Lab 9:
Macroalgae and Seaweed

Introduction

The macroscopic attached marine plants are often lumped together as “seaweeds”, with little regard for the structural and functional variety actually present in these groups. The purpose of this laboratory exercise is to assist you in becoming more familiar with the taxonomy and diversity of the common plant members of intertidal and near-shore marine communities.

The majority of the attached marine plants can be placed in four divisions of the kingdom Plantae. Three divisions – the Phaeophyta, Rhodophyta, and Chlorophyta – are collectively referred to as seaweeds. The fourth division, Anthophyta, is mostly terrestrial but also includes several species of seagrasses. These four divisions of common benthic plants are distinguished from one another by their different pigment systems and reproductive structures, as well as other structural differences. In these macroscopic forms, it is the pigment differences that are initially the most evident.

A. Division Phaeophyta (Brown Algae)

The brown algae are almost entirely marine and are quite diverse in form and structure. The characteristic brownish or olive drab coloration of this group results from several carotenoids that mask the green chlorophyll pigments. Some types of brown algae are massive and form extensive kelp beds. The giant kelp plant *Macrocystis* often grows to lengths of over 30 m and will live for several years. *Fucus, Alara, Desmerestla, Laminada,* and several other genera have representatives on both the Atlantic and Pacific coasts. They often form extensive mats of algae on wave-swept rocky shores, and some occupy quiet estuarine shores as well. *Ascophyllum* is a common Atlantic coast form. Members of the genus *Sargassum* are also found on both coasts in many diverse habitats wherever solid substrates are available. One member of the genus *Sargassum* occurs as large floating mats in the central North Atlantic Ocean (the Sargasso Sea).

Large members of the Phaeophyta attach to the substrate by means of rootlike **holdfasts.** Their leaflike **blades** are supported by stemlike **stipes**. Although these plants appear to have the same morphological features as the roots, stems, and leaves of terrestrial plants, their cells are not organized with the same degree of complexity.

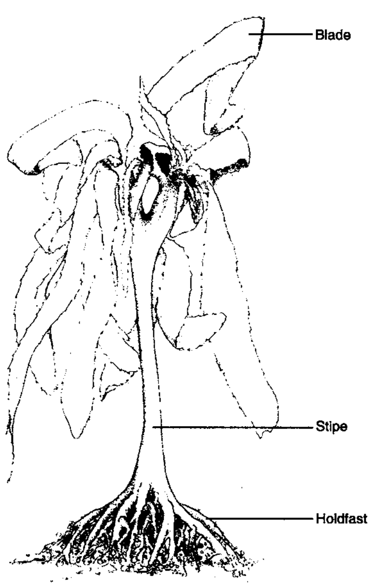

Fig. 1: Organization of a brown alga (kelp) thallus

B. Division Rhodophyta (Red Algae)

Like the brown algae, the red algae are almost entirely marine. Their size and complexity vary from thin films growing on rocks to complex filamentous and membranous forms growing to heights approaching one meter. Most red algae are small, and none rival the giant kelps in size, but they occupy a greater range of depths than do the brown algae. Because of their specialized pigment systems (chlorophylls *a* and *c,* phycobilins, and a wide variety of carotenoids), they often are able to photosynthesize in deeper water than either the Chlorophyta or the Phaeophyta. They are common inhabitants of the intertidal zones as well. Some forms of red algae secrete a stony $CaCO_3$ (calcium carbonate) skeleton. These calcareous red algae become prevalent in warmer tropical and subtropical waters, especially on coral

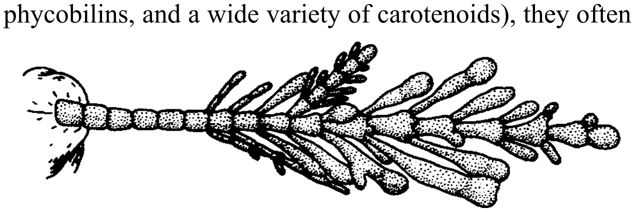

Fig. 2: Corallina, a segmented, calcareous red alga

reefs. Plants of the genus *Geildium* are processed commercially to yield a colorless gelatin, called **agar,** which solidifies as a gel at room temperatures. It is used as a growth medium for the culturing of bacteria. Many genera of red algae are also common inhabitants of rocky shores on both the Atlantic and Pacific coasts of North America. These include *Chondrus, Rhodymenia, Gigartina, Ceramium, Polysiphonia,* and *Porphyra.* Several *(Chondrus, Porphyra,* and *Rhodymenia)* are used directly as food or food additives.

