The Cyanobacteria or “blue-green algae”

The cyanobacteria, also known as cyanophyta due to their photosynthetic properties, were once viewed as primitive algae, also referred to as blue-green algae. However, the absence of a nucleus and circular arrangement of the DNA throughout the cell place them firmly within the Subkingdom Prokaryota. The cyanobacteria are therefore closely related to the bacteria (Division Schizonta) but are differentiated from the Schizonta by their ability to photosynthesize and their utilization of chlorophyll a. These features link the cyanobacteria to both the bacteria and the algae. The occurrence of accessory pigments such as phycobilins (red) and carotenoids (orange, yellow) in addition to the green chlorophyll a generally impart a blue-green appearance on the cells. Pigment percentages and distributions may also in rare instances produce species that range from dirty yellow to red, or dark brown to black. Whatever their color, cyanobacteria are photosynthetic, and so can manufacture their own food.

Similar to other bacteria the cyanos are ubiquitous due to their small size and resistant prokaryotic cellular organization. Explosive growths, mats or blooms are frequently found in soil and aquatic environments and some cyanobacteria may be toxic (Anabaena, Lyngbya and Nostoc) while others are used in nutrient supplements (Spirulina). Unlike other bacteria or algae the cyanobacteria are one of very few groups of organisms that can convert inert atmospheric nitrogen into an organic form, such as nitrate or ammonia. It is these "fixed" forms of nitrogen that plants need for their growth, and must obtain from the environment. Nitrification cannot occur in the presence of oxygen, so nitrogen is fixed in specialized cells called heterocysts. These cells have an especially thickened wall that contains an anaerobic environment. Diversity of morphology may be seen among the 150 extant genera, though this diversity is reasonable subtle compared to other organisms. Cyanobacteria may be uni-cellular or colonial and both versions may show a covering of mucilage. Colonial cyanobacteria may be filamentous (simple, mixed and branched) and in rare occasions form irregular or cuboidal cell aggregates and in same cases plates or sheets.

1. *Gloeocapsa* – a few-celled colonial cyanobacterium occurring in either single (rarely) or clustered cells enclosed in concentric layers of mucilage. Typically, clusters of two to four are seen. These organisms are predominantly terrestrial though there are also aquatic species (*Gloeocapsa crepidinum*).

2. *Oscillatoria* – Genus of blue-green algae most common in freshwater environments with some marine species (generally genus *Trichodesmium*). This unbranched filamentous alga, occurring singly or in tangled mats particularly found in polluted environments, derives its name from its motion, which is thought to result from a secretion of mucilage that pushes the filament away from the direction of excretion. Reproduction is by fragmentation in which dead concave cells (separation disks) separate sections of the filament (hormogonia). When present, the mucilage sheath is very thin. *Oscillatoria* [Trichodesmium] erythraea an unusual nitrogen fixing cyanobacterium that does not produce heterocysts.

3. *Spirulina* – An undifferentiated, helical filamentous cyanobacterium found in freshwater and marine waters. Previously this organism was thought to be uni-cellular because microscope resolution could not distinguish cellular cross-walls. Spirulina is a type of blue-green algae that is rich in protein (45-65% dry weight), vitamins, minerals, and carotenoids, antioxidants that can help protect cells from damage. It contains nutrients, including B complex vitamins, betacarotene, vitamin E, manganese, zinc, copper, iron, selenium, and gamma linolenic acid (an
essential fatty acid) and therefore provides a food source or nutritional supplement.

4. *Gloeotrichia* - Filamentous - colonial; trichomes heteropolar with basal heterocytes and apical hair-like ends with own sheaths, united radially into gelatinous, hemispherical or spherical colonies, which are microscopic up to several cm in diameter, olive green, yellow-green, brown or dark blue-blackish. The whole colony enveloped by a fine or firm slime; trichomes always oriented with heterocytes into the centre of the colony. Trichomes rarely false branched (during trichome division), the branches separate soon from the mother trichome, but remain parallel and radially located within the colonial slime and form their own gelatinous sheaths. Colonies are joined to the substrate or free floating. Trichomes uniserial, rarely with intercalar heterocytes (developing before or during trichome disintegration), constricted or unconstricted at the cross walls, more or less straight or coiled. Sheaths are always present, but sometimes gelatinize within the mucilage of colonies, especially near apical parts of trichomes (near the margin of a colony).

5. *Anabaena* – a differentiated, filamentous genus of nitrogen-fixing blue-green algae with beadlike or barrel-like cells and interspersed enlarged spores (heterocysts), found as plankton in shallow water and on moist soil. There are both solitary and colonial forms, the latter resembling a closely related genus, *Nostoc*. In temperate latitudes during the summer months, *Anabaena* may form water blooms and can be used as an indicator of pollution state of an aquatic habitat. *Anabaena* trichomes resemble a “pearl necklace” with the vegetative cells of similar size. In addition, you may find larger, empty looking cells, heterocysts, may be visible. If the colony you are looking at is aging you may also find another cell type, the akinetes, which are larger than the vegetative cells and yellow to reddish in color.

6. *Scytonema* – filamentous, thallous cyanobacterium forming solitary branched filaments or mats on the substrate. Filaments free or in fascicles, sometimes densely coiled, creeping on the substrate or with erected branches, commonly falsely branched, with one or (obligatory) two lateral branches. Branching initiates after trichome disintegration by help of necridic cells between two heterocytes, usually not at heterocytes; both branches grow parallel, aside or in crossing position; the filaments make sometimes typical loop-like lateral formations before branching, in which tops of the trichomes later divide. Trichomes isopolar, cylindrical, not diversified in basal and apical parts, uniseriate, usually with solitary heterocytes, constricted (rarely not constricted) at cross walls; terminal parts of branches cylindrical or slightly widened, with rounded apical cell; middle parts of trichomes sometimes with elongated, cylindrical cells. Sheaths firm, limited, parallel or diverging, lamellate, usually yellow-brown (colored by scytonemin) in some parts. Cells pale or olive-green, usually with solitary, irregularly disposed granules or with granular content, rarely yellowish or pinkish colored; apical cells sometimes with large vacuoles. Heterocytes intercalar, solitary, rarely in pairs, cylindrical or barrel-shaped. Akinetes were several times mentioned, but not proved and well described.

7. *Stigonema* - filamentous thallous cyanobacterium; thallus wooly or crusty, composed from free, coiled, true branched filaments, usually attached to the substrate, not diversified distinctly in basal filaments and branches. Trichomes two or multiseriate (only in young trichomes and at ends of branches uniseriate), sometimes very thick, irregularly laterally true branched (T- and V-type of branching), irregularly coiled, sometimes narrowed (with less number of cell rows)
towards the ends, apical cell is sometimes smaller than the other ones. Sheaths thin or thick, limited, later wide, lamellate and usually yellowish-brown; around cells in old parts of filaments sometimes special envelopes (trichomes disintegrating in separated cells within filaments). Cells barrel-shaped or roundly irregular, connected usually by one pore ("pit connections") one with another, which disappear in some segments of trichomes; cell content blue-green or olive green, usually with prominent solitary granules. Heterocytes intercalar, rarely lateral, in a similar form as neighboring vegetative cells. Akinetes not known. Sometimes chroococcoid cell clusters arise.

