone. Nearly all are marine. There are no unicellular genera. Forms vary from simple branched filaments to giant seaweeds over 60 meters long. Many of the giant species, especially the kelps, show a high degree of external differentiation into a root-like HOLDFAST, a short, stem like STIPE, and a long strap-like BLADE. All larger species have air bladders of various sizes.

Distinguishing characteristics are:

1. Pigments--chlorophyll a and auxiliary pigments, chlorophyll c, carotinoids and fucoxanthin (brown pigment).
2. Food reserve--laminarin and mannitol.
3. Cell wall of cellulose and algin (a commercially valuable compound).
4. Reproductive cells with two laterally placed flagella.
5. Well developed alternation of generations.

II. Examine the various specimens on demonstration. Describe one macroscopic and one microscopic sample on your paper. Use the following terms to help with the description;

Branching, cell shape, color, chloroplasts..(shape, size, location), presence of any reproductive structures, any outer sheath present and anything else you can describe it with!

DIVISION RHODOPHYTA

The red algae (Rhodophyta) are relatively small plants, most species being less than 0.7 meters long. Their growth forms are simple filaments, highly branched filaments or sheet-like bodies. They are abundant in warm marine waters. A few are fresh water. They are capable of living at depths greater than those of any other algae.

ALGAE LAB

Distinguishing characteristics:

1. Pigments--chlorophyll a and the auxiliary pigments, chlorophyll d, phycoerythrin, and phycocyanin.
2. Food reserve--floridean starch.
3. Cell wall of cellulose sometimes covered with gelatinous material commercially known as AGAR.
4. Absence of any kind of motile cells.
5. Complex life cycles in many.

III. Examine the various specimens on demonstration. Describe one macroscopic and one microscopic sample on your paper. Use the following terms to help with the description;

Branching, cell shape, color, chloroplasts..(shape, size, location), presence of any reproductive structures, any outer sheath present and anything else you can describe it with!

DIVISION CYANOPHYTA (CYANOBACTERIA)

The blue-green algae (Cyanophyta) are the most primitive of the chlorophyll bearing plants. In cell structure they more closely resemble bacteria than they do other algae. A general evolutionary trend of increase in size is evident among the blue-green algae. Colonial and filamentous forms are more advanced than the unicellular state.

Distinguishing characteristics are:

1. Pigments--chlorophyll a and the auxiliary pigments, phycocyanin (blue pigment), and phycoerythrin (red pigment); and carotinoids.
2. Food reserve--cyanophycean starch.
3. Cell wall surrounded by a gelatinous pectic sheath.
4. Absence of an organized nucleus or chloroplasts.
5. Absence of sexual reproduction.
6. Absence of flagella

IV. Examine the slides. Describe two microscopic samples on your paper. Use the following terms to help

ALGAE LAB

with the description;

Branching, cell shape, color, chloroplasts..(shape, size, location), presence of any reproductive structures, any outer sheath present and anything else you can describe it with!

DIVISION CHRYSOPHYTA (golden-brown algae (diatoms))

The golden-brown algae (Chrysophyta) possess evolutionary trends in size increase some of which are exhibited by filamentous and colonial forms. We will examine diatoms, either filamentous or unicellular forms. Diatoms are characterized by cell walls composed of two overlapping halves that fit together in a manner similar to the parts of a Petri dish.

ALGAE LAB MARINE SCIENCE 1 DRAWING SHEET NAME _____
PD _____

Label all parts with holdfast, stipe, blade, name of specimen, collection point if included.

Chlorophyta (Green Algae) Macroscopic _____ Chlorophyta (Green Algae) Microscopic

ALGAE LAB

-

Phaeophyta (Brown Algae) Macroscopic

Phaeophyta (Brown Algae) Microscopic

-

Rhodophyta (Red Algae) Macroscopic

Rhodophyta (Red Algae) Microscopic

-

-

-

-

-

-

-

-

-

-

ALGAE LAB

-
Cyanophyta (Blue-Green Algae) Microscopic Dinoflagellates Chrysophyta (Diatoms) Microscopic
-
-
-
-
-
-
-
-
-
-
-
-
-
-
-

Back side... Marine Angiosperms... marsh grass and complete any life cycle sheets.

