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Scope of Study: Biology is a study of living things and things that may have once been alive. It is "living things" which present the biology teacher with his problems, for the maintenance of living things requires time and care. With this in mind, this report has been written for the purpose of being used as an aid in helping to maintain a living laboratory. It presents successfully tried methods for collecting and culturing common laboratory animals according to works of leading authorities in the field. In addition, mention is made of the usefulness of each organism. In compiling the report, the data was gleaned from periodicals, biological supply house bulletins, and various texts which contained material relevant to the subject.

Findings and Conclusions: Many invertebrates and some classes of vertebrates can be cultured in the laboratory or classroom, providing living forms for activities in behavior, nature study, classification, reproduction, and variation, or comparative studies of organ systems, circulation of blood, and observation of the heart beat. Furthermore, many of these animals serve as food for other animals which often are reared in the laboratory.

The best ways for maintaining living things are those methods which reproduce the most favorable field conditions and eliminate natural enemies where possible. Diet and temperature control are the factors most responsible for success in keeping organisms in a healthy state. The methods given have proved successful, but this does not mean that other methods are not superior.

ADVISER's APPROVAL L. Herbert Bruneau.

COLLECTION, CARE, AND USE OF LIVING ANIMALS IN THE HIGH SCHOOL BIOLOGY LABORATORY

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COLLECTION, CARE, AND USE OF LIVING ANIMALS IN THE HIGH SCHOOL BIOLOGY LABORATORY

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CHAPTER I

INTRODUCTION

Biology is essentially a study of living things and things that have once been alive. It is "living things" which give biology its special flavor and interest. For many years the laboratory work in many high school biology courses has been confined largely to observation on dead organisms, including not only external features but also anatomy and even microscopic study of tissues. This is very interesting to specialists, but the average high school student is not much given to learning for learning's sake. He is not much interested in the fact that all the crayfish's many appendages are modified from one primative type, but he is immensely interested in watching a living crayfish as it uses its varied assortment of tools with expert handiness.

One may glue each appendage to a card, with beautifully clear statements about the appendages and little attention will be given to the display. But if one places a lively crayfish in an aquarium everyone flattens his nose on the aquarium and watches with attention every slow and clumsy movement of these same appendages. There is plenty of interest in biological subjects, if the subjects are living; and, when one thinks of it, it does seem logical that the study of life should deal largely with living things.

To have a laboratory of living things one should commence in a small way and add to it as new supplies of enthusiasm and materials accumulate. Never undertake the care of such a laboratory merely as

duty. If the teacher in charge is just trying to do his duty, without much love for the pupils or his subject, the work will be dead anyhow and may as well be taught from dead specimens. If, however, the laboratory is in charge of a teacher who loves living things, including live boys and girls, he will wish to conduct his work with living materials as soon as the idea occurs to him. As soon as he attempts to do it his troubles begin. His chief method of progress is by trial and error, with sometimes very discouraging results.

A living laboratory calls for constant care from the teacher in charge, as well as much patience and consideration on the part of teachers in the class rooms nearby. Snakes will escape at times, rabbits like to go exploring, noises can not be confined to the laboratory, and it is a regrettable fact that at times there are objectionable odors.

One of the first things that a laboratory teacher has to admit is that his room can not always look neat and tidy. If his pupils are interested they will bring in samples of all sorts of things and if he is interested in his pupils he will welcome all they bring. Sometimes the specimen will cause trouble, but it should be kept for a day or so, if possible, and then released.

This report will present successfully tried methods for collecting and caring for common laboratory animals. In presenting the most successful methods, the animal kingdom has been grouped into four main divisions according to similarity in culturing, habitat, or structure. The four main divisions; protozoa, fresh-water invertebrates, terrestrial invertebrates, and vertebrates, are subdivided into sections on the specific organisms most commonly encountered in the laboratory. In each section the recommended methods for collecting and culturing the various organisms are presented with a discussion of some of the possible uses of

of these animals. The methods given in this work have proved successful, but this does not mean that other methods are not superior. By trial and error, and by use of the data presented in this work, a biology teacher may arrive at the best method of care and collection for his laboratory.

CHAPTER II

PROTOZOA

Culturing protozoa is relatively simple if certain fundamental precautions are taken. Some environmental precautions which must be met are:

1. Keep cultures at a constant temperature within an optimum range of 18° and 21° Centigrade.

2. Keep cultures away from the fumes of concentrated acids such as nitric acid, hydrochloric acid, and sulfuric acid and such alkalies as ammonium hydroxide.

3. Try to keep the cultures at a hydrogen-ion concentration approximately neutral (pH 7).

4. Keep the cultures in medium light. Darkness is not necessarily detrimental. Direct sunlight is harmful, since the temperature of the culture may be raised above the optimum.

5. Avoid sudden drafts near the cultures, since they may carry contaminants. For the same reason the cultures should be covered.

6. Keep glassware clean. Wash culture dishes with soap, and rinse repeatedly. One of the best rinses is a baking soda solution (one tablespoon per gallon) followed by hot water from tap or distilled water.

Specific precautions for each type of organism discussed will be given in the section on the culturing of each.

