Labs

Frogs

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Rana pipiens, the leopard frog (or grass frog as many suppliers insist on calling it) has contributed much to our understanding of vertebrate biology. Besides being an important research animal, it is even more widely used in education. Millions of animals are used each year. Approximately half of the animals are preserved and used for dissection. Many of these specimens are frogs that do not survive the collecting and holding procedures. The others are killed by drowning and then preserved. The living frogs that are shipped by the supplier sometimes have only about a 50 percent chance of surviving long enough to be used in the laboratory. The majority of frogs that survive are generally in poor health.

Costs

Currently, a three-inch leopard frog costs about three U.S. dollars plus shipping which may bring the cost near to $5 if they are shipped air freight. If only 50 percent survive, the cost approaches $10 per usable animal. Because air freight charges often equal the cost of the frogs, in smaller shipments it may be more economical to order frogs in numbers that meet the minimum shipping rates and maintain them for longer periods of time.

The cost of preserved frogs is less because of lower procurement, maintenance and shipping costs. Small, uninjected frogs often sell for less than one dollar each in large quantities. Double injection raises the cost about 50 percent. A three-inch, double-injected leopard frog costs about two U.S. dollars plus shipping.

Mexican Frogs

Many of the preserved frogs come from Mexico, where collecting costs are low. The use of living Mexican frogs has raised a variety of questions which need more consideration. Of prime importance is the fact that Mexican frogs are a different species, usually *Rana berlanderieri forreri* but sometimes *Rana magnaocularis* and *Rana berlanderieri berianderieri*. Because these species differ biologically from *Rana pipiens*, it is important that biologists be aware that they are using a different organism.

Epinephrine, for example, causes the skin of northern frogs to lighten but causes darkening in southern species. The reproductive cycle of southern frogs makes them useful for inducing ovulation in the summer rather than spring as in the northern species. And, most importantly, differences in temperature tolerance require that southern frogs be maintained quite differently from northern frogs.

Many biologists have had disastrous results trying to maintain Mexican frogs, sometimes losing most of the animals within a week, because they treated them like northern frogs. *Rana berlandieri forreri* is often found out of water, covered with dust and dirt, at temperatures approaching 100 degrees Fahrenheit. It does not survive well in cold water and should not be kept below 55 to 60 degrees.

Because Mexican frogs come from a variety of collecting sites their health varies. Pesticides and nitrogen fertilizers are used extensively in many of these areas and if the frogs come in contact with these substances their health can be seriously affected. The southern species also are more heavily parasitized.

Suppliers of Mexican frogs give the above reasons for the large decrease in their use as living material. However, if proper attention is given to their care, particularly feeding, as indicated below, the southern frog may become more widely used. I first used Mexican frogs around 1970 when northern frogs were very scarce. The frogs I obtained were very large, healthy individuals, the best frogs I've ever used. Later on, I had trouble with some batches, and shipping costs began to make their use more prohibitive. These problems have made the Mexican frog mainly a dissection specimen.

When they require living frogs, most biologists now use northern leopard frogs, *Rana pipiens*. However, their health varies during the year. Animals collected in spring, summer or fall are usually very healthy and survive collection and shipping well. The academic year, however, is mainly in winter and this causes many problems.

Winter Problems

Because it is not cost effective to feed the animals, they are maintained in hibernation. The best approach seems to be to keep the animals contained under the ice, in ponds, similar to natural hibernation. Artificial hibernation systems also work. Hibernating animals gradually become less tolerant to shipping, and 50 percent mortality rates before use are not uncommon in midwinter and early spring. Frogs are particularly susceptible to disease at this time. Suppliers often use a variety of agents to prevent, and occasionally treat, infection. Most of the treatments I've tried do not work well with unfed animals. Conditions here are not too different from the tropical fish industry—after the animal arrives alive it's your responsibility!

Xenopus

Several suppliers have tried to use the African Clawed Frog, *Xenopus laevis*, because it appears to be a much hardier animal and can easily be cultured. There has been considerable interest in using *Xenopus* for all the exercises where leopard frogs have been traditionally used, in addition to using it for the study of development. This interest seems to have decreased, however, since the cost of laboratory raised animals appears not to be competitive with field collected material. This may change as more cost effective methods develop or as demand increases. Suppliers are also considering the use of Asian frogs.

The above problems are known to most biology teachers but are particularly difficult ones for those of us who have had to procure, maintain, and use hundreds of frogs each year in large university courses. I have found some solutions. Many of you have un-

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doubtedly solved some of the same problems in other ways. I would be delighted to hear about your solutions and to convey them to others via this column. Please take a few minutes to write!

Obtaining Frogs

Some of you may live in areas where frogs are normally collected and can procure them easily yourself by using local or student help. I have never lived where this was a reasonable alternative but I have collected frogs on a number of occasions. The most efficient way I have found to collect them was at night, when it was raining, and they were on the road. Leopard frogs may be called grass frogs by suppliers because they collect them in grassy fields rather than in ponds. Anyone who has tried to catch a frog near the edge of a deep pond knows how difficult it can be because frogs dive down into the mud. Pond collecting is much easier at night when males are calling, but it is still not easy unless frogs are extremely common. Conservation laws now require collecting permits in many areas so it would be best to check with your wildlife agency before collecting large numbers of animals.