8. *Rivularia* - heteropolar filaments, differentiated into basal and apical parts, simple, joined parallel into firm strata. At the beginning of the vegetation cycle hemispherical or spherical, later sometimes vast, flat, macroscopic, irregular strata, up to several cm or dm in diameter and several mm thick strata are layered (sometimes with several layers), with densely agglomerated trichomes, oriented by their bases with heterocytes to the substrate and by the apical hair-like parts to the surface of the colony. Strata are gelatinous up to leathery or intensely incrusted by calcium carbonate, always covering the substrate. Trichomes +/- cylindrical, constricted or unconstricted at the cross walls, dividing at intercalar heterocyst: the divided trichomes separate one from another, but remain parallel located within the mother sheaths in the colony. Hairs are composed from the narrow, long, hyaline cells. Sheaths firm, sometimes lamellate, colorless or yellow-brown, enveloping all the "daughter" trichomes.

9. *Nostoc* – filamentous, thallous cyanobacterium. The thallus may be micro- or macroscopic, gelatinous, amorph or spherical, later irregularly spherical, lobate, smooth or warty on the surface, filamentous or forming flat gelatinous or "paper-like" (when dry) colonies, usually with distinct periderm on the colonial surface. Filaments within colony irregularly coiled and loosely or densely agglomerated, sometimes more gathered in peripheral layer; sheaths around trichomes present, but visible usually only in the periphery of colony or in young colonies, wide, fine mucilaginous, confluent with colonial mucilage, sometimes yellowish-brown. Trichomes isopolar, of the same width along the whole length, apical cells morphologically not different from other cells; cells cylindrical, barrel-shaped up to almost spherical (forming moniliform trichomes; variability of cell-size and -shape sometimes distinct in one and the same species). Heterocytes solitary, develop in trichomes terminally or intercalary. (their frequency or absence is dependent on nitrogen metabolism); trichomes in principle metameric. Akinetes arise apoheterocytic, oval, little larger than cells; almost all cells between heterocytes change successively in akinetes towards heterocytes. Nostoc has special life (vegetation) cycle, during which forms several special and characteristic stages.
The Phytoplankton: Euglenophyta, Pyrrhophyta and Stramenopiles

Organisms termed planktonic include both “plant” and “animal”-like organisms and span from the Monera (cyanobacteria), Protista (euglenophyta, pyrrhophytae and stramenopiles) into the Plantae (chlorophyta). Since the surface of the earth in its majority is covered in marine/brackish waters (more than 70%) these types of life forms are innumerable.

In this course we will, of course, cover the autotrophs or phytoplankton, which is responsible for the genesis of approximately 70% of the world’s atmospheric oxygen. For these organisms to inhabit the open water, it is pertinent that they remain in the upper waters that have the highest light intensity (euphotic zone). However, since the euphotic zone is prone to turbulence (induced by wind, tide, etc.) remaining in the euphotic zone is an active process. It may be accomplished by many adaptive mechanisms alone or in combination, including flagellation, microscopic size (high surface area/volume, sinking increases with increased diameter), adaptive cell shape and reduced cell densities (lighter). Therefore the majority of organisms in the plankton are unicellular. The most prevalent in the marine phytoplankton are the dinoflagellates (10⁻⁸⁰ µm) and the diatoms (20⁻¹⁰⁰ µm). Morphological designs that deviate from the regular centric or spherical shape reduce sinking rates, which is especially obvious in the diatoms. Other unrelated algal groups such as the dinoflagellates and euglenoids have evolved lighter, “naked” or unarmored unicellular organisms, some of which are capable of shape changes. Additionally, large nutrient quantities and other optimal conditions will lead to generation times between 4-24 hrs which may be translated into changes in cell shape, size or densities. Many species also include lifecycle stages that are induced during less than optimal environmental conditions. These stages, i.e. cysts, will sink, become dormant and “germinate” following a sediment upwelling allowing these germinated stages to surface again.

I. Euglenophyta – a small phylum (division) of the kingdom Protista, consisting of mostly unicellular aquatic algae. Living in fresh and marine waters many are flagellated and therefore motile. The outer part of the cell consists of a firm but flexible layer called a pellicle, which cannot properly be considered a cell wall. Some euglenoids contain chloroplasts that contain the photosynthetic pigments chlorophyll a and b, as in the phylum Chlorophyta; others are heterotrophic and can ingest or absorb their food. Food is stored as a polysaccharide, paramylon. Reproduction occurs by longitudinal cell division. The most characteristic genus is *Euglena*, common in ponds and pools, especially when the water has been polluted by runoff from fields or lawns on which fertilizers have been used. There are approximately 1,000 species of euglenoids.

1. *Euglena* – is a common unicell found in the planktonic zone particularly of stagnant waters. *Euglena* is a protist that can both eat food as animals by heterotrophy; and can photosynthesize, like plants, by autotrophy. When acting as a heterotroph, the *Euglena* surrounds a particle of food and consumes it by phagocytosis. When acting as an autotroph, the Euglena utilizes chloroplasts, (hence green color) containing Chlorophyll a, Chlorophyll b, and some carotenoid pigments, to produce sugars by photosynthesis. Each chloroplast has three membranes that exist in thylakoid stacks of three. The number and shape of chloroplasts within the euglenophyta varies greatly due to environmental conditions and evolutionary history. *Euglena* are able to move through aquatic environments by using a large flagellum for locomotion. To observe its environment, the cell contains a red eyespot (stigma), a primitive organelle that filters sunlight into the light-detecting, photosensitive structures at the base of the flagellum allowing only
certain wavelengths of light to hit it. This photosensitive area detects the light that is able to be transmitted through the eyespot. When such light is detected, the *Euglena* may accordingly adjust its position to enhance photosynthesis. The mobility of Euglena also allows for hunting capability, because of this adaptation, many *Euglena* are considered mixotrophs: autotrophs in sunlight and heterotrophs in the dark. *Euglena* also structurally lack cell walls, but have a pellicle instead. The pellicle is made of protein bands that spiral down the length of the *Euglena* and lie beneath the plasma membrane. Reproduction is completely asexual, following intra-nuclear mitosis. As is typical of animals rather than plants, cytoplasmic division occurs by longitudinal furrowing of the protoplasm beginning at the anterior end of the organism (flagellum). *Euglena* can survive in fresh and salt water. In low moisture conditions, a *Euglena* forms a protective wall around itself and lies dormant as a spore until environmental conditions improve. *Euglena* can also survive in the dark by storing paramylon granules in pyrenoid bodies within the chloroplast.