Rhizopods (Amoebae and Allies)

Collection: Amoeba may be found in ponds or pools which do not contain much organic matter, where the water is clear and not too alaline, and where exceedingly swift currents are absent. Both Amoeba proteus and Amoeba dubia are found among aquatic plants such as elodea and Myriophyllum. Often scrapings from the base of a stalk of a cattail or from the underside of a leaf of the water lily will yield amoebae.[21]

Culturing: Temporary cultures may be prepared by placing small amounts of plants with amoebae into finger bowls, large petri dishes, or flat jars. The material is covered with pond water in which the plants had been growing, or spring water. The culture jars must remain at room temperature and to each two or four uncooked rice grains must be added.

Amoebae congregate on the bottom or sides of a container. They may be removed in either of two ways. Carefully pour off the excess fluid and then with a pipette pick up the amoebae, or if the animals are not congregated, swirl the container with a rotating motion. Then amoebae may be collected with a pipette at the center of the container.

Continuous cultures may be prepared by two methods. The first method involves the use of hay influsion. Place some timothy hay stalks in spring water. Boil this for 10 minutes and let it stand for 24 hours. Then add large quantities of Colpidium or Chilomonas. Let this medium stand for two or three days, then inoculate with amoebae. As the culture develops, the food organisms decrease in number. When this happens, remove half of the culture medium and add an equal amount of fresh hay infusion to which Colpidium or Chilomonas have been added. Add two grains of uncooked rice or boiled wheat, or four one-inch

lengths of boiled timothy hay for every 50 cc of culture medium which has been added. Cultures may last as long as six months.

Another method proposed by Brandwein[4] involves the preparation of synthetic pond water and agar medium. The preparation is made by weighing out the following salts and dissolving them in distilled water to make one liter of solution:

NaCl	1.20 gm
KCl	0.03 gm
$CaCl_2$	0.04 gm
NaHCO	0.02 gm
Phosphate buffer (pH 6.9 to 7.0)	

This is a stock solution. For use it should be diluted 1:10 with distilled water.

A number of finger bowls are prepared by rinsing them in hot water, then in cold. The bottom of each bowl is covered with a thin layer of agar. While the agar is still soft, rice grains are embedded in it.

Introduce about 50 amoebae, together with 15 cc of the medium in which they have been growing, into each bowl and add about 30 cc of the dilute solution formerly prepared. For the three days, add 15 cc daily of the solution, until the total volume is 90 cc.

After about two months of growth the culture wanes and should be sub-cultured. This may be accomplished by dividing the contents of each finger bowl into four parts. Add each part to a prepared finger bowl, and equal volume of the dilute solution.

Chaos, a giant form of amoeba, can be cultured in Brandwein's solution for synthetic pond water, using the rice-agar method described earlier. In addition add a pipetteful of paremecium or stentor as food.

Arcella, a shelled relative of amoeba, may be cultured by either method.

Use of Rhizopods. Rhizopods are used mainly in illustrating

amoeboid movement and ingestion. Specimens are also useful in taxonomy.

Ciliates (Paramecium and Allies)

Collection. To collect ciliates gather submerged plants and decaying material from cloudy pond water. An abundance of ciliates will be found wherever bacteria are plentiful.

Culturing. Ciliates may be cultured by the two methods given in the section on rhizopods or by the following.

A hard-boiled egg may be used in culturing paramecium and vorticella. A thin paste is prepared by grinding 0.5 gm of the yolk of a hard-boiled egg with a small amount of tap water or boiled pond water. This paste is added slowly and with stirring to 500 cc of hot tap water, boiled pond water, or Brandwein's synthetic pond water. Allow to stand for two days at room temperature. Then add seeding of organisms to be cultured. Paramecium need Chilomonas as a nutrient source.[4]

A medium can be prepared by the use of skim-milk powder. A pinch of skim-milk powder is added to 250 cc of spring water or boiled, filtered pond water. The protozoa reared in this medium are large with clear cytoplasm and clear food vacuoles.[13]

Another medium may be prepared by adding one-fourth package of dehydrated yeast to 250 cc of pond water, spring water, or tap water. Mix well and allow this culture medium to stand exposed to the air several hours. Inoculate with the culture of protozoa one plans to maintain. Rich cultures develop within a week at room temperature. Keep the cultures covered after they have been inoculated with protozoa to prevent evaporation and contamination.[21]

A method similar to hay infusion uses lettuce leaves. Rub the outer dry leaves of lettuce through a fine-meshed wire strainer. Boil

pond water, and when it cools, add one teaspoon of the lettuce to one liter of pond water. Boil this for one minute; let the jar stand covered over-night. Later divide the medium into finger bowls or baby-food jars and inoculate with a culture of paramecium. Keep the containers covered. A pinch of powdered milk is added after 12 days and repeated weekly.[21]

Horse and sheep manure pellets covered with pond water work well for culturing protozoa as does dead fish or waste from frog tanks.

Flagellates (Euglena and Allies)

Collection. The best source for collecting flagellates is the alimentary tract of termites. In addition the green surface which may be found in ditches or ponds may often contain large numbers of Euglena and similar flagellates.

Culturing. In general, methods which were described previously in the sections on rhizopods and ciliates are recommended for flagellates.

Use of Flagellates. Euglena can be used to illustrate the response of animals to light. Euglena can also be used as an example of a protist, organisms which illustrate both animal and plant characteristics. Volvox is an example of colonial behavior in microorganisms.

