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13-1017

Micro Aquarium™

Instruction Manual



MicroAquarium™ Instruction Manual

Table of Contents

Overview	3
Content Standards	3
Materials	4
Background	4
Guidelines for Use	7
General Methods	8
Viewing Tips and Observational Techniques	11
Other Manipulations	13
Culturing and Observing Protozoa	15
Culturing, Observing, and Keeping Small Animals	17
Culturing and Observing Fungi and Plants	19
Establishing a Pond Water Microcosm	20
Feeding Your Pond Water Microcosm	22
Discovering Aquatic Life in Terrestrial Environments	23
Additional Resources	24
Related Products From Carolina	26

Overview

The MicroAquarium™ is a slide-mount and culture vessel in one. Small aquatic organisms within its chamber can be examined with or without a microscope. Manipulated in simple yet unique ways, it is a versatile, easy-to-use tool for conducting experiments, investigating natural environments, and studying the natural history of cyanobacteria, protists, plants, animals, and fungi. This sturdy water chamber has macroscopic appeal and is designed to double as a microscope slide wet mount. Macroscopically, living organisms are displayed within a thin, easily backlit microcosm which at arms length allows small organisms (e.g., single-celled *Paramecium*) to be seen with the naked eye. Under a microscope or hand lens, the structural details of many organisms are revealed. Many of the classic “pond water” organisms studied in the biology curriculum can be conveniently cultured and/or maintained within the MicroAquarium™. Displayed as a classroom aquarium or as a personal desktop aquarium, the MicroAquarium™ is sure to provoke interest.

Content Standards

The MicroAquarium™ is appropriate for students of any grade level and addresses the following National Science Content Standards:

Grades K–4

Life Science

- Characteristics of organisms
- Life cycles of organisms
- Organisms and environments

Grades 5–8

Life Science

- Structure and function in living systems
- Reproduction and heredity
- Regulation and behavior
- Populations and ecosystems
- Diversity and adaptations of organisms

Grades 9–12

Life Science

- The cell
- Interdependence of organisms
- Matter, energy, and organization in living systems
- Behavior of organisms

Materials

13-1016 MicroAquarium™	13-1017 MicroAquarium™ (pack of 10)
MicroAquarium™ chamber	10 MicroAquarium™ chambers
MicroAquarium™ lid	10 MicroAquarium™ lids
MicroAquarium™ stand	10 MicroAquarium™ stands
MicroAquarium™ Instruction Manual	MicroAquarium™ Instruction Manual

Note: For your convenience, we have listed throughout this manual the catalog item numbers of products available from Carolina Biological Supply Company (with the prefix, RN-). For pricing and other information, please refer to the most recent *Carolina™ Science* catalog, call toll free 800-334-5551, or visit the Carolina Biological Supply Company Web site at www.carolina.com.

Background

The MicroAquarium™ is a 2- x 3-inch (50 x 75 mm) water cell with a capacity of ca. 5 mL. The small size is of great convenience in storing multiple cultures in a small amount of space. The MicroAquarium™ is similar to larger aquariums in that it is open across the top dimension. As with any aquarium, food, water, and individual organisms can be added or removed as needed. Due to the narrow opening (about 2 mm wide), it will accept only those organisms thin or small enough to fit through its narrow opening. The number of different types of organisms or species known that will fit within a MicroAquarium™ is in the hundreds of thousands! Many of these organisms are microscopic and can be observed only with a microscope. Many others are macroscopic and visible to the naked eye.

A MicroAquarium™ supported by a stand and resting on a desk or windowsill serves well for casual viewing and can be enjoyed just as one enjoys a larger aquarium. A closer look at the contents is possible with a hand lens (the base of the MicroAquarium™ stand serves as a brace to steady the hand lens at proper focal distance). The MicroAquarium™ is uniquely adapted for microscope use. Due to the capillary retention of

water, the MicroAquarium™ does not spill when laid flat upon a microscope slide. A MicroAquarium™ with animal and plant life can be maintained for days, weeks, and even years. Many natural phenomena, including the interactions between living organisms, can be observed and photographed at any time—especially as seen through a microscope. Established cultures are instantly and always ready to be observed. Given no wet mounts to prepare and no cleanup afterwards, more time is available for observations.

Within an established MicroAquarium™, organisms interact with one another in an environment similar to their natural environment. Interstitial spaces, a benthos of sediment, and biofilms—each an aspect of microcosms found in nature—characterize the artificial habitat within the MicroAquarium™. Observing the artificial microcosm within a MicroAquarium™ does little to disturb the spatial organization and active interaction between organisms and their physical environment. It is common to find individual organisms seemingly content, browsing for food in familiar surroundings such that slowing agents (often recommended for standard wet mount preparations) aren't needed. In addition, organisms can be seen in various perspectives as they move about, and upon, surrounding substrates and one another.

These features make the MicroAquarium™ a remarkable device. Simple and easy to use, the MicroAquarium™ can empower more of us to connect with worlds beyond our normal experience. When viewed with a microscope, ordinary places harbor extraordinary life. The simplicity of the MicroAquarium™ allows even children to discover life at small scale. Richard Louv's inspiring book, *Last Child in the Woods: Saving Our Children from Nature-Deficit Disorder*, is full of reasons why we, and especially children, need to connect with nature. The MicroAquarium™ can help fulfill this need and allow more people to affiliate with even the tiniest of creatures.