PLANKTON LAB

INTRODUCTION: Nearly every creature in the sea spends either a part or all of its life drifting about. If we add these "part time" drifters to those plants and animals that spend their entire existence as drifting plankton we see a population or organisms so immense that it defies counting. In this exercise you will have a opportunity to examine this vast assemblage of plankton. While you will be observing microscopic plankton, remember that some of the plankton are as large as two to three feet. Among the members of the animal plankton (zooplankton) eggs, larvae, and juvenile forms of most invertebrates and fishes are common. Copepod crustaceans (related to crabs and shrimp) are the most abundant and widely distributed zooplankton.

ALGAE LAB

Phytoplankton (plants) are more numerous than zooplankton and are best represented by the diatoms that form the vast bulk of the ocean's vegetation.

Plankton forms the basis of life in the sea. All forms of life in the marine world depend either directly or indirectly upon them for food. Without plankton the seas would be barren. In this activity you will have a chance to observe and analyze a sample of these dynamic little creatures.

MATERIALS:

Compound microscope, dissecting microscope, petri dish, slide and cover slips, eye dropper, sample bottle and label, concave slides, preservative (3-5% formalin), plankton sample.

PROCEDURE:

PART 1

1. You will be provided with the recorded conditions under which the plankton sample was made. Enter this information on the data sheet.
2. Use the eye dropper to draw up a few drops of the concentrated plankton. Take the sample from the TOP one third of the bottle of concentrated plankton (if the sample is still alive).
3. Place one drop in each of four or five locations on your petri dish.
4. Observe each drop with a dissecting microscope. Since the plankton can move up and down in the drop, you will need to focus up and down with your microscope to see the plankton at different levels.
5. Many organisms are too small to see with the dissecting microscope. Make wet mounts of the sample and observe under a compound microscope.
6. Look for:
 - a. most abundant organisms
 - b. variations in shape, color and swimming abilities

ALGAE LAB

- c. types of appendages
- d. chlorophyll-containing organisms
- e. eggs
- f. larval and juvenile forms of crustaceans and fish.

7. If not preserved, preserve the sample in 3-5% formalin before leaving. Use 3 drops of formalin per 120 ml of plankton sample. Label the bottle with your name.

PART 2 IDENTIFICATION

The two major divisions of plankton are phytoplankton (plants) and zooplankton (animals). Within these two groups exist a number of subgroups:

1. Phytoplankton-able to produce their own food through photosynthesis.

A. Diatoms- often called the golden-brown algae, have a transparent silicon skeleton called a test. The tests have a precise geometric design.

B. Dinoflagellates-have a cellulose cell wall and, in most species, two whip-like flagella used for mobility.

II. Zooplankton Obtain their food by eating phytoplankton or other zooplankton. All of the major groups of animals are found within the zooplankton.

A. Permanent zooplankton-spend their entire existence as drifters (HOLOPLANKTON). Most are crustaceans .

B. Temporary Zooplankton-spend part of their life cycles as drifters (MEROPLANKTON). The most common temporary zooplankton are the larvae (in some ways equivalent to "baby") forms of the following groups:





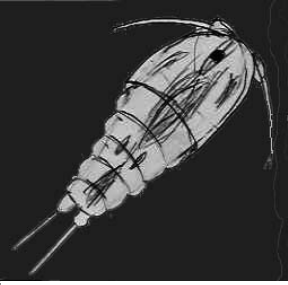



- 1. Cnidaria or Coelenterate (corals, jelly fish, sea anemone) larva-PLANULA larva.
- 2. Platyhelminthes (flatworms) larva- MULLER'S larva.

3. Molluscan (snails, clams, chitons) larva- VELIGER larva.
4. Annelid (segmented worms) larva- TROCHOPHORE larva
5. Crustacean (barnacles, crab, shrimp) larva- NAUPLIUS
6. Echinoderm (sea star)-BIPPINARIA larva.