CHAPTER III

FRESH-WATER INVERTEBRATES

Many invertebrates can be cultured in the laboratory or classroom, providing living forms for activities in: behavior, nature study, classification, reproduction, and variation, or comparative studies of organ systems, circulation of blood, and observation of the heart beat.

In this chapter, methods for collecting and cultivating common fresh-water invertebrates will be described. As each animal is mentioned, possible usefulness of the animal in the classroom is indicated.

Hydra

Collection. Fresh-water hydra may be found in lakes or ponds, attached to submerged stems of water plants, on the under-side of floating leaves of water lilies and water hyacinths, or attached to the surface film of the water itself. Transfer the plants upon which hydras are found into finger bowls or small aquaria. Be certain to use the water in which the hydras have been living, if available, or the water from a thriving aquarium. Add the aquarium water slowly, a glassful per day, to the original water, so that the organisms will become acclimated. Brandwein's solution (described in the section on rhizipods) may be used in the absence of satisfactory aquarium water.[21]

"Keep the containers in medium light or semi-darkness, at a temperature below 20° C. Within a day or so the hydras will be found on the surface of the water."[21] They can be picked up with a pipette and transferred into new containers to start fresh cultures.

Culturing. About twice a week feed the hydras Tubifex or Daphnia. Well-fed hydras grow rapidly and reproduce readily. Adequate green water plants are needed for a rich supply of oxygen.

"At times periods of depression beset hydras; tenacles are contracted and the body becomes shortened. Sometimes depression may be avoided by frequent changing of the water."[21]

Use of Hydras. Hydras may be used to show how coelenterates use their nematocysts in food-getting. The coelenterates also can be used in the studies of regeneration, tropisms, reproduction, and grafting.

Planaria

Collection. These small flatworms can be found on the underside of submerged logs and under stones in ponds and lakes. Several varieties may be found in clear, running water, but the usual forms are the small black or brown types. When one finds some on a submerged log, wrap the whole log in wet newspaper and bring it into the laboratory. Submerge the log in a white enamel pan of water and peel off sections of the wood. Usually the planarians float to the top. Planarians may also be baited by submerging a piece of raw beef liver or hard-boiled egg yalk (tied in cheesecloth) attached to a string, in a cold stream or lake. Brush off the gathered forms into collecting jars and submerge your bait in another part of the lake or stream.[21]

> Culturing.
>
> Because planarians are photonegative, they should be maintained in black or opaque containers. Enameled containers are excellent. Frequently change the water with fresh additions of aquarium water. Keep them at a temperature below 35° C. Once a week feed them a diet of finely chopped raw beef liver, or bits of Tubifex. At other times, feed them bits of hard-boiled egg yolk. Remove the excess food with a pipette after several hours to avoid fouling the water.[21]

Use of Planarians. The chief use of planaria is in the studies of regeneration. They can also be used to show different types of tropisms.

Rotifers

Collection. Half fill several jars with submerged plants, and fill them with pond water. Place the jars in moderate light and after a day or so these many-celled microorganisms may be found congregated on the surface where there is an abundant supply of oxygen. This is also a good method for collecting Hydra and planaria.

Culturing. Change the culture water frequently. Feed them cultures of Euglena.

Use of Rotifers. Rotifers have limited use but are often encountered in laboratory work. Rotifers can be used in studies of taxonomy, anatomy, and parthenogenesis.

Tubifex

Collection. Tubifex may be collected from the muddy bottom and decaying leaves of streams and ponds, or from sewage disposal plants. They form tubes of mud held together by a secretion from epidermal cells.

Culturing. Tubifex can be cultured in well-established aquarium tanks which contain an inch or so of muddy soil.

Use of Tubifex. These aquatic worms can be used for regeneration experiments, for asexual reproduction studies, and a food supply for laboratory animals.

Snails

Collection. Among these fresh-water mollusks there are wide variations in shape, size, and habitat. Such egg-laying forms as Physa,

Planorbis, and Lymnaea may be found attached to water plants in ponds and lakes. A larger form, Campeloma, is a live-bearer and may be found in lakes or rivers attached to rocks or plants. Helix, the land snail, may be found in moist, but not too acid, soil such as that in gardens or some wooded areas.[21]

Culturing. Raise Helix in a cool place in a moist terrarium with occasional feeding of lettuce. Keep aquatic snails in an established aquarium. They normally feed upon algae, but when they increase in number they feed upon the aquatic plants. Supply them with lettuce and the aquarium plants will be spared.

Use of snails. Snail eggs can be used in embryo studies. Snails should be kept in aquaria to hold down the abundant growth of algae and to remove decaying materials.