Microscopic organisms exist all around us. In the words of E. O. Wilson (pp. 177–178), "They are everywhere but it takes a special eye to find them." Unfortunately, far too few people have meaningful, positive encounters with tiny organisms that are common in ordinary habitats. To the contrary, most people are quick to equate microscopic life with "germs"—a sentiment in conflict with reality. While there are germs and parasites that can and do harm humans, most micro-life is incapable of inflicting direct harm upon us. Rather, micro-life can be a source of enjoyment because life at this scale offers many opportunities for discovery. As a culture and observation chamber the MicroAquarium™ makes it relatively easy for one to observe and discover life history traits of

many organisms. To quote Thomas Eisner in his extraordinary book, *For Love of Insects* (p. 268), “To discover for yourself what is already known can still be a source of wonder—which is why the study of nature can never disappoint.”

In exploring the educational uses of the MicroAquarium™, several hundred cultures, many taken from field habitats, have been established. Following is a list of the major types of organisms maintained for extended periods.

Zoological Organisms

- sarcodines (naked and testate amoebae)
- ciliates (many types)
- flagellates (esp. *Paranema* and *Euglena*)
- hydra (esp. *Chlorohydra*)
- planarians
- microturbellarians
- gastrotrichs
- rotifers
- nematodes
- tardigrades (water bears)
- annelids (both freshwater oligochaetes and marine polychaetes)
- mollusks (snails, fingernail clams)
- arthropods (crustaceans, arachnids, insects)

Botanical Organisms

- cyanobacteria (many types)
- bryophytes (both terrestrial and aquatic species of mosses and liverworts)
- angiosperms (esp. the carnivorous *Utricularia*)
- water molds
- slime molds
- algae (many types)

Guidelines for Use

- The MicroAquarium™ is easily broken by pressure exerted upon its face. To avoid breakage while handling, always hold the MicroAquarium™ by the edges. Do not press on the face of the chamber.

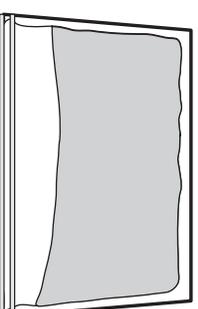


Do Hold by Edges

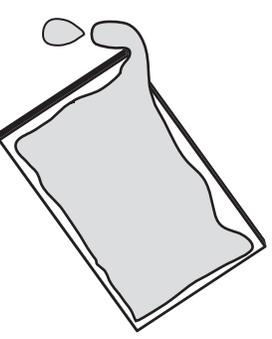


Do Not Press on Face

- When laid flat upon a microscope stage, even without a lid, the MicroAquarium™ will not spill, due to the capillary retention of water. However, tilting an open MicroAquarium™ too far or holding it upside-down will spill the contents. Therefore, use of the lid is recommended.



Do Lay Flat



Do Not Tilt

- Do not expose living cultures to excessive heat. Given the high surface area in relation to the small volume, the contents of the MicroAquarium™ will heat up quickly when exposed to warm temperatures. Limit the exposure time to heat from light bulbs below the stage in dissecting microscopes. Brief exposure to high temperatures (e.g., a hot car) will kill most invertebrates.
- Do not overfeed. Excessive feeding will favor bacterial growth and can result in the loss of organismal diversity. Most cultures will do well when “starved” for several weeks.

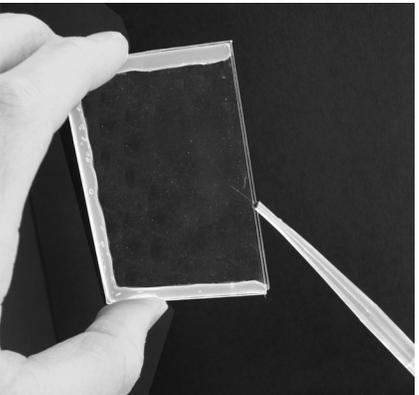
General Methods

As a precaution, rinse the MicroAquarium™ with tap water prior to initial use. When properly treated, the MicroAquarium™ will provide years of service. Placing the lid upon the MicroAquarium™ should facilitate handling in that it becomes a spill-resistant chamber that can be passed around and shared by multiple observers.

Stocking

The MicroAquarium™ may be stocked with an infinite range of materials for a multitude of purposes. The methods presented here are simply guidelines that may be modified to fit specific uses.

Use a pipet (plastic preferred) to fill the chamber and to transfer organisms (Carolina items RN-73-6986 and RN-73-6988 work well). Hold the pipet at a low angle with the tip resting upon, and cradled by, the opening of the chamber. Pipet tips cut to larger diameter are useful for transferring larger animals (e.g., scuds, flatworms). Plastic pipets whose tips have been stretched with pliers into long, thin extensions (“stretch pipets”) are useful for introducing and retrieving selected microorganisms. A plastic tool and fine forceps aids the addition of larger substrates and plant life (e.g., shoots of moss, pieces of substrate collected from aquatic environments).



While there is no wrong way to stock a MicroAquarium™, striking a balance between solid matter (sediment and substrates) and water is advised. Excessive solid matter can reduce visibility into the microcosm. A thin layer of sediment (0–5 mm) resting in the bottom of an upright MicroAquarium™ provides food and habitat for many invertebrates. A narrow, flat tool, several inches long (e.g., a six-inch manicure nail file, flat coffee stirrer, flat toothpick, or piece of thin plastic) can be used to position substrates in the lower half of the MicroAquarium™.

While it is generally advisable to completely fill the MicroAquarium™ with fluid, in cases where improved oxygen delivery is needed or a smaller volume is desired (as in establishing new cultures with a single cell isolated from a source culture), the chamber should be filled to the desired level.