C. Division Chlorophyta (Green Algae)

Botanists are particularly interested in this primarily freshwater group because the plants contain the same photosynthetically active pigments (chlorophylls *a* and *b)* as terrestrial plants. This division is considered to represent the ancestral forms from which terrestrial plants evolved. The green algae along the coast are of minor significance when compared with their overwhelming abundance in fresh water. However, a few coastal species of green algae do occur in abundance. The Chiorphyta occur primarily in the upper portion of the photic zone. Large, thin sheets of the familiar sea lettuce *Ulva* often totally obscure the muddy substrate in sheltered bay and estuary habitats. Other marine Chiorophyta exhibit filamentous or branched growth forms.

D. Division Anthophyta (Flowering Plants)

The division Anthophyta is a primarily terrestrial group that produces seeds from flowers. In contrast to members of the three previous divisions of marine plants, flowering plants do have true roots, leaves, and stems. A few seagrasses have adapted to marine conditions to form extensive near-shore beds in the sublittoral zone, occasionally being exposed for short periods during exceptionally low tides. Although their diversity is limited, they play an important role in the primary production of bays, estuaries, and other coastal communities. A few other flowering plants such as pickleweed *(Salicornia)* and the cord grasses *(Spartina)* are common inhabitants of salt marshes and estuaries. These species are limited to occasional inundation by seawater at high tides.

E. Pigmentation in Marine Plants

It should be apparent that there is a correlation between the pigmentation of marine algae and their gross morphology. You have already recognized this in previous exercises by grouping the attached macroscopic marine algae into the divisions Chlorophyta (greens), Phaeophyta (browns), and Rhodophyta (reds). This system of classifying algae is related to pigment differences between them.

The pigments of algae are divided into three major groups: the chlorophylls, the carotenoids, and the phycobilins. Not all of these pigments are directly related to photosynthesis; some are used for shading purposes. Others absorb energy from certain wavelengths of light and transfer it to those pigments that are capable of photosynthesis. Also, the role of some pigments in these groups is not yet known. It is known, however, that photosynthesis in algae, as well as in higher plants, is associated with the complex molecule chlorophyll.

Chlorophyll occurs in several slightly different molecular forms: chlorophylls a, *b,* and *c.* Only chlorophyll *a* is a common factor in all photosynthetic plants. It is *the* photosynthetic pigment. The other chlorophylls, as well as some carotenoids and phycobilins, function in photosynthesis as accessory pigments, being involved either as part of the light reaction pigment systems or as donors of light energy to chlorophyll *a*. The accessory pigments are particularly important in the light energy absorption in the region of the light spectrum that chlorophyll cannot absorb in. Absorption of chlorophyll is essentially limited to blue and red light, and most accessory pigments exhibit their highest absorbance in the green range of the spectrum.

The carotenoids may be separated into two major groups, **carotenes** and **xanthophylls.** The carotenes are usually divided into several types. One type, β-carotene, is found in all groups of algae. Xanthophylls include some 20 different pigments. Two xanthophylls, **fucoxanthin** and **lutein,** are important in attached algae. Fucoxanthin is found only in the Phaeophyta, whereas lutein is a common pigment of green algae.

The phycobilins occur as a blue **phycocyanin** and a pink-red **phycoerythrin.** Both are major pigments of the Rhodophyta. Slightly different molecular forms of phycocyanin and phycoerythrin are also found in Cyanobacteria. The phycobilins are water-soluble pigments that cannot be extracted with organic solvents (acetone) as the other pigments.

Lab Work

A. Diversity and Organization of Macroalgae

Attached marine plants are the major primary producers in many coastal communities. Recognition and identification of intertidal plants is a necessary first step in developing an awareness of the interrelationships that exist between plants and animals in intertidal zones.

Your instructor will provide a collection of live brown, red, and green algae. The collection will comprise both macroscopic, thallus-forming species as well as filamentous species. The collection of live specimen will be complemented by permanent microscope slides. Whereas some algae can be identified by their macroscopic appearance (color, thallus form), other forms, particularly filamentous forms, require studying their organization and celling forms under the microscope. Permanent microscope slides will also be provided to study the cellular thallus organization of big macroalgae such as the brown alga *Fucus*.