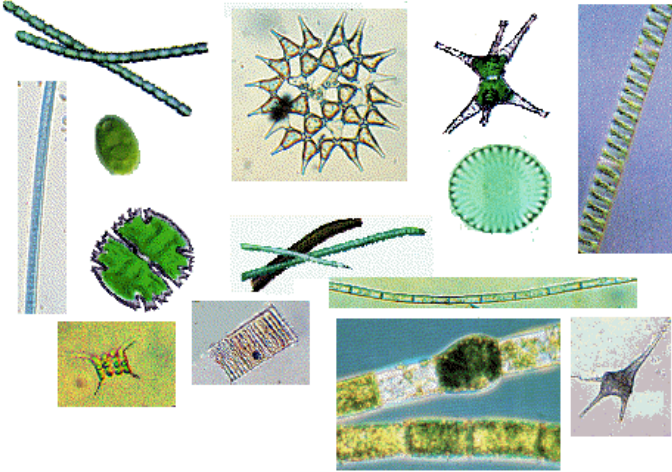
Use the descriptions on the preceding page and the figures which follow to help you identify the organisms in your plankton sample.

Simple Plankton Key

Guide to Marine Plankton

	Phytoplankton - (plant plankton)	SHAPES KEY	 Star	 Circle	 Cone/Triangle
	Zooplankton - (animal plankton)		 Oval	 Worm Like Elongate	 Pennate

Plankton generally greenish in color, if present small microscopic appendages



PHYTOPLANKTON

OR

Blue-green pigment diffuse through cells



Anabena



Oscillatoria

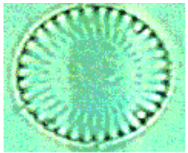
Blue Green Pigment

OR

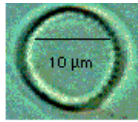
grass-green, yellowish-green, or brownish, localized in chromatophores

Pigment yellowish-green or brownish, cell wall composed of two nearly equal halves

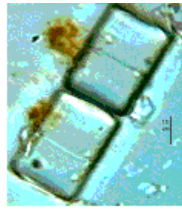
ALGAE LAB



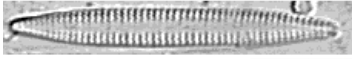
Cyclotella



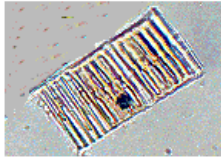
Stephenodiscus



Melosira



Fragilaria



Diatom sp.

DIATOMS

OR

Pigment grass-green, cell wall not subdivided

Flagella Present

Transverse furrow generally present, body with armored plates



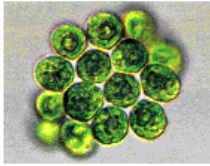
Ceratium

DINOFLLAGELLATES

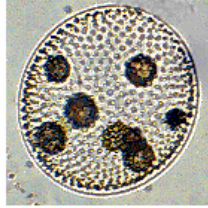
OR

ALGAE LAB

Color Bright green, cells in small colonies, flagella in pairs



Eudorinia



Volvox

COLONIAL GREEN ALGA

OR

Cells divided into distinct halves by median constriction



Staurastrum

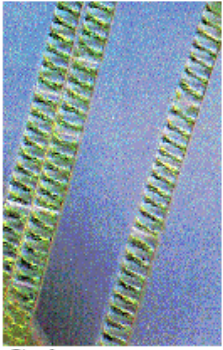


Mirasterias

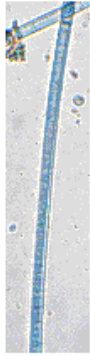
DESMIDS

OR

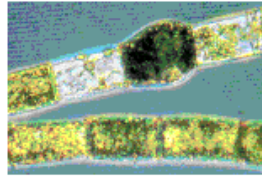
Cells arranged in long filaments



Spirogyra



Ulothrix



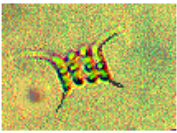
Oedogonium

FILAMENTOUS GREEN ALGAE

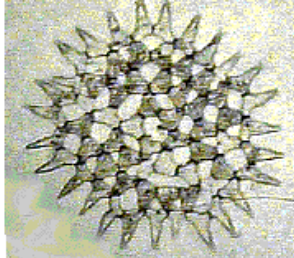
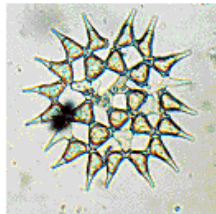
OR

