Lab 4:
Phytoplankton Diversity and Taxonomy

Introduction

Photosynthetic marine organisms, as the primary producers of the marine ecosystem, are the first link in the marine food web. They convert solar energy to an energy form usable by themselves and, in turn, by other marine consumers. Their importance to the biological economy of the sea cannot be overstressed. The most significant contribution to the primary productivity of the world oceans does not come from the obvious macroscopic attached plants that occupy the fringes of the continental masses. Rather, it is the microscopic, free-floating forms, collectively called **phytoplankton,** that are the dominant primary producers.

Marine phytoplankton include representatives from several classes. Among the most common and important phytoplankton are the diatoms and the dinoflagellates (Dinophyceae) in the microplankton (20 – 200 μm) size range, and coccoid cyanobacteria in the picoplankton (0.2 – 2.0 μm) size range. Small nanoflagellates (2 – 20 μm) can play an important role in tropical and subtropical oceans and in temperate oceans during summer stratification. Each group of phytoplankton exhibits characteristic colors, depending on its relative abundance of the major groups of photosynthetic pigments: green *chlorophylls*, yellow *carotenes*, or pink or blue *phycobilins*. The relative abundance of phytoplankton groups varies seasonally and geographically, so representatives of all groups are seldom found in the same plankton sample.

A. Cyanobacteria (Blue-Green Algae)

Members of this group are extremely small and are not easily collected with standard plankton nets. They are usually studied and counted by filtration onto black polycarbonate filters of 25 mm diameter and with a pore size of 0.2 μm. These filters are placed on a microscope slide, covered with fluorescent-free immersion oil, and studied under blue or green light excitation on an epifluorescence microscope at maximum magnification (1000× to 1200×). Their relative contribution to the total primary productivity of marine communities may be of considerable importance – an importance overlooked for a long time because of their very small size. It was only in 1979 that the ubiquitous abundance of the coccoid cyanobacterium *Synechococcus* (0.8 – 1.5 μm) was realized, and the even smaller (0.5 μm) *Prochloro-coccus* was only discovered in 1989.

Cyanobacteria are prokaryotic, with few cellular features visible with light microscopes. They may occur singly or form simple linear chains of cells. They contain chlorophyll a, carotenoids, and phycobilin pigments. Living cyanobacteria taken from natural ocean water samples are difficult to observe with light microscopes, so you will be provided with prepared slides of some representative species for observation of general characteristics. A number of the provided species will be freshwater species, which can sometimes be found in estuaries and brackish water as well. Among the provided slides are also filamentous species, which possess the capability of nitrogen (N_2) fixation. Nitrogen fixation is a high-energy process, and the responsible enzyme (nitrogenase) is highly oxygen-sensitive. To spatially separate oxygen production by photosynthesis and N_2 fixation by nitrogenase, nitrogen fixation in filamentous cyanobacteria is often located in special, thick-walled cells called heterocysts. Heterocysts are some times not only distinguished by their thick cell wall, but also by their larger size as compared to the vegetative cells of the filament. While studying microscope slides of cyanobacteria, pay special attention to whether your specimen possesses heterocysts or not.

B. Diatoms (Bacillariophyceae)

The diatoms are the most important planktonic primary producers in the ocean. They contain the chlorophyll pigments *a* and *c* and a wide variety of carotenoids. As a consequence, diatoms often appear brown. Diatoms are usually single celled but frequently occur in chains of cells. The cell shape of the different species varies greatly, but some generalizations can be made. Diatom cell shape follows one of two basic forms, *centric* or *pennate*. Centric diatoms exhibit some form of radial symmetry and are most commonly found as members of the phytoplankton. Pennate types are bilaterally symmetrical and have a structure called a raphe that is used for a simple gliding form of locomotion. Pennate diatoms are often found on solid substrates, such as rocks, animals, or larger algae.

Both types of diatoms have an external cell wall, or frustule, composed of SiO_2 (silicon dioxide). The frustule is usually in two parts, with a slightly larger *epitheca* fitting snugly over the *hypotheca*. The cell contents are contained completely within the silicate frustule. Most diatoms exhibit fine lines, or *striae*, on the frustule surface. These striae are actually rows of very small pores. Exchange across the cell wall occurs through these pores. During the class, you will be able to observe both live diatoms (or preserved material containing diatoms with cell contents in their frustules) and so-called frustule preparations, in which the organic cell content was digested for clarity of frustule structures. Some delicate diatom frustules are often used to test the quality of microscope optics, i.e. whether the microscope can resolve the fine striae or not. Upon your studies, distinguish pennate from centric forms and pay particular attention to striae and other frustule structures (spines, pores, raphe).

