Protocol for Field Treatment to Reduce Individual Fungal Loads During a Bd Outbreak

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Supplies, equipment, and consumables:

- Permits, protocols, and paper-work.
- Site map with lake IDs
- PENS (**Figure 1**)
    - Large mesh pens (2 x 2 x 0.5 meter), with zippers on one and two half top edges.
    - Cage support poles (metal with plastic coating, 4-6 per pen).
    - Twine (several meters pre-attached to each pole).
    - Laminated signs ("Do not disturb!", **Figure 2**).
    - Zip ties (4"/10cm, UV resistant preferred).
    - Binder clips (approximately 1" mouth).
- FROG Processing
    - Smartphones for data entry, with MLRG Fulcrum app.
    - Swabs and swab vials, with pre-printed labels.
    - 8mm PIT tags, previously entered into database.
    - Compatible PIT tag readers.
    - Frog processing kits:
        - surgical scissors
        - ethanol
        - calipers
        - Pesola scales (several 60 g, with on 100 g and one 30 g)
    - frog weigh bags (snack sized Ziplocs)
- TREATMENT
    - **1: 150 mL bottle Itraconazole/Sporanox®** (Figure 8)**, wrapped in foil, bubble wrap, and a Ziploc.**
        - Optionally, prior to trip: aliquot Sporanox® to smaller glass bottles, parafilm lids, wrap in foil.
    - 7-10: disposable plastic transfer pipettes (one for each day of treatment).
    - 1: One-liter graduated Nalgene bottle (1-2).
    - 1: large water storage container, such as a collapsible 2.6 gal/10 L cubitainer.
    - 2-4: treatment tubs, each with 2 lids and one screen (**Figures 3-6, 9**). Each treatment tub requires:
        - Tub: plastic under-bed storage tubs approximately 60 cm x 40 cm x 15 cm (for 50 frogs; use smaller tubs if treatment groups contain far fewer frogs); see Figures 6, 9.
        - Outer lid: a tub lid, drilled with air holes and a frog sized "trap door" or "hatch" with Velcro closure; see Figures 3-4.
        - Screen: One piece of plastic window screening fabric, large enough to "cover" the inside surface of the tub, with some excess (approximately ≥ 90 cm x 70 cm); see (Figures 3-6, 9).
        - Inner lid: an additional tub lid, cut to nest inside tub at a depth equivalent to being filled with 5 L of water, drilled with air/water holes, with small loops of twine near the corners and center. See Figure 9.

- OTHER EQUIPMENT
    - Amphibian capture nets (one per person); square corners preferable for capturing frogs in corners of pens. Remove any sharp or pointy bits (like a snipped-off zip-tie) to avoid damaging pens.
    - Disinfection dry bag and quaternary ammonia (with plastic dropper).
    - Latex or nitrile gloves for handling Itraconazole and treated animals, many of common sizes.
    - Many Ziploc bags of various sizes (snack, quart, and a few gallon) for trash.
    - Pocket knife with scissors (for odd jobs, NOT PIT tagging).
    - Optional: float tube, mini crush proof frog cups with air holes.
    - Foam sleeping pads.
    - Extra bungees, accessory straps, old bike innertubes, or twine for attaching gear to packs.

Strategy and schedule:

When initiating a Bd treatment, time is critical to saving a Bd-naïve population that is experiencing an outbreak. Treatment should be conducted as soon as possible after the Bd outbreak is detected. Delay of even a week can make the difference between having hundreds of frogs to treat and having very few frogs still alive. Given that several days to weeks can elapse between swab collection and receipt of swab results (which indicate the severity of Bd loads on frogs), average Bd loads will increase, and dramatic population declines may occur in this brief timespan. Thus, a critical first step when arriving at a treatment site should be visual encounter surveys (VES) to ensure that sufficient adults remain to justify a treatment (***Appendix 1***). Visual surveys may only represent 10 to 30 percent of the total adults that may occur at a site.

Number of frogs in the treatment: The number of frogs to be treated will depend on the number extant at the site, their catchability, and crew resources. In our experience, a large, experienced, and efficient crew of 4-6 can capture and process up to 200 frogs per day; crews with less experience will be slower. This suggests that the maximum treatment size will be 500-600 frogs, even if the abundance of frogs extant at a treatment site is much higher. Thus, if treatment is effective, several hundred frogs may survive the Bd outbreak and treatment. See ***Appendix 1*** for comments on number of frogs. If greater than 150 frogs are available for capture, **control groups** will be established (see ***Appendix 2*** for a description); if many more frogs are available, a non-capture control group will also be established (***Appendix 2***).

