

# ***Manual for Rearing Gray Tree Frogs***



***Michael J. Smith and Allison M. Welch  
University of Missouri – Columbia***

## 1.1. Tadpoles

Tadpoles are raised in 1.5 liter rectangular plastic containers (Figure 1) with 1 liter of carbon-filtered tap water. Tadpoles are fed finely ground fish food in individually measured rations.

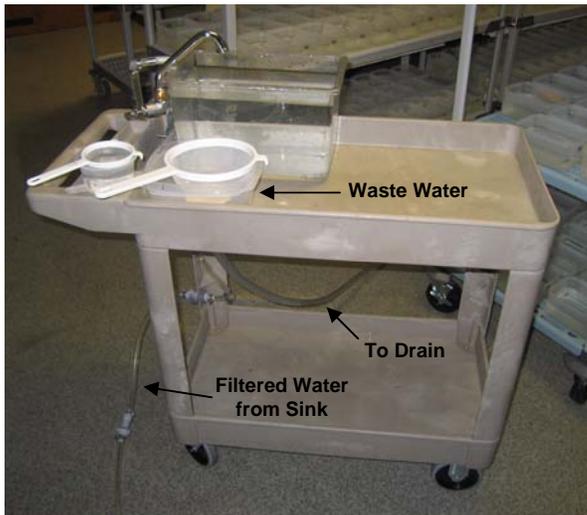
*Step 1:* Check all tadpoles daily. Record any deaths and any that have metamorphosed. Remember to initial the animal care log daily. Containers from dead tadpoles should be scrubbed in hot water using a bath pouf or scrub brush to remove buildup, then rinsed in filtered water and allowed to dry *thoroughly* before being stacked. Containers should be scrubbed each day, both to keep dishes from accumulating in the sink and because it's easier to remove buildup if it's not allowed to dry. Never use detergents to clean frog containers.



**Figure 1: Tadpole container**

*Step 2:* Tadpoles' water is changed every three to six days. Tadpoles cannot tolerate chlorine, so water must be carbon-filtered to remove chlorine. Tadpoles may also be affected by heavy metals; to avoid heavy metals that may leach into the water from pipes, let the water run for at least 10 minutes before using it for animals. (During this time water can be used for dishwashing, etc). Once a week, test filtered water for presence of chlorine; if any chlorine is present, request a service call from Culligan and use an alternate source of chlorine-free water in the meantime.

Water is changed by pouring out old water through a plastic sieve (to catch the tadpole) and refilling the container with carbon-filtered water to the fill line. The



**Figure 2: Water change cart**

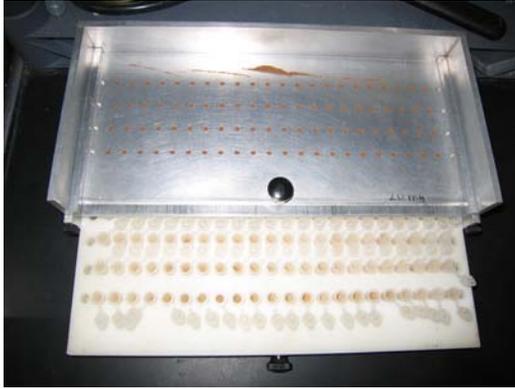
container should be rinsed with fresh water to remove buildup. Periodically, buildup can be removed physically with a scrub brush or bath pouf. A cart set up with a drainage system (plastic container plumbed with tube that can be directed to the floor drain) and faucet facilitates water changing at the tadpole racks (Figure 2). Connect the hose from the filtered-water tap at the sink to the connector (which prevents water spillage) and then to the cart.

After a water change, double check to make sure that all containers have been changed.

Rinse all equipment (sieves, larger plastic tubs, etc.) with filtered water, pour excess water off the cart near the floor drain, and squeegee the floor. Periodically rinse the drainage system with regular (chlorinated) tap-water to inhibit mold. Record water change on animal care log and initial.

*Step 3:* Tadpoles are fed every three days, including after each water change. Tadpole food is made by finely grinding fish flakes (Tetra tropical) in a blender. It takes several minutes of intermittent grinding to achieve a fine powder. Sift the ground food through a small wire mesh sieve to remove larger pieces. Ground food can be stored in a plastic container or baggie in the freezer.