Daphnia

Collection. These small, laterally-compressed water fleas may be collected from ponds, lakes, or streams by means of fine-mesh nets. During the Spring and summer, females are usually found. In the autumn, males appear and the "winter eggs" are fertilized. Collecting is frequently better at night as many of the water fleas rise to the surface waters after sunset.[3]

Culturing. Fill large batter jars with tap water and let them stand overnight to permit evaporation of gases which may be harmful. Then put the battery jars in strong sunlight and inoculate them with nonfilamentous algae from an aquarium. After this "green water" has been standing for two or three days, add Daphnia and several cc of hard-boiled egg yolk mashed into a paste with a bit of culture medium. A suspension of yeast may also be added to stimulate growth. The temperature range

may vary between 12° and 24° Centigrade.[22]

Chipman's method[7] culturing Daphnia involves the using of bacteria. A rich growth of Bacilus coli is used as the food source. First filter pond water through coarse filter paper. Then add about 90 gm of garden soil and 17 gm of cottonseed meal to one liter of this filtered pond water. Stir the mixture well and set it aside at room temperature for at least five days. Fermentation takes place and gases are formed. At this time, decant off the supernatant fluid and strain through muslin. An almost pure culture of Bacilus coli is produced in the supernatant liquid. Correct the pH to 7.2 by adding sodium carbonate. Use hydrion pH paper for testing the pH.

Now dilute this fluid with pond water (1 part of strained fluid to 100 parts of pond water). Inoculate this culture medium with Daphnia. Keep the culture in large batter jars. Each week prepare fresh stock of cottonseed meal and garden soil. Then add a small amount of the old culture each time a new medium is established.

Use of Daphnia. Daphnia can be used as food for small fish, tadpoles, and hydras. Other uses of Daphnia are to study circulation, respiratory system and peristalsis. A minor use of water fleas is to clear aquaria which have become soupy green.

Cyclops

Collection. This elongated crustacean lacks a shell and has no abdominal appendages. It is characterized by the single compound eye located in the center of the head; it uses antennae for locomotion. During the summer months, females can be found carrying two brood pouches posterior to the body. Cyclops may be found in brackish water as well as fresh-water streams and lakes.[21]

Culturing. Culture methods are similar to those described for Daphnia.

Use of Cyclops. These forms are interesting for laboratory study and for introductory work with a microscope; they can be used as food for small invertebrates, fish, and amphibia.

Crayfish

Collection. Crayfish inhabit the limy regions of quiet streams and ponds, concealing themselves under stones or burrowing into banks. Collecting crayfish is usually a simple matter, for they can be taken with an ordinary long-handled net when seen near the shore or with a large seine-net. Baiting is also another method used in the collection of crayfish. It consists of attaching a piece of raw meat to a string and placing it in front of the organism. After the crayfish grasps the bait with his pincer, he is drawn by the string into the collecting vessel.

Culturing. A large enameled dishpan with about two inches of water, sides high enough so the crayfish can't climb out, is all one needs for an aquarium. It is advisable to have a single crayfish per acquarium, for when several are kept together, they frequently get entangled with pincers in a fight and many part with limbs or lives. A flat rock put in the crayfish dishpan and rising slightly above the water will enable the creature to come up when it wants to try the air.[30]

Crayfish can be fed bits of raw meat, chopped raw fish, earthworms, and water insects.

Use of crayfish. Crayfish can be used in anatomical studies to show the specialization of structure and complexity of body systems. They are especially good for the study of respiration by gills.

CHAPTER IV

TERRESTRIAL INVERTEBRATES

The best ways for maintaining living things are those methods which reproduce the most favorable field conditions and eliminate natural enemies where possible. Diet and temperature control are the factors most responsible for success in keeping invertebrates in a healthy state. This chapter will deal with the collection and care of a few common terrestrial invertebrates which may prove interesting in the laboratory.

Earthworms

Collection. These annelids are readily collected at night and after a good rain, when they come to the surface. Earthworms can also be found by use of a spade in damp areas of chicken yards or areas of decaying vegetation.

Culturing. Place several worms in plastic or glass ice box dishes. In the containers place four to six inches of moist rich soil or peat moss. Dampen the soil but avoid excess moisture. Keep the animals covered in a cool place (at a temperature about 15° C. About twice a week feed them lettuce and bread soaked in milk. Bury the food.

Use of Earthworms. Earthworms are excellent organisms to use in studies of tropisms. Earthworms are also favorable material for dissection in many classrooms over the country. Of course, earthworms may be maintained as food for frogs, fish, and reptiles kept in the laboratory.

Woodlice

Collection. The sow and pill bug are all well adapted for their way of life and are widespread. They may be found under stones, boards, logs, in places which are dark, moist, and undisturbed.

Culturing. Their usual ecological conditions are best duplicated in the laboratory in a terrarium containing damp, rich humus with small rocks or logs under which the organisms can hide. Supply these isopods with bits of apple, bits of lettuce, and at times pieces of raw potatoes. They will also accept small earthworms and insects.[21]

Use of Woodlice. Isopods are interesting to have on hand in studies of adaptation of animals to show how their compressed bodies fit them for their environment. They can be used in tropism studies and their intestines can be examined for flagellates.

