II. AMPHIBIANS*

A. INTRODUCTION

1. General Considerations

Considering the wide variety of adaptations and the diversity in environmental requirements found among amphibian species and even sub-species, the most important single requirement in establishing proper conditions for their maintenance in captivity is that the investigator be properly and fully informed on the biology and environmental needs of the species in its natural habitat. The importance of this point and of assuring in advance that sources from which such information can be obtained are readily available, cannot be overemphasized.

The list of general references, books, and identification manuals in the Appendix to this chapter will prove useful as a biological data source and for the identification of most North, American amphibians. Specific reports on nutrition, health, handling, behavior, etc., of various amphibians, both in the wild and under captive conditions, are published from time-to-time in a number of scientific journals (see Appendix) and should be available to the investigator working with these animals.

2. The Captive Environment

The homeostatic abilities of amphibians are less well developed than those of reptiles, birds or mammals. In nature, amphibians maintain their body temperatures within a preferred range by selecting microenvironments in which they can gain or lose heat as required. In captivity, temperatures must be controlled and maintained within the range which will allow the particular species to function. A simple listing of optimal temperatures has not been attempted here, as these vary markedly among and within species, and with such widely divergent functional states as torpor, mating, feeding and digestion.

Water loss is an especially critical problem for captive amphibians. As a general rule, terrestrial and semiterrestrial amphibians should be provided with a water dish large enough to allow them to submerge. The water provided for this purpose, as well as that to the tanks and aquaria for the more highly aquatic amphibians, should always be from a clean source and be oxygenated to reduce the possibility of putrefaction. Where domestic supplies must be used, the water should be dechlorinated.

Some amphibians, particular smaller species and those from humid environments such as tropical rain forests, will require nearly saturated humidity levels in their terraria.

3. Nutrition

Amphibian species generally exhibit extremely narrow feeding adaptations: most are predators, many of which will fail to show a feeding response to anything except live, moving prey. Because of this, the nutritional requirements are either poorly known or completely unknown for most species, and there is a real danger of nutritional deficiencies developing in individuals maintained on diets which differ appreciably from their natural foods. Furthermore, those that can be induced to take non-living food often require extensive conditioning before these foods can be relied upon to elicit a feeding response. During this period of adaptation, the nutritional reserves of the animal may become dangerously depleted.

B. HOUSING AND CAGING

1. Housing

The most practical and effective way of providing suitable holding conditions for amphibians within an animal facility is firstly, to establish a set of general environmental conditions for the room(s) as a whole, for such parameters as day length and temperature range. Secondly, each terrarium or tank can be established as an individual environmental chamber in which temperature, light level and humidity can be adjusted to suit the requirements of each species being held.

2. Aquatic Caging

a. Size Grading and Population Density of Larvae

Cannibalism may occur, particularly among larval salamanders and spadefoot toad tadpoles. It is so frequent among both larval *Ambystoma* and *Scaphiopus* that they are usually best maintained individually in small aquaria, or in screen baskets suspended in larger aquaria. Cannibalism can be reduced or eliminated in most other larval amphibians by size grading. With the exception of Scaphiopus, most other larval amphibians are herbivores or detritivores and will not show any tendency to cannibalism. A further consideration in providing caging for the aquarium rearing of amphibian larvae is that of density dependent growth inhibition in tadpoles (Aikin, 1966; Rose and Rose, 1961). This is caused by an unidentified, possibly species-specific factor, emitted into the water by growing tadpoles. The effect maybe eliminated if a flow-through water supply system is used, rather than recirculation. If filtered, recirculating water must be used, tadpole densities should be kept at one per litre or lower, and individuals showing large differences in size or growth rate from that of the majority should be removed.

b. Water Temperature for Larvae

Water temperatures of 10^{o}-12^{o}C are generally suitable for rearing larval salamander species found in Canada, and temperatures of 18^{o}-22^{o}C are suitable for most species of tadpoles. However, there will be exceptions which affect some species, or even specific populations of a species. Again, the importance of proper identification of the animals and ascertaining their exact biological requirements is emphasized.

c. **Cool Flow-Through Water System (Frogs)**

Leopard frogs (*Rana pipiens*) may be kept in fairly large numbers in large, shallow, flow-through tanks. This system is commonly used in holding frogs required for teaching laboratories, where large numbers may be needed. In such instances, frogs should be maintained in a state of simulated winter torpor, as they will not usually feed on anything but live, moving prey. Because of this, it is neither effective nor practical to attempt to provide them with food.

