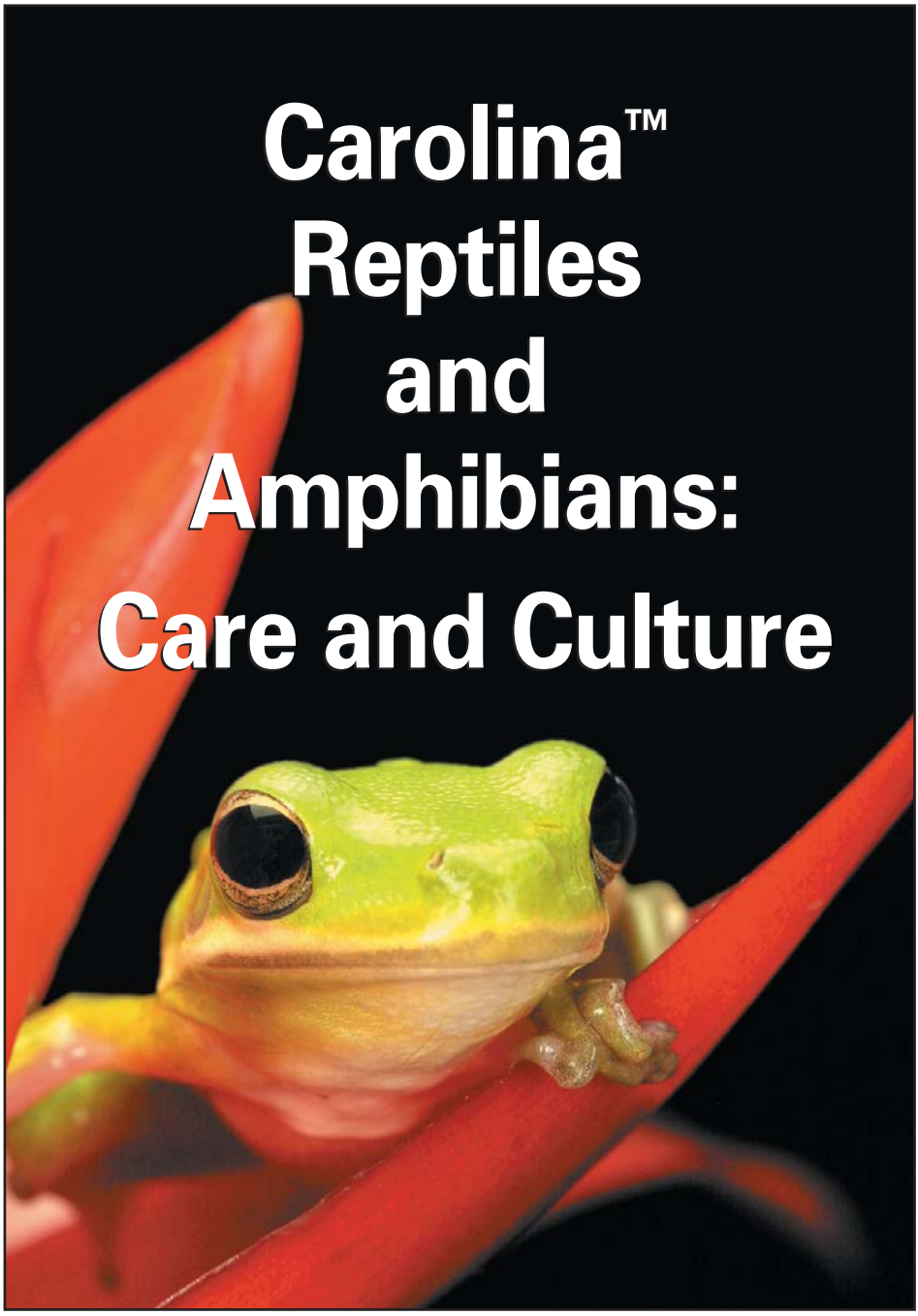
Carolina™ Reptiles and Amphibians: Care and Culture



CAROLINA®

World-Class Support for Science & Math

This booklet provides a brief description of the general characteristics and care requirements of the amphibians and reptiles supplied by Carolina Biological Supply Company. Throughout the booklet, you will find Carolina item numbers in parentheses following the mention of a product that we carry. If you have questions about any of our products, please call 800.334.5551.

Note: Under no circumstances should any animal be released into an area where it is not native. If you no longer need or want a classroom pet, investigate alternatives such as sharing them with other classes, giving them to other caring individuals, or disposing of them as humanely as possible.

Safe Handling of Reptiles and Amphibians

Whenever handling any reptile or amphibian, or any part of their habitats, be sure to wash your hands thoroughly with soap and warm water afterwards. The feces of these animals may harbor *Salmonella*, the bacterium responsible for the disease salmonellosis. Immunocompromised individuals, the elderly, and children under five years of age should avoid contact with reptiles and amphibians. These animals should not come in contact with food preparation areas, such as the kitchen sink or countertops. Additionally, reptiles and amphibians should not be allowed to roam freely in your home. With minimal precautions, both reptiles and amphibians are safe for classroom use. For more information, please refer to the Center for Disease Control's December 12, 2003, article: "Reptile-Associated Salmonellosis—Selected States 1998–2002" (http://www.cdc.gov/).

CAROLINA BIOLOGICAL SUPPLY COMPANY
2700 York Road
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Carolina™ Reptiles and Amphibians: Care and Culture

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Amphibians

Amphibians are a class of "cold-blooded" (poikilothermic or ectothermic) vertebrates including caecilians, frogs, toads, and salamanders. The females lay their eggs in or near water since the larval stage is usually spent in the water. The name "amphibian" derives from the Greek words *amphi* meaning "both" and *bios* meaning "life," or "living both on land and in water."

Amphibians have smooth or warty skin with no scales but with many mucous glands that keep the skin moist. These glands may be modified to exude poisons for self-defense. Although few amphibians are dangerous to humans, we recommend that you wash your hands thoroughly each time after handling amphibians. With only a few exceptions, amphibians lack claws on the toes.

Terrestrial Salamanders

Keep salamanders in a covered vivarium if you are going to keep them for more than a few days. They can be held temporarily in a slanted container with a small amount of water at one end. The temporary container should have a tight-fitting lid that allows air circulation.

Salamanders require high humidity and cool temperatures. Hot, dry conditions cause salamanders to become much less active than normal. Sprinkle water on the leaves of plants and the side walls of the tank every other day, and maintain the temperature at 18–21°C (64–70°F). A small fluorescent bulb placed near the vivarium provides adequate light without creating excessive heat.

Feed the salamanders mealworms, crickets, whiteworms, earthworms, or soft-bodied insects of appropriate size. Eating a variety of foods keeps the salamanders healthy. Remember that overfeeding causes stress and that larger food organisms may harm the salamanders if they are present in large enough numbers. Remove dead and uneaten food after the salamanders have finished eating. Provide fresh drinking water at all times.

Keep salamanders (14-6070) in a vivarium with high humidity and cool temperatures.

If you are raising terrestrial salamanders from larvae, you will need an aquatic habitat. The larvae have gills, which they will gradually lose over the course of several weeks. You must provide something for the larvae to crawl onto as their lungs develop, and you will eventually convert the aquatic habitat into a vivarium as described above for the adult salamanders.

Aquatic Salamanders

Necturus (14-6030) is a large neotenic salamander.

