

A Presentation of the United States Fish and Wildlife Service Survey Methods for Frog Abnormalities on National Wildlife Refuges

Narrator: Introduction

Amphibians, the group of animals that include frogs, toads, and salamanders, are found in forty-nine states. Amphibians are quite sensitive to changes in their environment and can be considered indicators of environmental quality for several reasons. First, during various life stages, they live both in the water and on land. Their skin is highly permeable to water and air. Thus, they can absorb gases and other constituents, such as pollutants from their environment. Amphibians go through a process called metamorphosis where by they change from their larva stage into adults. During this part of their life cycle, the changes in these animals are extreme, making them particularly vulnerable to disturbances in their environment. For example, during metamorphosis, the immune system is temporarily suppressed, thereby increasing the chances for infection or disease. Because of their sensitivity to changes in their surroundings, amphibians can be considered the modern day canary in a coalmine.

This training video is designed for Fish and Wildlife Service biologists and technicians who will be asked to conduct frog surveys primarily at National Wildlife Refuges. The purpose of this video is to show the proper survey methods, handling techniques, processing and shipping methods. The U.S. Fish and Wildlife Service maintains over 500 National Wildlife Refuges, which provide important habitat for amphibians and other resources.

In the Northeast and Midwest regions, biologists began surveying in 1997 to gather basic information on the occurrence of abnormal frogs on refuge lands. In 2000, the survey was expanded nationwide. The Service has worked closely with our partners including the U.S. Geological Survey, universities, and state agencies in developing the project, analyzing data, reporting and sharing our findings.

The phrases abnormality, malformation, and deformity are often used interchangeably. For our purposes, “abnormality” is defined as missing, extra, or unusual body parts based on field observations. A malformation occurs when something goes wrong during developmental stages, causing an organ or body part to form improperly. A deformity occurs when a body part that already exists becomes disfigured. There are different habitats where frogs and other amphibians can be found. They are found at different times of the year according to your locations. You should be familiar with the species and life histories of the frogs living in your area.

Although the focus of this video is for sampling on refuges, the basic skills would apply to other lands as well. The most important contacts for your survey are the refuge manager and refuge biologist. Talk with them early in the season and visit the refuges that possess the habitats where frogs are found. Carry a species key or guide with you so that you know which species are likely to be found in your area and what are the appropriate size ranges for metamorphs.

This is the equipment you will need and you should find this list in your training guide:

- Hip boots or waders-have both.
- Long-handled 24” deep mesh net-one per person.
- Plastic containers with tight fitting lids with holes punched in them.
- Clear plastic bag or plastic containers with tight fitting lids without holes for tadpoles.
- Data Sheets.
- Rulers (metric).
- Field Guide to Malformations of Frogs and Toads.

- Hand lens or magnifying glass.
- Field vest for holding plastic containers.
- Two-foot deep plastic container with lid for holding frogs.
- Five-gallon bucket with lid for holding frogs.
- Camera.
- GPS recorder.
- Hydro lab or similar device.

Two biologists, Fred Pinkney and Sherry Krest, will be demonstrating the techniques for capturing and handling frogs in this video. (0:46-5:43)

Fred: Well I've got all our equipment together and I think we're just about ready to go out and collect some frogs. So we're just going to review all the equipment we've got and make sure we have everything. You see with these long handled nets which are good, we like good deep nets so when we get a frog or a toad it can't hop out on us. Once we get them, we'll go over the technique once we get in the water. I like to store them in these little plastic containers – what we did we punched some holes in them ... These vests are handy because -- not all you guys have vests -- but you can put them in your pocket and then just keep going – it doesn't slow you down.

Sherry: And this is another type of container you can use – prefer the smaller ones, but these will do too.

Fred: We'll record the site with a GPS just so we have the location. I like to record what grid we're using and then we'll take a picture, a couple pictures, of the site. We'll also have the camera ready once we examine the frogs. If we see anything that looks unusual we can take a picture now. We can also take the frogs back to the lab with us and use a better camera with the macro lens and try to really get some good photographs if we see something interesting. So we're going to go out and try to get at least fifty metamorph frogs of the same species and what we'll probably get here are green frogs. We may get southern leopard frogs and Sherry and I can go over what you've got in case there's any doubt. The size we're looking for is forty millimeters -- snout to vent length -- so that's about this big for the green frogs and southern leopard frogs. We're also looking for cricket frogs, which are much smaller, and the snout to vent length on those is 15 millimeters so they're pretty small. Basically in the field catch whatever you can; if it's an obvious huge adult – let it go and then we'll sit around and do the measurements as a group later.