Praying Mantis

Collection. Collect egg masses in the fall or early spring. The egg cases are recognized as tan, foamlike masses attached to twigs.[21]

Culturing. It is well to place the eggs in a cage or terrarium containing some twigs, grass or growing plants on which the young mantes can climb. The cage may be any kind of a glass jar or terrarium tank which can be kept covered with cheesecloth or fine-meshed wire screening. Being carnivorous, the mantes do not require fresh vegetation, but the presence of growing plants maintains a natural humidity which is desirable.[15]

After the eggs are placed in the cage, it should be kept in a warm location until the young mantes begin to emerge; and should then be

moved to a part of the room that does not get any direct sunlight. This precaution is necessary to prevent the rpaid drying of the casts which are left by the mantes when they moult. If moulting occurs in a dry room or in direct sunlight, the case may become dry before it is entirely shed, causing the death of the animal.[15]

The young mantes are not only carnivorous, but are also cannibalistic. They have enormous appetites and, if not given plenty of food, will eat each other until only one remains. Their natural food is living insects. Fruit flies make an excellent food to begin with, and the older mantes will eat cockroaches and grasshoppers. If living insects are not available, fresh liver may be substituted. Small bits of liver placed on the end of a toothpick and held before the mantis will be devoured quickly.[14]

Use of Praying Mantis. Can be used to show the moulting process and gradual metamorphosis in insects. These organisms can also be used to show adaptations.

Tenebrio - Mealworms

Collection. Mealworms are larvae of the black beetles, commonly found about grain bins that are but seldom disturbed. The larvae have a shiny yellow to brown body armor.

Culturing. The size of culture one wishes to maintain determines the size of receptacle in which to house it. If a moderate culture only is required, then use a jar of about one-half gallon capacity. A Mason jar is suitable as is a crock. Clear glass jars should be wrapped with black paper. Punch a few small holes in the lid or cover top with fine screen. Fill the jar about half-full of ordinary bran; the breakfast food will do. Add dry bread crusts and a crushed sheet of newspaper and insert this. Place a carrot or a half of an apple in about the center

as this supplies some moisture and also serves as food. Damp overlying burlap covers are sometimes used to furnish moisture. Now add the mealworms to the culture jar.[15]

Very little further care is necessary. Add a crust of bread occasionally and a carrot or apple as the other becomes dry or is consumed.

Use of Mealworms. Mealworms are perhaps the most widely used living food for various laboratory animals, particularly lizards, toads, snakes, and salamanders. Also these insects can be used to show complete metamorphosis.

Drosophila

Collection. Fruit flies are attracted to soft grapes, plums, bananas, in fact, any fermenting fruits. Larvae feed freely on yeast and other microorganisms in the fermenting fruit juice; clearly, a fermenting medium must be prepared.

Culturing. For rapid, temporary cultures, where little handling will occur, the simplest medium is prepared by dipping a piece of ripe banana into a suspension of yeast (made from a quarter of a package of yeast dissolved in 100 cc of water). Insert this piece of banana along with a strip of paper toweling into a clean glass vial or bottle. In season, this may be left open to attract fruit flies, or you may introduce flies into the bottles. Then plug with cotton wrapped in cheese cloth, or with milk-bottle caps. However, this medium is not recommended for careful work or long term cultures.

Since many media have been successful for raising permanent cultures, no discussion will be given on what to use. One desiring information can consult Morholt's Resourcebook or Turtox Service Leaflets. From experience, media requiring propionic acid seem to be superior in preventing growth of molds. "Should molds appear in the culture of Drosophila,

paint the surface of the culture with alcohol or with a solution made from one part carbolic acid and eight parts of water."[21]

Use of Drosophila. The importance of using Drosophila lies in genetic experiments. The laws of dominance and segregation are easily demonstrated. Drosophila also are a good source of food for other laboratory animals.

Ants

Collection. Among our North American ants, most of those that build some form of the well-known "mound" are suited to classroom use. The mound-builders can be found on grassy meadow land, on the southern slope of a hill, or in fairly open woodland glades. Many nests are also started under stones. The best hunting time is the period from late July to early September, since then the winged males and females are to be found in the nests, in addition to the "common" workers.[15]

With a trowel or spade, dig up a scoopful of soil containing about 50 adult ants, several dozen larvae (like polished rice), and pupae (like wheat grains), and empty it into the ant house. If a queen is captured, splendid; but so long as larvae and pupae are obtained the queen is generally not essential. She will develop later.[10]

Culturing. Feed ants on lettuce, carrots, potatoes, and bread crumbs as well as dilute molasses or honey. On occasion add some dead insects, but remove all excess food to prevent the growth of molds.

Use of Ants. Ants can be used as a study of social behavior in insects and tropisms.

Termites

Collection. A colony consists of a wingless large queen

winged males, wingless workers, and wingless soldiers. Inspect old tree stumps and wet logs for termite galleries. Gently strip off sections of the bark and wood to expose the insects and their eggs. Collect all stages with the wood in which they were thriving. In the laboratory remove the forms with a camel's-hair brush to avoid injuring them.

Culturing. Keep the insects, along with wood fragments, in covered finger bowls or Petri dishes. Add strips of moistened filter paper, then store in a dark place at room temperature. Keep moist by adding a few drops of water twice a week.[21]

DeLong and Keagy describe an excellent method[9] for making observation termite colonies in the laboratory. This simple method recommends the use of flat battery jars of the Delco type. A piece of balsa wood is placed inside along each of the two wide sides of the jar. Then the jar is filled about one-fourth full of earth. Use thin strips of balsa wood to keep a space to accommodate free movement of termites between the balsa wood layers and the glass walls. When termites are introduced into the jar, they establish themselves within a few hours. Tunneling may be seen in a short time.

Use of Termites. These insects are good examples for a study of social life. They can also be used as a source of the symbiotic flagellates which are found in their intestines.