Frog capture and frog pens: On arrival at the treatment site, frog holding pens (Figure 1) should be installed at the lake with the highest frog density (if multiple lakes are to be included in the treatment). Pens should be positioned along the shoreline, partially in the water and partially on land (to allow frogs both basking and deeper water habitat). Grassy shorelines with sandy/silty littoral substrates are preferable for installing the pens. Typically, some crew members can begin installing pens while other crew members begin visual surveys. Pens should be installed with the Velcro on the bank and the zippered edges extending from the bank into the water. One support pole should be installed with zip-ties (top and bottom) on each corner of the pen. When zip tying the poles to the mesh, make sure you capture the bias taps along edge seams, and try to capture all edges leading into a corner; do not zip tie only to the mesh itself. Additional support poles may be needed to keep the top of the mesh off the water. Attach rocks to guy lines and position the rocks adjacent to the pens such that mesh is relatively taut. Binder clips can be used to close gaps at the top corners where zippers and Velcro meet, to ensure frogs cannot escape the small gap. When garter snakes are present, be aware that smaller snakes will be able to get into the pens; remove them. Do not place rocks inside pens, and beware of sharp/pointy substrates or other equipment that may tear the mesh. Lastly, place laminated signs on the pens.

The number of frogs anticipated for treatment will determine how many pens are required (see ***Appendix 1***). No more than 100 frogs should be held in a single mesh pen. In addition to the frog-holding pens, it is often useful to have an additional pen installed for placing frogs in before and after processing (swab, weigh, measure, pit tag). If obviously sick frogs (lethargic, no righting reflex, etc.) are encountered when collecting frogs for treatment, exclude these frogs from the pens. Their presence in the pens has the potential to increase the Bd loads of penned frogs and such sick frogs are beyond the help of treatment.

When collecting frogs, do not hold multiple frogs together in a frog net. Unfortunately, this rule makes it onerous to collect one frog, walk it back to the pens, then continue searching, especially if a site is complex or terrain especially rugged, or frogs are well distributed around the shoreline and far from pens. This rule decreases efficiency and overall capture rate, BUT it protects individual frogs. At sites with extremely challenging terrain, consider placing captured frogs in individual cups (Figure 7). However, crew members must take

extreme care to keep those frogs cool and hydrated as they move around the lake, and to make sure those cups are well rinsed between frogs. At most sites, this may not be worth the potential stress to frogs, but at a few sites, it could help increase the number of frogs captured and treated. Lastly, between each frog delivery to the pens and the next frog capture, **frog nets should be vigorously swished and rinsed in lake to remove sloughed skin and mucus**.

Frog mortality will occur throughout the treatment, in both untreated and treated frogs. As sad as this can be for crewmembers to observe, do not waver from the protocol. This is especially important if your treatment design includes control animals (See ***Appendix 2***)

Timeline:

An entire foray to treat a population will take 7-11 days (Table 1). To ensure maximum effectiveness, **frogs must have a minimum of seven days of antifungal treatment**, and up to nine days of treatment is acceptable. Each frog (and group of frogs) must receive 7+ treatment days. Days 8 and 9 may not be required if frogs were only collected on Day 1, or on Day 1 and Day 2. For example: Group 1, captured and first treated on Day 1, can be released post-treatment on day 7, but *not after* Day 9; Group 2, captured and first treated on Day 2, could be released as early as post-treatment on Day 8; and Group 3 can be released post-treatment on Day 9. The eleven-day estimate includes one day to hike in to the site, 9 treatment days and one day to hike out of the site.

