

## Vivarium Biosecurity

Written by Chase Jennings

Biosecurity is something typically thought of only in regard to laboratories, but biosecurity actually plays one of the most critical roles in the success or failure of amphibian keepers, both new and experienced. The criteria for vivarium biosecurity may be a bit less stringent than that practiced in our laboratory at Houston Frogs, but the concepts remain the same. You want certain desired organisms to live and thrive, while eliminating or preventing the establishment of others that may cause harm to those organisms you desire.

By biosecurity, we are specifically focusing on methodology with which to protect our animals, plants, and ourselves from potentially harmful viruses, bacteria, fungi, and other organisms. From a laboratory approach, this is typically implemented via sterile technique and the use of ultraviolet radiation, ozone gas, extreme heat/pressure (autoclaving), and various chemicals, but this doesn't quite work for vivariums or for home hobbyists. For vivariums, we must take a more naturalistic approach in many aspects, sometimes even using some beneficial organisms to combat those which may be harmful.

Biosecurity is extremely important as a single harmful microorganism can cause the death of your animals and can necessitate the disposal of all the materials in the vivarium in addition to all of the hard work and frustration dealing with it.

Basic vivarium biosecurity can be broken down by the various critical vivarium components below:

**Animals:** The pets you buy are the most critical component of your vivarium and, even if your vivarium is seemingly pristine, putting an animal in your vivarium containing parasites or fungal/bacterial/viral infections will lead to contamination of the vivarium and the potential death of any other inhabitants, the disposal of all affected materials, and potentially the destruction of your entire collection. It is critical to get your animals from a reputable amphibian business (such as the dart frog businesses listed on [www.dartfrogbusinesses.com](http://www.dartfrogbusinesses.com)) who practices proper husbandry and who cares about providing you with healthy animals. If you buy from a disreputable business, or one which does not practice proper biosecurity, you may be introducing deadly pathogens to your collection such as *Batrachochytrium dendrobatidis* (BD or Chytrid for short) and Ranavirus (a group of viruses from the Iridoviridae family). If you do buy an animal from a business other than those known to be reputable, it's important to both quarantine (for 3-6 months) and test your amphibian (free emergency kits are available from Houstonfrogs.com for this purpose) before moving them into the vivarium via taking skin swabs and fecal samples and sending them to a qualified laboratory for testing to ensure your animal(s) is/are free of harmful pathogens. Depending on the test results, the animals may then require treatment; although, Ranavirus is a death sentence and no treatment is currently available to cure your amphibian(s) of this virus.

**Tank:** If you get a new tank, you have little to worry about. If buying a used tank, you will need to completely throw away any contents, clean it as thoroughly as possible until visually clean, then decontaminate the tank using F10SC (Veterinary disinfectant) or via using a multi-step process with various household chemicals such as vinegar (1<sup>st</sup> step, also removes calcium deposits), H<sub>2</sub>O<sub>2</sub> (3% Hydrogen Peroxide)(2<sup>nd</sup> step—will dislodge remaining particulates and will cause oxidative stress on even fungal spores and bacterial cysts, “softening them up” to be fully destroyed with the isopropyl alcohol), then rinse with water and dry, then use 70% isopropyl alcohol (step 3), then rinse with water and dry. DO NOT MIX CHEMICALS! Wear gloves and other proper personal protective equipment while doing this as H<sub>2</sub>O<sub>2</sub> will oxidize your cells as well.

**Leaf Litter:** The layer of leaf litter is another extremely important aspect of biosecurity as it can help to protect your frogs against any potential contamination in the soil and allows an area in which to forage for microfauna and to both thermoregulate and conserve moisture if humidity levels fall too low. It’s important to ensure your leaf litter comes not only from a reputable source, but that it’s sanitized before putting in your vivarium to kill any unwanted fungi, bacteria, or pest eggs. This can be done by boiling for 30 minutes, then drying before use or by baking at 250F for one hour. A wash in 3% hydrogen peroxide will help to further clean the leaves by dislodging dirt and small debris in addition to killing many types of bacteria and fungi via oxidative stress. Once your leaf litter is installed (ensure all substrate is covered with a recommended .5-1” of leaf litter), it is very important to keep the leaf litter relatively dry. If the leaf litter becomes soggy via over misting/too frequent of misting or by using the archaic method of using sphagnum moss between the leaf litter and soil, your leaf litter can easily grow large colonies of bacteria, which your frogs can absorb directly through their skin, causing bloat and other bacteria infections. Ensure that while maintaining your humidity between 70-100% that you have natural fluctuations in humidity falling below 100% and that your leaf litter becomes completely dry to the touch before misting again. Allowing your leaf litter to naturally dry out in between misting cycles will prevent the saturated conditions conducive to excessive bacteria growth. Let it be emphasized that you should Never use sphagnum moss under your leaf litter as this creates a constantly wet layer that will begin to decay within a few weeks of installation, creating an ideal environment for the proliferation of many strains of potentially harmful bacteria.