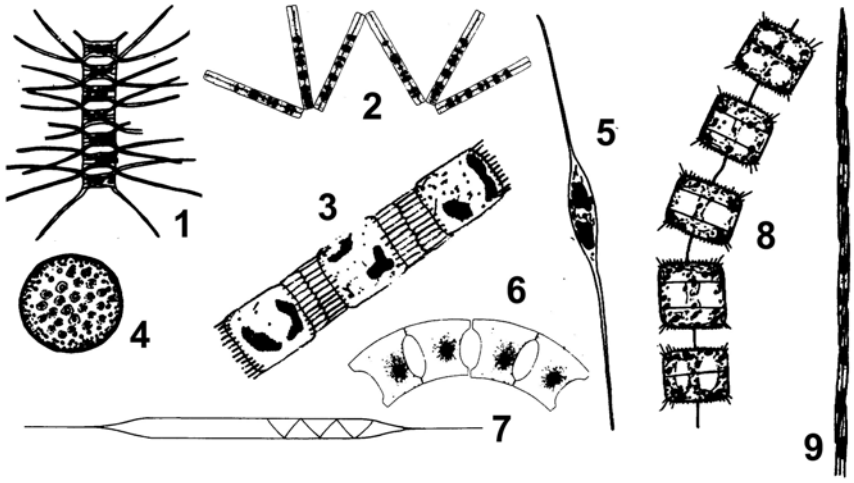

Fig. 1: Some common diatoms that might occur in your field samples. (1) *Chaetoceros* sp.; (2) *Thalassiothrix* sp.; (3) *Skeletonema costatum*; (4) *Coscinodiscus* sp.; (5) *Nitzschia* sp.; (6) *Eucampia* sp.; (7) *Rhizosolenia* sp. (note: scale for this species reduced; very long cells with little cytoplasm inside; often occur broken in net samples); (8) *Thalassiosira gravida*; (9) *Nitzschia pungens*, chain.

C. Dinoflagellates (Dinophyceae)

The Dinophyceae are predominantly marine and are second only to the diatoms in importance as marine primary producers. They contain pigments similar to those of diatoms and mostly appear brown. Many dinoflagellates are noticeably luminescent and glow in the wake of a boat and in breaking waves. Roughly half the dinoflagellates are strictly heterotrophic and lack chlorophyll. Of the chlorophyll-containing species, a high number exhibits mixotrophy, i.e. they perform photosynthesis and are able to feed on bacteria or other phytoplankton.

Dinoflagellates usually have a cellulose cell wall, perforated by many pores. Most forms have an equatorial groove that contains a ribbon flagellum. This groove separates the dinophyte's cellulose cell wall into two portions, the *epicone* and *hypocone*. Spines, wings, and horns may decorate the cell wall. Another groove perpendicular to the equatorial groove contains a longitudinal flagellum**.** The long longitudinal flagellum gives the cells mobility.

Fig. 2: Some common dinoflagellates that might occur in your field samples. (1) *Dinophysis* sp. (note the girdle is moved closed to the front end of the cell; (2) *Ceratium* sp. (note the long horns; length varies among species); (3) *Gyrodinium* sp. (note the spiral of the girdle gaps on the ventral side of the cell); (4) *Gymnodinium* sp. (the spiral of the girdle gaps only little as compared to the previous species; common example of naked dinoflagellates, both photo- and heterotrophic forms); (5) *Prorocentrum micans* (the epicone is reduced to a spine-like feature).

For more detailed information on these groups of phytoplankton and for information on other algal groups that occur in freshwater and marine plankton, refer to the lecture notes of the FIU Phycology course (BOT 4404) web page starting at http://www.jochemnet.de/fiu/bot4404/BOT4404_LN.html. These lecture notes also contain photographs and drawings of most common representatives of the different algal groups.

Lab Work

1. Study of representative specimens of phytoplankton groups

Permanent microscope slides and live and/or preserved phytoplankton net samples will be provided by the instructor for this exercise. The net samples were collected at different stations in Biscayne Bay and offshore by a 20 µm plankton net. Preserved samples are fixed with 1% (final conc.) formaldehyde. Avoid inhaling the formaldehyde (harmful!) and keep sample bottles closed if not in use. You will use the provided microscope slides and plankton samples to study the diversity of phytoplankton and to prepare detailed drawings of representatives of the different groups of phytoplankton. Details are given below for using either permanent slides or plankton samples. Include your sheets of drawings in your lab report. Your lab exercise should comprise at least 6 different species from at least three different groups of phytoplankton. But take the chance to observe many more different species form the collection and the samples. You should be able to get out of class and know more than six species of phytoplankton. Combine documentations from the permanent slides and the plankton samples for your lab report collection.