Water

To fill initially, use source water from the culture or environment being investigated. If source water is unavailable, use any of the following: bottled springwater (RN-13-2450), distilled water, aged tap water, sterilized pond water, or any aquarium-safe water. **Chlorination alert:** Some tap water now contains chloramines as disinfectant agents. Chloramines are not removed by aging tap water. Instead, treat the water with a dechlorinator specifically formulated to remove chloramines (i.e., Carolina's RN-67-1939 or RN-67-1985 water conditioners). If in doubt, contact your local water treatment plant. To minimize mineral deposits in the evaporation zone, distilled water is recommended to replace evaporative water loss.

Labeling

An individual MicroAquarium™ may be labeled with an adhesive label strip cut from a mailing label or with “color coding labels” (adhesive paper dots, 1/4” diameter). Labels should be placed where they will not interfere with the viewing area, i.e., near the top of the chamber in the evaporation zone, or near the edge of the chamber over the sealant under the glass.

Placement and Storage

In general, organisms within the MicroAquarium™ are best maintained at room temperature in diffuse light. Room lighting is adequate; however, placement near a window receiving indirect sunlight is ideal. MicroAquarium™ chambers without stands may be conveniently stored and transported in test tube racks or modified slide boxes (with the aid of a small chisel or sharpened screwdriver, remove every other partition in the Carolina™ Blue Box, RN-63-4200). To transport for use in the field, take care to pack MicroAquarium™ chambers carefully in a crushproof container so they do not rattle.

Care and Feeding

Replenish evaporative water loss as needed (ca. every 10 days) with distilled water or any aquarium-safe water. To minimize mineral buildup in the evaporation zone, distilled water should be used to replace lost volume. Otherwise any aquarium-safe water (bottled springwater, aged tap water) is appropriate. A small bottle of refill water placed near the MicroAquarium™ cultures is recommended.

The rate at which water is lost due to evaporation varies greatly depending on the humidity where stored and the amount of substrate exposed (exposed substrate acts as an evaporative wick). The lid of the MicroAquarium™, applied loosely to allow gas exchange, can be used to retard evaporation. Alternatively, multiple MicroAquarium™ chambers can be partially sealed in a clear plastic bag and left for one to several months without their drying out.

Depending on one's objective, food comparable in size to a grain of Betta fish food pellets (up to 2 mm in diameter) may be added once or twice a month. For transfer, small particles of food will adhere to one's fingertip when gently pressed. Excess food results in blooms of foul smelling anaerobic microbes and will kill aerobic life. In general, the addition of appropriate food can produce blooms of protozoans and metazoans (e.g., rotifers, gastrotrichs, and microcrustaceans). Foods (nutrients) that have been added to MicroAquarium™ chambers with success include:

- poppy seeds
 - sesame seeds
 - grits (crushed corn)
 - fragments of rice, wheat, and other seeds
 - drop of fresh skim milk (Deliver less than one drop by dipping a toothpick moistened with skim milk into the MicroAquarium™.)
 - pine pollen
 - spider silk (collected from spider webs using a toothpick)
 - rabbit food pellets
 - fragments of Protozoan Pellets (RN-13-2360)
 - Betta fish food grains
 - fragments of other fish food (e.g., algae wafers, shrimp pellets)
 - Timothy hay (RN-13-2385)
 - active dry yeast granules
 - bits of hard-boiled egg yolk
 - Liquid Protozoan Nutrient Concentrate (RN-13-2350)
 - fresh carrot
- For ease of transfer, a small amount of crushed Protozoan Pellet, rabbit food pellet, or fish food pellet may be dissolved in water and then transferred with a pipet.

Cleaning

Algal films can become a nuisance in cultures designed to display macroscopic life (e.g., crustaceans, planarians, annelids, etc.). To clean the aquarium and restore a clear view, pour the contents of the MicroAquarium™ into a small dish for later retrieval. If necessary, rinse the MicroAquarium™ to remove all of the desired organisms. Once emptied of fluid contents, wipe away the obscuring films with a pipe cleaner or piece of tissue pushed by a flat tool (a six-inch manicure nail file is excellent for this purpose). Rinse with water and return the organisms to the MicroAquarium™ with a pipet.

A small magnet (a few mm in diameter) can also be used to manipulate a small piece of magnetic metal dropped into the aquarium chamber. The magnet is pulled across the outer glass face while the attracted piece of metal is moved across the inner walls, scrubbing obscuring films away from the glass. Take care not to crush larger organisms with the metal inside the chamber.

Prior to starting a new culture in a previously used MicroAquarium™, it is recommended to partially sterilize the aquarium by soaking it for several hours in a 1:9 ratio of bleach and water. After soaking, rinse well multiple times with warm tap water. Pipe cleaners and stiff, flat, narrow tools several inches long (e.g., nail files) are useful for removing particulate matter.

Viewing Tips and Observational Techniques

Various microhabitats exist within the MicroAquarium™. The upper water column near the surface may harbor life different from that found near the bottom or along the sides. The zone along the edges of the inner silicone sealant is often a region of interesting activity and good visibility, particularly in the bottom of the MicroAquarium™. The use of a hand lens or dissecting microscope can help you quickly access where particular organisms are located. Other organisms are found only with a compound microscope.

