

The following protocols for dealing with frogs are based on the assumption that the care and welfare of the frogs is a communal responsibility that all frog users share. The frog colony must be kept clean all the time. If frogs had been abandoned after ovulation or in used frog products for large periods of times, massive microbe growth would eventually overcome even the frogs stupendous resistance to infection and death. To avoid this tragedy in our community we here set out guidelines for practising frogologists. The list of responsibilities is intended to help us look after our little buddies.

Chain of command

Queen

Elly Tanaka, her word is law ("my rule is the rule")

Frog Czar

Heino Andreas, commander in chief of frogs. To be obeyed unquestioningly in all matters of frog care.

Serfs

All those how use frogs, however infrequently.

Responsibilities

Frog Czar

1. Feeding frogs
2. Taking care of water conditions
3. Organizing frog colony
4. Priming (PMSG injection)
5. Overall physical and mental well being of frogs

For PMSG Injection

Wednesday

Frog Users should send the e-mail to Heino (andreas@mpi-cbg.de) to inform how many frogs will be needed next week. 20 frogs are maximum per week for whole the lab.

Thursday

Picking up required number of new frogs and injecting them with PMSG.

Put PMSG-primed frogs in the tank tower, and label it with date of PMSG injected.

HCG Injector

Day of injection

Check with other frog users to see if use can be consolidated. Inject frogs and place in white square containers in 1x MMR. Label container with date and name, remove old labels. If you take the last frog out of a container of PMSG-primed frogs, remove label and look about/make inquiries to make sure that there are other primed frogs available for the next user. If you use the last of the HCG, make up a new vial and date it.

Day after injection

After use place frogs in white square containers (labelled with your name and date) in water overnight to allow any terminal ovulation to occur.

2nd day after injection

Remove extra eggs, put back frogs to empty tank and label it with date of HCG injection. Ask Heino to which tank you should put back frogs. Don't mix your frogs with those which was injected more than 2

weeks before yours. If tank contains more than ten frogs after the addition of your valiant aquatic collaborators, start to use another tank and label it. Wash white square containers and lids and put them back.

ALL FROG USERS

General points to notice

- Clean up the sink every time after your use.
- Throw away all eggs in the sink in your own labs (NOT in frog colony).

Ultimately, we are responsible for the proper care of all the frogs in the CBG frog colony whether or not we have used a particular frog. Therefore as you stroll about the lab:

- Look for frogs abandoned after ovulation. Any frogs that you see in water containing large numbers of necrotic eggs should be immediately transferred either to clean water or to the bucket.
- If you find dead frogs: Dispose of the dead frogs with the same rapidity you would use if you found it in your bed by placing in a plastic bag and putting in -20 freezer in the colony. If there are live frogs in same tank, exchange water and clean it thoroughly.
- When you are done with frog containers remove all labels, wash out the tanks (scrubbing if necessary to remove eggs stuck to them), and store them stacked underneath the frog bench.
- Keep the frog bench clean. This means disposing of surplus oocytes, eggs and embryos upstairs, putting the dishes in used dish tray, wiping up spills on the frog bench and generally tidying up. The first step in this process is obviously cleaning up after yourself the same day you do an experiment. But we all also have to look out for each other.

Identifying and treating sick frogs

Especially in the summer months "red-leg" a virulent, highly infectious disease of frogs is an ever present danger. Epidemics can be prevented by caring for frogs properly and by the looking out vigilantly for infected individuals. Tell tale symptoms are (in order of increasing severity):

1. Haemorrhaging in foot webs (also caused by insufficient salt in water and some other forms of stress)
2. Rough skin
3. Distended stomach, difficulty in remaining submerged
4. Severe emaciation (this may not be red leg, but sure is sick!)
5. Uncoordinated swimming
6. Visible redness on stomach
7. Visibly cloudy cornea

Frogs displaying any of these symptoms should be removed from the population. Those with cloudy corneas, severely distended and red abdomens, or severe emaciation should be euthanized. Those with milder symptoms should be quarantined in clearly marked tanks in water plus 100 mM rock salt. This solution should be changed daily. If condition worsens appreciably euthanize frogs. If condition improves keep frogs in quarantine until they are asymptomatic before returning to used frog tanks. Any frog quarantined must spend at least one week in quarantine.

If an epidemic occurs

Successful treatment of epidemics requires the concerted effort of all frog users. The colony is kept in 150 mM rock salt containing 10 ug/ml tetracycline and streptomycin which is changed daily. During initial change frogs are held in buckets and tanks are drained, scrubbed with bleach, swabbed with ethanol and then washed extensively with water. Each day visibly sick frogs are removed from the colony and euthanized or quarantined.

