The Chlorophyta or green algae

Chlorophyta are commonly known as green algae. This is the most diverse group of algae, with over 10,000 species. The majority of organisms in this group are unicellular, but there are some multicellular species. Some are free-living, some are colonial, and others are coenocytic. When flagellae are present they are usually biflagellate. Many multicellular adult forms are not flagellated, however, the gametes of all chlorophyta do display flagellae. Like other green plants, Chlorophyta contain chlorophylls a and b, although the major pigment is chlorophyll b. In addition, some tropical species are pigmented by siphonoxanthin and siphonein and some contain large quantities of \( \beta \) carotene. They store starches in double-membrane bound chloroplasts. The chloroplasts may have a variety of different shapes and also generally contain pyrenoids (organelles of carbon fixation). Cell walls are present in most chlorophytes (with the exception of the order Prasinophyceae which are identified by 1-5 layers of scales instead of cell walls) are composed of cellulose.

The chlorophytes reproduce both sexually and asexually, but usually sexually. Asexual reproduction can occur by fission, fragmentation, or zoospores. Sexual reproduction can be isogamous, anisogamous, or oogamous. The species *Ulva lobata* experiences alternation of generations, alternating between haploid and diploid phases. In the haploid phase, gametes are formed; in the diploid phase, zoospores are formed. Not all species have this, however. For the species without alternation, meiosis occurs in the zygote. Chlorophyta are adapted to shallow water, and live in both freshwater and marine habitats. 90% of Chlorophyta are freshwater species. Those that live in marine habitats largely inhabit tropical environments. There are a small number of terrestrial species; these largely dwell on rocks or trees. Some species form symbiotic relationships with fungi, producing lichens. There are a few instances in which Chlorophyta have formed symbiotic relationships with animals.

Green members of the Phytoplankton

I. Chlorophyceae

The Chlorophyceae are a large and important group composed mainly of freshwater green algae with some marine and terrestrial representatives. They include some of the most common species, as well as many members that are important both ecologically and scientifically. There are approximately 350 genera and 2650 living species of chlorophyceans. They come in a wide variety of shapes and forms, including free-swimming unicellular species, colonies, non-flagellate unicells, filaments, and more. They also reproduce in a variety of ways, though all have a haploid life-cycle, in which only the zygote cell is diploid. The zygote will often serve as a resting spore, able to lie dormant though potentially damaging environmental changes such as desiccation.

Perhaps the most famous and important chlorophyceans are *Chlamydomonas* (freshwater, terrestrial and marine) and *Volvox* (freshwater only). Both are important research organisms in biological laboratories. Many other species of Chlorophyceae are common. *Oedogonium* grows in freshwater worldwide, usually attached to other plants or algae. It has been a subject of intense study for its unusual cell division method.

The oldest fossils resembling Chlorophyceans come from the Proterozoic Bitter Springs Formation of central Australia. There is insufficient preservation to make a firm identification of these fossils, but other multicellular algae are known from this time, so the identification is not
unreasonable. More likely fossils of this group have been found in Middle Devonian rocks from New York State. Among these is *Paleooedogonium*, a fossil with striking resemblance to modern *Oedogonium*. The presence of this fossil and those of other green algae in the Devonian suggests that the group was quite diverse by this time. Tertiary fossils of modern chlorophycean genera are also known, such as *Scenedesmus, Pediastrum* and *Botryococcus*, and *Tetraedon*. The last of these is believed to be an important component in the formation of oil shales of the Tertiary, such as those in the Messel Grube, Darmstadt, Germany.