Try to put effort and patience into your drawings and document as many details as you can observe. Refer to Fig. 3 as an example for a good (Fig. 3a) and a poor (Fig. 3b) documentation. Although microphotography has replaced a good deal of hand-drawing these days (particularly since the introduction of digital photography), the old law of microscopy still applies today: You only have really seen what you have drawn! Drawing enhances the quality of your observations and deepens your acquaintance with the specimens. Also remember that this exercise is not a sketch-race; good quality documentations take their time, and fewer good documentations are worth more than a lot of poor sketches. Interested students can extend their study time beyond the official class time if so wished.

1.a. Study of microscope slide preparations

Microscope slides will be laid out sorted in groups of phytoplankton. Take one slide at a time and observe the specimens under the light microscope. Prepare drawings of the observed specimens on white paper using a soft pencil. Use as high microscope magnification as needed to access cellular structures of your specimen and prepare a detailed drawing of one representative, well-preserved specimen. Provide ca. ¼ of a letter sized page for each drawing and try to document as much cellular structure and characteristics as you can observe. Some preparations are better than others; if you feel your slide is of too poor quality fr appropriate observations, contact your instructor for advice. Next to the drawing, include the species name (given on the slide label), the systematic group this species belongs to, and some cellular features that characterize this species, distinguishes it form others, and that are characteristic for the group of phytoplankton this species belongs to. Remember that any drawing without correct and detailed annotations is worth nothing! After completion, return your slide to the central desk and place it back in the column of slides for the group your species belongs to (this will make the study of your colleagues much easier!). Please, handle the permanent slides with care and always return them to the central desk; these preparations are quite costly, and future courses will be grateful if we keep the slides in good shape.

1.b. Study of plankton samples

Start your observation of the plankton samples with the stereo microscope. Take the plankton sample bottle to your desk and use the provided plastic Pasteur pipettes to fill one or two pipette loads into the center of a plastic petri dish. Close the plankton sample bottle and return it to the central desk. Place your petri dish under the stereo microscope and commence your observations. The plankton samples will also contain some zooplankton (small crustaceans, rotifers, ciliates); you might ignore these small animals, as we will come back to the zooplankton in detail during a dedicated lab class. Try to get an overview of what groups of phytoplankton occur in your sample.

For a detailed study and preparation of drawings and documentation, use the plastic Pasteur pipette to capture your specimens of interest while observing through the stereo microscope. You will see the tip of your pipette; once you have focused the microscope on your specimen of interest, use your second hand to

hold the other arm, thereby providing stability to your pipetting hand. After you have captured your specimen from the petri dish, place it with a little drop of seawater (not too much) on a microscope slide, cover with a cover slip (never press the cover slip down, it will smash your specimen), place slide on the compound microscope and commence your observation and drawing as described in section 1.a.

Since phytoplankton species composition differs among stations, and each sample contains a multitude of species, the plankton samples are not labeled with species names. For your documentation, we will develop an identification collection on the classroom's blackboard. Whenever you discover a "new" species that you are interest in observing and documenting, contact your instructor. We will gather a collection of sketch drawings on the blackboard and assign the appropriate genus (and where possible species) names; this list will help your colleagues to document their observations. Be aware, though, that these sketch drawings shall not serve as an example or excuse for the quality of your documentations; you should be able to observe and document much more cellular structure than what will be drawn on the blackboard.

2. Semi-quantitative analysis of phytoplankton community structure

After having studied your plankton samples and the permanent slides, you should be acquainted with the most important groups and species of phytoplankton. Use this knowledge to assess the phytoplankton community composition in the net samples of your field station.

Return to the plankton sample of the field station of your group. Use the plastic Pasteur pipette to fill the bottom of a plastic petri dish with sample and study your sample under the stereo microscope at different magnifications. After you have studied your specimens under the compound microscope in detail, you should be able to recognize the different taxa even under low magnification on the stereo microscope. Prepare a semi-quantitative list (highly abundant, abundant, occasionally found, rarely found) of the taxa you can differentiate and recognize. Group your list of taxa according to the major classes of algae (cyanobacteria, green algae, diatoms, dinoflagellates, others). Discuss your observations with the members of your research team to come up with a final assessment of which taxa are more or less abundant in your sample.