A typical schedule follows (also see Table 1):

- **Day 0:** *(optional)* Hike into site and establish camp. Conduct VES if time. Also, one crew member could hike to the potential treatment lake, conduct VES, and send a satellite-text-message to the crew to relay the number of frogs seen in the VES.
- **Day 1:** Hike into site, **conduct VES**. Upon arrival at the outbreak site, decide:
    - **Are sufficient frogs available for treatment?** See *Appendix 1*.
    - **Can we collect, process, and treat a group of frogs before end of workday?** If early enough in the day, frog collection, processing, and treatment may begin on day 1. If the group is not extremely experienced or efficient, make this decision conservatively.
    - **It is critical that frogs receive treatment on the first day they are captured and penned. If frogs cannot be captured, processed, *and* treated on Day 1, do not capture any frogs on Day 1.**
    - If the decision is to catch and treat, catch as many frogs as possible and place in a pen (or pens, if more than 100 frogs were captured). These frogs are Group 1. At some point during the afternoon and before **treatment** (see below for treatment details), begin processing frogs. Each frog should be pit-tagged, weighed, measured (SVL), sexed, and condition described. Enter this data into the MLRG Fulcrum app on the smartphone. UTM coordinates and location of capture need not be included for treatment frogs. Treat all Group 1 frogs with itraconazole (details provided below) in the last hour of the workday.
- **Day 2:** Catch, swab, and process as many frogs from the site as possible. If the site contains hundreds of frogs, you may want to limit the number of frogs to the number that can be processed in that day by the available personnel. All frogs captured on Day 2 should be placed in a "Day 2" pen (or pens). Treat all frogs, including those in Day 1 and Day 2 pens, at the end of the day and return to their original pen.
- **Day 3:** Same as day 2, but place frogs in a "Day 3" pen (or pens). This is the final date on which new frogs are captured at the site.

- **Day 4-8:** Continue to treat all frogs collected on days 1, 2, and 3. Timing of treatment may be advanced to earlier in the day.
- **Day 9:** Treat all frogs in the morning and release back into pens. Allow frogs to swim and rinse themselves between treatment and additional handling. In the afternoon, swab, weigh, and measure frogs.
    - Measure all frogs, or,
    - If greater than about 150 total frogs (including controls) remain, swab and measure a subset of frogs. If sub setting, swabs and measurements from 25 frogs from each treatment group (and controls) is a good goal.
    - Ideally frogs should be treated in the morning on the last day and swabbed, weighed, measured, then released later the final day. Trip logistics will determine how this is done. For example, frogs could be processed frogs on the second-to-last day and treated and released early on the following day.
    - Release all frogs back into the lake!

Itraconazole treatment details:

The following is for the 10-minute treatment of batches of 50 adult *Rana muscosa and R. sierrae*. For treatment of adults, use a ratio of 0.15 mL of itraconazole/Sporanox® to 1 Liter of lake water. This is equivalent to 3 drops Sporanox® - from a standard disposable transfer pipette – per liter of lake water, or 15 drops per 5 liters. If much smaller groups of frogs are being treated, revise to use smaller treatment tubs and lower total volumes. If a task requires more than one person, it is noted.

0. Store the itraconazole/Sporanox® in the coolest, shadiest, dry location you can find at the treatment site. Under the north side of boulder is often the best you will find. Surround with snow if available.
1. Assemble treatment tubs: remove any objects from within the tub. Place the outer lid on the tub. Set the screen fabric and the inner lid nearby.
2. Move 50 frogs from one pen and add them to an **EMPTY** (contains no Sporanox® solution) treatment tub via the hatch (Figure 4). Two people.
    a. If the pen contains >50 frogs, then two treatment tubs should be filled simultaneously, or another pen used to help sort treated/untreated frogs.
3. Slide the fabric screen between the outer lid and the treatment tub (Figure 4), taking care to keep frogs from escaping and not to pinch any toes. Two people.
4. Set the treatment tub on a level surface or prop it with rocks to make it as level as possible.
5. Mix 5 L of Sporanox® treatment solution.
    a. Measure 1 L of clean, clear lake (not stream) water in the graduated Nalgene.
    b. Add 3 drops of itraconazole/Sporanox®.
    c. Add to bucket/cubitainer.
    d. Repeat 5x times (5x total, for 5 total liters).
    e. Fully mix itraconazole and water before adding to frog-containing treatment tub. Mix the solution in the large water storage container. Use a gloved hand to mix.
6. Pour mixed solution into the treatment tub, either through the hatch, or by setting the outer lid askew to expose one corner of the tub, while keeping the fabric screen snugly covering the tub to prevent frog escapes. Two people.
7. Start the treatment timer, for **10 minutes.**