CHAPTER V

VERTEBRATES

Precautions similar to those for protozoa and other invertebrates must be taken in caring for vertebrates. Here again, diet and temperature control are the factors most responsible for success in keeping vertebrates in a healthy state.

The purpose of this chapter will be to describe general methods which may be followed to advantage in the laboratory. No attempt will be made to describe the methods of caring for many different kinds of vertebrates.

Tropical Fish

Collection. In most areas of the United States, tropical fish can not be collected in the field. They can be bought from a tropical fish dealer.

Culturing. To keep fish in the laboratory, one must have containers. As far as the fish are concerned any container which will hold water without contaminating it will serve, but for purpose of study the fish should be easy to observe. For general purposes a tank holding ten or twelve gallons is a good size. Such tanks are convenient to place and will accommodate almost any of the commonly kept aquarium fish. Tanks and jars holding only a gallon or two change temperature more rapidly than larger ones do and are more affected by such accidents as a dead snail or a neglected accumulation of debrish. There is always a tendency to overload a tank and this is especially true of small ones. A safe

rule for any aquarium is an inch of fish to each 12 square inches of water surface, although one will find that some liberties may be taken with this rule. For instance, fish need more water per fish in warm weather.[24]

The tanks to be used, at least if they are large ones, should be set where they are to remain before they are filled with water. They should not be where direct sunlight falls on them for more than an hour or two a day, preferably not at all. They should, however, receive light enough so that plants may grow readily. Most aquatic plants grow well several feet back from a window.

When the tank is in place it may be filled with water. Any clean water will do, although pond water is recommended if one can get it. Water from a city supply should be allowed to stand 48 hours before the fish are introduced.

Many kinds of tropical and sub-tropical fish have been raised in aquaria. The most satisfactory are the small and beautiful guppies. These little fish are live-bearing and are not so likely to eat their young as many fish are. They like living food but will live on prepared foods. They will live in small aquaria and stand crowding although like other fish they seem to grow better and larger in larger tanks.

Other tropicals which are easy to rear are the platy and swordtail. If kept in the same tank, the two will cross and give interesting and pretty hybrids. They will eat prepared food, shredded shrimp, and daphnids.

Goldfish will thrive under the same conditions as tropical fish. Small goldfish are preferable since the larger ones require larger tanks. They are omnivorous and feed on plants in the tank and bits of boiled spinach in addition to other food.

Use of Tropical Fish. Most tropical fish have little usefulness except to add color to the aquarium and to show some of their life processes, such as reproduction. However, fish can be used to demonstrate blood circulation through arteries, veins, and capillaries in the fins and tail.

Native Fish

Collection. When collecting put only one kind in a tank, if possible, and keep the number down. Most of them will not endure crowding. The forms from still and sluggish streams adapt themselves to aquarium conditions, and some forms from rapid streams are fairly adaptable. Banded sunfish, blue-gill sunfish, bullheads, and darters may prove interesting to keep.

Culturing. The above specimens should be kept separately in tanks well supplied with vegetation. In one sense they are easier to keep than tropical fish, since they can withstand a wide range in temperature.

In general, these fish will not accept the prepared foods although they may do so after some time. All living food and bits of raw meat, raw fish, or raw liver are readily accepted.

Use of Native Fish. The uses for native fish are the same as for tropical fish.

Salamanders

Collection. In the spring, eggs of salamanders are easy to obtain and may be hatched in the laboratory. As soon as they hatch, place a few in any long-standing aquarium which does not contain animals which eat little tadpoles. Salamander tadpoles are easily distinquished, because their gills are not covered over as they are in the other tadpoles, but develop into tufts which remain until long after the legs

have developed, even into the next spring.[24]

Culturing. Salamander tadpoles are meat eaters, and large ones eat small ones. They should be kept in large pans, in shallow water, with plenty of water plants and small stones to provide shelter, and they should be fed regularly with bits of beef, earthworms, or perhaps any other meat.

Adult salamanders should be kept in a vivarium. A vivarium may be prepared by using an aquarium tank and a water container. At one end of the tank put a small glass dish or non-corrosive metal pan to serve as a pool. Cover the remainder of the tank with coarse pebbles together with a few pieces of charcoal. Then build up this part of the tank with a loam soil rich in humus. Slant the layers of soil away from the small pool to a height of three inches and keep about two inches of water in the pool. Moss can be placed around the pool to form a beach. Place at least one rock in the water. Plant the rest of the vivarium with small ferns and mosses.[21]

Almost all salamanders require living food such as Tubifex and earthworms. On occasion, some forms may take fresh liver if it is dangled in front of them on a string. Salamanders snap at the food and can take it better if the food is brought up to the side of the mouth and touched to the lips. They seem to bite at it better when they feel it than when they merely see it.

Use of Salamanders. Salamanders are desirable to have on hand for studies in both natural history and in the development of eggs. Reproduction, respiration, and similar life processes may be demonstrated by them.

Frogs and Toads

Collection. Eggs and tadpoles, as well as adult frogs and toads, may be collected and cultivated in the laboratory. The time of egg-laying is different for each species and the surrounding temperature is a controlling factor. Therefore the time may vary, progressing from the southern to the northern states.