1. *Chlamydomonas* sp. – These members of the phytoplankton are unicellular and typically spherical to subspherical, but may be fusiform. Each cell typically contains two anterior contractile vacuoles, but these may be absent or numerous (depending upon species), with two anterior flagella. There is only a single chloroplast per cell and their shapes are extremely variable, variants species specific and provide basis for defining species group or "Hauptgruppen"(main groups). Chloroplasts variously cup-shaped (*Euchlamydomonas, Chlamydella, Bicocca, Pleiochloris*), band-shaped (*Chlorogoniella*) to indistinct (*Sphaerella*). The cells contain one to several pyrenoids with position variable depending upon the species basal (*Euchlamydomonas*), lateral (*Chlamydella, Bicocca, Chlorogoniella*), or axial. Eyespots (stigma) are prominent in most species and are generally located at cell anterior embedded in the chloroplast. The single, typically central nucleus is < 5 µm. The flagellar root system is cruciate and the basal bodies, connected by striated (proximal and distal) fiber systems, exhibit clockwise absolute configuration. Mitosis is characterized as closed spindle type, the spindle collapses during telophase. A phycoplast system of microtubules develops in the plane of cytokinesis. Asexual reproduction occurs by zoosporogenesis. Motility is variable (from "zoospores only motile stage" to "all stages motile") and species specific. Sexual reproduction may be isogamous, anisogamous, or oogamous, depending on the species. Gametes may be naked or walled with the cell wall discarded during fusion. Gametogenesis triggered by sub-optimal environmental conditions (loss of nitrogen). In *C. reinhardtii*, mixture of opposite mating types leads to immediate adhesion of flagella from two cells of each mating type. Glycoproteins (agglutinins) excreted by gametes into the media induce union. Agglutination reaction is specific to gametes; active molecule present on flagella of gametogenic cells only. Mating structures are activated and a fertilization tube, derived from the mt+ cell, connects the two cells. Fusing cells are quadriflagellate and may remain motile for several hours. The zygote wall is either ornamented or unornamented and it germinates to form 2-8 meiospores. Chromosome number is variable (n = 8-38) depending upon the species. Although these specimens in many ways resemble the previously studied members of the phytoplankton it, with all other representatives of the chlorophytes is placed in the kingdom Plantae (in monophyletic taxonomic relationships) due to the following features: (a) plastids with chl a and b, (b) production and storage of amylose starch as a reserve photosynthate and (c) the presence of true cellulosic cell walls containing traces of hydroxyproline proteins.

A. Use the preserved slides to observe the organelles (as appropriate) in *Chlamydomonas*.

Where are the two contractile vacuoles found in comparison to the location of the flagellae? Regarding the flagellae, are these structures equal und unequal in their length? Are the flagellae located at the anterior or posterior end of the organism?
The preserved slides, when stained, should outline a clear nucleus but may obscure the chloroplast, which should be visible in live organisms.

B. Prepare a wet mount of *Chlamydomonas* cells in culture. Observe under 10x and then 40x magnification.

Does the cell shape and size compare to other flagellated unicells we have studied in previous labs? To compare you will want to draw a *Chlamydomonas* at either 10 or 40x magnification and compare them to drawings done at the same magnification of other flagellated members of the phytoplankton.

How do *Chlamydomonas* differ compared to other members of the marine phytoplankton, i.e. the diatoms.

How many chloroplasts are visible in the unicell *Chlamydomonas* and what is their shape? Can you see the pyrenoid inside these cells? Can you distinguish the chloroplast from the pyrenoid in live *Chlamydomonas*? Observe the carotenoid-containing eyespot near the anterior end of the chloroplast.

In order to determine the function of the carotenoid packed stigma or eyespot turn the light on the microscope as high as you can while still watching the organisms move. Following this observation turn the light down as much as possible while still being able to visualize the unicells and again observe their motion. How does directionality and activity of the organisms change under the different light levels?

After you have observed the live, motile cells place a drop of Lugol’s near the edge of one side of the coverslip and a paper towel near the opposite edge to draw the solution under the coverslip. Though the Lugol’s will kill the cells it will aid you to see the pyrenoid.

Make a composite diagram of the entire *Chlamydomonas* showing the general shape, plastic structure, stigma position, nucleus and flagellae.

2. *Dunaliella* - Unicellular, biflagellate and uni-nucleate algae without cellulosic cell walls. Cells ellipsoid, ovoid, globose or depressed-globose. The anterior end is usually rounded while the posterior end is rounded, or in some cases, caudate. The chloroplast is parietal and principally cup-shaped with pyrenoid and stigma present. The nucleus is typically at the anterior end along with two contractile vacuoles. *Dunaliella* spp.is widely distributed in marine or brackish water habitats.

A. Prepare a wet mount of the *Dunaliella salina* culture and observe the organisms under 10x and 40x magnification.

Do the movement of *Dunaliella* and *Chlamydomonas* compare or differ? Why?

What are the differences in the chloroplasts between these two single celled chlorophytes (pay close attention to coloration)? What is the reason for this difference?
After you have observed the live, motile cells place a drop of Lugol’s near the edge of one side of the coverslip and a paper towel near the opposite edge to draw the solution under the coverslip. Though the Lugol’s will kill the cells it will aid you to see the pyrenoid.

