

Protista

Protozoa: General Information

The protozoan body consists of a single cell, but it is a mistake to think of these organisms as “simple”. Without the benefit of multi-cellular tissues or organs, many protozoa achieve structural complexity that rivals that of some multi-cellular animals.

Most protozoans are microscopic, but certain amoebae reach 4-5 mm in diameter. Many protozoa’s are agents for disease: malaria, for example is caused by the protozoan *Plasmodium vivax*.

The protozoan can be classified according to their means of locomotion. The shape of *Amoeba proteus*, a sarcodinian, constantly changes as it puts out false feet (pseudopodia) from any part of its body. The pseudopodia also engulf particles of food (phagocytosis). The flagellates, including *Euglena* and *Volvox*, move by means of one or more whip like structures, flagella, which propel them through the water.

Euglena, move in a spiral path. Ciliates, such as *Stentor*, *Vorticella*, and *Paramecium*, possess short, hair-like cilia; which beat in unison, moving them rapidly through the water. Paramecia can swim either forward or backward while simultaneously rotating on their long axis.

Observation of Protozoa

Direct microscopic observation of protozoa is easier if their movements are slowed down. To do this, place a drop of water containing the organisms in the center of a ring of two percent methyl cellulose on a glass slide, and carefully cover the slide with a cover slip. Methyl cellulose, which is sometimes marketed under trade names, is readily available from biological supply houses.

Sources of Protozoa

Collecting samples from the field is challenging and fun to do; but this method is often unreliable, especially if a particular species is sought, and the yield may be poor. It may be easier to request your cultures from the Region 20, Living Science Materials Center. *Amoeba*, *Blepharisma*, *Chlamydomonas*, *Euglena*, *Euplotes*, *Mixed Protozoan*, *Paramecium caudatum*, *Paramecium multimicronucleatum*, *Spirostomum*, and *Stentor*, are available. The organisms from a shipment can be used for class study and to start a mass culture.

For those who enjoy studying their own environment, the best natural sources of protozoa are ponds and streams containing abundant plant and organic life. *Euglena* are abundant in stagnant contaminated pools near manure piles, or in duck ponds. *Paramecium* occurs in freshwater ditches, in garden pools rich in decaying matter, and in neglected flower vases. *Amoeba* and *Stentor* are found in clean ponds. Search for *Amoeba* on aquatic plants such as water lily, *Elodea*, or *Cabomba*. Both *Amoeba* and *Stentors* are abundant around sphagnum. Since many crustaceans are competitors of amoeba for food, pools containing crustaceans will usually not contain amoeba.

Culturing Protozoa

Protozoans, except the photosynthetic forms, which can manufacture their own food, must prey on other organisms to provide their energy. To culture protozoans successfully, you must provide them with food by setting up a short food chain with the protozoan of interest at its top.

For example, when a new culture is inoculated with *Paramecium*, small amounts of bacteria and *Chilomonas* (a tiny flagellate) are included in the inoculums. The bacteria multiply around wheat grains placed in the medium, and the *Chilomonas* feed on the bacteria. *Paramecium* feed on the increased numbers of *Chilomonas*, but as they have no predators in the culture dish, the *Paramecium* increase rapidly and soon exhaust their food supply.

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The maintenance of any organism for a few weeks is quite different from culturing it over a period of years. Media that give excellent results at the start may not be satisfactory for continuous cultures over long periods. You may find the following hints helpful even for brief culture periods:

1. Choose water with good biological properties. Tap water is usually not suitable, so use spring water or filtered pond water. We do not recommend using the spring water available at most grocery or drug stores.
2. Pasteurize all media ingredients and allow them to cool to room temperature before inoculation.
3. Keep cultures covered to exclude dust (but not air).
4. Be sure culture vessels are clean. Traces of chemicals, soap, and soap powders may be toxic to organisms.
5. Always subculture just before the parent culture reaches its maximum population. Inoculate heavily using dropping pipettes, which are biologically clean.

Protozoa are best cultured under conditions of dim to moderate light, a neutral or slightly alkaline pH, and temperatures of 20° to 21° C. Amoebae are especially prone to lose their vitality and die at higher temperatures. Cultures can be maintained in clean baby food jars with the lids loosened on the top.

Recommended Media for Protozoans

Protozoan	Recommended Protozoan Medium
Sarcodinids	
<i>Amoeba</i>	Wheat Medium
Flagellates	
<i>Algae</i>	Alga-Gro Freshwater for freshwater algae
<i>Euglena</i>	Euglena Medium
Ciliates	
<i>Blepharisma</i>	Wheat Medium
<i>Euplotes</i>	Wheat Medium; Protozoan pellets
<i>Paramecium</i>	Double Wheat Medium; Protozoan Pellets
<i>Spirostomum</i>	Hay-Wheat Medium; Protozoan Pellets
<i>Stentor</i>	Wheat Medium; Protozoan Pellets

Wheat Medium: For most protozoa, place three or four grains of previously boiled wheat in each culture dish. For paramecia, use six to eight grains (double wheat medium). Pasteurize spring water and while it is hot, pour about 200 ml into a dish. Cool at room temperature and inoculate. If *Amoeba* Medium or *Paramecium* Medium is used, heating is not necessary.

Hay-Wheat Medium: Pasteurize spring water and while it is hot pour about 200 ml into each culture dish. Add two grains of wheat and two 3-cm stems of timothy hay, which have been previously boiled. When cool, inoculate.

Euglena Medium: Combine 1 liter spring water, 40 wheat grains, 35 rice grains and 5 cm³ dry skim milk. Boil for 5 minutes. If *Euglena* Medium is used, heating is unnecessary. Let stand overnight and inoculate. Deep culture vessels or wide-mouthed gallon jugs work well. Keep *Euglena* cultures in a well-lighted area, but out of direct sunlight because high temperatures are harmful. Artificial illumination is adequate.

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Concentrated Paramecium: Thick cultures of *Paramecium caudatum* are required. With a dropping pipette, remove a large number of paramecia without getting too much fluid. Place in fresh spring water. Taking a cloth handkerchief, strain to remove debris and large masses of bacteria. Let the filtrate stand until paramecia congregate in white masses on the bottom of the dish. With a dropping pipette, transfer the concentrated paramecia to fresh spring water in another dish and inoculate with *Didinium* or *Bursaria*. More paramecia must be added every few days, and subcultures should be prepared about every two weeks.

Sand-Spirogyra Medium: Place about 0.5 g of clean sand in a culture dish containing 200 ml of heated spring water. After the water cools, add a generous portion of healthy *Spirogyra* (preferably a single chloroplast species). Inoculate