Food is measured out using the food-measuring device (Figure 3; design courtesy of Ray Semlitsch, University of Missouri): choose the appropriate measuring plate,



**Figure 3: Food machine**

place small microcentrifuge tubes into the holes in the plastic tray, slide the plastic tray under the metal platform, then spread food into all the holes in the plate (except the outer four on each side). Using a smooth plastic edge, like a credit card, smooth over the food several times to be sure that all holes are completely filled. Then pull the plate forward to the stops; if necessary, knock the food out of the holes into the tubes below using something non-marring (like a credit card or roll of tape). If the ration is larger than can be delivered with a single

dose, continue measuring food using the appropriate combination of plates to achieve the desired ration. Then remove the plastic tray and close the tubes. It's a good idea to measure out food ahead of time, particularly when the feeding will follow a large water change.

Deliver food to each tadpole by opening a tube with the appropriate ration and dumping it into the tadpole container. The food should float on the surface. Larger rations may need to be loosened by tapping or inverting the tube before opening to prevent the food from falling to the bottom of the container. Double check to be sure all tadpoles have received food. Record amount of feeding on animal care log and initial. Tadpoles initially receive a ration of 10 mg, which is increased in steps (10, 15, 20, 30, 40, 60, 80, 90 mg) when at least some individuals within the experiment can eat the entire ration before the next feeding.

*Step 4:* For individuals that have metamorphosed (defined as emergence of at least one forelimb; Figure 4) on any given day, dump out water as for a water change, rinse and scrub buildup from container, then add a small quantity of filtered water (~¼ cup) to the container. Tightly seal a lid with holes onto the container, label the container with the date of metamorphosis, and prop up one side of the container so that the metamorph has easy access to both water and dry surface. Record the date of metamorphosis in notebook and spreadsheet. Metamorphs are easier to keep track of if they're kept together on a shelf, grouped by date of metamorphosis.



**Figure 4: New metamorph**

*Step 5:* At the end of the experiment, tadpole containers need to be acid washed to remove hard water deposits. Acid wash using 10% hydrochloric acid (HCl) from a

squirt bottle. The acid is corrosive, so you absolutely must wear tall dishwashing gloves (not just regular lab gloves). Be careful to avoid breathing fumes. If acid contacts skin, rinse affected area with lots of water. After the acid has contacted all interior surfaces of the container and removed buildup, rinse thoroughly at least three times and allow to dry completely. Be sure to dispose of HCl properly.

## 1.2. Metamorphs

*Step 1:* Three days after forelimb emergence, tails should be resorbed (Figure 5). At



**Figure 5: Metamorph with tail resorbed**

this point, metamorphs are weighed on an analytical balance. To standardize hydration level, push the metamorph into the water to get it wet, then gently blot on a paper towel to remove excess moisture before weighing. Record the mass in the notebook and spreadsheet. It's best to have all weighing done by a single researcher to avoid error that could be introduced by differences in drying by different individuals. If this is not possible, the two researchers who will be doing weighings should standardize their techniques by practicing on a common set of individuals.

*Step 2:* After weighing, metamorphs are transferred to metamorph containers consisting of two plastic deli cups, moss, and cotton fabric (Figure 6). A 1-inch layer of long-fiber sphagnum moss is placed in the bottom of a deli cup (with or without holes). If the moss has not already been wetted, add filtered water. Wear gloves when handling wet or dry moss, and wear a dust mask when handling dry moss. Place a 6" square of cotton fabric over the deli cup and then insert another deli cup (without holes), from which the bottom has been removed. This will secure the fabric in place, resulting in a barrier between the moss and the frogs which will prevent insects from hiding in the moss, allowing the frog access to controlled amount of food. Pour off excess water. Label the container with the tadpole's identification number and date of metamorphosis.



**Figure 6: Metamorph container (without lid)**

The lid of the metamorph container is a deli cup lid with a 2.5" hole removed and replaced with a 3" square of cotton fabric. An additional 1/4" hole provides a port for adding food and water, and should be securely taped over with lab tape except when adding food and water. A strip of fiberglass screen is closed into the container to provide a perch for the frog. Metamorphs are very mobile and can easily get from the bottom of a container to the top just in time to get smashed in a closing lid. Be *very* careful that frogs are deep in the container before closing the lid.