To reveal life hidden in the benthos debris, gently shake or agitate the MicroAquarium™ thereby loosening and spreading the debris across the inner chamber. As the MicroAquarium™ is laid flat after agitation, many organisms respond by scurrying across the chamber when so disturbed. Others organisms will be made visible as they are no longer hidden within a thick layer of sediment.

Light

Light is the key to good viewing conditions. One can obtain quality lighting simply by learning to manipulate common light sources for desired effects.

Daylight as seen from indoors through a window, ceiling lights shining from directly overhead, and lamplight from behind or below may serve well for viewing the aquarium contents with a hand lens or naked eye. For naked eye viewing, even the light located below the stage of a compound microscope can be useful in illuminating a handheld MicroAquarium™ positioned directly over the light source and under the stage. Another light source commonly available in schools is an overhead projector—the light is bright and useful for viewing a handheld MicroAquarium™.

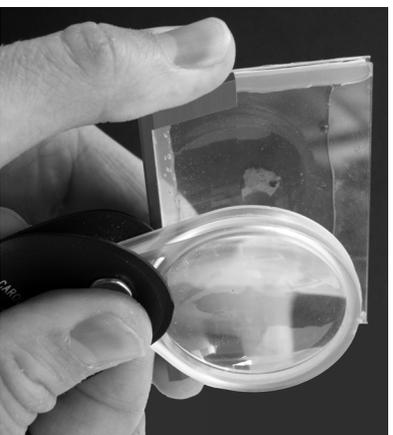
In a dark room or with a dark backdrop, light projected from directly above, below, or from the sides provides for dark-field illumination. A simple technique to achieve this effect is to lay a flashlight to the side of a MicroAquarium™ in front of a dark background. Minor changes in the position of the flashlight can bring about desired lighting. An elevated table surface makes for comfortable viewing: your eyes should be level with the MicroAquarium™.

Naked Eye

When properly presented with favorable lighting, the naked eye alone allows one to see clearly, and with some detail, features of macroinvertebrates. Blooms of one-celled, tiny ciliates can be seen as glistening specks moving through the water. Individual paramercia are easily seen without magnification.

Magnifiers

A large magnifying lens positioned in front of the MicroAquarium™ can serve as a viewing aid. A great variety of handheld magnifiers may be used in conjunction with the MicroAquarium™. Perhaps the best magnifier is a quality hand lens. A hand lens allows for detailed observation that can rival that provided by a microscope. Bristles, hairs, color patterns, eyes, legs, and mouthparts of invertebrates along with cell



shape, ornamentation, and movements of algae and protozoa become clearly visible with skillful manipulation of a hand lens. Positive attributes of hand lens use include: organisms can be quickly located in the wide field of view; lenses can be conveniently carried in pockets or worn with a lanyard around the neck and can be used virtually anywhere; use in the field during field trips and nature hikes greatly enhances one's experience with the natural world; ease of manipulating lighting effects, e.g., alternating between dark field and bright field with just a turn of the head to affect angle of lighting; observing natural colors of organisms that are typically lost in bright-field microscopy; 3-D shapes are readily evident; ease and convenience of use makes more-frequent observations likely.

The stand provided with the MicroAquarium™ serves as a brace upon which the housing of a hand lens can be rested. The focal length required to form a sharp image is obtained by slightly tilting the lens towards or away from the MicroAquarium™. Sliding the hand lens along the base of the stand maintains a sharp focus as the contents of the chamber are visually scanned.

Microscopes

The 2 x 3-inch size of the MicroAquarium™ fits the mechanical stage of most compound microscopes. The thickness of the MicroAquarium™ allows the use of 4x, 10x, and 20x objective lens of most compound microscopes. Dissecting microscopes are excellent for general observations and for use in locating specimens to be viewed with the compound microscope.

Multimedia

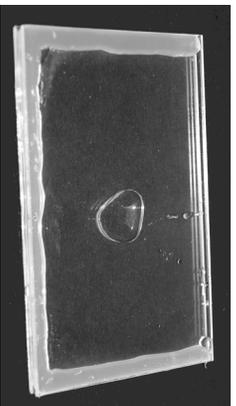
Document cameras, visual presentation cameras, digital magnifiers, and overhead projectors all can be used in conjunction with the MicroAquarium™. The live action of flatworms, mosquito larvae, and other organisms can be presented to an entire class or other audience with the aid of a document camera and projector. Digital magnifiers, such as those used by sight-impaired individuals, are excellently suited for use with the MicroAquarium™.

Other Manipulations

Suspended Drop Mount

Similar to the hanging drop mount, the suspended drop mount may be used to isolate organisms within a capillary cage so that they may be

easily relocated and studied over a period of several days. To prepare a suspended drop mount, gently squeeze a small amount of water containing selected organisms from a pipet into an empty MicroAquarium™. The water entering the chamber should adhere to both sides of the inner glass. Before the suspended water reaches the bottom, lay the MicroAquarium™ flat. The suspended drop can be manipulated experimentally through the addition of nutrients or additional organisms by using a slender probe or stretch pipet.



Irrigation and Aeration

While generally not required, it is possible to either irrigate or aerate the MicroAquarium™. Two pieces of plastic tubing (Tygon® microbore tubing, size 0.020 x 0.060, formulation 5-54-HL) are needed for irrigation. One piece of tubing siphons fresh, aerated water into a MicroAquarium™ from an elevated container (delivery rate about one drop per second) while the second piece of tubing siphons away from near the water's surface the upwelling water. To adjust the rate of inflow and outflow, simply adjust the height of the peak curve in the tubing. To aerate, one piece of the small tubing connects an air source to the lower water column. Tiny air bubbles will rise in succession just as in a larger aquarium. These techniques may be useful for the culture of less tolerant organisms that require higher oxygen levels (e.g., bryozoans). Thun (1966) originally proposed the method of using capillary tubing to deliver aerated salt water in his microaquarium used in the culture of marine nematodes.