**Substrate:** It’s advisable to get your substrate from a reputable source, or otherwise to pasteurize it and, in either case, to inoculate it with beneficial microorganisms (such as those in the Houston Frogs Beneficial Microbial Inoculant) to prevent the spread of later introduced, potentially harmful microorganisms. You can pasteurize your substrate by placing it in a pillowcase or similar fine mesh bag, then submerging it in water and boil it for 1 hour, then spread it on a tarp to dry. Inoculating your substrate is extremely important as you’ll be able to control the exact strains of fungi and bacteria in your substrate instead of playing “microorganism roulette” with later introduced bacteria and fungi from your hands, the air, plants, and other materials placed in the tank. Beneficial fungi, especially endomycorrhizal and

ectomycorrhizal fungi, will establish throughout your substrate, killing other fungi with their mycotoxins. These mycorrhizal fungi will defend your plants against harmful pathogens either by forming a sheath around their roots (ectomycorrhizal) or by colonizing the interior of the roots (endomycorrhizal) and will additionally gather micronutrients for the plants in exchange for some sugars from the plant roots. Beneficial bacteria, such as *Bacillus subtilis*, will compete with other bacteria for nutrients and space, interfering with the colonization of pathogenic microorganisms and even killing other bacteria, reducing instances of root rot and the potentiality for bacteria related diseases such as foot rot or bloat in amphibians. Competition from beneficial bacteria and fungi can effectively prevent the overtake of your substrate by harmful microorganisms which would otherwise thrive on this mostly “blank slate”.

**Drainage Layer:** Your drainage layer is considered a line of defense against the proliferation of harmful bacteria in your substrate, especially many strains of anaerobic bacteria. Ensure that the water level in your drainage layer is always below your substrate. Waterlogged substrate is frequently the cause of amphibian bacterial infections, which is why the drainage layer is recommended to sequester any excess water. This is additionally why it is very important to use a well-draining substrate so that any excess water will drain into this last layer. It's ok to have standing water in the drainage layer as nitrifying bacteria will feed upon nutrients washed down through the substrate to this layer, but it's also perfectly ok for your drainage layer to be completely devoid of water as well.

**Plants:** Plants can carry a wide variety of pathogens, which is why you should NEVER use plants harvested in the wild (especially mosses!) or from less than trusted sources. To eliminate the threat of harmful bacteria, viruses, fungi, and pests such as snails, slugs, nematodes, and predatory flat worms, it's best to use Tissue Cultured Plants as these are prepared in a laboratory under sterile conditions and will be free from all contaminants. Some rare exotic plants are not currently in tissue culture, but you can still sanitize them enough to be used in your vivarium. Bryophytes (mosses and liverworts) are near impossible (even near impossible with an advanced laboratory) to sterilize without killing them and even difficult to sanitize. Bryophytes lack lignin and other protective features of vascular plants, therefore allowing the absorption of any sterilization agents directly into their cells, killing them. Luckily, vascular plants (just about every plant you will use in your vivarium other than mosses and liverworts) can be relatively easily sanitized (killing most but not all microorganisms) using a solution of 5% Sodium hypochlorite ( $\text{NaHO}_2$ , also known as household bleach) and water to soak and thoroughly clean the plants for up to 30 minutes to kill most bacteria, fungal spores, viruses, and pest eggs. You'll want to thoroughly rinse the plants with clean water afterwards, then acclimate them under a humidity dome outside of the vivarium for a few days to a couple of months before planting in a vivarium. This method of sanitization is particularly harsh on cuttings as the solution will be absorbed at the cut portion, often leading to the failure of the cutting to survive. Cuttings should first be established in a quarantined humidity dome for 1-2 months until fully rooted, at which point they can be sanitized using this method. To increase the efficacy of this method, you can add a couple of drops of Tween 20 as a surfactant.