Make a composite drawing of the overall shape of Dunaliella and the presence and shape of the chloroplast and the pyrenoid.

3. *Scenedesmus* - single celled or colonial, forming 2- to 32-celled, usually 4- or 8-celled coenobia or colonies. A surrounding mucilaginous matrix may be present or absent. Cells arranged linearly, alternating or in 2-3 rows and touching with the lateral walls only. Cells are nearly spherical to ellipsoidal, elongate or fusiform and between 2-10 µm in diameter. The cell wall contains a hemicellulosic and sporopolleninic layer and is usually smooth. Cells are spineless, uninucleate and chloroplasts are single and parietal with a single pyrenoid. Asexual reproduction is conducted by production of autospores, sexual reproduction, though observed, is extremely rare. Gametes are biflagellate and isogamous. *Scenedesmus* is planktonic mainly in eutrophic freshwater ponds and lakes and brackish water and been reported world-wide in all climates. Species tolerate/prefer eutrophic water with slight acidity and low salinity (up to 18ppt). Temperature optima for this genus are at 28-30°C, but with some species may survive and flourish at 36°C or above. Some species of *Scenedesmus* may be highly polymorphic in culture with variation induced by various culture conditions. Polymorphism has been studied for about 100 years but remains incompletely understood. Variability includes cell number per coenobium, cell arrangement, cell size and especially expression of ornamentation (spines, ribs, granulation). Some species change the ultrastructural features of their cell walls from generation to generation based on nutrition. Species are distinguished mostly based on differences in cell size and shape, coenobial morphology and patterns of cell wall ornamentation. Some species of *Scenedesmus* are produced in mass culture and used as food because of their protein and mineral content, or used for other purposes in biochemical industry.

A. Observe a prepared slide of *Scenedesmus* at 10 and 40x magnification.

What is the overall shape of the cells? Are the organisms on the slide single celled or colonial? Prepare a drawing of the organisms highlighting the cell shape, organization and the absence/presence of mucilage.

Are there any other identifying characteristics regarding these organisms, such as spines? What would spines be used for in these organisms (look at larger magnification of the spines and see whether you find any internal structures or whether the spines are “empty)? Would you imagine these organisms to move actively (like *Chlamydomonas*) or passively?

Can you see the cell nucleus, the chloroplast(s) and the pyrenoid and distinguish between them? What are the differences to other organisms such as *Chlamydomonas*?

Human uses and issues with *Scenedesmus* – Freshwater representatives of *Scenedesmus* are used in sewage purification processes, since they will provide oxygen for the bacterial breakdown of organic matter, which in turn helps to eliminate other harmful substances. In the marine environment this positive collaboration between bacteria and chlorophytes may actually have a
negative impact. *Scenedesmus* in marine environments may provide oxygen to metabolic bacterial processes in coliform bacteria (any suggestions what coliform bacteria are and how they get into the marine environment), which may lead to increases in bacterial populations and contaminations of marine bivalves as well as the closure of their fisheries.

4. *Pediastrum* – A colonial chlorophyte, comprising 4-64 (-128) celled coenobia. The cells are 15-400 µm diameter, arranged in a flat, circular to oval plate, one cell thick. If 16 or more cells, cells tend to be in concentric rings; each ring with definite number of cells; disc continuous or with perforations between cells. Cells walls are smooth, finely reticulate or highly granulate. Cells are generally multinucleate with a single, diffuse chloroplast and one or more pyrenoids per cell. Asexual reproduction occurs mostly via coenobial formation. Every cell in a colony is capable of coenobial formation but this process is rarely synchronous. Zoospores are released from parental cell wall in a vesicle that persists throughout period of swarming and shortly after a new coenobium is formed. Asexual reproduction by thick-walled resting spores may occur in old cultures. These resting spores are spherical, 8-50 µm in diameter and orange with roughened cell walls. After transfer to fresh medium the spores turn green and produce zoospores. Cells in the initial coenobium are spherical. Sexual reproduction in *Pediastrum* has only been reported infrequently. Isogamous, spindle-shaped, biflagellate gametes (2.5-8 µm) are equipped with single chloroplast and eyespot. The cells walls are unusual among green algae in having a significant component of silicon. Species are distinguished based on cell size and shape, especially peripheral cells, and colony morphology.

