

level biology course in 62 high schools across the nation. This program is designed for students who have successfully completed the 10th grade program and who have demonstrated the interest and ability to pursue the science of biology in greater depth. Special materials have also been prepared to assist the minority of students who have difficulty reading at the normal 10th grade level.

I am sure that Dr. Weaver is aware of these broad aspects of the BSCS program and I hope that this letter will serve to make clear the role of the BSCS in helping to bring about a general improvement in the teaching of secondary school biology.

Norman Abraham
Yuba City Union High School,
California

Parasitism, Hyperparasitism, and Commensalism

- *A. Amaro, Colégio Estadual Paulo de Frontin, Rio de Janeiro, Brasil*

The following laboratory exercise has been submitted by one of our Brazilian readers. In it he describes an interesting exercise in parasitology using frogs.

Presentation of some associations among living beings, including the study of adaptations and the collection and classification of specimens.

Materials

Recently captured frogs and toads
Dissecting microscope or hand lens, microscope slides, and cover glasses
Thick pasteboard or a wooden plank (30 x 20 cm.)
Entomological pins, dissecting forceps, scissors, camel's hair brush
Alcohol lamp
Large mouth jar (500 ml.) containing some cotton soaked with ethyl ether
Petri dishes, small bottles
Saline solution (0.7% NaCl)
Aqueous solution of Neutral Red (1/5.000) or other vital stain
Lugol's iodine solution (1 gm. iodine, 2 gm. potassium iodide in 300 ml. distilled water)
Formalin-acetic solution (5 ml. formalin [commercial], 2 ml. glacial acetic acid in 93 ml. saline solution)

Procedure

(1) Observe the animal's skin to look for ticks (ectoparasites). To anesthetize the animal, put it into a large mouth jar containing cotton soaked in ethyl ether. When anesthe-

tized, attach the animal to the pasteboard, belly side up, by pinning through the feet.

(2) Cut off one of the fingers and collect a drop of blood on a microscope slide. Place under the microscope and search among the red blood cells for small worm-shaped organisms, the embryonic filaria (microfilaria).

(3) With the scissors, make a longitudinal incision all along the trunk. If microfilaria were present in the blood, seek the adult forms between the skin and muscle wall (under the cutaneous tissue), in the abdominal, inguinal, and axillary regions, or in the thoracic-abdominal cavities.

(4) Cut the membranes holding the organs so as to free them, and remove the entire mass of internal organs, leaving only the animal's carcass. Separate and identify each organ, placing each in a petri dish with a small amount of saline solution. Tear the organs into fragments and examine with the naked eye or the microscope for the presence of parasitic organisms.

(5) Using the brush, transfer mucosities adhered to the body to petri dishes containing saline solution to prevent dehydration.

Observations

(1) To examine parasitic forms alive, place in the saline solution a few drops of Neutral Red which will better show structures without killing the animals.

(2) Draw the parasites, attentively observing manner of locomotion and whether they are free or attached by special organs. Study carefully the digestive apparatus, determining the buccal formation, pharynx region, and whether or not the intestine has a definite termination. Confirm the existence of sex organs, including the ovary, testes, uterus, and eggs. Determine if the individuals have definite sexes or if both sexes are present (hermaphroditic). Note the great development (hypertrophy) of the reproductive apparatus, the increased number of eggs, and determine whether the animal be oviparous or the embryonic form of viviparous parents.

(3) Note the presence of organs of attachment such as suckers, hooks, and the like.

Identification

(1) With reference books, identify the parasites found, most of which are nematodes or trematodes.

(2) The intestinal contents of the amphibian will be rich in micro-organisms such as bacteria and protozoa. These are difficult to separate into endoparasites or endocommensalists.

The protozoans are almost always *Nyctotherus*, *Zelleriella*, and *Trichoma*. Under the microscope observe the cilia, cytopharynx, and the nucleus which will stain reddish from the Neutral Red.

The *Zelleriella* present a curious case of hyperparasitism, for they themselves are parasitized by another parasite, an amoeba. The amoeba is localized in the cytoplasm, appearing similar to a rounded corpuscle.

The protozoans move rapidly, and to study them better, place a drop of Lugol's iodine solution at the edge of the cover glass. This will kill and stain them, showing more clearly structures and hyperparasites.

Fixation

To preserve the parasites, use formalin-acetic fixing agent. Decant as much saline solution as possible away from the material to be fixed, and place the material immediately into a quantity of the fixing agent such

that it will not be greatly diluted by the remaining saline solution.

For nematodes, use the fixing agents heated to more than 70°C.

To fix trematodes and cestodes, compress them between two plain surfaces such as glass plates tied with a cord. After compression, place the material to be fixed in the fixing agent. The time of fixation will vary with the dimensions of the animal, normally taking a few hours.

After fixation, the material should be deposited in small bottles with labels, done in pencil, accompanying the specimen in the preservative. The label should mention the host and its origin, the organ where the parasite was found, the date, and the collector's name.

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