Each student will prepare detailed drawings and documentation of a minimum of 6 macroalgal species, two of each group of brown, red, and green algae. Devote one half letter-sized page to each species and label your documentation with the species name *and* the division name (Phaeophyta, Rhodophyta, Chlorophyta). Start your documentation by drawing the macroscopic appearance of your specimen and label most obvious characteristics that may help identifying this species. Next to this drawing, add a drawing of the cellular organization of the thallus/filaments of your specimen. Use the compound microscope to gather this information. Take notice of how many cell layers produce the thallus; how many rows of cells create the filaments; do the cells in the inner and outer layers differ in size, form, and pigmentation? For larger kelp such as *Fucus*, refer to the permanent slide collection. The permanent slide collection also comprises a number of filamentous algae that cannot be provided as live specimens.

B. Paper Chromatography of Algal Pigments

The different pigments of algae can be separated by chromatography methods after extraction in a suitable organic solvent. As the solvent is running through a chromatography paper (paper chromatography) or chromatography column (column chromatography or High-Performance Liquid Chromatography, HPLC), the pigments will move along but at different speeds depending on their molecular size and polarity (electron charge). The pigments will, thus, reach the end of the chromatography column/paper at different times, one after the other, which is used to separate the pigments for quantification of qualitative description (for example absorption spectra). If the chromatography is stopped before the solvent reaches the end of the chromatography paper, pigments are visible as discrete bands on the paper.

Each group will perform one paper chromatography from one macroalgal species provided by the instructor. All algal groups, brown, red, and green algae, will be presented in the lab class and students will compare the paper chromatograms from the different algal species at the end of the class to elaborate the differences in pigment composition among the used algae. Dried algae for paper chromatography will be provided in porcelain mortars. Prepare the paper chromatography following the below protocol:

1. Add 10 ml of 20% ethanol/80% acetone solution and grind with pistil until the plants appear completely homogenized. (Chlorophyll and carotenoids are soluble in organic solvents but not in water.)
2. Centrifuge this mixture to remove the cellular debris. (Be sure the centrifuge is properly balanced.)
3. If necessary, pour the green supernatant (floating on the surface) through a general-purpose filter paper to remove any suspended material.
4. Apply a small pigment spot about 2 cm from the end of a 15-cm-long strip of filter paper (Fig. 3). The pigment spot should be as small as possible (less than 4 mm is best). Use a capillary tube to transfer the pigment solution to the filter paper. To make the pigment spot as dense as possible, you will need to reapply the pigment solution about 50 times. Patience must be exercised between applications to allow the pigment spot to completely dry. If the pigment is reapplied before the spot dries, a very wide spot is formed.
5. Suspend the paper in a test tube containing a 10% acetone/90% petroleum ether solution. The chlorophyll spot must be about 1 cm above the solution (Fig. 3).

6. Allow to stand for approximately five minutes, then observe. β-carotene will appear as a rather narrow, chrome-yellow band. The xanthophylls will appear greenish yellow. Chlorophyll *a* is blue-green, and chlorophyll *b* will separate out as a yellow-green color.
7. Use a pencil to outline the areas of pigmentation on the paper strip and label the pigments found (some will fade as they dry). Also, mark the edge of the solvent front. All groups will bring their final paper chromatograms together to compare and discuss differences among the used algal species.
8. Take notes of the results and discussion for your lab report. Also prepare sketches of the different chromatograms to be included with their discussion in your lab report.

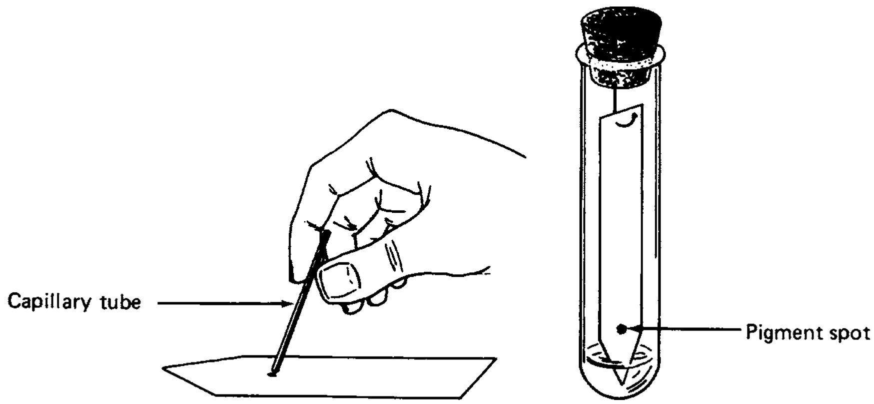

Fig. 3: Paper chromatography of algal pigments. Left: Technique for applying a pigment spot to a strip of filter paper. Right: Correct position of paper with pigment spot when placed in test tube.