A. Make a wet mount of live *Pediastrum* cultures and observe them at 10x and 40x magnification.

Compare the cell structure to those of the remaining unicellular and colonial chlorophytes. Is there a difference in cell at the center of the colony and the edge? What is the reason for these differences?

Compare the structure of the live *Pediastrum* to the live desmids (Chlorophyta, Charophyceae). What do these structures tell you about using morphology as a sole characteristic to base algal relationships on?

After you have observed the live, motile cells place a drop of Lugol’s near the edge of one side of the coverslip and a paper towel near the opposite edge to draw the solution under the coverslip. Though the Lugol’s will kill the cells it will aid you to see the pyrenoid. What is the location of the pyrenoid?

5. *Pandering* – A colonial chlorophyte with ellipsoidal to spherical, often flagellated colonies, mostly of 8 or 16 *Chlamydomonas*-like cells of similar size, arranged in a common gelatinous matrix. The colonial boundary is smooth, part of it penetrating between cells into the central region of colony forming individual sheaths. Colonies are much larger than the unicellular *Chlamydomonas* and avoid sinking by rolling movements produced by the beating of each individuals’ flagellae. The cells are mostly closely packed, flattened by mutual compression and contiguous in the center (any intercellular connections resulting from incomplete cleavage). The cells are obovate or wedge-shaped or more rounded, bearing 2 equal flagella. Chloroplasts are
generally cup-shaped, sometimes longitudinally striated, with one to several pyrenoids. Eyespots may be present and larger in anterior cells. The nucleus is central. Two contractile vacuoles are located on the anterior end. Sexual reproduction is isogamous or anisogamous. Reproduction of new colonies occurs by mitosis of each individual cell of the old colony producing a miniature replica of itself. Therefore, in 16-celled colonies, 16 new miniature colonies, each consisting of 16 cells may be produced asexually. Colonies of this type are called coenobia (coenobium = singular) and the reproductive process is called autocolony formation. A coenobium is defined as a colony with the cell number fixed at origin and will not increase or decrease subsequently.

A. Obtain a prepared slide of *Pandorina* and locate and study a coenobium (colony) under 10x and 40x magnification.

Draw a diagram of a coenobium of *Pandorina* as it appears on the slide. Compare the individual cells of the colony to the unicellular *Chlamydomonas*.

How many daughter colonies can this one colony produce through autocolony production? How do you determine this?

B. Make a wet mount of living *Pandorina* cultures in a depression slide in order to observe the movements of the cultures.

How do they move? What is the directionality and ability depending on light intensity? Do you detect a difference depending on light intensity?

Can you determine shapes of the individual chloroplasts? Are the shapes the same within the different cells of a colony.

6. *Tetraspora* - Spherical to amorphous or highly elongate cylindrical to irregular colonies with tens to thousands of cells embedded in mucilaginous matrix 10-40 µm thick. Some species may appear saccate or perforate when mature. Cells are scattered or arranged in groups of 2 or 4 often with sheath boundaries remaining distinct within the wall matrix, although becoming more confluent as each cell generation produces more mucilage. Cells are spherical to oval 6-12 µm in diameter with a pair of anterior pseudoflagellae. In some species pseudoflagellae project beyond the colony’s envelope. Cells are uninucleate with two anterior contractile vacuoles, a single cup shaped chloroplast and a basal pyrenoid. Stigma are generally absent. Colony growth is accomplished by mitotic division of cells. Mitosis is similar to *Chlamydomonas* with cytogenesis via typical phycoplast. Tetraspora may be benthic (attached or unattached) or planktonic, mostly in freshwater lakes and ponds and brackish estuaries. The genus is cosmopolitan although some species apparently with restricted distribution. Species may be distinguished based on colony morphology, structure of mucilaginous matrix and form of starch sheath around pyrenoids.

A. Observe the colony structure of *Tetraspora* on a prepared slide. Observe the cell clusters within the entire large colony. What clustering do you observe? Draw a picture of the clusters and observe and note the cell shape. Determine the shape and size of the mucilage surrounding the cell cluster.
Can you visualize the presence and shape of the chloroplast? Make a drawing of the chloroplast in comparison to the overall size of the cells.

*Tetraspora* may also have anthropogenic uses. Polysaccharides extracted from certain species of *Tetraspora* have been observed to have antibacterial or antifungal properties.