Agar

As a semi-transparent medium, a layer of 1.5% non-nutrient agar in the lower portion of the MicroAquarium™ can provide interesting views of those organisms that penetrate the semisolid medium from the water column above. Various types of nutrient agar can also be used. To add agar, use a plastic stretch pipet or glass Pasteur pipet to deliver hot, molten agar to the lower portion of the MicroAquarium™. Add agar to a level of 1 cm above the lower chamber. After the agar has cooled, add pond water or other source material to the chamber. Examine periodically over the next few weeks for developing biota.

MicroAquarium™ as a Trapping Device

To demonstrate the dispersal of microorganisms downward over a tree trunk, empty MicroAquarium™ chambers can be positioned upright with the opening flush against the bark of a tree where rainwater is expected to flow and fill the MicroAquarium™. Crotches near the lower trunk are convenient places to station microaquaria. After a rain, examine the MicroAquarium™ for the presence of life. Water bears, nematodes, and rotifers have been captured using this method.

As a means of sampling microlife in aquatic habitats, let the MicroAquarium™ remain submerged for a month or more in the habitat being investigated. When retrieved, the outer faces should be wiped clean and the contents examined. It should be interesting to note which forms of life die off soon after removal from the field environment and which organisms prosper within the MicroAquarium™ maintained indoors.

Culturing and Observing Protozoa

The MicroAquarium™ is an excellent chamber for studying and culturing many types of protozoa. With experience, one can with relative ease obtain specific protozoa from natural habitats. For convenience and to save time, cultures of protozoa may be ordered to arrive several days (or weeks) in advance of planned laboratory use. The quickest way to produce well-stocked microaquaria containing the desired protozoa is to fill each MicroAquarium™ with purchased cultures. A typical Carolina culture will fill several microaquaria with abundant protozoa. Alternatively, a single Carolina culture may be used to inoculate many microaquaria with fewer protozoa. The inoculated microaquaria should contain more abundant protozoa after one or two weeks provided nutrients were also added. The use of a dissecting microscope can help you locate the protozoa in the source culture. If a dissecting microscope is not available, retrieve fluid from the bottom of the culture dish and from around any included food substrate. If the purchased culture contains wheat or rice grains, these grains should be cut with a razor blade into several thin fragments and one fragment added to each MicroAquarium™ subculture. Stored at room temperature in diffuse lighting, MicroAquarium™ cultures may last for weeks, even months. Adding nutrients to older cultures typically results in population growth. Older cultures may also be used to establish new cultures. Old cultures will eventually die out if not managed carefully. Nutrients should be added to protozoan cultures about once or twice a month, depending on the culture's vigor (overfeeding a vigorous culture may hasten the buildup of harmful waste products).

Sarcodines

Actinosphaerium (RN-13-1302). Add microcrustaceans and observe these sun amoebae prey upon them.

Amoeba proteus (RN-13-1306). Renowned for being difficult to find, *A. proteus* upon the inner face of the MicroAquarium™ are visible with a hand lens or microscope.

Arcella vulgaris (RN-13-1310).

Centropyxis aculeata (RN-13-1320).

Chaos (RN-13-1324).

Diffugia lobostoma (RN-13-1334).

Ciliates

Blepharisma (RN-13-1430). Both the smaller cells and the “giant” cannibalistic cells that develop in aging cultures are easily seen with a 14x hand lens.

Bursaria (RN-13-1434). This large ciliate is easily accommodated within the MicroAquarium™. Aging cultures may develop large, resting cysts that readily break dormancy when introduced into fresh culture.

Colpidium (RN-13-1452).

Didinium (RN-13-1460).

Dileptus (RN-13-1466).

Euplotes (RN-13-1480). *Euplotes* uses leg-like cirri to walk across the inner face of the MicroAquarium™.

Paramecium sp. (RN-13-1540).

Paramecium bursaria (RN-13-1548). Containing the green symbionts zoochlorella, this species performs well in neglected cultures.

Paramecium caudatum (RN-13-1554). The star-like radiating canals of the contractile vacuole are readily evident when viewed at 100x with a compound microscope. Also, demonstrating the effect of voltage on cell behavior is easily done by inserting the wire leads of a battery clip attached to a 9-volt battery [Paramecia visible to the naked eye instantly begin moving towards to cathode (-) wire].

Spirostomum (RN-13-1590).

Stentor coeruleus (RN-13-1598).

Flagellates

Euglena (RN-13-1768). The phototropic response of *Euglena* can be easily demonstrated by shielding part of the MicroAquarium™ from light with a piece of black construction paper or aluminum foil.

Paranema (RN-13-1838). Though small, the flagellum is easily seen at 100x. *Paranema* does well for months within a MicroAquarium™.

Volvox sp. (RN-13-1860) and *Volvox globator* (RN-13-1864). The MicroAquarium™ is an excellent temporary viewing chamber for these dynamic colonies.

Culturing, Observing, and Keeping Small Animals

Maintaining reproductive animals and keeping selected nonreproductive individual animals for extended periods (weeks to years) is fairly simple and requires little space.