Necturus is an aquatic salamander found in rivers, lakes, and streams throughout North America. *Necturus* is neotenic, retaining the larval form throughout life.

Keep *Necturus* in a large container of water that has a tight-fitting lid to prevent escape. Provide hiding places for the salamanders by piling rocks to form crevices and caves. Spring or pond water (16-3380) is best, but tap water can be used if it is treated with a water conditioner capable of removing both chlorine and chloramines, as well as detoxifying harmful ammonia (67-1985). Salamanders should be housed individually to prevent fighting and spread of disease. Although *Necturus* is neither poisonous nor aggressive, handle them carefully to avoid bites.

To prevent temperature shock when transferring *Necturus*, place the shipping bag in or beside the holding tank until the temperature of the water in the shipping bag matches the temperature of the water in the holding tank. This may take 1 or 2 hours. The best temperature at which to hold *Necturus* is 4–10°C (39–50°F), but they can be held at room temperature (20–26°C [68–79°F]).

Necturus will eat earthworms, tadpoles, small grass frogs, and small crayfish. Waste materials and uneaten food must be removed from the tank daily.

Newts

Newts (14-6050) are aquatic salamanders that do not retain external gills as adults. Newts can live in aquaria or any other containers of water. Most often, the newts shipped from Carolina Biological will be broken-striped newts (*Notophthalmus viridescens dorsalis*). A floating platform must be provided so they can crawl out of the water and rest. Newts are non-territorial and non-aggressive, so several may be housed in one container. Newts eat small tadpoles, small insects, worms, and bits of raw meat, or canned, all-meat dog food. Do not foul the water with too much food, and remove all uneaten and dead food promptly.

Frogs

Carolina ships frog eggs, live frogs for breeding, and frogs for general use. When the frogs arrive, empty the box into 15–25 cm of cool water (10–15°C [50–59°F]) in a deep container (such as a bucket or plastic trash can) and wash the frogs free of the packing material, which kept them moist in transit. We recommend that you

use the frogs for experimental purposes as soon after arrival as practical; the shock of shipping is hard on frogs and reduces their defenses against endemic microorganisms.

Reddened legs on a frog may be a symptom of environmental shock. Even healthy frogs may be traumatized by shipment and arrive with reddened ventral surfaces; we can professionally pack healthy frogs, but we cannot control their exposure to stressful conditions or handling while in transit. We do guarantee to ship healthy frogs, but the recipient must provide reasonable care. Be sure to tell us the expected use date of the frogs so that we can package and ship accordingly. If you need to maintain the frogs for some time, be prepared to provide very good care.

If an arriving frog is to be added to a tank with other frogs, we recommend that you quarantine the new frog for several days before adding it to the established tank. Generally, illness in frogs results from unclean habitats, malnutrition, or excess stress. Lethargy, thinness, and cloudy eyes are all signs of illness. Any frog you suspect of being sick should be quarantined.

Quarantined frogs can be kept temporarily in shallow water in aquaria or plastic containers. Keep the water 2–5 cm deep and provide a platform or rocks so the animals can get completely out of the water. Use chlorinated tap water to reduce bacterial growth, and change the water daily.

Very sick frogs can be treated with an antibiotic such as tetracycline. Many home remedy suggestions may be found both in literature and online, but it is best to take any sick animals to a veterinarian for treatment.

Carolina ships both northern and southern grass frogs (also known as leopard frogs), depending on the season. We recommend a maintenance temperature of 10–15°C (50–59°F) for northern grass frogs and 15–20°C (59–68°F) for southern grass frogs. Outside these temperature ranges, the frogs tend to have more

The mature southern grass frog, *Rana sphenocephala* (left), is generally larger than the northern grass frog, *Rana pipiens*, as illustrated by these two females.

disease problems. Most of the grass frogs shipped from Carolina Biological are northern grass frogs. These frogs tend to be less active than their southern counterparts, making them well suited to captivity. You can differentiate between the two species by examining their spots. The northern grass frog has dorsal spots outlined with a light color, while the southern grass frog has solid dark spots.

Adult frogs will eat most animals that move above the water and are not too large, such as sowbugs, flies, mealworms, crickets, caterpillars, moths, and earthworms. A variety of food items may be offered to your frogs. Frogs should be fed in a dry area to allow them to capture the food easily and to prevent food organisms from drowning and fouling the water. Offer food daily.

Bullfrogs

Bullfrogs are also available from Carolina. Bullfrogs should be maintained at 20–25°C (68–77°F). Most often we ship *Rana catesbeiana*, but this may vary with availability. Adult bullfrogs eat a variety of foods, from crickets to small turtles, snakes, and even other bullfrogs—basically any moving thing of edible size. Excess food items and waste materials should be removed often, as tank cleanliness is essential for bullfrog health. Bullfrogs are native to the eastern United States but can be found in the wild as far west as the Rockies. Bullfrogs are known for their ability to colonize new areas and endanger native species. For this reason, never release any bullfrog or their progeny into the wild. Male bullfrogs have a loud, bellowing call.

If you plan to house a single bullfrog for any period of time, an aquarium size of at least 20 gallons is suggested. Five additional gallons should be allotted for each individual added. Floating plants, such as those found in Carolina's Floating Plants Set (16-1790), are a good addition to any bullfrog tank.

Bullfrog, *Rana catesbeiana*

All stages of bullfrog development, from egg to adult, are available. For tadpole care requirements, please refer to the section Amphibian Larvae, in this manual.

Bullfrog Eggs (200–300)	14-6540
Bullfrog Tadpoles (Up to 2″)	Pack of 6: 14-6550 Pack of 12: 14-6552 Pack of 50: 14-6554
Bullfrog Tadpoles (2″ to 4″)	Pack of 6: 14-6560 Pack of 12: 14-6562 Pack of 25: 14-6564
Bullfrog Tadpoles (Hind Limb Bud Stage)	Pack of 12: 14-6470 Pack of 100: 14-6472
Two-Legged Bullfrog Tadpoles	Pack of 6: 14-6570 Pack of 25: 14-6572
Adult Bullfrogs	3″: 14-6530 4″: 14-6532 5″: 14-6534

Breeding Frogs

Either northern or southern grass frogs can be obtained year-round. A certain number of female southern grass frogs reach sexual maturity during every season, so we can ship mature southern grass frog females any time except during winter months when it is too cold to collect them. During the winter, northern grass frogs are available for breeding experiments. Northern grass frogs normally reach sexual maturity in the fall after a summer of feeding. Mature frogs are collected in late fall and held at low temperatures (8–12°C [46–54°F]) during the winter months with little harm. Southern grass frogs cannot withstand such cold temperatures.

In the wild, southern grass frogs ovulate throughout the year, while northern grass frogs ovulate only in the spring. In either species, ovulation can be induced in mature females by artificially increasing the level of pituitary hormones. The eggs can then be stripped from the uterus by hand, as described later in this manual.

The female's pituitary level is increased by injecting whole frog pituitaries, frog pituitary suspension, or a pituitary progesterone mixture into the body cavity. Carolina offers preinjected frog sets, pituitary extracts, gravid female frogs, mature females with eggs (14-6350), and mature males (14-6356). The species of frog supplied depends upon the season. The Preinjected Frog Set (14-6300) includes one injected female frog and two male frogs for sperm. The Pituitary Extract (14-6330) includes six dried frog pituitary glands for inducing ovulation and yields the best results when used with the northern grass frog. Gravid female frogs (14-6340) have been injected with pituitary extract and are available individually.