2. *Phacus* - oval-shaped or spherical cells, often flattened and leaf-like. Some species may be twisted throughout the cell (*Phacus helikoides*) or only at the cell posterior (*Phacus tortus*). The pellicle is quite rigid and is composed of wide proteinaceous strips that prevent the elastic metabolic movements seen in *Euglena* and other euglenoids. The cells instead move by gliding and swimming with their single emergent flagellum. The chloroplasts may be small and spherical without pyrenoids, or large and discoidal with pyrenoids present. The cytoplasm of euglenoids contains many paramylon starch storage granules, which are usually donut-shaped in *Phacus* cells. Like other euglenoids, *Phacus* cells have contractile vacuoles and may have a red-pigmented stigma to sense light.

II. Pyrrhophyta - The division Pyrrhophyta (from the Greek "pyrrhos" meaning flame-colored) comprises a large number of unusual algal species of many shapes and sizes. There are about 130 genera in this group of unicellular microorganisms, with about 2000 living and 2000 fossil species described so far. The name "dinoflagellate" refers to the forward-spiraling swimming motion of these organisms. They are free-swimming protists (unicellular eukaryotic microorganisms) with two flagella, a nucleus with condensed chromosomes, chloroplasts, mitochondria, and Golgi bodies. Biochemically, photosynthetic species possess green pigments, chlorophylls a and c, and golden brown pigments, including peridinin. Dinoflagellates primarily exhibit asexual cell division, some species reproduce sexually, while others have unusual life cycles. Their nutrition varies from autotrophy (photosynthesis; in-nearly 50% of the known species) to heterotrophy (absorption of organic matter) to mixotrophy (autotrophic cells engulf other organisms, including other dinoflagellates). Free-living dinoflagellates are an ancient and successful group of aquatic organisms. They have adapted to pelagic (free-floating) and benthic (attached) habitats from arctic to tropical seas, and to salinities ranging from freshwater, to estuaries, to hypersaline waters. Many species are found in numerous habitats, living in the plankton or attached to sediments, sand, corals, or to macroalgae surfaces or to other aquatic plants. Some species are present as parasites in marine invertebrates and fish. Some even serve as symbionts, known as *zooxanthellae*, providing organic carbon to their hosts: reef-building corals, sponges, clams, jellyfish, anemones and squid. Dinoflagellates exhibit a wide variety in morphology and size (from 0.01 to 2.0 mm). They commonly have a cell covering structure (theca) that differentiates them from other algal groups. Cells are either armored or unarmored. Armored species have thecae divided into plates composed of cellulose or polysaccharides, which are key features used in their identification. The cell covering of unarmored species is comprised of a membrane complex. The theca can be smooth and simple or laced with spines,
pores and/or grooves and can be highly ornamented. In systematics, dinoflagellates have been claimed by both botanists and zoologists. Dinoflagellates share features common to both plants and animals: they can swim, many have cell walls, and both photosynthetic and non-photosynthetic species are known. Botanists have grouped them with the "microalgae" and zoologists have grouped them with the protozoa, and both have produced classification schemes for this diverse and confusing group. Dinoflagellates have attracted a lot of negative attention from the general public in recent times. For example, blooms (population explosions) of dinoflagellates can cause the water to turn a reddish-brown color known as "red tide". Red tides can have harmful effects on the surrounding sea-life and their consumers. Additionally, certain species of dinoflagellates produce neurotoxins. These toxins are carried up the food chain, ultimately to humans and can, sometimes result in permanent neurological damage or even death. Yet dinoflagellates are important members of the phytoplankton in marine and freshwater ecosystems.

1. *Peridinium* – Although most dinoflagellates are marine, *Peridinium* is a biflagellate unicell that may live in both freshwater and marine environments. *Peridinium*, like many dinoflagellates, is heterotrophic with autotrophic capabilities. Therefore, the organisms contain many small chloroplasts, several carotenoids (responsible for the reddish-brown color) and usually small drops of oil and starch grains alongside the chloroplasts. Like all other dinoflagellates, *Peridinium* has a mesokaryotic nuclear organization, chromosomes lack histones and remain permanently condensed. During cell division chromosomes attach to the nuclear envelope, replicate and separate without the production of mitotic spindles. These primitive nuclear features are not visible unless special stains were employed. At the light microscopy level *Peridinium* is most readily recognized by its “armor” (you might think of them as fierce, armored plankters) and flagellar position. The armor, composed of cellulosic plates, may bear various types of ornamentation. The two flagellae emerge through a lateral pore (unlike the apical flagellum in euglenoids) with one flagellum extends as a cingulum in a groove called the girdle surrounding the organism. The other, shorter flagellum, extends posteriorly in a groove referred to as the sulcus. The two flagellae are both synchronized to direct the organism’s movement with the cingular beat propelling the cell forward and the shorted flagellum “steering” the cell. Though most dinoflagellates lack the light sensitive stigma, *Peridinium* is an exception and possesses a plastid bound eyespot near the location of the flagellar emergence. *Peridinium* reproduces both sexually and asexually. During sexual reproduction the theca or armor is being discarded (similar to a molting process) after which nuclear and cytoplasmic divisions occur. Sexual reproduction is rarely observed and involves zygotic meiosis.

2. *Ceratium* – one of the most spectacular dinoflagellates, they are covered with an armor-like cell wall, made out of polysaccharide. The most distinguishing characteristic are the spines (also known as horns), which increase the organisms surface: volume ration. The arms help *Ceratium* float, but prevent them from moving very quickly. Another important feature is that they contain small plasmids (minicircles). *Ceratium* have two flagella. These wind around the cell body. The flagella each have different movements and shapes. The transverse flagellum beats in a spiral motion, while the longitudinal flagellum pulses in waves. Most *Ceratium* species also contain chloroplasts. Certain species are bioluminescent. Under adverse conditions, *Ceratium* are able to encyst themselves as a form of protection. *Ceratium* are mixotrophs, obtaining food both through
photosynthesis and phagocytosis. Asexual reproduction is most common in Ceratium. However, sexual reproduction is also possible, usually taking place under adverse conditions. Ceratium are aquatic organisms, living in both marine and freshwater environments. They are most common in temperate areas and are particularly common in the water of the North Sea, but they can be found all over the world. Unlike other dinoflagellate species such as Alexandrium, Ceratium are relatively harmless organisms. They are non-toxic, and are necessary for the food web. However, they can cause a red tide if conditions allow for excessive blooming. While this red tide is not toxic, it can deplete resources in its environment, causing strain on the ecosystem. In general, though, Ceratium are necessary components of their habitats. They serve not just as nutrients for larger organisms, but they keeps smaller organisms in check through predation.

3. Amphidinium - Small to large (<10-100 µm) unarmored free-living cell, predominantly motile, sometimes enclosed in a hyaline cyst. Cells globular to fusiform, laterally or dorsoventrally compressed. Epicone small and hypocone large, as the cingulum is located in the anterior part of the cell. Some cells have larger epicones. Cingulum circular or little displaced with the cingulum making one or a just over one turn. Sulcus extends from cingulum to antapex or posterior portion of the organism, in some species to apex. Typical dinokaryon located in the hypocone. Chloroplasts may be either present or absent. Nutrition is phototrophic or phagotrophic either by ingestion of whole particles or by myzocytosis (“cellular vampirism”. Chloroplasts of some species may be derived from cryptophycean endosymbionts. Cytoplasm is hyaline (glassy) or of various colors, dense granules may be present. Some species form temporary cysts. Vegetative reproduction is conducted by binary fission. Sexual reproduction known but rare. Some species produce unusual sterols, others toxic substances. Amphidinium is cosmopolitan, marine, brackish or freshwater, planktonic or sand-dwelling and some species may be endosymbiotic.

