

# Grass Frogs

## Tadpoles and Frogs

**Species:** *pipiens*

**Genus:** *Rana*

**Family:** Ranidae

**Order:** Anura

**Class:** Sarcopterygii

**Phylum:** Chordata

**Kingdom:** Animalia



### **Conditions for Customer Ownership (per USDA permits)**

We hold permits allowing us to transport these organisms. To access permit conditions, [click here](#).

**Never purchase living specimens without having a disposition strategy in place.**

A USDA permit is required to ship grass frogs and all other vertebrates to Ohio. This organism must be housed in an escape-proof habitat. In order to protect our environment, never release a live laboratory organism into the wild.

### **Primary Hazard Considerations**

- Always wash your hands thoroughly before and after handling frogs, for your protection and theirs. Chemicals and toxins can be absorbed through their skin and can poison your frog.

### **Availability**

- Adult grass frogs are collected from the wild and seasonal. They are available from September through April. Grass frog tadpoles are seasonal and only available in late April and early May.

### **How Will Animals Arrive and Immediate Requirements**

- Grass frogs will arrive in a waxed cardboard box with moist styrofoam packing. Open the container when it is received and place the frogs into a habitat. If you don't have a habitat set up yet, you can store the frogs in the shipping container for 2-3 days as long as you mist them daily with de-chlorinated water.
- Tadpoles will arrive in a plastic bag with water. They can live in their shipping container no longer than one day due to build-up of wastes and depletion of oxygen. When you receive your tadpoles, take the bag and float it for 15-30 minutes in the aquarium in which the tadpoles are to be housed, to allow them to acclimate to the temperature of your aquarium water. Then open the bag and add an equal part of your water to the water in the bag; continue to let the bag float on the surface for another 10-15 minutes. After the time has elapsed, you may release the tadpoles into their new habitat. If your tadpoles are cold when you receive them, their movement will be very slow or nonexistent. This does not indicate poor health; they will behave normally once they adjust to a higher temperature. Once the tadpoles are acclimated, carefully cut open the bag and release them into the aquarium.
- Frogs are green or greenish-brown dorsally, with round, brown spots arranged on their back, sides, and legs. A white line runs on either side of the mouth, from the nose to the shoulder. The underside is white or greenish white. Tadpoles are greenish or brown, with yellow or black speckles frequently visible. The belly is white and somewhat transparent, with the intestinal coils visible through the skin.

### **Captive Care**

#### **Habitat:**

- The most convenient method of "holding" adult frogs (*Rana pipiens*) until needed is to render them inactive by reducing their metabolic rate. This method makes feeding unnecessary, reduces waste build-up, and reduces the occurrence of various frog diseases. In addition, this method will maintain the frog in a specific physiological state (e.g., maturation, pre- or post-ovulation, etc.). The metabolic rate is lowered by refrigerating the frogs. If the frogs are to be held for less than a week, refrigerate them at 2° - 7°C (35° - 45°F) in a shallow pan of water. Long term storage should be in an aquarium/terrarium (at least 2 gallons of space per frog) or a large plastic bin. Be sure to have a cover on the top of the container with holes or screening for air exchange. You can have a half land/half water setup or the habitat can be entirely aquatic. To set up a land mass, use topsoil or moss. Change the water twice a week with de-chlorinated tap water or pond water.

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- **Housing Tadpoles:** Tadpoles can be kept in an aquarium that has an air pump and stone for aeration. The bottom of the tank should be covered in coarse gravel. Live or plastic plants are beneficial for tadpoles to rest on when they are not active.
- As metamorphosis proceeds, a provision must be made so that the young frogs (now air-breathing) may climb from the water. This occurs very shortly after the front legs erupt from under skin flaps that have been concealing them during their development.

#### Care:

- At metamorphosis, *R. pipiens* shift from an omnivorous to a carnivorous diet comprised of food that must be moving. They differ from *R. catesbeiana* and *R. clamitans* in that they do not or cannot take food while submerged. If animals are not held in hypothermic conditions, they should be fed 2-4 full-grown live crickets, 2 – 3 times per week. Other food that is recommended includes sow bugs, beetles, moths, and earthworms. Fly maggots are not recommended, since such ingested maggots may destroy the frog.
- **Feeding Tadpoles:** Since tadpoles are omnivorous, many types of food have proved suitable. Prepared fish food works well as does ground dry dog food, mashed hardboiled egg, chopped liver (raw or cooked), and boiled lettuce leaves. The diet should be varied from time to time. Only those foods which the tadpoles readily consume should be given. As with any aquarium set up, overfeeding must always be avoided. If the normally clear water starts to turn cloudy the tadpoles are either being overfed or have not consumed the particular type of food they are being fed. This is a dangerous condition. If this happens, do a partial water change and add dechlorinated water. Be sure that the food given is being eaten and use only that amount of food which can be consumed in a short time.
- After the tadpoles metamorphose into air-breathing young frogs, they will be strictly carnivorous, eating small crickets and bugs.