A flow of 10 to 15 litres per minutes of water at 4^{o}C supplied to a 1 m^2 tank will suffice for up to 50 frogs. If possible, however, holding densities should be kept to between 20 and 30 frogs per m^2. The light level should be low, comparable to that under snow and ice cover at the bottom of a pond, with day length constant at 8-9 hours. These conditions will maintain fall collected leopard frogs in a state of near torpor, so that they can be held without feeding for as long as four to five months. Increasing day length and/or warming temperatures will induce the onset of sexual maturity, activating the frogs and increasing their food requirements.

Consequently, frogs collected while emerging in the spring will have depleted fat reserves and, in adults, mature gonads. They cannot be maintained without feeding until they have bred and had sufficient feed to build up their body fat reserves. A further advantage of a cool, flow-through water supply system is that it appreciably reduces the hazard of such diseases as red leg.

d. **Other Aquatic Amphibians**

Highly aquatic amphibians, such as mud puppies (*Necturus*), larval salamanders and frogs, and wholly aquatic adult frogs (Xenopus, for example) are best held in tanks or aquaria provided with circulating, dechlorinated water of suitable temperature, a compressed air supply and airstones for aeration.

3. **Terrestrial Caging**

a. **Humidity and Substrates**

Non-aquatic amphibians should be kept in terraria in which air circulation is long enough to allow a near-saturated humidity to develop. A water container large enough to allow submergence, should be provided.

The substrate used should vary with the type of amphibian. Most toads do well with a clean sand substrate deep enough in which to burrow. Small salamanders, such as most plethodontids, seem to survive best in moist sphagnum moss. Soil, with or without growing plants may be used, but must be removed if fungal growth or bacterial decomposition show any signs of developing from the death of plants or other contained organisms.

Temperatures, again, will be determined by the needs of the species being held.

b. **Simulating Winter Torpor**

Desiccation can become a severe problem if captive terrestrial amphibians are to be maintained under simulated winter torpor conditions, as air chilling units effectively condense most of the water vapor in the air. This can be compensated for by making sure that the substrate in the chambers is kept continuously moist. A wide-mouth glass bottle with a few perforations in the lid to allow limited air circulation makes a suitable chamber for a terrestrial amphibian in simulated winter torpor. Moisture can be provided by pads of moist blotting paper placed on the bottom of the bottle and above the animal. Continuous darkness and a temperature between 1^{o} and 4^{o}C, should be maintained.

Amphibians brought in from the wild, which have just emerged from torpor in the spring, have depleted body fat reserves and are beginning sexual maturity. It is important to realize that such animals will not usually survive any further extended period of cold and food deprivation.

C. **FOODS AND FEEDING**

1. **Feeding Behavior and Response**

With relatively few exceptions, captive amphibians should either be maintained on their natural foods or on foodstuffs closely approximating those of their natural diets. Disease, apparently resulting from nutritional deficiency, is frequently encountered when the feed for these animals differs appreciably from that of their natural diets. It seems unlikely to expect any more precise data on the nutritional requirements of most captive amphibians to become available in the foreseeable future because of the following factors:

a. The majority of amphibians are predators, sometimes with extremely narrow and specific diet specializations.
b. Many species exhibit specific feeding behavior with a strong orientation to movement as a means of recognizing prey. Consequently, captive amphibians often will not recognize food that does not closely resemble a natural prey item, and thus will fail to give a feeding response.
c. Those species which can be conditioned to take dead food items or foods which differ from their natural ones can still rarely, if ever, be induced to feed on anything approaching a nutritionally defined (or definable) diet.

2. **Natural Diets**

Data on the composition of natural diets is available for many of the more common North American and European species and has been summarized by Porter (1972a). Diet information for specific species is also available in identification manuals and in occasional articles in the appropriate scientific journals (see Appendix). This, again, underscores the importance of searching the relevant literature on each of the species to be maintained in captivity.

Daily feeding is usually not necessary; however, food should be offered between once and three times weekly, in most cases. The best survival and growth seem to result if the animal is fed to satiation at each feeding.

a. **Order *Urodela* (Salamanders)**

Aquatic adult salamanders and larval and neotenic salamanders feed on a wide variety of aquatic invertebrates, such as leeches, snails, crustaceans, and aquatic insect larvae. Larger forms may also consume fish or other amphibians, a fact that emphasizes the need for precautions against cannibalism (see Aquatic Caging). In captivity, frozen brine shrimp, frozen whole minnows and pieces of fish fillet have been fed successfully to several species of aquatic salamanders.