III. Stramenopiles - Stramenopiles are a "crown" taxon that evolved about 300 million years ago and radiated after the Cretaceous Period. They include both photosynthetic and nonphotosynthetic taxa. Photosynthetic members include brown seaweeds, diatoms, chrysophytes and several other groups varying in morphology from simple unicells to more highly complex structures. These autotrophic eukaryotes impact many of the earth's biogeochemical cycles (e.g. sulfur and nitrogen loading) and serve as primary producers that fix a significant portion of the total CO\(_2\) processed on earth. The stramenopiles represent a major eukaryotic group that is taxonomically distinct from the chlorophytic or rhodophytic lineages of autotrophs.

III.1 The Diatoms - Diatoms are single-celled organisms, which secrete intricate skeletons. Diatoms are one of the predominant autotrophic organisms, particularly in temperate water, with approximately 10,000 species of various morphologies described. Most taxa are unicellular and planktonic, though some may live attached to submerged vegetation and others form colonial aggregations. Diatom walls are composed of pectin material with large quantities of silica, the latter accounting for about 50% of the cellular dry weight. This skeleton is divided into two parts, one of which (the epitheca) overlaps the other (the hypotheca) like the lid of a box or petri dish. Observe the diatom frustule below at right, in which the two halves have been pushed slightly askew. Both epitheca and hypotheca are made up of two or more parts: the valve, a more or less flattened plate, and at least one cingulum, a hoop-like rim. As is visible in the photographs, both parts of a frustule may be highly perforated. Pennate diatoms show a long slit,
the raphe, along the long axis. Through the raphe, the living diatom secretes mucilage, with which it may attach to a substrate or move by gliding over the substrate. Within their silica walls, diatoms show a typical level of eukaryotic organization. Living diatoms contain several chloroplasts, where photosynthesis takes place. Diatoms may be of two body symmetries, either radial or bilaterally symmetric. Radial symmetry genera are called centric diatoms and are always associated with marine waters while bilateral symmetry is found in pennate diatoms, which may be found in both marine and freshwater habitats. Many diatoms are slightly asymmetrical, though they generally fall into one of these two categories. Diatom reproduction takes on different forms (it may be anisogamous with conjugation or oogamous) depending on the overall shape of the diatoms.
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 β 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.

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, Chlamydaella, 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 (*Chlamydaella, 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.

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.

3. *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*.

4. *Pandorina* – 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.

5. *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.

6. *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.

7. *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 cytokinesis 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 distributions. Species may be distinguished based on colony morphology, structure of mucilaginous matrix and form of starch sheath around pyrenoids.

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. *Chlorella* reproduces asexually.

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. *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.

2. *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.

3. *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.

4. *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.

5. *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 diplobiontic 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.

6. *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 laciniae. 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.

IV. Charophyceae

*Micrasterias* is a genus whose members are considered placoderm desmids, those that feature a cell wall composed of two sections that attach in the mid-region. This type of desmid is also characteristically furnished with pores, spines, granules, or other protuberances. Contrariwise, saccoderm desmids typically possess a smooth, unornamented cell wall that consists of a single piece. As placoderm desmids, the more than 40 known species of *Micrasterias* exhibit a substantial amount of diversity in the adornment of their lobed, disclike shapes, and many are among the most picturesque microscopic life forms in the world. Most often found in acidic waters and bogs, the organisms may grow between 80 to 200 micrometers in diameter. The majority of desmids are constricted in the center of the cell, creating two symmetrical semicells connected by an isthmus. Generally, located within the isthmus is the nucleus, and inside each semicell are one or more pyrenoids and chloroplasts. The chloroplasts enable photosynthesis and the pyrenoids are involved in the storage of starch. Reproduction of desmids may involve the sexual act of conjugation or may take place asexually, individuals dividing along the isthmus and each half developing a new semicell. The organisms seem to thrive best in locations that are nutrient poor. *Micrasterias* can produce both asexually and sexually. Asexual reproduction occurs via mitosis where the genetic material of *Micrasterias* is duplicated and two small semicells grow between the original semi-cells, gradually increasing in size. Sexual reproduction occurs through conjugation whereby two organisms come together and fuse their haploid cells to form a diploid zygote. This zygote typically forms a thick protective wall that allows the organism to remain dormant to survive cold temperatures and droughts.
The Phaeophytes or Brown Algae

Phaeophytes are the largest of the chromists, and are among the largest photosynthetic organisms on earth. The largest kelps may grow to more than 100 meters in length, forming dense underwater forests. The group is found primarily in colder waters of the northern hemisphere, with the largest forms occurring in cooler waters, rather than in the tropics. Many familiar species, such as rockweed, are intertidal, and are exposed to the air at low tide. Though the giant forms receive more notice, there are also many microscopic brown algae. These may grow as epiphytes on underwater vegetation, forming networks of branched filaments, or broad encrustations. Among all this variation, there are no unicellular or colonial phaeophytes -- all are multicellular, and have a large surface area. They depend on this to acquire dissolved nutrients from the surrounding water, in much the same way that animals use the large surface area of their gills in order to obtain oxygen.

New tissues are added by a variety of processes, each involving cell division within a relatively localized part of the body. Some have intercalary growth, in which divisions of cells may occur in multiple directions. This kind of growth produces broad sheets of tissues, or thick regions. Most kelp renew their damaged tissues through intercalary growth, in which the cell divisions occur in the middle of a set of tissues, usually near the base of the stalk. This is in contrast to the apical growth in plants, which among phaeophytes is found only in a few derived forms of Fucales and Dictyotales.

The overall thallus including photosynthetic structures is composed of, the lamina, or blades, branching from the stipe, or stalk. At the base of the stalk is the holdfast, which cements the alga to the sediment (most commonly rock for the phaeophytes) and prevents it from being washed away. This has allowed kelps to grow in the turbulent surf zone and intertidal regions. A close-up of such a holdfast is shown below; you may click on the image to see an enlargement.

Phaeophytes have a number of compounds that help them to retain water. This might seem unnecessary for an alga, since it is immersed in water, but many brown algae live in the intertidal zone, where they may become exposed to the air at low tide. Alginic acid, along with cellulose, is a component of phaeophyte cell walls that aids in water retention. In addition, many phaeophytes have lifecycle and habitat strategies that aid in preventing desiccation.

Though there are a few rare freshwater species, the brown algae dwell almost exclusively in marine (or coastal) environments. Members of the group dominate many benthic marine biotas, sometimes reaching from the ocean floor to its surface. In general, they are not free-floating organisms, but are attached to rock, coral, or other firm surfaces.

