PRESERVATION OF ALGAL SAMPLES

For the purposes of this course, we recommend that you store algal collections in the glass-door refrigerator in the teaching lab as soon as possible after sampling, in lieu of adding chemical preservatives. We have found that most algal forms will survive long enough to complete projects within the scope of a semester, and you will not be exposed to toxic preservatives. If large zooplankton are present, it is a good idea to filter them out so they won't eat all the algae before you get a chance to see them. Cheesecloth works well to filter zooplankton. The rest of this document provides preservation information that you may find useful outside this class.

Storage periods require addition of chemicals that reduce bacterial activity and autolysis of algal cells. The major preservatives in use today (formalin, Lugol's solution, and glutaraldehyde) are discussed in Wetzel & Likens (1990), Limnological Methods, Springer-Verlag, on page 140. The discussion below is based on our personal experience in use of these preservatives.

The single best preservative, in terms of cell structure retention, is buffered glutaraldehyde. Wetzel & Likens recommend use of 3% glutaraldehyde, neutralized to pH 7 with NaOH, and suggest that the glutaraldehyde should be filtered to remove particles. Glutaraldehyde may be purchased in various grades--if you use high grade 70% glutaraldehyde in ampules, which is designed for use in ultrastructure studies, there will be little problem with particles. It is not desirable to filter glutaraldehyde because it is highly irritating to mucous membranes. Filtration (if done) should occur in a chemical hood. Also, 3% glutaraldehyde is too high in concentration for retention of normal cell shape of wall-less flagellates, which may shrivel or disintegrate completely (0.5% or less is better for these forms). We recommend that you try to match the pH and osmolality of the system sampled by use of appropriate buffer. When you are ready to count/identify algae that have been preserved in glutaraldehyde, you should wash the glutaraldehyde off with use of a micropore filter apparatus and buffer wash, if you are using a filter-based method for counting/identification. If you are using a settling chamber, merely cover the chamber with glass so that you are not exposed to glutaraldehyde fumes.

Many published sampling protocols make use of Lugol's solution. You add this preservative in amounts to achieve a 1% final concentration (1 part per 100). To make Lugol's, dissolve 10g I2 (pure iodine, which is toxic) and 20g KI in 200 ml distilled water along with 20 ml concentrated glacial acetic acid. Solution should be stored in the dark, preferably in a glass bottle with ground glass stopper. The iodine in Lugol's is effectively bacteriostatic, but it causes a number of changes in algal cells. For example, iodine will bind with starch to form a blue-black complex. This reaction is useful in identifying starch, which is present in some algal groups, but not in others. On the other hand, green algal cells often accumulate so much starch that treatment with Lugol's renders the whole chloroplast or whole cell so dark in color that identification is difficult or impossible. Moreover, in our experience, Lugol's solution does not preserve cell structure well in many cases, making identification difficult. We prefer to preserve in glutaraldehyde, and use Lugol's as a reagent to identify starch when necessary. Though formalin is often used to preserve certain marine algae, we do not recommend the use of formalin to preserve freshwater algae because cellular features required for identification are often not retained.