**Invertebrates:** Never collect invertebrates from the wild as they can be carriers for a wide variety of pathogens. Cultured isopods, springtails (Collembola), and some other potential tank cleaners such as micro-roaches can be extremely beneficial to the biosecurity of your vivarium. Springtails will predate upon fungi (though some species of springtails will not eat certain fungi, which is why it is best to have a variety of springtails when possible), preventing fungi from overtaking your vivarium or its décor, such as botanicals/wood. Springtails, isopods, and micro-roaches will feed upon bacterial biofilms, frog feces, fly corpses, rotting leaves from plants, and even the corpse of any animal that dies in the tank. This detrital consumption is extremely beneficial in the reduction of nutrients that would otherwise be primarily colonized by bacteria, causing a large bloom in potentially harmful strains of bacteria that can be walked upon and absorbed through the skin of your amphibians, causing many illnesses such as bloat. These small organisms will additionally help to aerate the soil of your vivarium, allowing beneficial fungi, beneficial aerobic bacteria, and your plants to thrive (fungi need oxygen to thrive and to protect plant roots more effectively).

**Hardscape Materials:** It goes without saying that you should refrain from using hardscape materials that have not been properly sanitized. Most wood on the market has been heat treated and sometimes additionally sandblasted and stored for months before sale, but this is not always the case. Rocks should be cleaned using the same procedures as outlined for tanks with the exception of those composed primarily of calcium carbonate (vinegar will partially dissolve them), or less consolidated rock such as sandstone (H<sub>2</sub>O<sub>2</sub> will cause the loose particles to break apart). Silicon dioxide (SiO<sub>2</sub>) composed, well consolidated rocks such as quartz, quartzite, and amethyst are the safest to use in vivariums as they are inert and the easiest to clean. Do not bake rocks as air pockets can expand and cause the rocks to explode. Wood can be sanitized through washing and scrubbing in addition to either baking (250F for 1 hour) or boiling for one hour followed by drying.

**Water Features:** Water features are typically not necessary or recommended for most dart frogs (although most Epipedobates and Ameerega are adept swimmers as they naturally occur near streams and can do well with water features so long as the majority of the tank is still terrestrial), but are generally necessary for many tree frogs and newts. Water features can include anything from a shallow water dish to a deep paludarium (again, the water feature design will depend upon the animal). Water features can quickly become a biosecurity hazard if not designed or maintained properly. Often bacterial infections are seen from improper sanitization of water bowls or the installation of water features with no active filtration or aeration. For water dishes, it is extremely important that the water is changed daily to every other day at most. The dish should additionally be scrubbed using 3% hydrogen peroxide weekly (use gloves) to help sanitize and clean the bowl. As frogs hop into the water (depositing soil and bacteria), fruit flies and microfauna die in the water, and frogs defecate in the water, bacteria can easily bloom due to the nutrient rich environment, which can then easily be absorbed through amphibian skin causing bacterial infections. For any permanent water features, a filtration system is extremely important to remove decaying debris from the water and to

sequester nitrifying bacteria, which will help to remove nutrients from the water which may otherwise be utilized by other harmful bacteria. A filtration system can be as simple as a sponge filter with an air pump or as complex as a canister filter or under-tank sump system. The inclusion of live plants in the water including fast growing mosses, liverworts, floating plants, etc. will help to reduce nutrient levels and thereby reduce the chances of a harmful bacterial bloom.

**Humans:** We are the final potential breach in biosecurity! Even if you've done everything right in setting up your vivarium and buying healthy animals, you can later introduce pathogens via your clothing, hands, plants, or décor moved from one tank to another! Ensure that in between touching tanks, your hands are thoroughly washed with an antimicrobial soap, or wear disposable gloves in between touching tanks. Do not move décor or plants from one tank to another. If you have conducted high risk activities that could expose you to any potential pathogens (such as doing yard work or touching animals), change clothes and wash your hands with an antimicrobial soap or wear gloves before working on/touching your vivarium(s).

In conclusion, if we are mindful of the biosecurity of our vivariums, will have a significantly reduced risk of contamination from harmful pathogens such as Chytrid or Ranavirus, a significantly reduced risk of our animals contracting bacterial infections, and even a reduction in the risk of introducing pests that could attack our rare plants and frog eggs.