A slapping-down technique can be used for adult frogs or toads that freeze in place instead of dashing away. This consists of slapping your flattened hand over them, pinning them down while grasping a leg or legs with your other hand. Don't slap too hard, or there will be casualties.[8] A bright light and a large net is useful in collecting bullfrogs from the edges of ponds and streams at night.

Culturing. When a few frogs are kept for display purposes, a woodland or beach vivarium is desirable. Such forms as bullfrogs are an exception; they fare better in two inches of water in a clean aquarium.

The problem of handling large quantities of frogs in the laboratory is a difficult one. The survival rate is high when they are kept at 10° C. in a granite sink containing one inch of water. Cover them with wire mesh; change the water daily. Keep frogs away from zinc.[21]

Where a sink is not available, an alternate, but less desirable, method may be used. Keep the frogs in large aquaria with water at a level of one inch. Flush the frogs with a stream of water when the water in the tanks is changed each day; the water becomes fouled quickly.

Frogs and toads require a diet of living material. Leopard and green frogs will accept small earthworms, flies, and similar living materials. Bullfrogs readily accept living things smaller than themselves, such as smaller frogs and earthworms. Spring peepers and tree frogs

may be fed on fruit flies and small mealworms.

Use of Frogs and Toads. These amphibians can be used for studies both in natural history and in the development as well as for physiological experiments in circulation and reflex behavior.

Turtles

Collection. Aquatic types of turtles can be collected from most streams and ponds. Box turtles, or terrapins, can be collected from gardens, flower beds, or along roadsides.

Turtles can also be trapped. A simple efficient device can be made from a chicken wire box and a board. The box must be constructed in such a manner as to prevent the turtles from climbing over the side. The board must be fairly wide and be attached to the rim of the box in such a manner as to form a teeter. The box is submerged to within one or two inches of the top rim. As the turtle climbs upon the board to sun, he passes the teeter's fulcrum point and is dumped into the box. If desired, bait, such as raw fish or chicken entrails, can be placed at the end of the teeter to attract the turtles. Information concerning the construction of such traps can be obtained from most state wildlife departments.

Culturing. Aquatic turtles should be kept in aquaria containing two to four inches of water. Cork floats can be added or a flat rock placed in one corner of the vivarium as a useful resting place. The water should be changed twice weekly to keep the water clear. Painted turtles, wood turtles, and box turtles may be kept in water in a beach vivarium. However, box turtles seem to prefer a moist terrarium or vivarium rather than water. Segregate snapping turtles, although the small ones may be kept with other species.[21]

Most of the aquatic forms will accept bits of fish, grown raw meat,

earthworms, or dead frogs put in the water. Most turtles will accept hard-boiled egg cut into slices, as well as lettuce and slices of apples. In addition, box and wood turtles take snails and slugs.[21]

Use of Turtles. Turtles are useful in anatomical and physiological studies.

Lizards

Collection. Different species requiring different types of care can be collected in different regions and habitats. Lizards can be gathered by hand, nets, or noosing. Slingshots or rubber-band guns can be employed to stun lizards, but some risk is involved; a few may be killed or permanently injured instead of just being rendered temporarily immobile. Noosing lizards is safer. Attach a small noose of horsehair or fine thread or wire to the end of a pole measuring a few feet in length. Slip the noose over the lizards head and let it come to rest around the neck. Jerk the pole quickly upward and the lizard is caught.[8] The horned "toad, the skink, and the chameleon are the lizards most useful in school.

Culturing. Chameleons and the larger skinks should be housed in a large terrarium. Include some twigs so the animals have room to climb. Spray the plants in the terrarium daily to supply water for these lizards, since they seldom drink from a dish. They subsist mainly on live insects. It may be necessary to raise fruit flies or mealworms for this purpose, especially during the winter months.

Horned "toads" are maintained best in a desert vivarium containing about five inches of sand for burrowing, along with several rock piles for hiding. Embed a bowl of water up to the level of the sand. Several hours in direct sunlight should be provided but the vivarium should be ventilated to prevent overheating. The lizard feeds upon live insects.[21]

Use of Lizards. Uses of lizards are limited. Of course, they can be used in taxonomy and natural history studies.

Snakes

Collection. Large harmless snakes can be caught without equipment after a little practice. One method is to immobilize the snake by stepping on it gently while one reaches for the nearest stick with which to pin down its head. Grasp the snake just back of the head with one hand and the body with the other hand to prevent the snake from jerking loose.[8] Place the snake in a cloth flour, salt, or sugar sack for transporting.

Snake tongs may be used for collecting. These tongs are a mechanical device three or four feet long that depend upon spring action. By pressing a hand grip, rubber-padded jaws can be made to grasp the body of a snake. Releasing the grip frees the snake. Such tongs are available commercially.[8] A snake stick, an instrument which resembles a golf putter, is also used to capture and handle snakes.

Culturing. Mesh cages much like those used for mammals are best for housing snakes, although the mesh should be of smaller gauge so that the smallest snakes cannot escape. In addition, bottom pans of zinc are needed. Door openings at the top of the cage are the most convenient to facilitate handling the animals. Into such a vivarium place a plan of water and a few rocks. Keep the snakes at a temperature between 70° and 80° F.[21]

The most desirable forms are those which are easy to keep and handle. These are the garter, ribbon, hognose, black, and ring-necked snakes. Many others such as the bull, milk, water, and green snakes may also be maintained. Ring-necked snakes feed on insects and small earthworms;

hog-nosed, garter, and ribbon snakes accept entire, large earthworms as well as insects, frogs or other amphibia, and lizards. Black snakes need live mammals. They may be fed a small rat every two weeks.