*Step 3:* The moss in the metamorph containers should not be kept too damp, but will need to be moistened once or twice per week. Water is added via plastic transfer

pipet through the port in the container lid – one pipet’s worth is enough. It is better to add water on days when frogs are fed flies, as flies can climb up the container walls and not get stuck in excess water, while crickets cannot. The goal is to keep the moss and fabric damp, but not wet or sopping. Too much moisture will promote mold growth. If the room becomes less humid, water may have to be added more often.

*Step 4:* Metamorphs are voracious and should be fed daily. Food is administered by counting the appropriate number of insects into a plastic dixie cup, then dumping the



**Figure 7: Feeding metamorphs**

contents of the dixie cup into a small funnel fitted to a section of a plastic pipet inserted into the port in the lid (Figure 7). If the hole in the lid is of the right size and shape, the funnel will stand up on its own. The funnel and dixie cups should be kept dry to keep insects from sticking. ReptoCal calcium/vitamin powder should be added to food before feeding. It’s easiest to get a bunch of insects in a cup or jar, add a bit of powder, swirl them around to coat the insects with the powder, and then count out insects into individual rations.

For the first 3 to 4 days after weighing, metamorphs should be given wingless fruit flies (15 per day), which are small enough to be eaten easily. Once the metamorph can eat all of its daily ration of wingless flies (it will take 3 or 4 days), it can be put on the standard diet of 20 small crickets (about 1 ½ weeks old) every other day, alternating with 25 flies. The larger, winged but flightless flies should be used as much as possible at this stage, but the smaller wingless flies can be used if necessary.

Flies are easier to handle if they are chilled first. Chill a smaller jar in the freezer for about 5 minutes, and then knock a bunch of flies into the jar, using the jar lid fitted with a funnel and plastic pipet; the flies will slow down quickly when they hit the cold jar. If they’re still crawling up the jar, chill them for an additional 1-2 minutes in the freezer. After they’re chilled enough, add the ReptoCal and distribute them to individual cups.

It will be necessary to increase the size of crickets and the number of flies as the frogs grow. When many individuals of a particular age are eating all of the administered insects before the next feeding, increase the size or number of prey. When increasing prey size, you’ll need to carefully monitor whether the larger prey are getting eaten – give only 10 larger crickets so that you can determine whether some are being eaten. If less than ½ to 2/3 of the prey are eaten, they may be too large for the frogs to handle. It will be helpful to organize metamorphs by date of metamorphosis to facilitate feeding older metamorphs larger or more food. Record feed amount on animal care log and initial.

*Step 5:* Metamorph containers should be checked and, if necessary, cleaned after about two weeks and every week thereafter. Containers should be cleaned if there is any mold growing on the fabric (a little mold on the feces is okay). Most cleanings will involve replacing the inner deli cup and cotton fabric, while leaving the outer deli cup and moss. Once a month, the container should be entirely replaced, including the outer cup, moss and screen (the lid can remain). (Be sure to transfer the label!) Used

containers are scrubbed in hot water and rinsed in filtered water. Fabric squares are scrubbed in hot water, then autoclaved. Used moss is discarded. For moss changes, it will be easier to soak a lot of moss and add wet, squeezed-out moss to the containers. Any wetted but unused moss can be wrung out and either left out to dry or refrigerated for use within a couple of days. Remember to wear gloves when handling wet moss and gloves and dust mask when handling dry moss.

*Step 6:* Metamorphs should have some exposure to UVB light. ReptiSun 2.0 or VitaLites can be used (the low levels of UVA and UVB are not enough to be of concern for human health). Lights should be dated with permanent marker when installed, and replaced at 6-month intervals, as UV production diminishes over time even though visible light is still produced. Because these bulbs are expensive, we're only using one per fixture; the other light in the fixture is an older bulb and thus produces little or no UVB. UV levels are negligible more than a few inches from the bulb, so it's important that frogs be kept close to the bulb. The tops of the containers should be within 3-6" of the bulb. Lights will need to be lowered to an appropriate height for metamorph containers. Keep frogs as close to directly under the lights as possible (e.g., not out to the edges of the shelves), and rotate frogs at least once a week so that those further from the light are closer to the light, and vice versa.