Hydra

Green hydra can be added to microaquaria and maintained for months when exposed to 8–10 hours of indirect sunlight or fluorescent light daily. Feeding green hydra microcrustaceans or fragments of the aquatic oligochaete *Lumbriculus* may induce budding.

Hydra (RN-13-2800).

Green Hydra (RN-13-2810).

Mixed Hydra (RN-13-2814). Culture contains green and brown specimens.

Planarians

The MicroAquarium™ is an excellent chamber in which to house and feed individual planarians. Planarians may reproduce by fragmenting but are typically maintained as individuals, sometimes living for nearly a year within the confines of a balanced MicroAquarium™ that contains photosynthetic organisms (algae, aquatic moss). To observe planarians feeding, add (with the aid of a pipet) a freshly cut fragment of *Lumbriculus* to a MicroAquarium™ containing one to three planarians that ideally have been starved for a week or two. Beta fish food grains and injured live organisms (scuds, mayfly larvae, etc.) have also been used with success. After planarians begin to feed, flip the MicroAquarium™ to the appropriate side to observe the ventral side of the planarian from which the pharynx emerges. Dissecting microscopes work well, but care should be taken to minimize exposure to bright light and heat from the light source. A hand lens and compound microscope are also useful. A water change may be required in order to remove excess food, especially if the MicroAquarium™ contains only planaria.

Mixed Planaria (RN-13-2958).

Dugesia dorotocephala (RN-13-2970).

Rotifers, Gastrotrichs, Tardigrades, Copepods, Ostracods, and Small

Cladocerans

These animals may be cultured as described under “Culturing and Observing Protozoa.” Like protozoa, they will frequently move across the surface of the inner face of the MicroAquarium™, where they are easily viewed. These organisms may be collected in the field or purchased from Carolina Biological Supply Company.

Rotifers, *Philodina* (RN-13-3172).

Gastrotrichs, *Lepidodermella* (RN-13-3100).

Tardigrades (RN-13-3960).

Copepods, *Cyclops* (RN-14-2365).

Ostracods (RN-14-2370).

Daphnia pulex (RN-14-2314).

Mixed Crustaceans (RN-14-2350).

Annelids

Small, aquatic annelids commonly reproduce by the asexual method of fragmentation and frequently multiply within the MicroAquarium™. A new appreciation for these animals will emerge as you observe their behavior and form within MicroAquarium™ habitats. Small, marine polychaetes may be obtained from sediments of marine aquaria or from natural coastal habitats where they are quite common within many submerged substrates. Small, freshwater oligochaetes may be obtained from various pond water habitats or purchased from Carolina Biological Supply Company.

Aeolosoma (RN-14-1748).

Lumbriculus variegatus (RN-14-1720).

Mixed Annelids (RN-14-1740).

Insects

Many larval forms of insects collected in pond water can be maintained for extended periods within the MicroAquarium™. Midge and mosquito larvae are particularly well suited and frequently metamorphose into adults.

Culex Egg Raft (RN-14-4470).

Culex Larvae (RN-14-4476).

Mosquito Diet (RN-14-4485).

Amphipods and Aquatic Isopods

These relatively large aquatic crustaceans make extraordinary classroom pets. Feeding behaviors and interactions with other organisms are easily observed, and small individuals will molt and grow larger (1–4 animals per chamber recommended). Note that the size of these animals varies, and larger specimens may not fit within the chamber.

Amphipods (RN-14-2355).

Aquatic Isopods (RN-14-2360).

Culturing and Observing Fungi and Plants

Fungi

Water molds frequently develop in just a few days on poppy seeds and Betta fish food grains added to freshly collected pond water that contains a little sediment. The *in situ* observation of these tender organisms is in many ways superior to the traditional wet mount preparation.

Achlya (RN-15-5901).

Allomyces arbuscula (RN-15-5910).

Saprolegnia (RN-15-6270).

The slime mold *Physarum polycephalum* is easily grown in a “dry” MicroAquarium™ previously coated on the inside with non-nutrient agar (add molten agar to the MicroAquarium™, let set for 30 seconds, and then pour the agar out). Transfer either a sclerotium or a piece of active plasmodium to a prepared MicroAquarium™. Add an oat flake to maintain the plasmodium for a week or longer. Rinsing the plasmodium every other day is recommended to keep it hydrated and vigorous. This technique is outstanding for microscopic study of cytoplasmic streaming; it also demonstrates slime trails left behind the advancing plasmodium.

Physarum polycephalum Sclerotium (RN-15-6190).

Physarum polycephalum Plasmodium (RN-15-6193).

Plants

Carrot seeds have been shown to readily germinate underwater. Root hairs and the root cap are easily observed with a hand lens. Duckweeds such as *Wolffia* and *Wolffiella*, the smallest angiosperms, grow very well. Many aquatic and terrestrial bryophytes survive for long periods underwater within the MicroAquarium™. Many produce interesting structures

(rhizoids, protonemata, gemmae, gametangia) while underwater. Perhaps the most successful true plant grown in the MicroAquarium™ is the carnivorous aquatic bladderwort, *Utricularia*. This hardy plant will survive and grow for many months, producing its characteristic bladders. Within the MicroAquarium™, it is relatively simple to trigger the bladders (with a slender probe) while observing the trapping mechanism with a dissecting microscope.

Aquatic Bladderwort, *Utricularia* (RN-15-7200).