OTHER GREEN ALGAE

Cells not arranged in filaments but small colonies



Scenedesmus



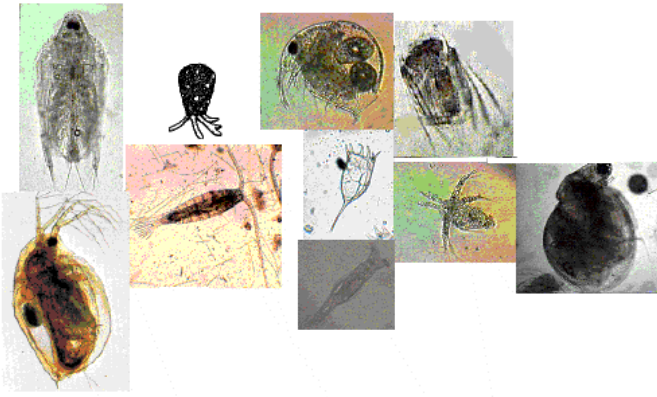
Pediatrum spp.

OTHER GREEN ALGAE

OR

ZOOPLANKTON

Greenish pigment absent, cilia or flagella may be present



Body organization unicellular



Epistylis

CILIATES

OR

Body Organization multicellar

Jointed appendages absent, body with ciliated rings, may be encased in a shell



Kellicotia



Keratella



Brachionus



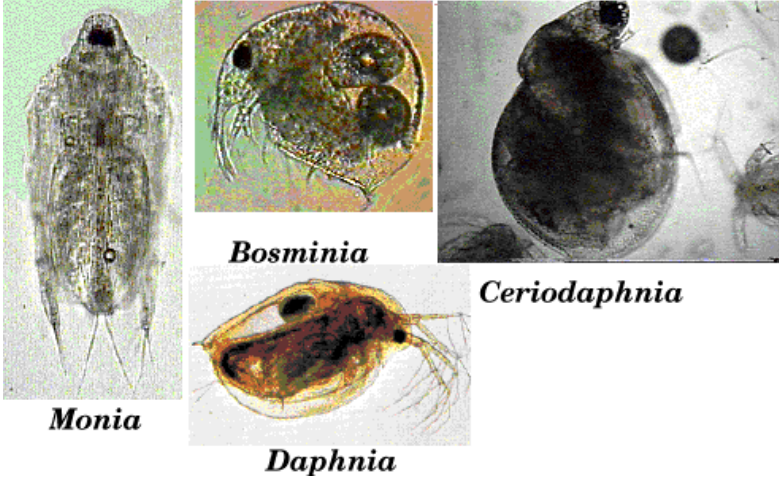
Polyarthra

ROTIFERS

OR

Jointed appendages present

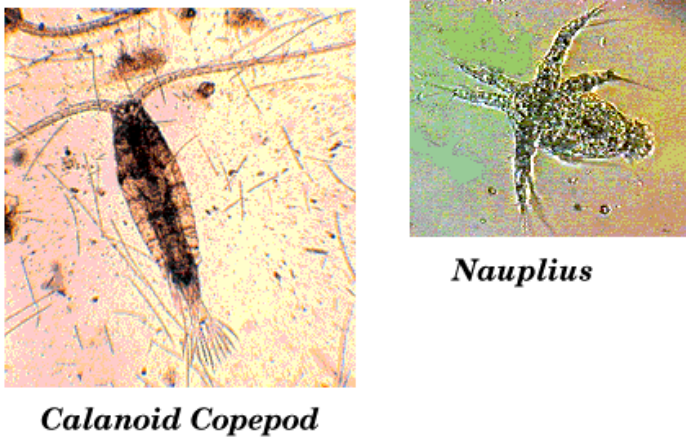
Trunk appendages flattened and mostly encased in paired valves, body bird-like



CLADOCERA

OR

Body not enclosed in paired valves, segmentation obvious, body elongated, appendages for swimming



COPEPODS

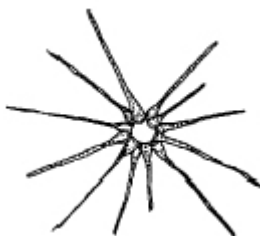
The Diatom Game

OBJECTIVE:

To introduce the two types of phytoplankton: diatoms and dinoflagellates. To explore the specific adaptations of the shapes of diatoms, which are effective in keeping some of them in the upper, sunlit area of the ocean.