Sherry: And make sure it has all four legs – it can have a tail, but all four legs.

Fred: If you happen to get tadpoles, while you're scooping in the water, a tadpole that has both four limbs and hind limbs is considered a metamorph for this study, so if you have something that looks like a tadpole but you see four legs on it – that's good. If you have a tadpole, we'll have a container that doesn't have holes that you can carry with you for the tadpoles. So the frogs will go in the containers with the holes, the tadpoles in the containers without the holes. Once we get back here, we'll sit in a group -- probably two groups of three -- and we'll have a recorder whose going to record the data on these data sheets and an examiner whose going to measure the frogs and we'll go through these check lists and get everything recorded. And if there is any doubt of what we're seeing we can use this field guide that has a lot of good pictures and x-ray pictures of the frogs. Once we finish, we'll also go and use the Hydrolab to record water quality. I think what will probably work best is if we start out in three groups of two.

This is Blue Gill Pond. You can see it's got a lot of submerged aquatic vegetation. There's a lot of frogs that are going to be on the edge here and I think the best way to do this is to work in pairs and basically the two things you want to do is you want to try and have a quick linear motion after the frog, not a big looping thing cause you'll never get them. You can work in pairs effectively. Sometimes by having one person hold the net and the other person put their foot behind the frog and chase it into your net. You'll just sort of develop your own style as you work together. So let's give it a try.

Metamorphs

Sherry: discusses the southern leopard frog-Rana sphenoccephala; cricket frog-Acris crepitans; gray tree frog-Hyla versicolor; green frog-Rana clamitans melanota (11:58-12:48)

Tadpoles

Narrator: Although you will be collecting metamorphs, you should also be familiar with tadpole and adult morphology. (12:48-12:55)

Sherry: discusses tadpoles of the green frog-Rana clamitans melanota; bull frog-Rana catesbeiana; northern cricket frog-Acris crepitans (12:57-13:43)

Adults

Sherry: discusses adults of the green frog; southern leopard frog; cricket frog (13:48-14:25)

Measuring & Handling

Sherry: We've been surveying for about an hour now and we've collected all of our frogs in this little tub and we're going to put them in the bigger tub and it has some water in the bottom approximately half an inch to an inch and a cold pack to keep the frogs comfortable so they don't over heat. We've got several species in here. You need a tub with very high sides because as you can see they immediately start to come to the top.

Fred: We've collected about fifty metamorphs from this pond and now we are going to do our measurement and examination of each animal. We take the frogs from our individual plastic containers and place them in this large rubber enclosed bucket and we have about one half inch to one inch of pond water in the bottom to keep the animals moist. Now Sherry has just reached in and collected this green frog. She's moistened her hands to keep them wet so she won't injure the frog during handling. The first thing she's going to do is to turn the frog over and measure the snout to vent length. We have operationally defined 40 millimeters as the largest size for a green frog metamorph. What is the length of this one?

Sherry: 42 millimeters.

Fred: By the terms of our study this animal would not be used and would be released.

This is a metamorph cricket frog. These are much smaller than the green frogs and we've operationally defined the maximum size for a cricket frog metamorph at 15 millimeters. Sherry is now measuring the snout to vent length.

Sherry: 14 millimeters.

Fred: I record that on the data sheet. Since this still has a tail we take a separate measurement of the tail length.

Sherry: 21 millimeters.

Fred: She starts the examination looking at the head. Is the frog missing any eyes?

Sherry: No

Fred: Is there a small eye?

Sherry: No

Fred: Is the iris normal?

Sherry: Yes

Fred: Is there a displaced or malpositioned eye?

Sherry: No

Fred: Is the head small or domed?

Sherry: No

Fred: Is there a lower and upper jaw?

Sherry: Yes

Fred: Is there any pigment lacking?

Sherry: No

Fred: Is the pattern abnormal?

Sherry: No

Fred: Is there a curvature of the spine?

Sherry: No

Fred: Is there an extension of the spine beyond the rump?

Sherry: No

Fred: Now we move on to the examination of the limbs.

Are there four limbs?

Sherry: Yes

Fred: And two hind limbs?

Sherry: Yes

Fred: Is the hind limb complete?

Sherry: Yes

Fred: Is there a complete foot?

Sherry: Yes

Fred: Is there any skin webbing?