Establishing a Pond Water Microcosm

“Microscopic” life in pond water has entertained many since the 1600s, when attention was first turned to the microworld. Biology students and pond water enthusiasts no longer need to make repeated temporary wet mounts to gaze into these microscopic worlds. A MicroAquarium™ stocked with pond water is constantly ready and waiting to be viewed. Large enough to support microhabitats representative of those found in nature, the MicroAquarium™ provides a unique cross-sectional view into these microecosystems. A succession of changes typically develops within a newly stocked pond water MicroAquarium™ as various ciliates and other organisms bloom and wane. In time, the aquarium becomes a balanced ecosystem with its own characteristic mix of organisms. Some aquaria may become dominated by ostracods or harpacticoid copepods. Others may support long-lasting populations of microturbellarians, protozoa, or other life.

Collecting Methods for Pond Water Organisms

For ethical and legal considerations in making field collections, see Darmono (1995).

Tools. No tools are needed to stock a MicroAquarium™ with pond water and sediment. Simply scoop up water and sediment in the palm of your hand and pour over the MicroAquarium™ opening. By chance, you are sure to get a number of invertebrates. A more discriminating approach can also be interesting. A pipet is required to choose organisms and transfer them to the MicroAquarium™. Collecting jars and a white collecting bowl or pan help considerably in locating and transporting material from the field. A turkey baster is also useful in making field collections.

Field Orientation. Organisms are not uniformly distributed along the margin of ponds, puddles, or other aquatic habitats, so develop your field eye for variations in microhabitat. When possible, take samples from a

variety of microhabitats (sunny vs. shaded areas; muddy vs. rocky bottoms; dead wood vs. green vegetation; and ad infinitum). When collecting, remember that you are accessing various microhabitats for the presence of microlife. With practice and experience, your ability to locate specific organisms in the field will greatly increase.

Habitats. Microinvertebrates can be found almost anywhere. Habitats often rich in invertebrate life include long-standing pools or puddles, pond water, pond water sediment, and surfaces of submerged objects in creeks, ponds, and rivers. If natural habitats are difficult to visit, collect from residential birdbaths or fish tanks. Pet store employees typically are willing to donate scum from aquarium filters if you provide a collecting jar. Terrestrial moss collected from tree bark or rock (concrete) walls also contains abundant aquatic invertebrates.

Panning. This method is similar to panning for gold, only you are searching for living treasure. Any type of white, shallow dish or container will work (e.g., plastic food containers, small dish pans). Dip the dish along the margins of ponds and wet ditches and then stare—patience is rewarded—at the water and the bits of sediment retrieved. For best viewing and ease of capture with a pipet, the water in the dish should be an inch or less deep. As the sediment settles, the activity of arthropods (copepods, daphnids, insect larvae, etc.), mollusks, and worms should soon become apparent if the habitat is rich. If one dip doesn't turn up anything, dip again in another place. Also, try shaking out submerged substrates and vegetation into a pan. Simply place a handful of floating algae, submerged leaves, etc. in the white dish, agitate, and then remove the large matter, leaving organisms behind that have fallen from the larger substrates. Try dipping to acquire sediment from the bottom of the water body. Sediments are often teeming with observable life. If muddy sediments obscure your view, do like a gold miner panning for gold flakes and gently add more water to dilute the mud. Decant the muddy water while leaving the animals behind. Examine closely for flatworms and other organisms moving across the bottom of the pan.

Looking Under Rocks and Submerged Leaves. Pick up partially or wholly submerged rocks and leaves along the edges of springs, creeks, and ponds. Examine the undersurfaces closely for small invertebrates. Many invertebrates cling tightly to these undersurfaces. To dislodge arthropods and planarians from the surface of these substrates, hold the rock or leaf in the air for a short time and then dip it briefly into a pan filled with water. Planarians and other organisms will release their hold and fall to the bottom of the pan where they are readily visible and easily captured with a pipet.

Baiting Traps. Set jar traps baited with food. A small glass jar whose lid has been punctured several times makes an excellent trap (a Phillips-head screwdriver serves well as a hole punch). Add a thumbnail-sized (or smaller) piece of raw liver or other food to the jar. A string can be tied between the holes in the lid before submerging it along the edge of a pond, ditch, or creek. Check after half a day or the next day. **Important:** Before leaving the collecting site with captured organisms, drain the nasty water from the bait jar and dispose of the bait; add clear water from the collection site.

Stagnant Pond Water Jar. Fill a jar half full with pond vegetation, algae, submerged leaves, a bit of mud, etc., and top off with pond water. Store the jar with no lid (or with a loose lid) out of direct sunlight and away from strong heat. Hours (or days) later, examine the upper column of water for aerobic organisms forced to the surface where oxygen is more available (material in the lower water column becomes anaerobic). Organisms are best seen by looking through the side of the jar such that your eye is at or below the water level. Any jar of pond water kept for a day or two will reveal organisms not noticed on the first day. Organisms that survive in a stagnant jar of water probably will survive for long periods of time in a MicroAquarium™.

Vortexing Concentrate. To concentrate small, planktonic organisms in the center of a bowl or pot, simply stir collected water in a circular fashion. The vortex created by stirring pulls small organisms and particulate matter to the center of the bowl for efficient retrieval with a pipet. This technique is quite useful to demonstrate the aerial dispersal of rotifers, water bears, and nematodes in forested regions of the country where snowfall accumulates. A pot of snow collected several days after a snowfall is allowed to melt and the particulate contents are collected as described above.