In addition to frogs and appropriate containers, the following materials are needed to demonstrate induced ovulation:

*heavy scissors (62-1775)
*fine scissors (62-1810)
*forceps (62-4504)
*medicine droppers (73-6903)
*dissecting needles (62-7231)
culture dishes (at least 12) (74-1004)
hypodermic needle
syringe

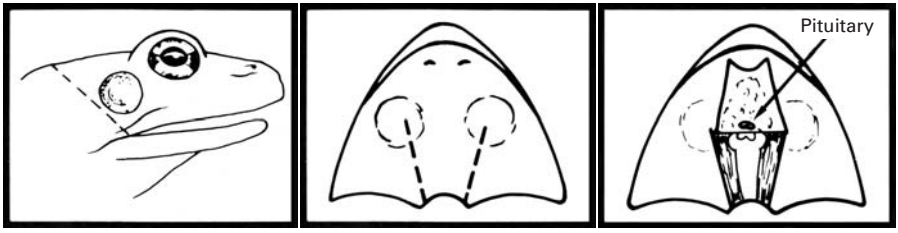
*Carolina offers a wide variety of dissecting instruments and can customize a dissecting kit for you if needed.

All instruments that come in contact with the eggs must be biologically clean. Glassware should be washed in hot, soapy water and rinsed thoroughly. Avoid glassware that has been used with fixatives. Chromium-plated instruments should not come in contact with living frog eggs.

Pond water or springwater known to support frog eggs is used for culturing (see Amphibian Eggs, in this manual). Tap water may be used if it is treated with a water conditioner (67-1985). Distilled water does not contain the salts necessary for normal development of the eggs. The water used should be at room temperature.

Removing Pituitary Glands

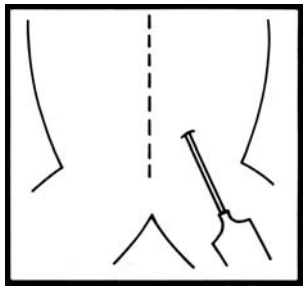
Carolina offers preinjected frogs from November to March, as well as prepared dried pituitary glands, but you may wish to collect pituitary glands yourself. To remove the pituitary gland from a frog, first cut off the frog's upper jaw and head, using heavy scissors and cutting along the line indicated in the following figure; this will kill the frog. The brain and part of the medulla will be intact. Insert the point of the fine dissecting scissors into the back of the cranial cavity. Keep as far as possible to the right side to avoid damaging the pituitary gland. Cut through the bone. Withdraw the scissors and cut along the left side in the same manner. This should release the floor of the cranial cavity as a small flap. Pull the flap up with a pair of forceps to expose the pituitary, which will be either adhering to the flap or lying on the ventral surface of the brain .



Cut lines

Site of Injection

The pituitary gland is a small, kidney-shaped organ often embedded in a mass of white lymphatic tissue. Remove the lymphatic tissue by carefully rolling the gland on a damp paper towel. Do not rupture the gland, as it will lose much of its effectiveness if broken. Place the gland in a small dish with 1 mL of aged tap water or springwater.



Site of injection

When all pituitaries are removed, draw them into the syringe with a small amount of water. Put a needle on the syringe and inject the pituitaries intraperitoneally. Be sure that the pituitaries do not cling to the sides of the syringe. It is best to inject through the wall of the lower abdomen, directing the needle anteriorly. Be careful not to damage the ventral abdominal and lateral veins or the internal organs.

For best results, a female northern grass frog should be injected with six whole pituitary glands at once; this is best done at some point between November and April. In October, November, and December, ovulation requires 72 hours. During January and February, ovulation occurs in 48 hours, while in March and April, eggs can be obtained in 24–36 hours. Female northern frogs that have ovulated should not be held in the refrigerator more than 48 hours.

The female southern frog also requires six pituitary glands for artificial induction, but the injections are split into two sessions (as described in the following section, Using the Pituitary Extract).

Place the injected female in a container in a quiet, dimly lighted, undisturbed area. When the eggs are ready for release from the ovary, the lower abdomen will bulge on both sides when the upper abdomen is pressed.

Artificial induction of eggs from a frog

When the female is ready to release eggs, pith a male frog and remove the testes. The testes are fairly large, oval, yellowish bodies on the dorsal surface of the peritoneal cavity posterior to the kidneys. The testes should be thoroughly chopped or crushed in about 10 mL of pond water. Sperm activity begins approximately 5 minutes after release from the testes and can be observed with a compound microscope.

It may be desirable to strip the eggs from a single female in several batches (e.g., for two or three different embryology laboratory sections). If so, simply remove a few eggs at each session. To strip the eggs, hold the female firmly in the palm of one hand while holding her legs in the other hand. With

your thumb, apply firm pressure and a milking motion to the abdomen to force the eggs from the uterus. Milk the eggs into a clean culture dish.

Gently pipet the sperm suspension over the eggs. A fresh sperm suspension must be used for each batch of eggs. Let the eggs stand for 5 minutes, then add about 50 mL of water. Change the water after 20 minutes, adding just enough to cover the eggs.

If the eggs are fertilized, after about an hour they will rotate, such that the animal hemisphere (dark side) is up. As soon as the jelly membranes have enlarged, the eggs may be separated. Any flat, nonmetal object can be used to cut the jelly and lift the eggs from the bottom of the dish. A small flexible ruler is ideal.

Distribute the eggs in culture dishes in lots of 25 to 30 eggs each. Add water to each dish to a depth of 1 to 2 cm. This should be done before the first cleavage, which occurs about 2 hours after fertilization. If the eggs are overcrowded, abnormalities will appear in just a few hours.

Using the Pituitary Extract

Frog pituitary extract (14-6330) is packed in a vial and contains dried pituitaries from six frogs. This material is sufficient for inducing ovulation in one northern grass frog or one southern grass frog. Suspend the powder by injecting 1 mL of aged tap water. Gently rotate the vial to disperse the powder.

Inject the northern frog with 1 mL of pituitary suspension. Ovulation occurs 48–72 hours after the injection.

The southern frog will also respond to the injection of pituitary extract. Withdraw 0.5 mL of suspension from the vial and inject a female frog. Place the remaining 0.5 mL of suspension in the refrigerator. After 24 hours, inject the remaining suspension into the frog; wait an additional 24 hours for ovulation to occur. Eggs should be stripped as described above.

Xenopus

The South African clawed frog, *Xenopus laevis*, is a stout-bodied, long-lived, fully aquatic frog. It is tetraploid, carrying four sets of chromosomes. For over 30 years, *X. laevis* has been a model organism for education and research in embryology, physiology, anatomy, morphology, and the amphibian life cycle. A closely related diploid species, *Xenopus tropicalis*, is becoming increasingly important for research in developmental genetics. At this time, Carolina supplies only *X. laevis*. The female can be induced to release eggs every 2–3 months throughout the year, and the male can be bred every 7 days. The frogs will engage in amplexus in the laboratory, and, unlike other species, do not need to be sacrificed for breeding experiments. *Xenopus* develops from the egg stage to a small frog in less than 2½ months. Unlike most frogs, the adult *Xenopus* does not require live food and can live as an adult on a diet of food pellets.

Xenopus adult male (left) and adult female (right). Note the small papillae at the posterior end of the female.