8. Realign the outer lid loosely and slide the inner lid between the outer lid and the screen fabric. Two people.
9. Remove the outer lid.
10. Press down on the inner lid, until there is barely room over the solution (Figures 5, 9), so that frogs can keep eyes/nares/heads out of solution, but not so much space that frogs can climb on top of each other to get out of solution. Make lid is level around the entire tub – which is also level – so that frogs can be evenly distributed and equally submerged. Use the strings looped through the breathing holes or the tug on the screen to raise the inner lid as needed.
11. Reset the outer lid to lock the screen in place and help keep the tub from warping.
12. Take a breather.
13. Check the tub every two minutes or so, to make sure frogs are submerged and liquid is level. Give the tub a gentle slosh to move frogs and liquid around (Figure 9).
14. When 10 minutes expires, move somewhat quickly but carefully.
15. Remove the outer lid. Lift the inner lid out. Set the outer lid back on the tub loosely. Slide the screen out carefully, keeping all frogs in the tub. Lock the outer lid back on the tub. Two people.
16. Carry the tub to the pen. Have another person open the pen, and others on hand as available to help monitor the frogs in the pen and those leaving the tub, to prevent escapees.
17. Release the frogs into the pen. This is a many person task and can get nuts (Figure 6).
    a. Have two people support the tub while positioning it down, inside the pen.
    b. Have a third remove the lid. This person should have gloves on. They may need to stand in the lake to have best angle.
    c. Encourage frogs to jump out of the tub and into the pen or lift frogs out of the tub and place them in the water.
        i. Some frogs will be extremely lethargic. The anti-fungal bath is stressful. They may recover, so should be placed in the water in the pen, supported by the mesh.
    d. Frog monitors should help keep jumping frogs in the pens.
    e. Once all frogs are returned to the pen, lift the tub out, and close the pen.
    f. Take care not to pour Sporanox® solution into the lake.
18. Dispose of used Sporanox® solution, which should be disposed of similarly to Quat solution: on dry, organic soil > 100 m from water (e.g. in the duff below conifer trees); previously disturbed sites (campsites, trails) should be used when possible.
19. Repeat for all additional pens and groups of 50 frogs. Use new itraconazole/Sporanox® solution for each group of 50.

Other considerations and information to record:

- Consider conducting an additional VES at the site before departure to describe advancement of Bd outbreak.
- Daily descriptive summaries of observations and the progression of the outbreak, and of the tasks accomplished.
- Number of animals dying in each frog group/pen, and their PIT tags.
- Attempt to recover PIT tags from frogs found dead in pens.
- Record observations of animal behavior, condition, and unusual response to treatment. Issues should be reported to USFWS and relevant agency or institutional animal care and use committees.
- Any observations of predation on healthy or sick animals.

- You may choose to move gartersnakes out of the lake, to habitats > 1 km away and containing Pacific treefrogs.
- **Euthanasia:** Researchers, scientists, and managers should adhere to the euthanasia protocols established by their agency's or institutions animal care and use committees and guidelines.

Follow-up:

Following the treatment, the population should be revisited and assessed using a capture-mark-recapture survey approach, with concurrent swabbing. Ideally, these follow-up CMR surveys should begin one-month post-treatment (if not too late in the season) and occur one to three times per ice-free season until the population appears to stop declining, whether due to complete extirpation or to recruitment of new individuals and positive population growth rate. Typical CMR surveys occur over three consecutive days. Upon first capture during each CMR survey, each frog will have its PIT tag scanned, will be measured and weighed, and will be swabbed. Newly captured adult frogs should be PIT tagged.

Sample Treatment Schedule

Table 1. Timeline of tasks performed during Bd field treatment.

Day 1	Conduct visual shoreline surveys. Collect, process, swab as many frogs as possible. Treat frogs in group 1.
Day 2	Collect, process, and swab more frogs (group 2). Treat frogs in groups 1 & 2.
Day 3	Collect, process, and swab more frogs (group 3). Treat frogs in groups 1 & 2 & 3.
Day 4	Treat frogs from all groups.
Day 5	Treat frogs from all groups.
Day 6	Treat frogs from all groups.
Day 7	Treat frogs from all groups.
Day 8	Treat frogs from all groups. Weigh, measure, and swab all frogs (or a subset).
Day 9	Treat frogs from all groups. Weigh, measure, and swab (if not conducted on day 8). Release all frogs.