### Information

- Method of reproduction: Sexual. A male will hold a female in “amplexus,” using his specialized thumbs, and fertilize her eggs as they leave her body.
- Determining sex: Males are smaller than females. Males have thickened thumb pads and paired vocal sacs that inflate over their shoulders as they call.

### Life Cycle

- Mating occurs from March to June, but peaks in April. Females lay from 300 to 6,500 eggs in globular clusters in breeding ponds. After metamorphosis, sexual maturity is reached in 1 to 3 years, depending on conditions.
  - **Egg:** When laid, 1.7 mm; in water, 5 mm.
  - **Tadpole:** Hatches after 1-3 weeks.
  - **Froglet:** Metamorphosis occurs after 70-110 days as a tadpole.
- Grass frogs can live up to 9 years in the wild, but most do not survive this long.

### Wild Habitat

- Grass frogs are found in marshlands, brush lands, and forests. They are well-adapted to cold and can be found at elevations up to 3,350 meters. Grass frogs are found throughout much of North America.
- Predators of grass frogs include fish, herons, other frogs, snakes, raccoons, mink, and otters. Grass frog eggs are preyed on by leeches, newts, and turtles. Tadpoles are preyed on by insects and many of the same animals that predate upon adult frogs.

### Special Notes

- Grass frogs are eaten by humans (frog legs), and are used as test subjects in many biomedical research projects, both as adults and as tadpoles.
- Grass frogs are also known as leopard frogs.

### Disposition

- We do not recommend releasing any laboratory animal into the wild. As a laboratory animal, it has not encountered or learned wild survival skills and is therefore likely to come to an inhumane end.
- Adoption is the preferred disposition for a vertebrate.



- If the animal cannot be adopted by a capable owner, it may be surrendered to your local humane society.
- If the animal must be euthanized, we recommend consulting the AVMA guidelines on euthanasia ([American Veterinary Medical Association, http://www.avma.org/issues/animal\\_welfare/euthanasia.pdf](http://www.avma.org/issues/animal_welfare/euthanasia.pdf)). According to these guidelines, acceptable methods of euthanasia for an amphibian include exposure to CO<sub>2</sub> at >60% or treatment with tricaine methane sulfonate (also known as TMS, MS-222 and Biocalm 947-2100). TMS is an anesthetizing agent that will cause fish and amphibian death due to central nervous system depression and hypoxia with overexposure. Wear personal protective equipment (gloves, safety glasses, lab coat) when handling this substance. The fish or amphibian is placed in a solution of 5 g per 5 gallons of water for 30 minutes or until all motion has ceased. To make sure the animal is dead, check for reflexive movement when the eye is touched. If movement occurs, replace the animal in the TMS solution for another 30 minutes.
- A deceased specimen should be disposed of as soon as possible. Consult your school's recommended procedures for disposal. In general, a dead vertebrate should be handled with gloves, and wrapped in an absorbent material (e.g., newspaper), wrapped again in an opaque plastic bag, then placed inside a opaque plastic bag that is sealed (tied tightly) before being placed in a general garbage container away from students.

## Additional Advanced Investigations

### Induced Ovulation

- Pituitary Extract contains the lyophilized pituitary glands of seven female Rana frogs, in addition to 6 mg progesterone. Only gravid northern *Rana pipiens* should be used, and not the "southern" sympatric species which is not physiologically suited for induced ovulation protocols. The addition of the hormone progesterone has been found to be more effective in producing consistent ovulation success.
- Since seasonal changes in pituitary potency and female sensitivity occur, the following table will serve as a general guide on the use of either collected pituitaries or pituitary extract.