7. *Hydrodictyon* - coenobial, macroscopic, mostly 20 (-40) cm in length when mature. Comprised of cylindrical or broadly ovoid cells up to 1 cm long when mature. The cells are joined at the ends to form a cylindrical net closed at poles. Within network, individual net units are comprised of 3-9 (mostly 6) cells, with cell number species specific. Cells are highly multinucleate, chloroplasts form a parietal reticulum around a large central vacuole and pyrenoids are numerous. Nuclei are generally dispersed but aggregations are associated with localized growth. *Hydrodictyon* is a large coenobium similar to *Tetraspora*.

A. Observe the prepared slides of *Hydrodictyon* under 10x and 40x magnification. What are the differences in the cell shape and the overall coenobial structure between *Hydrodictyon* and *Tetraspora*.

8. Chloroplast comparison – use some of the prepared slides of the freshwater algae *Draparnaldia, Spirogyra* and *Oedogonium* to visualize the differences in chloroplast structures. What are the shapes of the chloroplasts in these organisms?

II. Trebouxiophyceae

This is another class within the division chlorophyta but the circumscription of these algae is not well established on a light microscopic level though the majority of these algae is found in freshwater and terrestrial habitats. Trebouxiophyceans generally occur as non-flagellate uni-cells or colonies, which possess a unique combination of plesiomorphic (sharing an character state with an ancestral clade) ultrastructural features, counterclockwise flagellar basal bodies, non-persistent metacentric spindles and the presence of a phycoplast at cytokinesis.

1. *Chlorella* - Cells are spherical or ellipsoid, single or forming colonies with up to 64 cells, mucilage present or absent. Chloroplasts are single, parietal, a pyrenoid is present and surrounded by starch grains. Organisms are planktonic, edaphic (soil dwelling) or endosymbiotic.

A. Prepare a wet mount of *Chlorella* cells and examine them first under 10x and then 40x magnification.

What features of this organism would allow it to be planktonic? List all that apply.

After you have observed the live cells place a drop of Lugol’s near the edge of one side of the coverslip and a paper towel near the opposite edge to draw the solution under the coverslip. Though the Lugol’s will kill the cells it will aid you to see the pyrenoid. Notice the location of the pyrenoid?
Prepare a complete drawing of a *Chlorella* cell including the cell outline, chloroplast and pyrenoid.

*Chlorella* has been used as an alternate food source or food supplement due to its high amount of protein and other essential nutrients. In addition, because of its ability to bind with mercury, lead, and cadmium, *Chlorella* has been increasingly used in alternative medicine to detoxify the body of certain heavy metals.

III. Ulvophyceae
Most representatives of the Ulvophyceans are marine, though few occur in freshwater and terrestrial habitats. Body types may be flagellate/non-flagellate unicells and colonies, branched and unbranched filaments, membranous sheets and coenocytes. The Ulvophyceans are characterized by the following ancestral characteristics: Closed mitosis, persistent spindles and furrowing at cytokinesis, flagellar bases that are counter-clockwise oriented and potentially flagellated gametes.

1. *Ulothrix* - Filaments flaccid, basically unbranched and uniseriate green algae. Cells are always closely adherent. The cell wall in juveniles thin and smooth while more thickened and sometimes roughened later on. Apical cell are rounded and sometimes slightly narrowed. Attachment occurs by simple or rhizoidal basal cells, sometimes by rhizoidal outgrowths projecting from both intercalary and apical cells. Cells are cylindrical and uninucleate with a single chloroplast that is parietal, napkin ring-shaped, partially or fully encircling cell circumference, usually lobed and not enclosed in young cells, usually more irregular and sometimes fully closed in mature cells. There is a single pyrenoid in more mature cells, surrounded by a thin or more conspicuous starch envelope. Asexual reproduction occurs by quadriflagellate zoospores, arising in each cell. Zoospores are positively phototactic, oval, enclosing a cup-shaped parietal chloroplast with distinct stigma. Sexual reproduction occurs by isogamous biflagellate gametes, arising in all differentiated cells. Filaments produced gametes that often coiled and yellowish-green. Life histories are basically heteromorphic and diplobiotic with multicellular filamentous gametophyte and generally a unicellular sporophyte. *Ulothrix* is cosmopolitan with wide ecological distribution especially in temperate and colder regions. It usually forms strands several cm in length with extensive populations forming tufts or mats of varying size, often in green belt with distinct seasonality. They are present in aerated localities such as shores of eutrophic lakes and are less abundant in stagnant waters such as ditches and pools, and almost absent in boggy habitats. *Ulothrix* also flourishes in brackish areas with extreme (daily) variation in environmental factors, e.g. near mouth of intertidal freshwater streams where plants are covered with seawater at high tide, in estuaries or tidal rivers, in supralittoral pools of rocky shores, and supralittoral pools exposed to freshwater drip. In marine habitats forming extensive sheets in upper fringe of littoral zone; in mid and low littoral zone of rocky shores often present as important component of pioneer vegetation. *Ulothrix* grows on hard substrata but is also abundant in salt marshes on soft bottoms.