Xenopus is extremely slippery because a mucous film covers its skin. It can be transferred from one container to another with a net if care is taken not to tangle its legs. To pick up a frog by hand requires a special technique. Grasp the frog from its dorsal side with your thumb and middle finger around its body and your index finger between its hind legs. Do not hold the frog too loosely as the claws on the back legs can scratch. If the frog is held too tightly, its internal organs can be damaged.

Xenopus is an extremely hardy frog, and it has been known to damage local ecosystems in areas where it has been released; be sure that all *Xenopus* that can no longer be maintained in the laboratory are destroyed and disposed of in accordance with local regulations, or given to an individual who will keep them through the frogs' remaining lifespan. Several states require that you have a permit in order to receive *Xenopus*. When you order *Xenopus* from Carolina, inquire as to the current regulations in your state.

Immediately upon receiving your frogs, check their condition. Leave the frogs in the shipping container (with the lid securely in place) until the temperature in the shipping container matches the temperature of the water in the holding tank, which should be room temperature (18–22°C [64–72°F]). Never place adult frogs in water that varies from their body temperature by 5°C (9°F) or more. Rinse the frogs with some of the holding water to remove any packing materials before placing them in the aquarium.

Maintain adult *Xenopus* in a container at least 20 cm deep and filled with approximately 10 cm of water. At least 2 L of water are required for each adult frog. A secure, ventilated cover is required to prevent adults from escaping.

Hold the adults in bottled spring or pond water (16-3380). If spring or pond water is unavailable, conditioned tap water may be used. To condition tap water, treat it with a water conditioner that removes both chlorine and chloramines. If locally collected pond water is the only source that can be used, it should be boiled to destroy any harmful organisms. Always allow water to reach room temperature before placing the frogs in it. Twice weekly, change the water in which the adults are held (unless it is continuously filtered). It is not necessary to aerate the water. Ideal holding temperatures are 18–22°C (64–72°F).

Dry foods have been developed to feed *Xenopus* of all stages. These foods provide a complete diet and are inexpensive. A pelleted form is best suited for the adults. Feed adult frogs twice a week, but do not overfeed—excess food quickly fouls the water. As a rule, do not feed them more than they can eat in 20 or 30 minutes. After a few feedings, you should be able to gauge the appetites of the frogs. Do not disturb the frogs for several hours after feeding, or they may regurgitate their food. Adults usually eat more readily if kept out of bright light. *Xenopus* Adult Food (14-6620) and *Xenopus* Tadpole Food (14-6650) are available from Carolina.

Breeding Xenopus

Xenopus laevis is easily sexed. At her posterior end, the female has small tail-like papillae called cloacal lips. These are absent in the male. Generally, the adult female is larger than the male, but you cannot rely on size alone in determining gender.

To condition the adults for breeding, feed them daily for 2 weeks. Avoid fouling their water during the conditioning period. Use an aquarium as a breeding/rearing tank. Cover it with a material that allows air exchange but keeps adults from escaping. Galvanized hardware cloth (10- to 15-mm mesh) with the edges bent around the top of the container is suitable.

The breeding/rearing tank must have a false bottom of screen mesh large enough for eggs to fall through, but small enough to keep the adults away from the eggs. The screen should be located about 2 cm above the tank floor. Secure the screen by weighing down the corners with glass culture dishes, so the frogs cannot dislodge it. We recommend the screen floor be made of plastic or plastic-coated wire.

The spawning response in *Xenopus* is initiated by injecting both the female and the male with chorionic gonadotropin (14-6610). The injection schedule depends upon when eggs are needed and in what stage they are required. Because *Xenopus* is extremely slippery, two people should cooperate in the injections. One person holds the hind limbs of the frog with a paper towel around each leg, while the other gives the injection. The person giving the injection should hold the front part of the frog's body still. Carolina's *Xenopus* Breeding Kit (14-6602) comes with everything necessary to breed *Xenopus*.

A vial of 10,000 IU of chorionic gonadotropin (14-6610) is diluted with 10 mL of bacteriostatic water for a concentration of 1,000 IU per mL. Injections are made into the dorsal lymph sac located beneath the skin of the back approximately 2 mm anterior to the hind limbs. Gently but firmly insert a needle just through the skin. The skin is resilient and sometimes tough, so use care not to stab and injure the muscles of the frog. Release the hormone slowly, tilting the frog slightly head-down. If the correct procedure is used, the hormone can be seen flowing under the skin toward the head. At all times during the injection, hold the frog firmly so it cannot injure itself with the needle. A properly injected female will develop dark red cloacal papillae within a few hours. An injected male in breeding condition will have black areas (nuptial pads) on the undersides of the front legs. Separate the male from the female until the injection schedule calls for them to be placed together.

Injecting chorionic gonadotropin induces *Xenopus* to breed.

Amplexus

When injecting *Xenopus*, we recommend that you inject 1 mL of chorionic gonadotropin into the females and 0.6–0.7 mL into the males. Keep the unused gonadotropin in a refrigerator so it retains its effectiveness. After injection, put both frogs in the breeding/rearing tank. Add enough water to the breeding/rearing tank so the water level is about 10 cm above the false bottom. Keep the tank in a dark, undisturbed area. *Xenopus* does not respond well to the hormone if disturbed frequently or if left in light. Covering the tank with an inverted cardboard box or a lab coat helps provide a good environment for breeding.

If the environment is favorable and the injections have been given correctly, amplexus occurs within a few hours. The male, sometimes emitting a mating call,

approaches the female from the rear and clasps her around the pelvic region. The male releases sperm into the water as the female releases eggs. The day after placing the pair in the breeding/rearing tank, remove them to a suitable aquarium. Some of the eggs may also be removed for study at this time.

If breeding results are not satisfactory, the frogs can be reconditioned for at least 10 days and then injected a second time. One pair of *Xenopus* may be injected as many as five times on this schedule.

Fire-Bellied Toad


Fire-bellied toad

This hardy and attractive toad is an excellent choice for a classroom habitat. With green and black on its back and vivid red-orange and black on its ventral surface, this toad is very colorful. This coloration has evolved as a warning system to predators; the toads can secrete a poisonous white substance from glands along their backs if provoked. Always wash your hands after handling these toads and do not allow young children to handle them at all. Unlike most other toads and frogs, the fire-bellied toad cannot extend its tongue. Instead, it leaps forward to catch its prey and uses its forelimbs to guide food into its mouth.

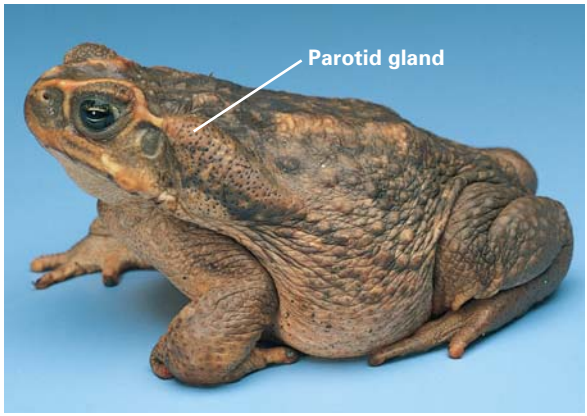
The fire-bellied toad has simple housing requirements. A glass or plastic aqua-terrarium with a screened lid is ideal. Using 1½ to 2½ inches of washed gravel as the substrate, create a sloping landmass and a shallow pool area with a depth of 3 to 4 inches. Use either springwater or tap water that has been treated with a water conditioner. You may wish to provide cork bark shelters and moss in the terrestrial part of the habitat and plants such as *Elodea* (16-2101) in the water. A fluorescent aquarium light can be used to light your fire-bellied toad's habitat. No additional sources of heat should be needed, as the toads will do well at daytime temperatures of 18–25°C (64–77°F) and nighttime temperatures of 12–15°C (54–59°F). Temperatures over 27°C (80°F) can kill the toads.