Terrestrial salamanders will feed on such surface and subterranean invertebrates as earthworms, slugs, insect larvae and nymphs, as well as on a wide variety of non-flying adult insects and other arthropods. In captivity *Ambystoma* and *Plethodon* species have been successfully maintained on earthworms. *A. tigrinum* will feed on grain beetle larvae (*Tenebrio mulitor*); however, it is preferable not to feed *Tenebrio* larvae as a long-term diet to amphibians, as the heavy, chitinous exoskeletons are indigestible and may cause bowel obstructions. It is possible, but not substantiated, that amphibians fed only on *Tenebrio* larvae may develop nutritional deficiencies.

b. **Order *Anura* Larvae (Tadpoles)**

Unlike other amphibians, most larval anurans (tadpoles) are herbivores or detritivores, feeding on aquatic algae and higher plants, and on organic debris in the substrate. In captivity, most tadpoles will feed on pieces of lettuce leaf softened by boiling. They will also consume most types of dry, commercially prepared aquarium fish foods. The normal aquarium practices of not overfeeding and of the immediate removal of uneaten food are necessary to prevent contamination of water by spoilage of the waste food.

A few species of tadpoles are predatory. The only such species in Canada is the larval form of the Plains and Great Basin spadefoot toads (*Scaphiopus bombifrons* and *intermontanus*, respectively), whose natural diet consists of aquatic insects, crustaceans and other larvae of their own species. Captive *S. bombifrons* tadpoles may be maintained up to transformation on a diet of live brine shrimp (*Artemia naupli*), followed, as the tadpoles grow large enough, by frozen adult brine shrimp.

c. **Order *Anura* (Frogs and Toads)**

Adult anurans are all predators. Under natural conditions their foods may range from earthworms, slugs and insect larvae for toads, through the various flying insects preferred by frogs, to other vertebrates, including fish, snakes, or occasionally small birds and mammals, taken by large leopard frogs and bullfrogs (*R. catesbeiana*). Toads (*Bufo*) and spadefoot toads are among the easiest to feed in captivity, as they will eat a wide variety of worms, slugs and insect larvae which are usually readily obtainable, even during winter. Again, *Tenebrio* larvae may be used; however, these should neither be the sole diet, nor should they be fed over an extended period.

Most true frogs (*Rana*) will not feed on anything except flying insects or other quite active prey; consequently, holding more than a few individuals in captivity becomes impractical unless they are kept in a torpid state, as described under Aquatic Caging.

Individual captive ranids may, with patience and luck, be induced to take dead food by dangling it on a thread or from forceps. Success will depend on the extent to which the animal has adapted to movement and disturbance. Unfortunately, many frogs will not tolerate the disturbance of human traffic around their terraria and continue to feed. In these cases, live food, usually in the form of flying insects must be provided. Very large frogs, such as bullfrogs, can sometimes be induced to take live crayfish or small fish, while occasionally live mice will prove to be the only acceptable food for a large bullfrog. The relatively large amounts of food needed by these animals make supplying them with insects and most other invertebrates impractical.

The African clawed frog (*Xenopus laevis*) and the closely related South American *Pipa* and *Hymenochirus* species, which frequently appear in the pet trade, are highly aquatic. All will readily take dead food. Fine scrapings from frozen liver can be offered to small juveniles, as can frozen brine shrimp. As they grow, they will accept strips of frozen liver of increasingly large size.

D. REPRODUCTION

1. Problems in Captive Breeding

There is much more variation in amphibian reproduction than is often realized. Unlike fish, birds and mammals, there are no captivity-adapted strains of amphibians available commercially. Almost all laboratory amphibians except *Xenopus* will have been either collected from the wild or reared from eggs of wild caught females. While a few types have been captive bred on a limited experimental basis, these have been maintained for only comparatively short periods and in small numbers. As a consequence, there has been no selection of stocks genetically adapted to captive conditions.

The lack of laboratory bred strains of amphibians is, in part, due to difficulties in establishing suitable environmental conditions to elicit sexual maturity and mating behavior in captives. These conditions are, in fact, often not known.

Even when reproductive activity can be readily induced, many amphibians will prove extremely difficult and expensive to rear successfully through their early larval and transformation stages.

With the exception of those studies specifically directed toward reproductive biology, there has been little reason to breed these animals in captivity. Most researchers are concerned more with behavior and/or ecological relationships under wild conditions than those exhibited in captivity. Where limited experiments under controlled captive conditions are needed, these can most easily be performed by using wild caught animals rather than resorting to a breeding program.

2. **Captive Breeding of Endangered Species**

In general, any project requiring consumptive use of significant numbers of wild caught animals from a species or population which is rare, or in danger of extinction or local extirpation, should require rigorous justification, in terms of research results and in terms of potential benefit to the population or species, before it is allowed to proceed. Captive breeding programs may be one way in which such research could be conducted with minimal or no damage to a wild population.