### 1.3. Juveniles, subadults, and adults

*Step 1:* When metamorphs have about quadrupled in size, they should be ready to transfer to larger containers. We have not yet worked out the precise size or age at which this transfer should take place, but the frogs will have to be large enough to forage for larger insects and avoid desiccation in a much larger container. They will likely take several months to reach this stage.

*Step 2:* Containers are large hard plastic "kriiter keeper" containers with colorful plastic mesh lids (Figure 8). The containers are lined with about an inch of fine sand (marketed as "play sand"), which is sprinkled with water to saturate. Holes drilled in one end of the container allow excess water to drain and thereby regulate the moistness of the sand. Water should be added three times per week (or more often, if necessary), using a watering can and filtered water. Try to leave enough time between watering and feeding so that prey won't drown in excess water. A



**Figure 8: Container for juveniles, subadults, and adults**

guttering system will be set up, which will drain the water dripping out of the containers. A long rectangle of fiberglass screen is closed into the container lid to serve as a frog perch. Container lids must fit securely, and should prevent frogs and their prey from escaping. The hatch on the lid must always be closed securely, and it may be necessary to close up any holes in the lid which could allow a frog to escape.

*Step 3:* Juveniles, subadults and adults will be fed crickets, the size and number of which will be determined by experience. Prey are added through the hatch in the lid. Be sure to close the hatch securely after feeding; both sides should pop into place. Prey should be dusted with vitamin powder and calcium:phosphorus powder before feeding.

## 1.4. Appendix: Sources for supplies

Organism	Item	Procedure
<b>General use</b>	Latex gloves	<a href="http://www.mugeneralstores.com">http://www.mugeneralstores.com</a> or scientific supplier
	Paper towels	<a href="http://www.mugeneralstores.com">http://www.mugeneralstores.com</a> or scientific supplier
<b>Tadpoles</b>	Fish food	Wal-Mart (Tetra tropical flake)
	Microcentrifuge tubes	<a href="https://www1.fishersci.com">https://www1.fishersci.com</a> ; Corning Costar 0.65ml fit in food machine (item 07-200-185)
	Chlorine test kit	<a href="http://www.hach.com">http://www.hach.com</a> ; product # 1454200 for entire kit, 1407099 for free Cl- refills, 1406499 for total Cl- refills, 2440400 for refills of both
	Water filter	Culligan
	Plastic containers	Lawnware (for large orders): 800-622-3400, item 43211LT – must set up an account in advance US Plastics (for small orders),: <a href="http://www.usplastic.com">http://www.usplastic.com</a> , item 85117
<b>Frogs</b>	Cotton fabric	Wal Mart – thin, white, and 100% cotton (e.g., muslin)
	Sphagnum	<a href="http://www.topiaryartworks.com/spmo10kgba.html">http://www.topiaryartworks.com/spmo10kgba.html</a> (10kg bale)
	ReptoCal	local pet store; use for metamorphs and small juveniles
	Calcium:phosphorus powder	<a href="http://www.flukerfarms.com">http://www.flukerfarms.com</a> ; product 73009; use for adults and large juveniles
	Repti Vitamin powder	<a href="http://www.flukerfarms.com">http://www.flukerfarms.com</a> ; product 73003; use for adults and large juveniles
	Fiberglass screen	Lowes or Home Depot
	Play sand	Lowes or Home Depot.
	Wide-spectrum lamps - must be replaced every 6 months	Duro VitaLites <a href="http://www.budgetlighting.com">http://www.budgetlighting.com</a> ; F40T12 Vita-Lite; product 06430
	Solo 16 oz tubs (without holes)	Industrial Soap Company: 314-533-5556, items 702606 (lids) and 702561 (16 oz cups)
	“Kritter keeper” hard plastic containers	I found the best price at All Star Pet Supply (913-764-4232); also try Pet City (573-334-6488) and General Pet (800-747-3826); item 20025, large rectangle, made by Lee’s Aquarium and Pet