The water in the toad's habitat should be changed once a week. Siphon a quarter to half of the water out of the habitat, making sure to remove as much waste material as possible. Replace the water with fresh, dechlorinated water of the same temperature.

Fire-bellied toads are insectivorous. Feed them vitamin-dusted crickets that are no longer than the width of the individual's mouth. Other live foods include waxworms and mealworms (14-4268).

Giant Toad

The giant toad, *Bufo marinus* is an enormous amphibian that requires little care beyond occasional feeding with live insects. Introduced onto sugar plantations for insect control, this toad is nearly ubiquitous in the American tropics. They are excellent colonizers and should never be released into the wild. Please inquire upon ordering as to the status of *Bufo marinus* in your state, as some states require a permit. *Bufo* is usually available September through April; availability is influenced by weather.



Giant toads have large parotid glands which exude poison when the toad is attacked.

Bufo marinus is easily maintained in an inclined tank with water in one end, or in a semiaquatic terrarium. Be sure the tank has a well-secured, ventilated top. The best temperatures to keep giant toads at are 24–27°C (75–80°F) during the day and 19–21°C (66–70°F) at night. Take care when handling these toads, and wash your hands thoroughly afterward since the toads secrete a toxin from the parotid glands at the back of their heads. Do not house the toad with any organism smaller than it is—the toad will eat it. Change the tank water once a week. Feed crickets, mealworms, and other soft-bodied insects to the toads. An occasional dusting of the insects with a commercial pet vitamin is recommended.

Small toads of mixed *Bufo* species (14-6760) are also available from September to March.

Green Tree Frog

An excellent choice for the terrarium, the green tree frog is attractive, readily available, and easy to care for. Native to North America, these brightly colored frogs range from 1¼ to 2¼ inches long at maturity. A glass or plastic terrarium is appropriate for tree frog housing. A 5½-gallon tank may house one or two frogs, but a larger tank is better. The tank should have a tight-fitting screen cover, as tree frogs require good ventilation to thrive. No special lighting is needed.

The ground medium for a tree frog terrarium may be as simple as newspaper or brown paper. Paper is inexpensive and easy to replace when housing a large number of frogs. For a display terrarium, we suggest using a 1- to 3-inch layer of peat-based potting soil (free of added fertilizers) over a 1- to 1½-inch layer of coarse aquarium gravel (for drainage). Tropical plants with low light

Green tree frog

requirements, such as *Pothos*, *Philodendron* (15-7658), *Croton*, *Rhoeo*, and ivy are good choices. A terrarium with native woodland plants and moss (15-6690) will also work.

Provide a porcelain, glass, or plastic container of clean, dechlorinated water at all times. The container should be big enough for the frog(s) to sit in, but shallow enough to allow the animal to get out easily. Tree frogs may drown if the water is too deep. It's a good idea to place a small rock in the water bowl so that the frogs can climb out easily.

Green tree frogs should be maintained at temperatures of 23–29°C (74–85°F). They can tolerate night temperatures as low as 16°C (60°F) when kept in a classroom. If the tank is in an area where temperatures will fall below this point, an under-tank heater (67-4411) or nocturnal heat lamp (67-4419) may be used to maintain an appropriate temperature. If you use an under-tank heater, position it at one end of the tank. Place a rock over the heater to absorb the heat and provide a warm resting place. If you use a nocturnal heat lamp, limit it to 25 watts. Position the lamp over a portion of the habitat where there is a high resting point, such as a branch. Humidity should be kept at or near 80%; a daily misting with dechlorinated water helps maintain proper humidity.

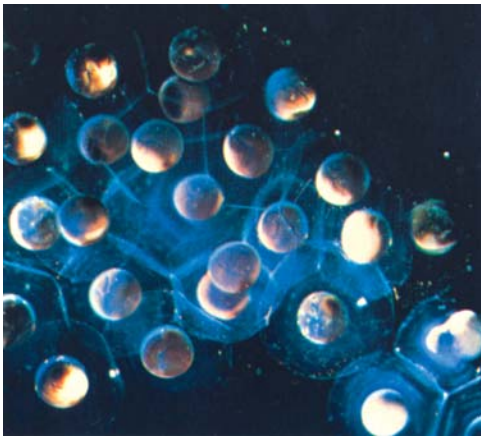
Feed the tree frogs live crickets two or three times a week. Once each week, dust the crickets with a calcium/vitamin supplement. You may also offer houseflies or other flying insects of appropriate size.

Dwarf Aquarium Frog

Carolina also offers dwarf aquarium frogs (14-6700). These small aquatic frogs are ideal for use in a tropical aquarium, and can be fed brine shrimp flakes (67-4753). Directions for properly acclimating the frogs should arrive with the shipment. Read and follow these directions as soon as you receive the frogs. Do not overfeed the frogs; remove any uneaten food 5 minutes after each feeding. Overfeeding and poor water quality are the main sources of trouble with maintaining aquarium frogs. We recommend a partial water change of about 25% of the total volume in the habitat two or three times a week. Replacing larger amounts at one time may shock your frogs.

Amphibian Eggs

Artificially fertilized frog eggs (14-6430), field-collected frog eggs (14-6435), and bullfrog eggs (14-6540) are available for study from Carolina Biological. Amphibian eggs (below) are shipped as soon after fertilization as possible. The extent of development during shipment depends on the temperature and the amount of time in transit.



Amphibian eggs are used in studies of animal development.

Upon receipt, frog eggs should be left in the plastic bag in which they are shipped to you. The bags are filled with oxygen, so it is safe to leave the eggs in the bag until tadpoles emerge. The tadpoles will be old enough to release when they begin attaching to the sides of the bag or actively swimming.

You may use tap water that has been treated with an appropriate water conditioner in the aquarium (or other tank) for the tadpoles. Pond or spring water may also be used, but do not use distilled water. Prior to their release into the aquarium, the tadpoles must be acclimated to the temperature, pH, and other conditions of the water in their new habitat. This may be accomplished by floating the shipping bag in the aquarium so that the temperatures between the

two can equilibrate. Next, remove and discard about one quarter of the water from the bag that the eggs were shipped in, and replace it with an equal amount of water from the aquarium. Wait 15 minutes, and repeat this step. Do not allow any shipping water to enter the tadpoles' new aquarium. After another 15 minutes, release the tadpoles into a small container of prepared holding water at the same temperature as the aquarium. There will be a clear, jelly-like egg mass with the tadpoles. Using a pipet, carefully remove all of the egg mass before transferring your tadpoles to the aquarium.