Most brown algae display alternation of generations between two different multicellular stages. The differences in life cycle define a number of orders, some with a dominant diploid phase, and some with isomorphic phases, that is, they are quite similar in appearance to each other. Some, such as the Fucales, have no free-living gametophyte stage at all.

The largest kelps are diploid, and release flagellated swimming sperm into the water to find egg cells. It has been shown that chemical signals called pheromones aid the sperm in their quest in at least some phaeophytes.

Distinct phaeophyte communities

Kelp forests dominate shallow rocky coasts of the world’s cold-water marine habitats. They comprise primarily brown algae in the order Laminariales and produce the largest biogenic structures found in benthic marine systems. Kelp forest ecosystems include structure-producing kelps and their myriad associated biota such as marine mammals, fishes, crabs, sea urchins,
molluscs, other algae and epibiota that collectively make this one of the most diverse and productive ecosystems of the world. Economically, kelp forest ecosystems have been significant to maritime peoples for thousands of years.

The Sargasso Sea is a free-floating (pelagic) kelp-dominated ecosystem in the western North Atlantic. It is bounded by the Gulf Stream, the Canaries Current, and other currents that together produce an ever circulating boundary. The local pattern of distribution appears to also be affected by both wind and thermal fronts, and more broadly may be affected by the action of storms. Attempts to quantify the amount and distribution of Sargassum in the Atlantic have met with many difficulties, including changes with seasonality and regional patchiness. Two species constitute the majority of the algae here, primarily *Sargassum natans*, and most of the rest is *Sargassum fluitans*. These two species apparently evolved from other anchored species of Sargassum, providing the basis of this bizarre ecosystem. Sargassum stays afloat by producing gas-filled bladders, which act like buoys. You can see these in the picture at left; the picture also shows the typical jagged-edged blades. Life here is precarious for animals who are poor swimmers -- they must maintain a firm grip on floating mats of kelp, or be lost to the ocean depths. Such a floating ecosystem of course will have difficulties in acquiring nutrients, and will therefore be severely limited by access to such nutrients. Many of the organisms which live here survive by being generalists, not limiting themselves to a single food source but making use of whatever is available. The most common crab is a generalist carnivore, eating many different kinds of prey. It is also interesting that this ecosystem has no animals that are strict herbivores, but rather they are omnivores, switching between diets of eating algae and animals. This may in part be due to the rubbery, chemical-laden nature of Sargassum. The accumulated mats of Sargassum support a wide variety of animal life, some of which depend on the kelp for only a part of their life. Other organisms spend their whole life among the algae, and this diversity of life has been called a "floating jungle". Some of the more unusual forms include fish and crabs that are camouflaged to look like Sargassum. Perhaps the best known of these is the pipefish *Syngnathus pelagicus*, a relative of the seahorse. This fish is brownish-green, and is covered by flaps of skin that resemble the kelp blades. There are more than 50 fish species whose lives are linked to Sargassum, and a myriad of invertebrates, including gastropods, polychaetes, bryozoans, anemones, and sea-spiders. The most numerous inhabitants are hydroids and copepods.

In Fucoid communities *Fucus*, or rockweed, is the organism that gives these communities their name. Communities dominated by members of the order Fucales are mostly subtidal and intertidal. In these regions, the stress of wave action makes life difficult for free-floaters; most organisms that live here attach themselves to the rocks. And it is not just the peaceful waves that must be withstood, but the powerful force of waves generated by storms as well. Those organisms not securely fastened to the rocks will likely be torn free and washed ashore or carried into the open ocean. Algae and plants that live here must also contend with many herbivores which live in these near-shore communities. Heavy grazing may damage and weaken the holdfasts that anchor the algae. Exposure at low tide also means that these organisms risk desiccation, which is reduced by the presence of gelatinous compounds such as algin.

I. Ectocarpales
The Ectocarpales are a very large order in the brown algae (class Phaeophyceae) which includes families with pseudo-parenchymatous or true parenchymatous tissues. Pseudoparenchymatous refers to a filamentous alga with cells packed very close together to give an appearance of
parenchymatous tissue. Filamentous algae are composed of cells that divide along a single plane, allowing only elongation to form filaments of one or more rows of cells. Algae that can divide in two planes can form sheet-like thalli or bodies and are considered as parenchymatous within the algae (true parenchymatous organisms divide in three planes, which is rare or absent within the algae). Parenchymatous algae generally have box-like cells characteristic in land plants though they lack the division in three planes.

1. Ectocarpus – *Ectocarpus* is a yellowish to dark brown, highly branched seaweed with tufts of erect, uniseriate filaments radiating from a prostrate filamentous portion which attaches the organism to the substrate. The laterals are always scattered and each cell contains 1+ ribbon-shaped chloroplasts. The growth is diffuse intercalary. Pseudo hairs are present. Plurilocular sporangia are terminal but they may also develop from normal vegetative cells below a pseudo hair. Unilocular sporangia are terminal on short laterals. Ectocarpus is a widely distributed genus which shows great phenotypic plasticity. The life history is an isomorphic to slightly heteromorphic alternation of generations, but asexual strains also exist.

II. Laminariales
The Laminariales are comprised of the brown algae that are commonly referred to as kelp. There are about 30 different Genera of organisms that live in the temperate to colder waters (4-14 degrees C, nutrient rich waters). Kelps grow to underwater forests and are thought to have appeared during the Miocene.

1. Laminaria saccharina – sweet or sugar wrack or sea belt
The genus may be used to characterize the largest of the brown seaweeds, the kelps. The dominant sporophyte phase of these sublittoral organisms consists of a differentiated thallus with a rhizoidal holdfast (not discoid like in *Fucus*), a slender stem-like stipe and from one to numerous leaf-like laminae. Chloroplasts abound only in the superficial cells of the thallus with photosynthesis occurring primarily in the uppermost laminal regions which project at or near the water surface. Translocation of the photosynthate mannitol to lower parts of the organism takes
place in centrally located sieve cells. While mature Laminaria are often 3 meters in length, the giant kelp Macrocystis may reach 100m or more, giving it the connotation “Sequoia of the sea”. In the larger kelp genera gas bladders or pneumocystis develop at the base of the fronds (divided “leaf”) to keep them floating closer to the surface of the water. In mature bull kelp Nereocystis these bladders can get 17-20cm in diameter with a wall nearing 3 cm in thickness. A few kelps such as Nereocystis are annuals but most are perennial, living 3-6 years. Growth rates are astounding and have been observed at 45cm/day for Macrocystis fronds, the most rapid growth rate of any known “plant” (do remember that these are still protists). Unlike Ectocarpus, kelps display a heteromorphic alteration of generations (What does that mean?).

1. Macrocystis sp. - thallus to 100 m long, perennial, typically 4-8 years. Holdfast conical and consisting of a few central erect stipes; holdfast prostrate. Stipes numerous, erect, branching 2-6 times near base. Each stipe branch contains a frond, which is composed of a stipe with attached blades and an apical meristematic blade. Blades numerous on each stipe, arranged at regular intervals with a short stipe and a pyriform to subglobose pneumatocyst subtending an undivided lamina, which is narrow to broad with tapering ends, smooth to rugose, and with denticulate margins. Macrocystis species occur in both the Northern and Southern Hemispheres, occurring along the Pacific coast of North America in the north and having a subantarctic distribution in the south. Many species were described based on morphological differences, later reduced to species based mainly on holdfast morphology.