A. Examine a stained, permanent mount with your 10x objective lens. You should be able to find vegetative and both zoospore- and gamete-producing filaments in the same preparation.
How can you distinguish between vegetative and zoospore- or gamete-producing cells? Draw and label all three filaments.

B. Switch to the 40x magnification and study both a vegetative and zoospore-producing cell.

Draw a single vegetative cell to show the following: nucleus, plastids, pyrenoids, terminal vacuoles and label the contents.

How can you distinguish zoospore-producing filaments from gamete-producing filaments? See the review of the *Ulothrix* life history.

2. *Rhizoclonium* - Filaments are very slender (60 µm in diameter) and loose lying with basal cells, or attached by holdfast with basal lobes. Plants unbranched or with one- to few-celled rhizoidal laterals. Cells are several to many times longer than broad. There are numerous nuclei per cell and chloroplast are reticulate, parietal, with pyrenoids, often densely packed with starch. Reproduction is done by fragmentation. *Rhizoclonium* is a cosmopolitan species in fresh, brackish and marine waters, often growing entangled with other algae and forms dense mats in salt marshes.

A. Examine the prepared and stained slides of *Rhizoclonium*. Draw a vegetative cell at 10 and 40X and observe and label the general outline as well as the organelles (nucleus, chloroplast and pyrenoid). What are differences between *Rhizoclonium* and *Ulothrix*?

3. *Cladophora* – With thalli of uniseriate branched filaments with apical and/or intercalary growth. Branches are sparse to profuse with branches inserted laterally below the apex of a cell or apically on cell. If attached, branching rhizoids arise from basal cell and other cells in basal region, or simple discoid holdfast produced. Chloroplasts parietal, either densely packed discoid and/or united in a reticulum. Pyrenoids are present in multiple chloroplasts and of bilenticular shape, flanked by two bowl-shaped starch bodies. Cells are multinucleate with nuclei dividing more or less synchronously with the nuclear membrane remaining intact. The cell wall is mainly composed of crystalline cellulose I, forming numerous lamellae of microfibrils in a crossed fibrillar pattern. Asexual reproduction by biflagellate or quadriflagellate zoospores is the only method of reproduction in some species while other species only reproduce by thallus fragmentation. *Cladophora* is cosmopolitan in temperate and tropical regions, occurring in freshwater, brackish and marine conditions. The various species are found intertidally in wave-exposed to very sheltered habitats, brackish pools, lagoons and mudflats, and in more or less eutrophic freshwater streams and lakes with pH > 7.

A. Examine the prepared and stained slides of *Cladophora*. Draw a vegetative cell at 10 and 40x magnification and observe and label the general outline as well as the organelles the chloroplast and pyrenoid.

After you have observed the live, motile cells place a drop of methylene blue near the edge of one side of the coverslip and a paper towel near the opposite edge to draw the solution under the coverslip. Though the methylene blue will kill the cells it will aid you to see the nucleus (nuclei).
What are differences to *Rhizoclonium* and *Ulothrix*?