Figures

Figure 1. Treatment pen. The fully zippered edge is positioned on the shoreline, and the opposite, closed edge is nearly fully submerged in the littoral zone. Corners are supported by 4' metal poles and guyed by twine tied to well-positioned rocks. From the shore, the biologist can reach all corners of the pen with a frog net.

Figure 2. An example of laminated signage. Some groups may wish to include contact information or agency logos.

Figure 3. Fifty-frog treatment tubs. Note screens between lids and tubs.

Figure 4. Frog tub lid. There is a 'hatch' in the left center of the lid (dashed line), held closed by velcro. Note the extensive air holes in the lid that are smaller than a small frog.

Figure 5. Five Rana sierrae *in a treatment tub, submerged in 5 L Sporanox® solution. This is ideal: the inner lid keeps frogs submerged, and the tub is flat so that frogs in any corner of the tub are equally submerged. Note the inner lid is on top of the screen.*

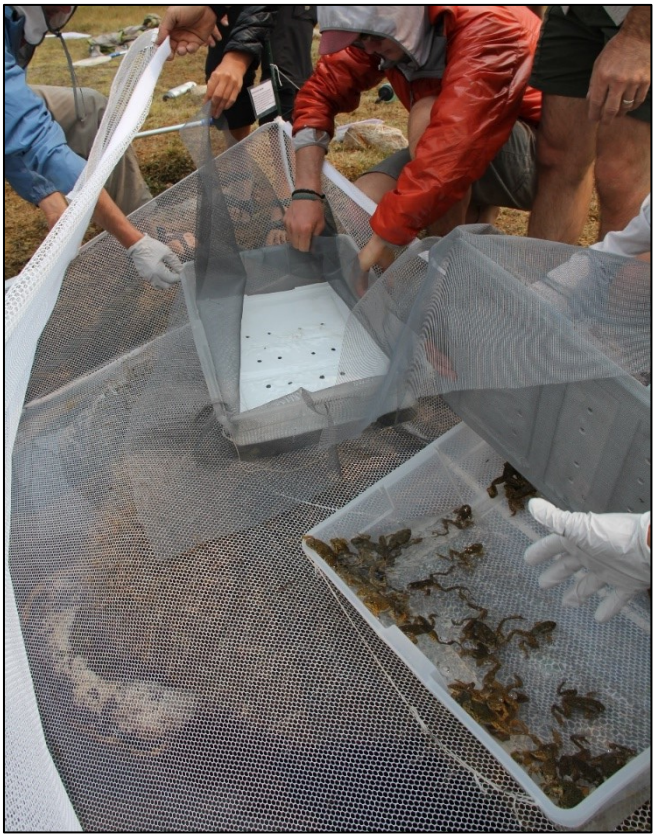

Figure 6. Releasing frogs into pens after a days' round of treatment. In the upper treatment tub, note the design and placement of the inner lid, on top of the screen. Note extra hands holding tubs to prevent spill of Sporonox solution in to lake.

Figure 7. Frog in a cup, with holes in the lid and some lake water.

Figure 8. Frog in treatment tub, with bottle of Sporanox. Generally, tub should be more level than in this photo, and Sporonox should be kept cool and shaded.

Figure 9. A biologist monitoring frogs in treatment tubs during 10-minute bath. Note placement of inner lids and screen fabric to keep frogs submerged. The biologist is lifting one end of treatment tub to gently slosh and mix Sporanox® and move frogs around.

Appendix 1: Are enough frogs available for treatment?

This is a hard question to answer. There is no decision tree, but there are guidelines. Ultimately the decision is likely to be made on the ground, in "real time", and will be made by the managers and scientists 1) with jurisdiction over the population, 2) with historical knowledge of that population's trends and characteristics, and 3) who are present at the outbreak site and armed with the most recent VES and swab information.