3. **Salamanders**

Most salamanders are internal fertilizers, the male depositing a spermatophore which is picked up by the female in her cloaca. Exceptions are the hellbenders (*Cryptobrenehidae*), Asiatic land salamanders (*Hynobiidae*), and the sirens (*Sirenidae*) which have external fertilization.

In Canada, salamanders of the genera *Plethodon*, *Enstatina* and *Aneides*, all of the family Plethodontidae, deposit eggs in protected, moist microenvironments on land. These genera have no free-living larval stage, and a terrestrial juvenile emerges from the egg.

4. **Frogs**

Anurans are nearly all external fertilizers with a few specific exceptions such as the tailed frog Ascaphus truei of British Columbia and the northwestern United States.

The technique of inducing sexual maturation and ovulation or sperm emission in anurans (chiefly *Xenopus laevis* and *Rana* species) by injection with frog pituitary gonadotropin or mammalian chorionic gonadotropin has been in use for some time (Welt and Wessels, 1967; Deuchar, 1972; Rugh, 1962).

Amphibians brought to maturity this way must usually be artificially spawned, as they do not necessarily show mating behavior with the artificially induced maturation.

Although the majority of frogs and salamanders familiar to Canadians have aquatic eggs and free living aquatic larvae, probably the majority of species of amphibians on a worldwide basis do not.

Among tropical and subtropical anurans and caecilians, there is a rich variety of reproductive strategies, ranging from ovoviviparity to specialized pouches in the female in which eggs and larvae are carried, to the use of water ponded in leaf axils and pitcher plants as egg deposition sites.

5. **Specialized Reproductive Models**

Special note should be made of two Canadian salamander species, *Ambystoma trembleyi* and *A. platineum*. The former occurs from southern Ontario through the Maritime Provinces; the latter may occur in southwestern Ontario. Both species exist as females only, and show a morphology intermediate between the bisexual species *A. laterale* and *A. jeffersonianum*. Their chromosome number is triploid with respect to the bisexual species. In each case, a triploid female will mate with a male of a bisexual species (*trembleyi*, with *laterale* and *platineum* with *jeffersonianum*. Fertile 3N eggs are laid which apparently contain no paternal DNA, and which hatch into female young which are apparently genetically identical with their mother. Additionally, it should be noted that both *A. platineum* and *A. trembleyi* are found in central Indiana, apparently in the absence of either of the bisexual species. These populations are considered to be truly parthenogenic (McGregor and Uzzell, 1964; Uzzell, 1964; Uzzell, 1970; Uzzell and Goldblatt, 1967), that is, reproduction occurs by means of unfertilized eggs (Porter, 1972b).

E. **DISEASES AND HEALTH CARE**

Like most other information on amphibians, that relating to diseases is largely empirical and usually relates to studies on only one or at most a few species.

1. **Red Leg (*Aeromonas* infection)**

The most common disease problem with captive frogs is red leg, a bacterial infection previously mentioned under Flow-Through Water System. Red leg like most other external infections probably begins with mechanically caused abrasions or wounds to the skin which become infected with *Aeromonas hydrophila*.

Even though an added advantage of a cool, flow-through water supply is that it appreciably reduces the hazard of such diseases as red leg, it is not a cure, nor will it prevent these diseases. Frogs held under these conditions should therefore be inspected daily for signs of infections (mainly red leg) and any diseased individuals removed immediately (Frazer, 1976).

Typical signs of red leg include cutaneous ulcers and characteristic pinpoint hemmorrhages over the abdomen, legs and tongue, with lethargy and emaciation; however, many of these signs may be absent in acute cases.

Segregation and individual antibiotic treatment (e.g., oxytetracycline by stomach tube twice daily at 150 mg/kg body wt. for a week) is effective but rarely considered practical under laboratory conditions (Siegmund, 1979; Frye, 1977). Tetracycline group treatments in the holding tank water are widely recommended for red leg and as a "cure all" for other bacterial infections in amphibians. In reality, the cutaneous absorption of these antibiotics is patently insufficient to provide a systemic therapeutic effect at the low (safe) tank water concentrations that must be used if adverse toxic skin reactions are to be avoided (Siegmund, 1979; Frye, 1977; Temple and Fowler, 1978).