ACTIVITIES:

Make paper diatoms and investigate how they travel.



Diatoms (*Chaetoceros sp.*)

BACKGROUND:

Phytoplankton are microscopic, single-celled plants that drift along with the ocean currents and tides. They are capable of photosynthesis and can only be found in the photic or sunlit part of the ocean. Phytoplankton are the base of all aquatic food chains and are also found in lakes, ponds, and rivers. The oceans produce 20 BILLION tons of phytoplankton per year—three times the amount produced by plants and trees on land. Phytoplankton produce 65-75% of the world's oxygen.

Phytoplankton are divided into two major groups: The diatoms, which make up 98% of the phytoplankton, and the dinoflagellates, which make up the remaining 2%. Diatoms are unicellular plants that average from 0.05 mm to 0.5 mm in size. Diatoms can form chains of single cells and some have small projections extending from the plant to bolster the plant's ability to float. Diatoms are characterized by a transparent shell, which is made of silica (glass-like). This allows the plant to photosynthesize. Diatom shells have been collecting on the ocean floor for millions of years. In some areas the shells are mined and used in silver polishes, toothpaste, and as an abrasive agent in flea powder.

PROCEDURES:

1. Begin this exercise by stressing that phytoplankton are plants but that they are very tiny—smaller than this dot (.). As plants, they provide us with the oxygen we need. Some diatoms are more successful at staying in the upper sunlit part of the ocean, due to their shape and the ocean currents. Others are not as successful. With this activity, try to find out which diatom shapes are successful in keeping the diatom afloat.
2. On the following page are several types of diatoms. The paper diatoms are 150 times larger than the real ones. Cut them out and drop them, one at a time, from the top of a set of stairs or other high place.

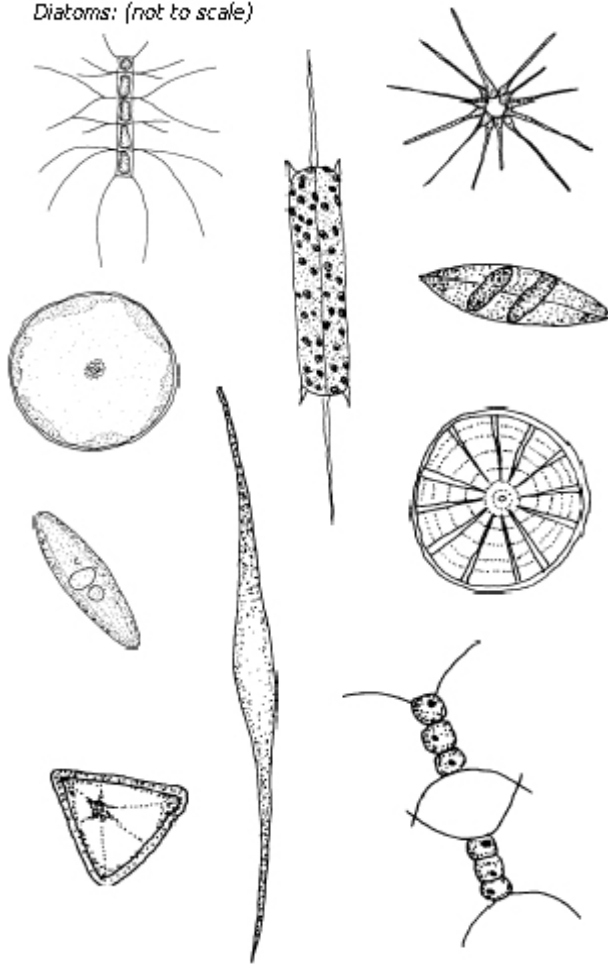
ALGAE LAB

3. Describe how the diatom falls-does it spin or fall like a maple seed? How long does it take for each paper diatom to reach the floor? Use a stop-watch to measure the time. Why do some fall faster than others?
4. Design your own diatom shape within the square provided. Have a contest to see which design takes the longest to reach the floor. Diatoms can also form chains. Is this useful in slowing their fall?

EXTENSION:

Try using aluminum foil diatom shapes in an aquarium. Which shapes are successful at staying afloat?

Diatoms: (not to scale)



Diatoms: (not to scale)

**From the Huntsman Marine Science Centre*