III. Fucales
The Fucales are the largest order of the Phaeophyceae and a sister group to the remainder of the brown algae. They are generally the toughest species of the brown algae, show large
morphological diversity and difference in lifestyles.

1. *Ascophyllum nodosum* – Knotted Wrack - *Ascophyllum nodosum* is one of the most important seaweeds on the more sheltered shores. It is a large brown alga, which can grow to 2m long and live for several decades. The large egg-shaped air bladders occur in series along the frond making it easy to recognize. The frond is olive-brown in color, somewhat compressed, thick and strap-like but without a mid-rib; it branches freely. ‘Hold-fasts’ attach it firmly to rocks and boulders. These hold-fasts simply attach the plant to the rock but unlike roots they do not in any way ‘feed’ the plant. The bladders are up to 5cm long and are not easily ‘popped’, but can be used by children to spray water or as whistles. Reproduction takes place in beautiful yellow receptacles in spring (below). These develop in response to short days in the autumn, mature during the winter, and are at their most prolific in spring. Eggs and sperm are released into the water, and the eggs release a low molecular weight sperm attractant known as Finnavarene, named for the village of Finavarra (“wood by the sea”) in north Co. Clare in Ireland. *Ascophyllum nodosum* commonly bears small tufts of what appears to be a parasitic alga, *Polysiphonia lanosa*, which is a small red alga that can grow to about 5cm long with dense branches and although it seems to be common on *Ascophyllum* it is rare elsewhere and is not a true parasite.

2. *Fucus vesiculosus* – The bladderwrack
*Fucus vesiculosus* can grow in the littoral zone, where the tide changes the depth of the water, and the sublittoral zone, where the organism is constantly submerged. Being in the littoral zone can cause a couple of complications for the thallus of bladderwrack. For example, when the low tide comes and the water recedes further back into the ocean, many organisms are left to "sunbathe" on rocks, possibly drying out. The common distribution for bladderwrack is around the north Atlantic, in more temperate waters with lower salinity. Some shores (rocky, boulders) may lead to the organism not developing the air bladders that are the signature of bladderwrack simply because they can be damaged to easily. A little more sheltered shoreline, where the tide doesn't vary as much, such as the one upwards and to the right, is where you can find *F. vesiculosus* with bladders. Bladderwrack is most commonly attached to hard substrata such as pebbles, rocks, and dense sea beds. The connection to the substrate is with a discoid holdfast, a root-like structure that simply aids in anchoring the organism and ensuring it does not get pulled out into the ocean. Notice the root-like structures that are all over the substrate. Thin and flat Bladderwrack has also evolved to be somewhat of a specialist in surface area to volume ratio. The alga has extremely flat blades that allow it to soak up as much sunlight as possible without having to sacrifice many nutrients, which would be the case if more cells were present in the blade. This flat morphology also allows local production of sugars, and also allows for the plant to simply get its water through osmosis. This is the reason why Bladderwrack hasn't evolved vascular tissue like the plants have. There is also a special pigment that has evolved in *Fucus vesiculosus* that allows for a greater absorption of light, fucoxanthin. This extra absorption makes photosynthesis a lot more efficient. You can find out more about photosynthesis and Bladderwrack's source of nutrients.

The famous bladders - As was stated on the home page, *F. vesiculosus* is known for the air bladders found in pairs on its blades. These bladders help keep the brown algae afloat when the tide comes in so photosynthesis can continue at a more productive rate. The bladders are filled with mostly O₂ that is a product of photosynthesis. The bladders are held together by a network
of white filaments that, as the organism gets older, break, and leave the bladder incapable of holding any gases. After only a couple years, F. vesiculosus will only have bladders towards the younger end of the thallus.

Bladderwrack has quite a few predators since it is a primary producer. Fortunately enough for bladderwrack, however, it has developed a way to prevent this herbivory. Fucus vesiculosus has developed a certain set of compounds called phlorotannins. Scientists have found that this resists grazing from herbivores by looking at the lower levels of damage of a bladderwrack thallus with higher levels of phlorotannins relative to thalli that have lower levels of these compounds.

Fucus vesiculosus inhabits a place where the waves can be especially harsh. Waves can smash the thalli against rocks and other algae in the area, which can cause damage to the bladders and the organism as a whole. To prevent damage from this constant motion while submerged and battering against other objects, F. vesiculosus has developed gel-forming polysaccharides that helps cushion the thallus of the organism. These polysaccharides also help prevent desiccation during the low tides when the organism is subjected to harsh sunrays, heat stress and air exposure.

Fucus vesiculosus only produces its gametes during two seasons out of the year, those seasons being spring and summer. The rest of the year the offspring grow and mature, and the mature bladderwracks just enter a vegetative state. The thing that is odd about this is that a species that is very closely related to bladderwrack, Fucus serratus, reproduces at the exact opposite time, possibly helping to prevent cross-fertilization. Sperm and eggs are produced in structures called conceptacles that are small structures on a receptacle. The overall structure is a receptacle, and the little bumps are the conceptacles. The conceptacles are swellings at the end of the thallus when the organism is about to reproduce. The structures that reside in the conceptacles that produce the female gametes are called oogonia and contain eight eggs each. The male reproductive structures are called antheridia, which also reside in the conceptacles. The antheridia carry 64 sperm cells each before they release the cells into the water. In addition to only releasing its eggs and sperm during summer and spring, F. vesiculosus also has a developed a timing mechanism for release of its gametes during the day. Relative to its very close cousin F. serratus, F. vesiculosus releases a lot more eggs later in the day and at lower tide than its cousin. Researchers speculate that bladderwrack has developed this because it is preventing rapid dilution of its gametes that require sperm to fertilize them. This fertilization process would become a lot less probable if the eggs were spread out and diluted, as would happen at higher tides. You may wonder how the sperm find the egg to fertilize it. The process is obviously not passive, like spores of certain trees, since the sperm are flagellated. It's actually a lot like how humans attract one another: pheromones. The female egg actually releases a species non-specific pheromone that attracts sperm in its direction. Because the pheromone is species non-specific, the egg may attract a male gamete that it is not compatible with. The way the sperm ends up knowing whether it can fertilize the egg are the oligosaccharides on the cell wall of the egg.