Add *Elodea* or similar water plants. As the aquatic tadpoles develop into frogs, they will need something to climb out of the water onto. A floating plant such as *Salvinia* (16-1860) works well for this purpose. Larger tadpoles (such as bullfrogs and toads) may require a sturdier surface to climb onto, such as a stick or rock that breaks the surface of the water. Feed the tadpoles Carolina's Tadpole Food (14-6500), pelleted rabbit food, fish food, and algae supplemented with finely powdered beef liver or powdered egg yolk. Each day, remove approximately one tenth of the water and replace it with fresh, prepared water. It is wise to have some conditioned tap water or other appropriate holding water set aside for use in these water changes.

Salamander Eggs

Salamander eggs (14-6100) are collected as soon as breeding occurs, usually in January or February. The exact time depends on weather conditions, and the eggs are shipped as available. We cannot accept orders for specific species; however, most of our field-collected salamander eggs are *Ambystoma maculatum* (spotted salamander) and occasionally *A. tigrinum* (tiger salamander). *A. tigrinum* lays its eggs a little earlier than *A. maculatum*. *A. tigrinum* egg masses contain 100 to 200 eggs, while those of *A. maculatum* rarely contain more than 100.

Salamander eggs are large (2.5 mm) and are excellent for embryological studies. The eggs typically hatch in less than 4 weeks, and metamorphosis occurs in 10 to 12 weeks.

Rana Eggs

Northern frog eggs (*Rana pipiens*, 14-6430) that have been artificially fertilized in our laboratories are shipped within 4 hours after fertilization. These eggs are shipped from early November through early April, depending on the availability of gravid females.

Frog eggs collected in the field (14-6435) from January through June are from available species. Those shipped from North Carolina include northern frogs (*Rana pipiens*), southern frogs (*R. sphenocephala*), green frogs (*R. clamitans*), pickerel frogs (*R. palustris*), and wood frogs (*R. sylvatica*). Wood frog eggs are 1.8 to 2.4 mm in diameter, while those of the other species are somewhat smaller (about 1.6 mm). We make every effort to ship field-collected eggs in the youngest stage possible.

The northern frog requires from 10 to 14 weeks to develop from egg to small frog under normal conditions. Early developmental rates at 18°C (64°F) are as follows:

Stage	Hours
2 cells	1.0
4 cells	4.5
8 cells	5.7
16 cells	6.5
32 cells	7.5

Stage	Hours
Late blastula	21
Crescent blastula	26
Mid-gastrula	34
Tail bud	84
Hatchling	140

Bullfrog eggs (*Rana catesbeiana*, 14-6540) are collected and shipped from late April through early August. These eggs are slightly smaller than those of the northern frog, usually about 1.2 to 1.5 mm in diameter. Hatching time for the bullfrog is 134 hours at 18°C (64°F). The fully grown tadpole is 10–15 cm long. In nature, the bullfrog may take 2 or 3 years to develop from egg to froglet.

Other Frog and Toad Eggs

Eggs of spring peepers (*Hyla crucifer*) are supplied from late January through early March depending on the weather. Fowler's toad eggs (*Bufo woodhousei fowleri*) are usually available later in the spring. Peeper frog eggs range in size from 0.9–1.1 mm in diameter; Fowler's toad eggs are slightly larger.

We recommend peeper eggs and toad eggs for demonstrating the frog or toad life cycle, but not for egg development. The eggs develop rapidly and will be near the tail-bud stage when received. Development from egg to small frog or toad usually requires less than 2 months in the laboratory.

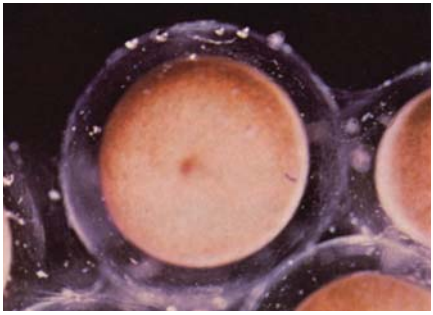
Xenopus Eggs

Xenopus eggs (14-6630) are excellent for embryological studies. Due to their rapid development, any *Xenopus* eggs that we ship will arrive in late stages of development. Generally, a large number of fertilized eggs results from one mating, and if properly cared for, the eggs can be studied from the one-cell stage through hatched tadpoles.

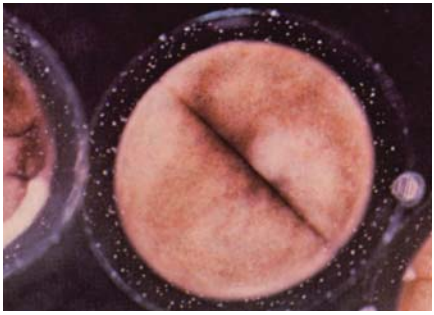
Xenopus eggs develop rapidly. If early developmental stages are needed for study, the chorionic gonadotropin injection schedule should be adjusted so the last injection is given late in the evening. Ten to 12 hours after the male and female are placed in the tank at 20°C (68°F), eggs from the one-cell stage to late gastrula can be found.

Change the water in the breeding/rearing tank immediately after the breeding adults are removed. Pour the water out slowly so the eggs, which are laid singly

Xenopus Life Cycle: One-Cell Stage–Yolk Plug

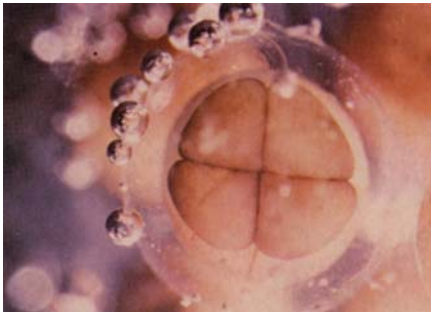


One-cell stage

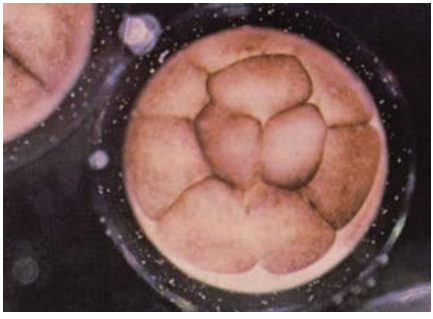


Two-cell stage

Four-cell stage



Eight-cell stage



Sixteen-cell stage

Blastula

Dorsal lip

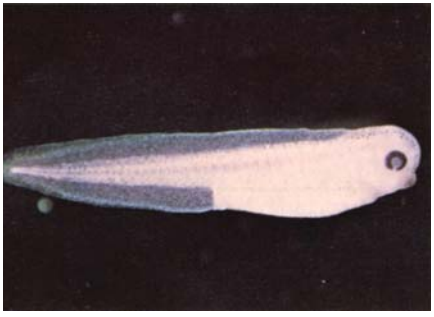
Yolk plug

Xenopus Life Cycle: Neural Plate–Young Frog

Neural plate

Neural groove

Neural tube



Hatchling

Tadpole hind-limb bud stage

Tadpole with hind legs

Tadpole with front and hind legs

Young frog

and are attached to the bottom of the tank, remain undisturbed. The replacement water must be at the same temperature as the water you remove since the eggs are particularly susceptible to temperature shock. Do not change the water again until the tadpoles begin swimming. Place an airstone in a corner of the tank 2–3 cm beneath the water's surface. This provides water circulation and aeration without disturbing egg development. After 5 days, remove the airstone and the false bottom.