Sherry: No

Fred: Is there any evidence of a bone bridge? (This would be an abnormal shape of the hind limb)

Sherry: No

Fred: Any evidence of rotation?

Sherry: No

Fred: The digits on the hind limb – are there five digits?

Sherry: Yes

Fred: Is there complete fore limb?

Sherry: Yes

Fred: Is there any evidence of skin webbing?

Sherry: No

Fred: Any evidence of a bone bridge?

Sherry: No

Fred: Rotation?

Sherry: No

Fred: Are there all four digits?

Sherry: Yes, all four there.

Fred: The examination is complete. We did not observe anything abnormal with this frog, and it would be released back into the pond. If there was any abnormal appearance to this animal, we would put it in a small jar with holes and moist vegetation, take it back to the laboratory for photography and then decide whether it would be shipped for x-ray analysis.

Narrator: Release any tadpoles, adult frogs, and normal metamorphs back to the pond only after you have collected 50 metamorphs. Hold the metamorphs in a cool, shady spot until you can release them. Set up your measurement station in the shade as well.

New Field Processing section shot in August 2004

Laura Eaton-Poole: Fred and Sherry showed us a way to process frogs in the field and that was a great way to do it when you're first getting started and you're not familiar with the types of abnormalities that you might run into, but you are processing fifteen to a hundred frogs per site, so it can be rather time consuming and laborious. So once you are familiar with the abnormalities, you might want to use a little bit of a short cut that is still systematic. What we often do is hold the frogs underneath the elbows so that their legs are dangling down, and then look at the body parts in the same order with each frog. I look at the eyes making sure they are symmetrical and look normal. Check the jaw. Check down the back for any potential scoliosis. Check the front toes – four on the front. Make sure they're all the proper length, symmetrical on both sides. Dangle the hind limbs so that you can see that they are the same length and that he has five toes on each foot and that the toes are symmetrical on both sides.

We do have a few abnormal frogs that we can share with you. These come from the state of Maryland. I like to point out that it can be highly unpredictable when you're going to run into abnormal frogs. We can sample a site for a number of years and think it's a reference pond, and then all of a sudden you've got abnormal frogs. We sample once in July – we have a limited season here in the northeast. We can sample once in July and have all normal frogs and then sample again in August and get a crop of abnormalities and the types of abnormalities we see can be highly unpredictable. This guy is particularly interesting it's actually a little amazing that he is alive and probably wouldn't be for long in the field. I'll show how we might process this fellow, trying to hold him underneath the elbow. You will note that we have no hind limbs what so ever. This fellow has a really interesting abnormality, example of an eye abnormality. As you can see his right eye is enlarged and all black. My guess is it's an injury but we may never really know for sure exactly what has caused it. Here we have another abnormal frog from the same site. He has a slightly shortened femur and below that the calf and foot are much reduced and disfigured. One last thing we liked to mention is the importance of having clean hands when handling frogs. They do have very absorbent skin, so if you have mosquito repellent on your hands, nicotine from smoking cigarettes, or hand lotion on your hands it could be a problem for the frogs. So make sure that you clean your hands before you go out in to the swamp and get them muddy and dirty. Now we're going to head back into the lab to prepare these frogs for shipping.

Narrator: If you have arranged with a pathologist to examine live frogs, the following section demonstrates how to ship those study animals. All of the SOP's are included in your manual. (19:38-20:05)

Packing & Shipping Live Amphibians

Laura Eaton-Poole: We've completed our field sampling, and since we did collect some abnormal frogs, we have decided to ship them for further analysis. Possible destinations include Parasol otology, pathology, and radiography. It is really important that you contact the people