What is enough? The goal is to treat a 'large' number of frogs so that, when accounting for capture probability, treatment mortality, and overwinter mortality, a sufficient number of frogs are treated and released to survive to the following season. These parameters vary by population, and we rarely have prior knowledge. Often, we rely on our intuition based on previous work at that lake or others like it. **Big enough:** If the VES reveals 50-100 **adult frogs**, there may be 150-1000 (3-10x the number observed) frogs at the lake (although Bd+ and sick animals may be more visible so the actual number could be lower, Joseph and Knapp 2018), and there could be one to several hundred frogs available for capture and treatment. **Too small:** If the VES reveals < 20-50 frogs, there are likely too few frogs to capture and treat and result in an ecologically meaningful cohort of survivors.

The grey areas: around these guidelines are conditions where local and real-time knowledge can help make the decision. For example, VES reveals 50+ frogs, but swab results indicated that most frogs had high Bd loads days to weeks ago, and the most recent VES indicates a large decline from the previous VES; these factors together might suggest that the outbreak is quite advanced, and treatments might not be effective or survival might not be high due to the stress of the infection and treatment. Alternatively, VES might reveal fewer than 50 frogs, but that number is similar to the previous VES, and you know that very recent swab results indicated relatively low loads indicating an incipient outbreak, and you know that at that site, detectability can be low but capture rates can be high with effort (e.g. frogs hide below undercut banks but can be 'noodled') and you might be able to catch >>50 frogs and their loads can still be reduced to sub-lethal levels by treatment.

Appendix 2: Evaluating treatment success: Control Frogs

If more than about 100-150 frogs can be captured for treatment, a control group (or groups) should be created. The inclusion of control groups provides important comparative data to evaluate the effectiveness of the treatment. There are two types of control groups to consider including:

First, a **"treatment-control" group** should be the priority. If there are sufficient frogs to create the treatment-control group, aim to designate about 20-33% of penned frogs as controls, up to about 100 frogs. The treatment-control group is treated identically to the treatment frogs, except for itraconazole treatment. Specifically, treatment control frogs are held in a pen for the duration of the treatment but are not treated. This control group has the same swabbing, pit tagging, weighing, and measuring schedule as the treatment group. If an additional clean and unused treatment tub is available, treatment-control frogs can be moved into this tub and held in lake water for 10 minutes using the same methods as the frogs being held in the treatment solution (i.e. they receive a 'sham' treatment). Treatment-control frogs are released back into the lake on the same schedule as treated frogs, at the end of the treatment. It is important to note that mortality of these frogs will be high - and highly visible - relative to treated frogs, but they play a critical role in our understanding of treatment success.

Second, a **"unpenned-control" group** should be created, when enough personnel, treatment frogs, and penned control frogs are available, and additional 'wild' and unpenned frogs can be captured and recaptured using a

capture-mark-recapture (CMR) approach. When possible, aim to include about as many frogs in the unpenned-control group as in the treatment-control group, or about 1/3 the number of frogs as in the treated group. This unpenned-control group will be monitored during the treatment but will not be held in a pen for the duration of the treatment. Frogs will be tagged, weighed, measured, and swabbed on the first or second day of the treatment, or as soon as the determination is made that 30-50+ frogs remain in the habitat after accounting for treatment and pen-control frogs. On all subsequent days of the treatment, CMR of the treatment site(s) will attempt to recapture as many unpenned-control frogs as possible. Upon recapture (maximum of once per day), a swab will be collected, and length, weight, and PIT tag id will be recorded. Note that the protocol for this unpenned-control group of frogs differs from normal CMR protocol: here, swabs and weight should be collected upon each capture of these CMR-control frogs (in normal CMR protocol, we swab and measure an individual only upon its first capture in a CMR survey period) because weight could decrease and Bd load will increase disease advances (but length need not be remeasured as it is unlikely to change over 7 days). Why swab unpenned-control frogs upon every capture? Because recapture is not guaranteed, and the sample size of these unpenned-control frogs is likely to be small.

Numbers of control and treatment frogs are provided (Table 2) for three examples of past rapid-response Bd treatments, as well as numbers for hypothetical future treatments.

Table 2. Actual and hypothetical divisions of treatment and control frogs.