When observing *Xenopus* eggs, be careful not to damage them. The tiny eggs, which are brown at the animal pole and yellowish-white at the vegetal pole, are 1 mm or less in diameter. They are also adhesive, so handling them requires care. To pick the eggs up, first scrape them loose with a flat-edged plastic instrument (a plastic, flexible ruler is ideal) and then pick them up with a wide-mouthed pipet. Chemicals, unclean utensils, rough handling, and excess heat from microscope lights can kill or damage the eggs.

Amphibian Larvae

Acclimating the Larvae

Twenty-four hours before expected delivery of amphibian larvae, prepare some culture water—either springwater or conditioned tap water. When the amphibian larvae first arrive, float the shipping bag in the culture water that you have prepared. This will allow the temperature of the two containers to equilibrate. Once you feel that the temperatures have equilibrated, remove and discard about one quarter of the water from the shipping container and replace it with an equal amount of prepared water from the holding container. Wait 15 minutes, and repeat this step. Do not allow any shipping water to enter your culture container. This gradual process acclimates the larvae to the temperature, pH, and specific gravity of their new environment. Culture dishes, shallow trays, and aquaria

Tadpole

make satisfactory containers for culturing amphibian larvae. Glass or plastic containers are good, but avoid copper. All containers must be biologically clean.

Maintain larvae in pond water, springwater, or conditioned tap water. Water from copper stills or water that has passed through copper pipes often contains enough copper ions to be lethal to the larvae. The depth of the water in the container should be 2–3 cm for frog tadpoles and salamander larvae, and 10–20 cm for *Xenopus* tadpoles.

If possible, rear amphibian larvae in water that is slowly flowing through the culture container. If this is not possible, change the water at least three times a week. Clean and rinse all culture containers and utensils once a week. Use a small aquarium net to transfer amphibian larvae. Never transfer larvae to water that differs in temperature by more than 3°C (5°F) from the water in which they have been held.

Amphibian larvae develop best at 18–20°C (64–68°F). Temperatures outside this range often damage the developing embryos. Salamander larvae can tolerate 48 to 60 hours of temperatures approaching 4°C (39°F), but this cold temperature is harmful to most tadpoles. Remove abnormal, diseased, or dead larvae from the cultures as soon as you notice them.

An important factor in rearing amphibian larvae is population density. For maximum growth, we recommend no more than 30 larvae for every 4 L of water. As the tadpoles mature, decrease the number of larvae per liter.

Begin feeding larvae as soon as they start actively swimming about. Most tadpoles are vegetarian and live on a variety of plant materials. Slightly boiled lettuce or spinach is a good food source, although tadpoles maintained exclusively on this diet may develop tumors. Carolina's Tadpole Food (14-6500), pelleted rabbit food, fish food, and algae supplemented with finely powdered beef liver or powdered egg yolk are recommended diets. *Xenopus* tadpoles are filter feeders and will eat Carolina's *Xenopus* tadpole food (14-6650), nettle powder, or pea soup.

Salamander larvae are carnivorous and must be fed living organisms. Aquatic larvae are given rich protozoan cultures, small mosquito larvae (14-4476), and young *Daphnia* (e.g., freshly hatched from culture kit 14-2300, since the hard shells of mature *Daphnia* can damage the larvae's stomach lining). Large aquatic forms are fed redworms (14-1650), *Tubifex*, whiteworms (enchytreids), and tadpoles.

Do not give amphibian larvae more food than they will eat in a few hours, and be sure to clean containers of uneaten food daily. If the cultures contain living aquatic plants for food, provide enough light for the plants to carry on photosynthesis, but avoid direct sunlight, which would damage the larvae.

Metamorphosis

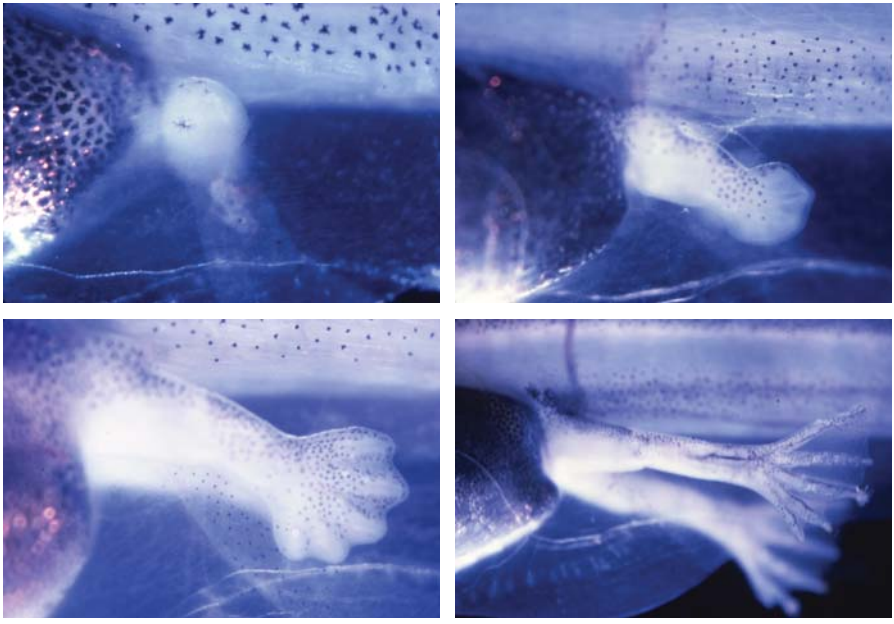
Amphibian larvae can be treated with a number of different compounds to hasten metamorphosis. Healthy larvae that are almost ready to undergo metamorphosis respond best to treatments.

To use thyroxine powder (14-6510), dissolve 10 mg of thyroxine in 5 mL of 1% NaOH and dilute to 1 L with glass-distilled water. This stock solution of a 1:100,000 concentration will keep indefinitely in the refrigerator. Treat tadpoles by placing them in thyroxine solutions of 1:1,000,000; 1:10,000,000; and 1:100,000,000 concentrations instead of normal culture water and compare the rate of metamorphosis at the different concentrations.

If you are using the Tadpole Metamorphosis Set (14-6475 or 14-6480), add 1 L of water to each of the 16 bowls. After acclimating the tadpoles as described above, divide the 32 hind-limb bud stage tadpoles equally among the bowls (2 to a bowl). Mark 8 bowls "Control" and 8 bowls "Thyroxine-Treated." Fill the dropping bottle with the stock solution and add 30 drops to each of the bowls marked "Thyroxine-Treated." Add three or four pellets of food to each bowl.

Every 2–4 days, change the water, replacing it with 1 L of clean culture water. Pour the water with the tadpoles slowly through the net. Do not interchange the tadpoles between the "Control" and "Thyroxine-Treated" bowls. Add 30 drops of thyroxine stock solution to the appropriate bowls and feed the tadpoles again.

After the tadpoles' first front limbs emerge, lean some stiff plastic netting in the bowl. The tadpoles can rest on the netting near the surface as their lungs develop



Hind limb development in *Xenopus*

and their gills become less efficient. *Xenopus* tadpoles do not need this netting because the young frogs are fully aquatic.

The experiment can be terminated at any time a difference becomes apparent in the rate of development. Most classes allow the tadpoles to develop into small frogs; however, as the treated tadpoles mature, the mortality rate increases because those tadpoles' physiological systems are stressed by their abnormally rapid growth.