before you ship to make sure that they will be available to collect the frogs at their destination. It's usually best to make sure the frogs arrive before Friday. The types of containers you might want to use for shipping include hard plastic Tupperware containers, which is what we've used in the past. You can use containers that you might have floating around the lab as long as you can perforate the lids. We have found these new throw away Tupperware containers to work very, very well for us and it's really easy to perforate the lids. It's also critical that you label each one of your containers with a frog number. This is the same frog number that would correspond to the frog number that you put in the database. As you put the frogs in the containers, give them their frog number, you also want to be filling out your shipping form that we're going to be including in this shipment. It's very important that the frogs stay very moist during shipping. One way of doing that is to use site water and site vegetation. If you don't have that available to you, you can use absorbent paper towels. We recommend that you use paper towels that don't have any coloring in them. Some people feel strongly that you not use paper towels that have bleach in them; however, they can be hard to find and are not very absorbent. We have been at times using the absorbent white paper towels, as far as I know they work all right. It is very important the type of water you use. You don't want to use any water that has chlorine or salt in it. So you can use well water or you can use drinking water that you got from the store. So you want to moisten your paper towel. Put it in the bottom of your container and get the frog in the container without mashing it with the lid. Once you have all of your frogs put into containers, size of the container does depend upon the size of the frog. For instance, I have an adult frog in this container and a very small pickerel frog in this container. As you prepare to ship, you want to put cold blue ice on the bottom of your containers. Cover the blue ice with flat newspaper. It's important that the frogs not have contact with the blue ice as it will freeze them and the idea is to get the frogs to their destination alive. There's a certain amount of common sense here in that you want to have the frogs in there snuggled up. I usually use crumpled newspaper to snug them up. And then I put more crumpled newspaper on top just in case the cooler gets tipped over or tipped on its side. Remember, whoever is shipping these frogs aren't going to know you have live animals in there that they need to keep alive. Once you've completed your shipping form, we usually fold it up and put it in a zippy bag to keep it from getting wet. And tape it to the lid of the cooler. And then you want to thoroughly tape down the cooler. Wrap tape around it. We label the coolers biological samples – we never write on them live frogs. Fedex usually doesn't appreciate live animals in coolers, but you can check your protocols for more details.

Preserving Euthanized Frogs for Shipping

Narrator: You should euthanize suspected abnormal frogs according to the method recommended by your regional coordinator. Not all survey teams will be required to conduct this step. This segment of the video guides you through the process of preserving euthanized animals.

Laura: You need a waxed bottom Tupperware container, ethanol, some surgical scissors, some pins, and surgical tape that I've already placed at the bottom of the table.

Now this container is essentially hand-made. We melted beeswax on the bottom of the Tupperware. The advantage of this over a dissection tray is that you can place a top over it which is, since we will be pouring ethanol in it-there will be fumes coming out of it, so we highly recommend having a top. If you can't find beeswax like the Chesapeake Bay office found, the New England field office melted the wax rings you use at the base of a toilet. I don't think it worked quite as well as the beeswax but in a pinch it did work. Now what's most important for radiology is that the frog be flat, but since we are preserving in ethanol, it is also very important to open the body cavity when your preserving informal and it absorb into the body cavity readily, and preserves the organs, but ethanol isn't absorbed as easily so we have to open the body cavity. Then we flatten the frog out as best we can. We try to spread the toes

since you can have abnormalities of the toes you want to try and get a clear x-ray of each of the toes. This requires a certain amount of patience. We are going to try and pin him flat. Pinning into the flesh of the frog just doesn't really work that well and the tape doesn't really hold to the wax all that well. So what we found we can do is tape the limb down and then pin the tape into the wax and that seems to work pretty well. I'm going to do that right next to the limb, and it helps to have cut your tape in advance because it's not that easy to work with, especially with gloves on. Now, we are working with a normal frog for this demonstration. It's recommended that you preserve the frog in 90% ethanol. We just need to cover him. You can leave him in here for a good two weeks and then move him to a jar with 70% ethanol -90% ethanol dries a little too much so use a more dilute solution for long-term storage. Keep the top on it-keep the fumes down.

Narrator: Any chemicals that you have used in either your euthanasia or preservation procedure should be captured and disposed of properly. Do not release any chemical into the environment. (29:50-30:03)

Decontamination

Sherry: Now we're going to begin the decontamination process. It's important to do this between each sampling location or refuge and the reason we do this, this eliminates the spreading of pathogens from one site to another site.

Fred: So the first thing we do is use a hose, a garden hose, to spray off the mud and the big pieces of debris that are on either the net or the waders, and then we want to make about a 5% solution of bleach, which we will pour into a garbage can and we will fill the rest of the garbage can with water with the hose. Then we just want the net and the boots to soak for at least 10-15 minutes in this solution to really make sure that we've decontaminated any disease organisms. So after the equipment has soaked for a while in the bleach solution, we use a brush, and we give it one final brushing to try and make sure we remove all the debris as best we can. Now once we finished, we take our equipment out and we hose it down again with the hose so that the bleach solution is gone. We don't want to be carrying around bleach in our nets when we're examining frogs. (30:28-32:47)

Sherry: If you have any further questions, please contact your Regional Amphibian Coordinator, the Washington Office Division of Environmental Quality, or Fred, Laura, or myself. (32:50-33:00)