4. *Acetabularia* – The thallus is an unbranched unicell (1-6 cm long), composed of a compact rhizoid, a tubular stalk ca. 1 mm diam., whorls of thrice-branched sterile laterals and a more or less flattened apical cap (0.5-1.5 cm in diameter). The mature cap is composed of 30-75 free or joined, terminally tapered or rounded elongate rays. The thallus may be lightly to heavily calcified. Thallus size, cap diameter and cap morphology are important species characteristics. Numerous parietal, discoid, grass green plastids without pyrenoids circulate in the peripheral cytoplasm that surrounds a large central vacuole. The chloroplast ultrastructure may be variable, without or with stacked grana and variations are generally species specific. Starch grains are present in the chloroplast and cytoplasm, the latter not membrane bound. *Acetabularia* distribution is pantropical and subtropical, commonly in brackish to hypersaline shallow waters. Thalli are firmly attached to solid substrates such as stones, coral rubble, shells as well as to wood and industrial detritus such as rubber. Seasonal life history is variable, especially at the cool extremes of the range. The development, nucleo-cytoplasmic interactions, biochemistry, photobiology, genetics and molecular biology of Acetabularia species have been extensively studied. Changes in microtubules and microfilaments associated with key phenomena during growth and differentiation. Their taxonomy within the Ulvophyceans remains uncertain.

A. Observe a live representative of *Acetabularia*. Observe and draw the rhizoid, stalk (with hair whorls) and the cap. Where would you expect to find the nucleus of the unicellular organism?

What is a difference between this unicell and the unicells we have previously observed?

B. Take a section of the rim of the cap and prepare a wet mount. You should be able to see a divided cell obscured by chloroplasts but with some bright, oval structures. Unlike in other unicells these organelles are not pyrenoids but cysts. Examine the lifecycle of *Acetabularia* present as a drawing to understand the significance of these cysts in the organism’s lifecycle.

What do you think might activate the cysts to germinate? Why? (Make sure that you check the answer with your lab instructor)

5. *Ulva* – The mature thallus consists of a flattened distromatic blade in which two cell layers are developmentally independent but closely adherent. Blades can be broadly expanded, irregularly lobed, cuneate, linear, lanceolate or deeply divided into linear lacinae. Growth of the blade occurs through diffuse cell divisions primarily along the margins. *Ulva* is usually attached to substrate by rhizoidal cells in a basal holdfast and/or rhizoidal extensions of cells in the lower portion of the blade or occasionally extending along part or the entire longitudinal axis of the blade. Rhizoidal extensions run between the two cell layers of the blade. Vegetative cells contain a single chloroplast and 1 or more pyrenoids and are generally uninucleate. In opposition rhizoidal cells are often multinucleate. *Ulva* is a cosmopolitan genus with species in all oceans and estuaries of the world. Species of Ulva have traditionally been based on morphological, anatomical and cytological characteristics such as shape, size, presence or absence of dentation,
thickness, cell dimensions and number of pyrenoids. Many studies have shown that these characteristics can be highly variable within species, varying with age, reproductive state, wave exposure, tidal factors, temperature, salinity, light and biological factors such as grazing. In recent years developmental patterns in culture, reproductive details and the apparent inability of species to interbreed have been used to evaluate species concepts based on morphological and anatomical characteristics.

A. Make a cross section of a piece of *Ulva* and prepare a wet mount. What is the cell arrangement in *Ulva*? What is the cell arrangement in *Ulva*? Draw a general outline of an *Ulva* cross section?

What is the chloroplast location in an *Ulva* leaf?

*Ulva* has a very high surface to volume ratio due to the fact that it is only a few cell layers thick. What might be the advantage of a high surface to volume ratio?

6. *Codium* – The thallus is spongy, anchored to rocks or shells by a weft of rhizoids, varying in size from 1 cm. to 10m long. Their habit varying widely their form may be digitaliform, globular, petaloid, membraniform, or dichotomously branched. The internal structure is composed of a colorless medulla of densely intertwined siphons and a green palisade-like layer of vesicles called utricles. Organelles, including innumerable nuclei and discoid chloroplasts (but no amyloplasts) are confined to a layer of cytoplasm pressed to the cell wall. Chloroplasts lack pyrenoids. Despite the ubiquity of Codium very little is known about its biology. Codium grows intertidally, and to at least -40m. It occurs in all marine waters except the Arctic and Southern Oceans. The largest numbers of species are found in floras that are transitional between temperate and subtropical, namely Japan, South Africa, Australia and California-Mexico. Each of these floras includes a member of most sections of the genus, indicating an ancient dispersal of the progenitors. Because of lack of calcification, there is no fossil record of the genus.

A. Take a thin section of a blade of *Codium*. Observe the multinucleate filaments and distinguish the medulla from the surrounding cortex. How do they differ? Why is their coloration as it is and not reversed?