To use thyroid tablets, grind 10 grains in 5 mL of glass-distilled water with a mortar and pestle. Add an equal weight of whole-wheat flour and grind again. Spread the mixture in a thin layer on a clean glass plate and allow it to dry. Chip the dried mixture from the plate and store it in the refrigerator in a tightly closed bottle. Treat the tadpoles by feeding approximately 50 mg of this mixture daily.

Fresh cow or rat thyroid tissue can be used by grinding it with a mortar and pestle and feeding the bits of tissue directly to the tadpoles. If frozen immediately after grinding, the tissue can be used over a period of several days.

Reptiles

Reptiles are covered with scales, and their toes (if present) have claws. Some scales are modified into plates and other types of shields. Usually the juvenile reptile looks much like the adult and does not go through a larval stage.

Turtles

Carolina supplies turtles (14-7050) of the genus *Chrysemys* (*Pseudemys*), which are known as cooters and sliders. Cooters are water-loving turtles often seen basking on logs, rocks, and the edges of lakes, ponds, and streams throughout the southeastern United States.

Turtles can be held temporarily in any inclined container with a tight but ventilated lid and a small amount of water in one end. Turtles can also be held in a semiaquatic aquarium if the water is well filtered. Turtles must be able to leave the water and dry completely. Keep them at a favorable temperature (24–30°C [75–86°F]) by using an incandescent hood or by placing a 40- or 60-W incandescent light near the aquarium. This will provide enough heat and light to keep the turtles healthy.

Cooters are mainly vegetarian and eat aquatic plants such as duckweed (16-1820), *Elodea* (16-2101), and *Cabomba* (16-2021). They also eat lettuce and other green leafy vegetables. Occasionally, supplement their diets with raw meat, fish, shellfish, insects, or all-meat dog food.

Keep the tank, and especially the water, clean at all times to prevent the buildup of wastes and spread of disease. Handle young turtles with care, and always wash your hands after handling them to avoid contracting salmonellosis.

Note: The US Postal Service cannot ship turtles. Carolina ships turtles only on Tuesdays via air freight, and a minimum shipping charge applies. Residents of Canada must apply for a Canadian Department of Agriculture permit to receive living turtles.

Snakes

Carolina Biological does not supply live snakes; however, since they are popular reptilian pets, we have supplied a brief overview of snake care below.

Garter snakes, water snakes, king snakes, and green snakes all make good vivarium specimens. Handle all snakes with care; a snake hook (65-1725) is recommended, particularly if you are handling snakes in the wild.

The best cage for holding snakes is a glass aquarium; however, a cage can be made of wood with a glass front. Good ventilation is a must. Cover the bottom of the cage with a layer of sand or newspaper. A hiding place such as a shoebox, a small wooden box, or other cavelike structure will give the snake a sense of security. Provide water in a small bowl; snakes drink by sucking the water between their lips.

Snakes molt several times a year. A few days before molting, the eyes of the snake turn a milky color because of changes in the eyeshields, which are discarded along with the skin. After two or three days, the eyes clear again. A branch, rock, or other rough edge must be provided so the snake can rub against it and begin the molting process. It is best to leave snakes undisturbed as much as possible during the molting period.

Keep snakes at temperatures of 24–30°C (75–86°F). Temperatures can be controlled by placing a lamp near the cage or by using an incandescent hood. Snakes with a balanced diet do not need sunshine. Overexposure to the sun can kill a snake by raising its body temperature too high.

Lizards

Carolina Biological supplies small anoles and a variety of larger lizards. These lizards are easy to care for and make interesting subjects for classroom discussion and study. Keep lizards in containers with or without plants. A base of sand or of paper towels is good for larger lizards. Both anoles and the large lizards need tree branches and stones for climbing and resting.

All of the lizards described below will feed on insects such as crickets, mealworms, flies, and lepidopteran larvae (e.g., tobacco hornworms). Feeding the lizards a variety of food helps keep them healthy. Dusting the insects with a commercial pet vitamin provides the lizards additional nutrition. Feed at least three times a week.

Provide water in a small bowl for the larger lizards, and add fresh water as needed. Anoles will lap water from plants and from the sides of the habitat if you provide it by occasionally sprinkling or spraying some drops inside.

Anolis carolinensis (14-7236) is a small lizard which can change from green to brown and vice versa.

Brown Chameleons, American Chameleons, and Mediterranean Geckos

The brown chameleon (14-7260, *A. sagrei*), the American chameleon (14-7236, *A. carolinensis*), and the Mediterranean Gecko (14-7620, *Hemidactylus turcicus*) all share the same basic habitat requirements. Control temperature by using an incandescent hood if the lizards are housed in a glass aquarium tank. If some other cage is used, a light bulb placed near the cage provides needed heat. Temperatures of 24–30°C (75–86°F) are sufficient. These lizards need a small amount of sunlight or its equivalent every day; however, do not allow the cage to get too hot if it is located in sunlight. The lizards should be able to choose sun or shade.

Mediterranean gecko

Leopard Geckos and Skinks

Leopard geckos (14-7625) and skinks (14-7500) make excellent choices for a classroom habitat. They are hardy, attractive, and easy to care for. The leopard gecko is distinguished from tree-dwelling geckos in that it has fully functional eyelids but lacks expanded toe tips. When young, the leopard gecko has a striking brown- and yellow-banded pattern, which becomes more spotted as the animal ages.

The leopard gecko lacks expanded toe pads.

Leopard geckos and skinks should be housed in a plastic or glass aquarium with 1–2 inches of small round pebbles or sand as a substrate (note: sand sometimes causes a problem for young geckos and skinks if ingested). Hiding places, such as a small section of PVC pipe or an inverted container with an entry way cut out, are a necessity. Provide a water dish, but place some pebbles in the bottom to help prevent live food from drowning. Supply a branch for climbing. Daytime

temperatures of 26–29°C (79–84°F) and nighttime temperatures of 21–24°C (70–75°F°) are sufficient. Position a light over one end of the habitat, near the climbing branch, so that the animal can bask as close to the heat as it likes.

Leopard geckos need a humid spot for shedding; a hiding container with some vermiculite or sphagnum moss works well. Limit handling of the geckos to avoid stressing them. If broken, the gecko's tail will regenerate, but it will be shaped differently from the original.

Preparing a Vivarium

A vivarium is a place for keeping and raising small terrestrial animals. It is most often made from a glass or plastic container that protects the animals and keeps them from escaping. The most commonly used container is a glass aquarium tank, but smaller, plastic habitats also work.

To prepare a vivarium, put 2–5 cm of moistened potting soil in the bottom of the tank. For animals from arid regions, make a desert vivarium by mixing a large portion of sand with the potting soil. Waterworn pebbles, quartz chips, bark chips, and sand placed on top of the soil layer hold the soil in place and decorate the vivarium. Place small branches and stones in the vivarium to provide hiding places and climbing surfaces. Bury a shallow bowl in the soil so the lip is just above the surface of the soil. The bowl holds water for drinking and swimming; its size should be proportional to the size of the animals and the vivarium. Small animals might need a bridge of stones, sand, or wood to get out of the water if the bowl's sides are too high.

Place plants in the vivarium according to the size and type of animals kept. Large animals need very sturdy plants. Plants for small animals include ferns and succulents. Desert vivariums should be planted with various species of cacti. Plants can be placed directly into the soil or left in pots placed on the soil layer.

See our current catalog or visit www.carolina.com for a complete listing of animals, habitats, and accessories.

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