For the lab report, include the semi-quantitative list of taxa and discuss which taxa were the most abundant in your sample. Which group of phytoplankton (if you consider all the taxa you reported) is the dominant in your sample? Report the most abundant taxa, and the most abundant class of phytoplankton to the classroom blackboard with your station number/location. After all groups have reported their findings to the blackboard, can we see differences in phytoplankton community structure among the sampled stations? Discuss these differences in your lab report, and also discuss the reason for these differences (such as nutrient concentrations, refer to lab 3, salinity, refer to the field trip, lab 2). Include these results and discussion in the final lab report of the field study [i.e. combine with results and discussion of hydrography (field trip, lab2), nutrient concentrations (lab 3), phytoplankton pigment analyses (lab 5)].

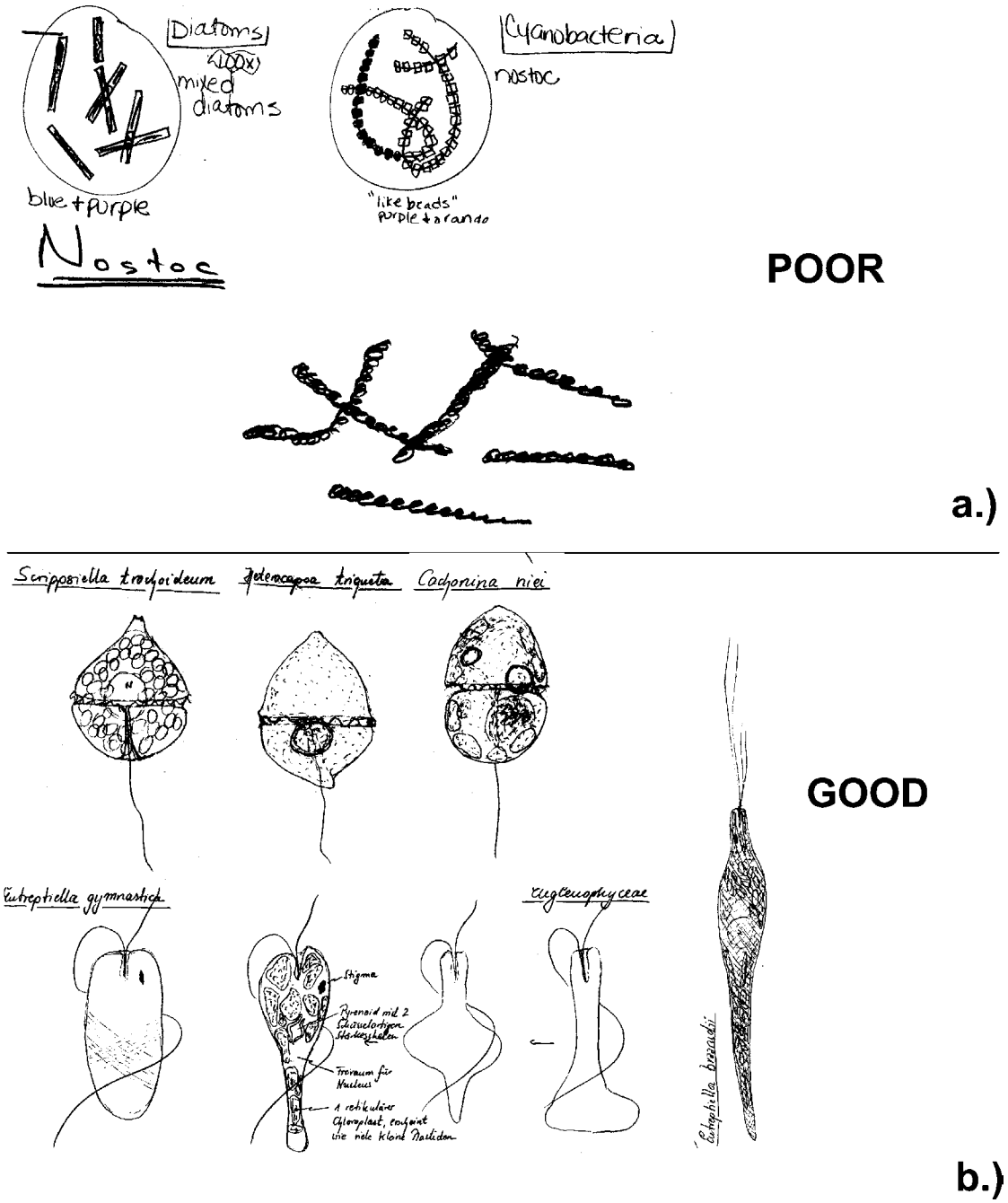

Fig. 3: Examples of poor (upper panel, a.) and good (lower panel, b.) documentation of phytoplankton cells upon observation by compound light microscopy (drawings taken from unnamed students' lab reports).