Month	Vial- Pituitary Extract	Collected Pituitaries*
Sept.-Oct.	1 vial per frog	10-12
Nov.-Dec.	1 vial per frog	6-8
Jan.-Feb.	1 vial per frog	4-5
Mar.-Apr.	1 vial per frog	1

\* two male pituitaries are equivalent to one female pituitary

- Please consult the separate product literature sheet accompanying the Pituitary Extract for re-hydration instructions. Please note that you will need a syringe with a #20 gauge needle to inject the female. Syringes and needles can be obtained at a local pharmacy with a prescription.
- Injection procedure for inducing ovulation is as follows: Carefully, so as not to injure the ventral blood vessel or internal organs, insert the needle just through the skin and underlying muscle wall of the lower abdomen and inject the pituitary-progesterone suspension. Be certain to choose a gravid female to inject. Females have reduced nuptial pads on the "thumbs" of the forelegs. Gravid females will be plumper than their non-gravid counterparts because the abdomen will be full of eggs. Place injected females in dechlorinated water approximately 1-1/2" to 2" deep and keep animals at a temperature between 18 - 21°C (64 - 70°F). Injection should be done 48 - 62 hours before scheduled artificial insemination. After 48 hours, check the enclosure for evidence of egg release. Eggs may require longer periods to "ripen" and observation of released eggs is the best indication that ovulation is proceeding. You may also "test" for ovulation by gently squeezing the abdomen towards the cloaca. To ensure a longer yield of mature eggs, do not extract them until 24 hours after the start of ovulation. Mature *R. pipiens* should yield between 1,000 and 2,000 eggs for fertilization.

### Artificial Insemination

- To obtain active sperm, either pith a male frog or overdose the animal using [Biocalm](#), an amphibian anesthetizing/euthanizing agent. Remove both testes, dissect away adhering tissues, and wash testes free of blood. Macerate in 10 - 20 mL of either pond water, dechlorinated tap water, or 10% Holtfreter's Solution\*. A convenient way to do this is to force the testes through a syringe with a #18 gauge needle. Large clumps may be broken by aspiration. Wait 15 - 25 minutes for full sperm activity, which can be determined by examination under a compound microscope. The sperm suspension may be diluted to a total volume of 100 mL, if necessary, for very large egg masses. However, better results (e.g., more consistent rates of fertilization) are obtained by using the sperm suspension as prepared, without further dilution. Note that polyspermy may occur in concentrated sperm solutions with October-caught or long-held animals.



- Following confirmation of sperm activity, eggs are stripped from the female by holding her and applying pressure to the abdomen with the force directed towards the cloaca, thus squeezing the eggs from the ovisacs into a dry Petri dish. This is accomplished by bringing the legs of the female forward parallel to the abdomen. This assures that pressure is not dissipated laterally. The third and fourth fingers are applied firmly over the throat and thoracic region to avoid dissipating pressure anteriorly. Remaining fingers are used to “milk” the abdomen toward the cloaca. Initially, rather sharp pressure may be needed to open the cloaca sphincter muscle; subsequently, gentle pressure will suffice to aid egg release.
- Eggs are expressed in a circular pattern on the dry Petri dish. Due care must be exercised so as not to cause rectal prolapsed by trying to obtain every “last” egg.
- Expressed eggs are inseminated by pipetting the sperm suspension over them. Make sure that each egg comes in contact with the sperm suspension, although moistening is all that is required. Application of sperm suspension must be done prior to egg jelly swelling. After 10 –15 minutes, flood the eggs with medium. Use of a dry dish is recommended because eggs stick to its surface. This facilitates changing the medium. The spiral or circular pattern prevents eggs from clumping. After an additional 15 minutes, pour off the medium plus sperm and replace with fresh medium. Set the eggs aside for 30 –50 minutes. They then may be scraped free from the Petri dish and transferred to culture bowls. A single-edged razor blade, tissue section lifter, or scalpel should be used. Do not attempt to pull eggs free. About one hour after fertilization, those eggs successfully inseminated will rotate so that the black animal hemisphere is uppermost. After several hours, remove the unfertilized eggs to prevent decay. At optimum development temperature (18 – 25°C, 64 – 77°F) cleavage will begin about 2-1/2 hours after fertilization, muscular response will be observed in about 96 hours, and hatching will occur in 5 – 7 days.
- \*Holtfreter’s Solution: Prepare a stock solution by adding 3.5 g NaCl, 0.2 g NaHCO<sub>3</sub>, 0.1 g CaCl<sub>2</sub>, and 0.05 g KLC to 1 L of glass-distilled water. Dilute this stock solution to 10% with distilled water before use.