Because the supply of frogs during winter is determined by how well the suppliers' stocks hold up in hibernation, it is best to make commitments as early as possible and on a regular basis with one dependable supplier. Occasionally, there are years when the whole frog population is down and it is extremely difficult to obtain frogs in February or March. When this happens the best customers get the frogs.

Because shipping is hard on the animals, one should attempt to find a source where shipping is minimized. This will also reduce cost. If you live in the north, there is little point in buying northern frogs from the south.

Keeping Frogs

Frogs can be maintained for a week or two without feeding and this is the general practice. However, their health does deteriorate without food, particularly if they have been in hibernation. The significance of feeding was not apparent to me until I began keeping a variety of animals in the laboratory and had to find a way of providing food. The difference in survival rate between fed and unfed frogs was amazing.

At the University of Calgary, because I developed a good relationship with a Wisconsin supplier, we always received the best frogs available. In the fall and late spring our losses were low. The frogs were kept in tilted, plastic rat cages with water covering one half of the bottom. The cages were kept in a large 10°C reach-in cooler and the water was changed at least every other day. However, when we received frogs in midwinter the situation changed. We lost a significant number of frogs if we had to maintain them for more than two weeks. One February I'm sure we lost nearly half of the frogs by the time we needed them. After that, I always ordered 50 percent more than we needed.

The last two years I was at the University of Calgary I developed a series of animal exhibits for demonstration and began receiving a regular supply of adult crickets for food from a cricket farm. I also had remodeled my bathroom at home and had a bathtub I didn't need. The bathtub ended up in my prep room, with the drain next to the sink, and supported at one end to tilt it. Large rocks were placed in the top end and enough water added to cover half of the bottom. The tub was covered with windows also left over from remodeling. In September, we put about two dozen frogs in the tub and fed them crickets a couple of times a week. The tub was rinsed and refilled with water when it got dirty, every two or three days. In four months, only a couple of frogs died. In February, we repeated the experiment with the same results. We had kept unfed frogs at room temperature before but discovered that survival was better if they were refrigerated. It was the feeding that made the difference.

Storage Devices

There are a number of frog storage devices that are available from cage manufacturers. Some are designed to allow water to flow through them and into a drain. Because frogs shed skin almost continuously overflows often become clogged and extensive water damage can occur. Therefore, it is important to check the design of the system to be sure that flooding cannot occur, i.e. there must be another route for the water as clogging occurs. Some devices use recirculating water that is filtered and, occasionally, sterilized with UV light. These devices are expensive and uncommon outside colleges and universities. One problem with devices of this kind is they encourage people to check the health of the animals less often. *If there is a single rule that applies to the care of living things, it must be they need to be checked on as close to a daily basis as possible.* Organisms may survive for long periods but when a problem occurs it usually requires immediate attention.

Survival rates of frogs increase greatly if they have a comfortable place to stay out of the water. Only hibernating frogs need to be in water continuously. Frogs have been successfully maintained by misting them with an automatic watering system, like that used in greenhouses. Putting a frog in a container with an inch of water in the bottom, in a conventional refrigerator, and leaving it there for days, is a sure way to produce a dead or dying frog.

Sexing Frogs

There are many subtle [to humans] differences between male and female frogs. As one works with frogs, sexing becomes easier, but one also gains some appreciation for the variability of these dimorphic characters. The size of the base of the thumbs seems to be the easiest characteristic to use. Males have enlarged bases, whereas, females have bases which follow the taper of the thumb. The largest frogs tend to be female. Has anyone discovered an easier way?

From the Frog's Point of View

The number of leopard frogs has decreased considerably over the past few decades and more conservation measures are needed if their numbers are to be maintained. Habitat destruction is the major cause of this decrease but over-collecting is also important. Consequently, the use of these animals must be carefully considered and justified. Is a frog required? Can the problem be studied in a laboratory raised animal, e.g. a mouse, which may also be less expensive and easier to maintain? The use of frogs for education seems more justifiable than as bait or food. Clearly, they are needed for basic research.

How can the least number of frogs be used most profitably for education? One can learn so much more from a living frog than from a preserved one that for me, at least, the use of preserved frogs is not justified unless they are a by-product of the collection process. If exercises are well planned, the number of living animals used can be reduced. For some exercises, demonstrations work better because the instructor can take the time to ensure

that students understand the point of the experiment. The traditional exercise on frog spinal reflexes is so often misunderstood that it may do more harm than good if the students feel they have abused the frog. This is a very meaningful experiment if it is properly taught. A thorough and well-explained demonstration may produce far more learning. In most cases, if students need to study the anatomy of the frog in more detail, animals frozen or preserved after physiological study can be used. The overuse of preserved specimens turns more students off than on to biology.

Once you receive living frogs, treat them humanely. Keep them at temperatures that are within their normal range and in containers where they can find comfort out of the water. Change their water when it becomes contaminated. And feed them, even if you're going to use them tomorrow. In addition to producing more healthy animals for study, feeding is interesting to watch. Finally, learn as much from them as you can, they've given their life for you and your students!

Next time I'll talk about some new ways to set up natural living exhibits.