Total frogs	Treated	Penned Control	CMR Control
Actual previous treatments			
388	286	102	0
201	127	74	0
52	52	0	0
Hypothetical treatments			
500	300	100	100
400	240	80	80
300	180	60	60
200	130	70	0
150	100	50	0
125	80	45	0
100	65	35	0
75	75	0	0
50	50	0	0

Appendix 3: **Budget**

ITEM DESCRIPTION / SPECIFICATION	QUANTITY	PER UNIT COST	EST. TOTAL COST
Sporanox® (itraconazole), 150mL bottle†	6	$280.50	$1,683.00
PIT tags (Oregon RFID 8 x 1.4 mm)†	400	$1.55	$620.00
Labeled swab vials (400 needed, 2500 vials/case) †	1	$366.86	$366.86
Swabs (400 needed, 1250 swabs/case) †	1	$307.55	$307.55
PIT tag readers*	4	$670.00	$2,680.00
Large mesh pens	5	$500.00	$2,500.00
Surgical scissors for PIT tagging	4	$100.00	$400.00
Pesola scales (60 g scales)*	4	$53.50	$214.00
Calipers*	4	$45.00	$180.00
Cage support poles (4' Sturdy Stakes, 5 per pen)	20	$4.17	$83.40
Plastic treatment tubs and lids	4	$15.00	$60.00
1 Liter graduated Nalgene bottle	2	$10.95	$21.90
Twine (attached to poles, ~15 m per pen, 1 spool)	1	$12.99	$12.99
Binder clips, package of many	1	$6.29	$6.29
Zip ties	1	$5.00	$5.00
Ethanol (about 120 mL)*†	1	$5.00	$5.00
Disposable plastic transfer pipettes	7	$0.10	$0.70
Amphibian capture net (one per person)*	8	$14.65	$117.20
Disinfection dry bag	1	$20.95	$20.95
A set of laminated signs	1	$5.00	$5.00
Frog weigh bags (1 pkg, snack size)	1	$5.00	$5.00
Quaternary ammonia (about 60 mL) *†	1	$5.00	$5.00
Latex/nitrile gloves*†	many	$5.00	$5.00
Resealable zip-top bags; many needed for trash	1	$5.00	$5.00
Fulcrum app licenses to access MLRG frog data entry forms.	4	$150.00	$600.00
		OVERALL TOTAL	**$9309.84**

**Commonly owned items that could be easily contributed/lent from team members' supplies.*

†Consumables.

Justification: The above equipment and consumables budget makes the following assumptions: 1) one complete treatment 'kit' (all of the listed supplies) exists with the Mountain Lakes Research group, 2) one complete kit is required, i.e. none of the listed supplies are available for loan from another group (e.g. UCSB, CDFW, NPS, USGS), 3) the kit is sufficiently supplied to treat a large number of frogs (n=400), 4) the kit is sufficiently supplied to enable four people to concurrently swab and PIT tag frogs, and to enable an additional four people to enter that swabbing data into smartphones as it is collected.

The budget is written for a treatment of 400 individual frogs, and each frog requires 'consumable' supplies. The most critical ingredient is the antifungal drug Itraconazole. Most treatments will not consume a whole bottle, but the drug has a one-year shelf life. Additionally, both frog treatment kits - the existing MLRG-based kit and the proposed Rapid Response Team kit – will require fresh Itraconazole each year, so the budget includes several bottles (2 purchased yearly for 3 years). To establish the fungal load of every treated frog prior to and

following treatment, strive to swab every frog upon capture and before release, which requires 2 swabs per frog (800 swabs plus vials for preserving; both are available in large cases). To track the survival and individual infection dynamics of treated frogs, each frog is PIT tagged, so the kit should be equipped with 400 PIT tags and several compatible PIT tag readers.

A large treatment requires 5 frog pens (and their accessories), where 100 frogs are assigned to each pen and one pen remains available to facilitate logistics during capture, PIT tagging, and treatment. Placing frogs in pens guarantees recapture of each frog on each day of treatment. Plastic storage tubs are required for the actual antifungal treatments, which does not occur in the pens. Several tools (e.g. scissors, calipers, scales) are used when measuring and PIT tagging frogs.

Lastly, to the extent that swabs are processed by and data is managed and analyzed by the Mountain Lakes Research Group (Roland Knapp, Tom Smith, Ericka Hegeman, and Max Joseph), it is strongly recommend that all data be entered directly into their data collection app (Fulcrum) at the moment that it is collected in the field. This allows data to move seamlessly, rapidly, and accurately through the established laboratory and statistical workflows. The app can be installed on any smartphone, so the budget includes fees for the software license.