Adding Submerged Surfaces. Adding small pieces torn or cut from submerged substrates (decomposing leaves and stems, submerged living green vegetation, or mineral and organic debris scraped from rock surfaces) will bring to the MicroAquarium™ much life, namely representatives of periphyton, the community of organisms that occurs on submerged substrates. For a long-lasting, interesting microcosm, adding pieces taken from submerged surfaces is strongly recommended.

Feeding Your Pond Water Microcosm

If the source sample placed into the chamber contains substrates and debris, no food is required during the first week following initial stocking; however, adding a small amount of food soon after stocking should do no

harm and may yield blooms of protozoa within a day or so. Copepods and other organisms can live and reproduce for weeks without additional food. Adding food (see “Care and Feeding” under General Methods) will increase the population growth of particular species. Many protozoa, annelids, and crustaceans respond well when nutrients are added. If crustaceans are present, ciliate diversity will remain low, as crustaceans tend to dominate the culture.

Discovering Aquatic Life in Terrestrial Environments

Much of the species richness of any environment resides in individual organisms too small to be seen without technical intervention. With a MicroAquarium™ and minor manipulation, at least some of the unseen diversity of life becomes visible. The discovery of aquatic microcommunities in terrestrial environments is one of the more novel uses of the MicroAquarium™. Terrestrial environments possess temporary waters that support abundant aquatic life on a micro-scale. During and after a rainfall, free water is retained by capillary forces within tiny crevices found within moss, tree bark, and soil. The term “limno-terrestrial” may be used to describe the unique nature of temporary aquatic habitats found within the myriad capillary interstices found in otherwise terrestrial habitats.

Aquatic life abounds in terrestrial landscapes. The mossy bark of an oak tree comes alive with aquatic organisms after a rain. Amoebae, one-celled ciliates, nematodes, rotifers, and water bears swim, crawl, and glide within the thin films of water retained within the mossy vegetation. Filaments of algae and tiny balls of colonial algae also reside within the watery gaps within mosses. The aquatic ecosystem is defined by the films of water held between moss leaves, rhizoids, and adjacent moss shoots, as well as the crevices of tree bark and the labyrinth of interstices between soil particles and decomposing forest litter. Within these capillary spaces rainwater may be retained for days, even weeks, during wet weather in many parts of the U.S. Even the face of a concrete wall can support aquatic life. Concrete darkened by “moldy growth” often is home to various types of algae and aquatic animals active only when wet. Other animal life such as springtails, pseudoscorpions, mites, and beetles live in mossy crevices, but these move through air spaces within the mossy mat or other humid crevices. Limno-terrestrial organisms must be submerged to be active. As the crevices eventually dry out, these animals and protists enter a state of dormancy either in an encysted form or a completely desiccated state.

To demonstrate microscopic life in mosses, tree bark, or soil, place a few pieces of moss, fragments of bark, or particles of soil into a MicroAquarium™ and add distilled water, bottled springwater, aged tap water, or sterilized pond water. It is recommended that small quantities of moss and/or bark be added such that there is at least 50% unoccupied space within the MicroAquarium™. There will be fewer obstructions in viewing the contents and less material that may decay, limiting available oxygen. Soil may be placed in a dish containing water and then transferred to the MicroAquarium™ with a pipet. The amount of soil added should cover the bottom of the chamber to a depth of 0–3 mm after settling. To increase the numbers of certain species, nutrients may be added (see “Care and Feeding” under General Methods).

Other means of extracting microlife from limno-terrestrial habitats include the use of Bearmann funnels or a nested screen sieve series (see “Related Products From Carolina”).

Many of the larger microinhabitants of limno-terrestrial “micro-widernesses” will be visible when established MicroAquarium™ cultures are examined with a microscope. It is common to find various algae, protozoa, nematodes, rotifers, and water bears in many terrestrial microhabitats. In locations seasonally wet or where the habitat is moist for longer periods of time, ostracods and copepods (microcrustaceans) are common, as are microturbellaria (nearly microscopic flatworms). Some interesting forms of amoebae, algae, fungi, and animals may not become apparent until after several weeks of culture.

Additional Resources

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Wilson, E.O. 1993. *The Diversity of Life*. W. W. Norton & Co.

Web Resources

At the time of this printing, the following Web sites are active. You may wish to perform an independent search for similar sites.

MicroAquarium™. Image gallery of organisms inside MicroAquarium™ chambers.

<http://www2.una.edu/microaquarium/index.htm>

Micro*scope. The Astrobiology Institute Marine Biological Laboratory, Woods Hole. “A communal web site that promotes information on the biodiversity of microbes.”

<http://starcentral.mbl.edu/mv5d/>

Micrographia. "...for students and teachers of (especially) fresh water biology in their search for creature identification..."

<http://www.micrographia.com/index.htm>

Micscape Magazine: The monthly online magazine of *Microscopy UK*. See especially "Pond Life ID Kit" and "The Smallest Page on the Web."

<http://www.microscopy-uk.org.uk/mag/indexmag.html>

Related Products From Carolina

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RN-13-2425 Wheat Seed

RN-13-2010 Microlife Mix Culture

RN-GEO9310 Screen Sieve Set

CD-ROMs

RN-40-1321 Carolina™ Gallery of Images: Protists

Books

RN-45-2005A *Guide to Microlife*

RN-45-3901 *Pennak's Freshwater Invertebrates of the United States*

The *How to Know* Series:

RN-45-4100 *The Protozoa*

RN-45-4412 *The Freshwater Crustacea*

RN-45-8200 *The Freshwater Algae*

Posters

RN-57-4015 Pond Life Poster Series Set

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