Fucus vesiculosus seems to thrive in areas where other marine species fail to reproduce. The reason why so many marine species are unable to live in these areas that Bladderwrack can survive in is because of the low salinity. Low salinity has multiple effects on the male gametes, which bladderwrack has seem to overcome. Low salinity can decrease the motility and length of life of gametes of most organisms, and also increase the rate of polyspermy. Polyspermy is lethal to almost all species, but bladderwrack has a few mechanisms to overcome this. The first is a fast block. The fast block is a change in membrane potential of an egg, and happens in a few
milliseconds of sperm-egg fusion. This reduces the chance of another sperm fusing with the cell membrane of the egg. Another polyspermy block is a slow-block, which happens over the course of a couple hours, and is basically the growth of a cell wall around the organism. *Fucus* eggs are released in excess of 1 million per season per organism. These gametes (both female and male) are not released individually into water but rather in “packets” in a drop of mucilage. As the tides go out, exposing the receptacles to desiccation, conceptacles with mature gametes begin to shrink squeezing the gamete containing mucilage droplets through the ostioles. When the high tide comes in, the first wave to wash over a receptacle will dislodge these packets, dissolve the “mucilage membrane” and disperse gametes for fertilization.

![Diagram of Fucus fertilization](image)

IV. Dictyota

The Dictyota are an order of dichotomously branched parenchymatous brown algae that grow from an apical meristem. Members of this order generally prefer warmer waters compared to remainder of the brown algae and dominate the benthos biomass of the Florida Keys. One of the genera in the Dictyota, *Pandina*, is the only calcareous member of the class Phaeophyceae.

1. *Dictyota* - Thallus flattened, ribbon-like, erect or prostrate, with smooth, dentate, crenulate or ciliate margins; attachment by basal rhizoids or marginal rhizoidal processes scattered along the edges of the thallus or restricted to the base, stoloniferous holdfasts present or absent; branching dichotomous, anisotomous or alternate, rarely falcate; apices obtuse, rounded, apiculate; phaeophycean hairs and superficial proliferations present or absent; thallus differentiated into a cortex and a medulla, the relative number of layers variable.

2. *Haliseris* – currently a synonym of *Dictyopteris*. Thalli erect or prostrate, attached by a matted rhizoidal holdfast, up to 60 cm long, twisted, sub dichotomously to laterally branched, branches 0.5-25 mm broad, with a conspicuous midrib, with or without fine veins in the lateral wings. Growth initiated by a marginal row of 3-15 apical cells. Thallus wings 1-8 cells thick with the midrib 8-30 cells thick. Thallus in multilayered species differentiated into a monochromatic cortex containing many chloroplasts and a medulla of slightly larger cells. Hair tufts scattered or in lines.
The Rhodophytes

Red algae are red because of the presence of the pigment phycoerythrin. This pigment reflects red light and absorbs blue light. Because blue light penetrates water to a greater depth than light of longer wavelengths, these pigments allow red algae to photosynthesize and live at somewhat greater depths than most other "algae". Some rhodophytes have very little phycoerythrin, and may appear green or bluish from the chlorophyll and other pigments present in them. When it comes to describing algae, the colors of the rainbow do just fine. For marine rhodophytes olives, red-browns, red and even near purple colors are present. But when it comes to freshwater algae, RED is rare.

In Asia, rhodophytes are important sources of food, such as nori. The high vitamin and protein content of this food makes it attractive, as does the relative simplicity of cultivation, which began in Japan more than 300 years ago. Amongst the algae the rhodophytes have to most food sources, including dulse (*Palmaria*), nori and laver (*Porphyra* sp.), carrageen and irish moss (*Mastocarpus stellatus* and *Chondrus crispus* respectively), *Eucheuma*, *Gelidiella* and ogonori (*Gracillaria*) to name a few. The rhodophytes certainly have the most well known representatives for human consumption.

Some rhodophytes are also important in the formation of tropical reefs, an activity with which they have been involved for millions of years; in some Pacific atolls, red algae have contributed far more to reef structure than other organisms, even more than corals. These reef-building rhodophytes are called coralline algae, because they secrete a hard shell of carbonate around themselves, in much the same way that corals do.

I. Class Rhodymeniophycedae
I.A. Order Ceramiales
1. *Antithamnion* - Plants of uncorticated, monosiphonous, indeterminate axes, prostrate with erect branches often consisting of free ends of branchings of the prostrate system. Axial cells each bearing an opposite pair of determinate branchlets, distichously or decussately arranged, and with a small basal cell devoid of branchlets. Branchlets may be further branched 1-several times. Cells uninucleate. Gland cells borne on special, short 2-4 celled branches or sessile on cells of branchlets, which are often reduced in length. Carpogonial branches borne singly on basal cells of 4-8(-20) successive branchlets at branch apices. The genus includes a phylogenetic gradation to species that are regular and consistent in form. Includes many species, widespread throughout world. Small delicate plants usually epiphytic on other algae, occasionally on sea-grasses, rocks and other substrates.
2. *Ceramium* - Thallus erect or prostrate, to 30 cm, axes monosiphonous pseudodichotomously or irregularly branched, cells ovoid or cylindrical, corticated by filaments of limited growth arranged in bands and forming partial or complete investment. Carpogonial branches 4-celled borne on first-formed pericentral (supporting) cells successively along branches. Subsequent to fertilization connection occurs between carposporangium and auxiliary cell, cut off from supporting cell, leading to development of carposporophyte with successive groups of carposporangia finally enveloped by an involucre of filaments formed from cells of the segment below. Spermatangia from apical cells of cortical filaments of limited growth. Tetrasporangia developed in cortical bands from filaments of limited growth, singly, in whorls or irregularly arranged, spores tetrahedrally or occasionally cruciately arranged. A large genus widely distributed on most coasts, occurring from intertidal to deep water situations under almost every ecological condition.

3. *Chondria* - Thalli with species of variable sizes, from a few mm to over 1 m long, highly branched, cylindrical to flattened, with thickened primary axes (up to approximately 5 mm
diameter); branching radial, sometimes secondarily distichous, smaller branches usually with proximal constriction; apices either pointed and attenuate or sunken within a terminal depression. Holdfast a basal crust or hapteroid prostrate branches; attachment of erect branches to other objects sometimes occurs and some species produce circinate tendrils. All branches polysiphonous with 5 pericentral cells, corticating filaments produced by pericentals resulting in layers of large pseudo-parenchymatous cells and an epidermis of small cells with rectangular, rounded or angular profiles. Proximally growing rhizoidal filaments often formed between pericentals and subcortical cells forming a sparse to extensive tissue; secondary cortex may develop from the epidermis in proximal parts of some species.

4. *Dasya* - Thallus erect, sympodially developed and radially organized, alternately to oppositely or irregularly branched, appearing virgate to bushy. Indeterminate axes spirally produce determinate pseudolateral branch systems (ramelli) from each polysiphonous segment. Pseudolaterals pigmented, monosiphonous throughout except in some species with polysiphonous basal segments, simple to dichotomously branched, often deciduous below. Axial cells surrounded by (4-)5 periaxials, with or without rhizoidal cortication. An intertidal to deep subtidal genus of sub-polar to tropical waters worldwide. Along with *Heterosiphonia*, one of the most common and widespread genera of the Dasyaceae.