Group treatment with tetracycline at safe levels may be of some assistance in limiting the spread of red leg, and control over outbreaks may also be enhanced by increasing the tank water salinity up to, but not exceeding 0.6% (Siegmund, 1979; Cosgrove, 1977). To this end, the following procedure** has been found to be effective: impregnate an absorbent mat, such as a piece of burlap, with crystalline sodium chloride and place on part of the tank bottom which is not water covered. Most frogs will come into contact with the salt impregnated mat fairly frequently as they move about in the tank, and the mat will serve as a prophylactic treatment dispenser. Since frogs cannot survive even brackish water, this treatment should not be used in a recirculating system or where the entire tank bottom may become water covered and the salinity of the water is likely to increase.

2. **Superficial Fungus Infections**

Superficial fungus infections, such as *Saprolegnia* can also start in minor abrasions. These can be a hazard amongst larval salamanders if several larvae in a single tank begin to nip at one another. Fungi appear as an opaque, usually fuzzy, white area of skin, often on an extremity, the median fin or on external gills. It has been demonstrated that fungus infections will respond to calcium propionate solution, used as a dip. Affected animals are dipped in an aqueous solution of 2-3% calcium propionate for one minute and immediately returned to fresh water. Treatment should be repeated once or twice daily until grossly visible signs of infection disappear. Topical painting of wounds and localized fungal infections with a 2% mercurochrome solution, followed in a few minutes by washing in flowing water has been recommended (Boterenbrood and Verhoff-De Fremery, 1976). Potassium permanganate dips at 1:5000 for five minutes have also proven useful against *Saprolegnia* infection (Temple and Fowler, 1978). The appearance of fungus infections, especially among larval salamanders, should serve as warning that attempted cannibalism is occurring and the larvae should be housed singly, if possible.

3. **Food Toxicity**

Occasionally, aquatic amphibians will develop a toxic syndrome that seems to be associated with the presence of uneaten, partially decomposed food in the tank. It can occur even in tanks with relatively high water flow rates and in which only small quantities of uneaten food can be found. What appears to happen is that fungal hyphae begin to grow in aggregates of uneaten food and probably have the effect of reducing water circulation so that anerobic decay can occur. The toxicity affects an entire tank, and (in the case of tadpoles or salamander larvae) all individuals will initially be found resting motionless at the surface. Their response to any disturbance will be an uncoordinated, violent swimming behavior, often involving body rotation.

Once these symptoms have appeared, death will follow within a few hours. It is sometimes possible to save a few of the least affected individuals by removing them to a separate, clean tank with a high water flow.

The problem can be prevented if care is taken to avoid overfeeding and to remove any aggregates of uneaten food as soon as they are detected (Siegmund, 1979).

4. **Parasitism**

Persons dissecting freshly killed frogs should note that these animals may be infected with *Alaria*, a parasite which may infect humans (Freeman, Stuart, Cullen *et al.* 1976; Fernandes, Cooper, Cullen *et al.* 1976). This dangerous zoonotic hazard will be more fully discussed in the chapter on Reptiles, in connection with garter snakes, which may also be infected with Alaria. With the above exception, no amphibian diseases represent significant zoonoses.

F. ANESTHESIA AND RESTRAINT

Frogs and toads should be picked up by the hind legs or, with experience, by the body; use of a cloth may be helpful. When it is necessary to pick up an axolotyl, or other urodeles with external gills, particular care must be taken not to put pressure on these.

Because of their permeable skin, amphibians can be easily anesthetized by placing them in a solution of anesthetic. Tricane methane sulfonate (MS-222), as a 0.01% aqueous solution will produce anesthesia within a few minutes, depending on the size of the amphibian.

Both chemical and physical restraint are discussed in more detail in the chapter on Wild Vertebrates in the Field and in the Laboratory.

G. EUTHANASIA

Euthanasia can be accomplished in several ways, depending on requirements. An elevated dose (0.1 to 1.0^{o}C) of MS-222 will anesthetize and kill amphibians in about a minute. If chemical anesthetics cannot be used, a blow on the head, crushing the skull, or pithing, preferably after immobilizing by chilling the animals to 4^{o}C, can be used. It is important that the proper procedure for pithing should be learned in advance by study of skeletal preparations and practice on freshly killed or preserved specimens. The pithing needle should be inserted into the *foramen magnum* and the rain destroyed quickly by lateral and vertical movement of the needle tip inside the brain case. Rapid decapitation at the first or second cervical vertebra may also be used.

Ether and heat shock are methods sometimes used in euthanizing amphibians. Both are rapid, but also produce some initial violent movements by the animal which might be subjectively interpreted as signs of distress. These techniques should be avoided if more suitable alternate means are available. Amphibians, even larvae, should never be killed by placing them directly in formaldehyde solution.

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APPENDIX

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