Use of Snakes. Every laboratory should have one or two snakes on display. It is one way to break down the inordinate fear many students have of snakes.

Rats and Mice

Collection. Rats and mice raised in the laboratory are most generally purchased from a biological supply house or donated to the classroom by a student's parent who is tired of rats. Wild varieties are less desirable since they carry diseases and will bite.

Culturing. Large cages are best for housing rats since they allow for exercise. The mesh should be large enough to allow droppings to fall through to a bottom pan.

The doors of the cage may be on the side or at the top. A door on the top is the most convenient since it allows for better handling. Cages should be cleaned daily and fresh newspapers placed in the bottom pan. If cedar shavings are used instead of paper, the offensive odor of rats and mice is not as noticeable.

Rats should be fed only once a day. They should be treated as pets. If they are, they respond satisfactorily and reproduce readily. Rough treatment may result in viciousness and cause the mother to destroy her litter. The rats should be handled gently and fondled from birth. When this is done they do not bite.

Rats can be fed a diet of bread, sometimes soaked in milk, in addition to lettuce, carrots, and other vegetables, sunflower seed, and similar seeds. The bread should be broken and the carrots cut into

portions equal to the number of rats in the cage. Rats also can be fed commercial rat, cat or dog pellets.

Use of Rats. Rats may be used in the study of digestive, reproductive, and respiratory systems. Rats can also be used to show conditioned responses and vitamin deficiencies.

Golden Hamsters

Collection. Golden hamsters may be obtained from a biological supply house or pet shop.

Culturing. Cages for hamsters should be made of strong, fine-meshed screening, for they will readily gnaw through a wooden container. The cage should be provided with a small covered box for a nest and some soft material for nest-making. A makeshift cage can be adapted from an old aquarium tank, provided it is at least 18 inches long and is covered with a wire netting top.[19] When two or more hamsters of different sexes are kept, separate cages are required for the males and females. Although two males may live together amicably, or even two females, it is more satisfactory to have one hamster per cage.

If a hamster is to be kept in good health it must be given a diet with a proper balance of protein, vitamins, and minerals. A good basic food consists of a reliable make of cat or puppy meal mixed with milk or water to form a stiff mash. Egg, cheese, peas, nuts, seeds, carrots, apples, and lettuce or cultivated greens are relished by hamsters.[19]

Use of Hamsters. Hamsters make good subjects for illustrating vitamin deficiencies, or as a host for parasites. Studies on reproduction have used this animal, and work concerning hormone reactions have been carried out by several investigators.

Guinea Pigs

Collection. Guinea pigs can be purchased from pet shops or biological supply houses. It is recommended that long-hair varieties not be used for the laboratory. Their long hair usually accumulates dirt, sawdust, feces, and becomes easily soiled, while the animals have a tendency to nibble each others hair.

Culturing. While guinea pigs are not particularly active animals and hence do not require large pens or hutches, they should have sufficient space in which to move about freely. During cool and wet weather and in winter time, they should be kept indoors where the temperature is fairly warm, in pens about three feet by two feet and a foot high for a pair. The floor of the pen should be covered daily with sawdust or sand to absorb any moisture and feces. During summer days, the animals should be kept outdoors on a plot of green grass in large wire pens having no bottoms.[20]

Guinea pigs can be fed oats or other grain, hay, fruits, rodent pellets, vegetables, or almost any kind of green food. Plenty of fresh water is necessary.

Use of Guinea Pigs. Their chief advantage is that they can be handled by children with little fear of being bitten or scratched. Guinea pigs make excellent laboratory subjects for heredity experiments and for testing the potency of vaccines, serums and germ cultures.

CHAPTER VI

SUMMARY AND CONCLUSION

No matter what the pattern or the kind of course adapted, the course in biology has its special flavor, and its special problems.

As stated previously, biology is essentially a study of living things and things that have once been alive. It is "living things" which give biology its special flavor. It is "living things" which also present the biology teacher with his problems, for the maintenance of living things requires time and care. If the course encourages the young people in class to take part in studying their own biology, the course not only concerns itself with study of living things but is alive as well. . .alive with the interests of young people in life and living. With this thought in mind, this report has been written for the purpose of being used as an aid in helping to maintain a living laboratory.

In Chapter II, methods were presented for collecting and culturing various types of protozoa. The methods presented were methods which have been tried and proven successful by various leading biologists. The writer does not want to imply that the given methods are the only methods. There are many methods, and some probably could prove more successful than the ones listed.

In Chapters III and IV, a discussion was given on the maintenance of living invertebrates in the laboratory. The material was limited to the types of animals which, in the writer's opinion, would be more beneficial to the average high school biology class.

Chapter V dealt with the care of vertebrates. In this chapter, comments on some common laboratory animals, such as chickens and rabbits, have been intentionally omitted. These animals can usually be obtained from a local source in the community which has better facilities for their rearing than can be furnished by the average high school laboratory.

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