5. *Polysiphonia* - Thallus developing a primary erect system of cylindrical polysiphonous branches, mostly having indeterminate growth; prostrate branches often developed secondarily; rhizoids, mostly unicellular, formed by pericentral cells, from which they are usually separated by a cell wall and pit connection, on ventral sides of decumbent and attaching branches in most species, sometimes epiphytic species forming a discoid cellular attachment derived from germling. Like many red algae, *Polysiphonia* displays a sexual cycle characterized by alternation of generations and sporic meiosis. The independent sporophyte thallus, referred to as tetrasporophyte, and diecious or unisexual gametophyte thalli are structurally alike. In most other red algal species the haploid/diploid generations are heretomorphic. The sexual cycle of red algae is also characteristically complicated by the insertion of an asexual diploid “phase” between fertilization (syngamy) and germination of the diploid thallus. The intermediate diploid structure, which remains attached to the haploid female thallus, is referred to as the carposporophyte. It usually consists of a cluster of diploid spores called carpospores. This cluster is often enclosed by a cup-shaped haploid structure produced by the female thallus termed the pericarp with the combined pericarp/carposporangial group collectively referred to as the cystocarp. Carpospores, upon release, produce the free-living sporophyte phase called the tetrasporophyte. The central cells in most tiers of the tetrasporangium functions as a sporangium, producing a tetrad of large, non-motile spores by meiosis. These haploid spores are dispersed and germinate to form separate male and female thalli that are structurally like the tetrasporophyte. The apex of the male thalli form spermatangial branches consisting of a central cell and pericentral cells that are subdivided to form thousands of non-motile spermia. Female thalli also produce short branches that bear the carpogonia. Mucous secretions of the trichogyne entrap the non-motile spermia as they float by. Following that contact the sperm nucleus moves down the trichogyne to fuse with the basal egg nucleus. This generally forms a typical, diploid zygote nucleus. Species of Polysiphonia occur in most marine regions of the world, often as epiphytes on larger plants in the sea but also epizoic on various invertebrates and on sea turtles. Many other species occur on rocks. Polysiphonia sometimes may be found in mangrove habitats as
well as in estuaries, sometimes in almost freshwater habitats.

II. Class Florideophyceae
II.A. Order Batrachospermales
1. *Batrachospermum* - Gelatinous gametophyte filaments, up to 40 cm long, with beaded appearance, varying from blue-green, olive, violet, grey to brownish. Uniseriate central axis with large cylindrical cells; 4-6 pericentrals produce repeatedly branched fascicles of limited growth. Rhizoid-like cortical filaments typically develop from lower side of pericentral cells. Cortical filaments grow downward and ensheathe axial cells; often produce secondary fascicle branches. Each fascicle cell contains several, ribbon-like, parietal chloroplasts with no pyrenoid. Few species form monospores in this stage. Spermatangia budded off terminal primary and secondary fascicle cells or in some species from filaments of carpogonial branch; spherical, colorless, 4-8 µm diam. Carpogonial branches mostly little modified but twist. Carpogonia with broad trichogyne sometimes stalked on small base containing the nucleus. Carposporophytes generally spherical, compact or loose mass of goniomblast filaments; carposporangia formed at apices. Carpospores germinate into chantransia (= pseudochantransia) stage, a crustose growth consisting of large basal cells and erect, sparsely branched filaments. Filaments can form monosporangia or divide meiotically, producing an attached gametophyte and 2 residual cells. Mucilage composed of neutral and acidic oligosaccharides.
Cosmopolitan genus in moderately flowing, reasonably unpolluted waters. Fertilization may occur in turbulent eddies; ca. 500 spermatia released for each carpogonium with a mean fertilization rate of 22%. Carposporophytes may be released and travel up to 35 m downstream.

II.B. Order Gigartinales
1. *Chondrus crispus* - Plants can exceed 20 cm in length and are erect from a discoid base, compressed to flattened, branched, and beset with varying numbers of marginal proliferations. Cystocarps are embedded and aggregated distally or confined to proliferations. Cystocarps lack a surrounding filamentous hull, the major generic defining character of *Chondrus*. Male plants are extremely rare. Tetrasporangia occur in branched chains formed laterally on cells of the inner cortex. The economic importance of this genus currently in eastern Canada and France, and formerly in Britain and Ireland has resulted in major studies of its biology. Much emphasis has been devoted to its cultivation in outdoor tanks as raw material for commercial utilization. Plants of *C. crispus* seem particularly susceptible to colonization by endophytes. It is almost exclusively restricted to rock and ranges from intertidal to 24 m depths.

II.C. Order Palmariales
1. *Halosaccion* - Thallus with erect tubular or saccate fronds arising singly or in small groups from a basal disc, becoming cartilaginous with age, unbranched or irregularly lobed, occasionally with irregularly arranged proliferations; construction multiaxial, cortex of 2-5 layers of small pigmented cells increasing gradually in size inwards and merging imperceptibly with medullary cells, medulla compact with 3-10 layers of isodiametric, multinucleate cells the lumina of which become increasingly stellate inwards. Mostly Pacific but also found in the Bering Sea.

II.D. Order Corallinales
Articulated corallines are structurally organized into cellular filaments. Thalli and developed stalks can be either uniaxial or multiaxial, depending on which genera one is taking into account. Algal size and thickness are usually determined by both genetic and environmental factors. The articulates are often referred to as geniculates due to their possession of flexible, uncalcified
genicula, which operate like joints. Organization of genicula differs between classes of corallines in the numbers of cell tiers or layers. However, the basic structure of these tissues is consistent throughout all articulates. The other primary tissues in the articulations are referred to as intergenicula. These calcified branch segments are extremely rigid, like bones. Most intergenicula have cuticles that cover their calcium carbonate walls. Intergenicula play a crucial role in the reproduction and growth of geniculates. Reproductive conceptacles develop only on intergenicula. The growth of articulates is dependent upon the elongation of intergenicula and division of these growing segments via the formation of new genicula. Decalcification of the intergenicula reveals underlying tissue just like that found on other red algae.

1. *Amphiroa* - Thalli comprising crustose or peglike holdfasts and one to several erect, branched, articulated fronds. Fronds of calcified intergenicula separated by uncalcified genicula. Branching dichotomous or varied. Intergenicula of arching tiers of medullary cells that are short (5-20 µm long) or long (40-130 µm long) surrounded by a photosynthetic cortex of short cells and a unistratose layer of epithallial cells.

III. Class Bangiophyceae

III.A. Order Bangiales

1. *Porphyra* - Thallus a foliose blade, one or two cells in thickness and ranging in size from a few mm to over 3 m in length. Blades arise singly from a small discoid holdfast. Stipe absent or minute. Basal cells have rhizoids. Blades ranging in morphology from orbicular to linear, with margins smooth, dentate, or ruffled depending on the species. One or two stellate chloroplasts with large central pyrenoid per cell. Contains R-Type II phycoerythrin. Pit plugs absent in the foliose phase. In early stages of development growth is apical followed by a shift to diffuse growth. The microscopic, filamentous conchocelis phase consists of short, uniseriate, branched filaments that penetrate calcareous substrata such as oyster shell and barnacles. Cells of the conchocelis have parietal, band-shaped chloroplasts. Pit plugs present.