Survey Methods for Frog Abnormalities on National Wildlife Refuges
Training Guide Companion to Video

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Chapter 1

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US Fish and Wildlife Service
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ABNORMALITY CLASSIFICATION SOP

A. Objective: To classify frog abnormalities for analysis.

B. Background: There is variability in what researchers term "abnormal" when reporting the prevalence of abnormal frogs. Researchers who focus on the prevalence of skeletal and eye abnormalities often exclude traumatic injuries, diseases, and surficial abnormalities or infections from their reports (Eaton et al. 2004; Helgen et al. 2000; Hoppe, 2000; Hoppe, 2005; Johnson et al. 2001; Levey 2003; Ouellet et al. 1997; Schoff et al. 2003, Taylor et al. 2005). Other researchers include abnormalities and diseases but not obvious injuries when reporting the prevalence of abnormal frogs (McCallum and Trauth 2003), and still others report all gross physical abnormalities (Dubois 1979; Johnson et al. 2002; Lannoo et al. 2003). Because of this variability, a decision was made in the USFWS Abnormal Amphibian surveys to categorize frogs with any gross physical abnormality as "abnormal" to ensure consistency in field data collection (USFWS 1999). Consequently, the results from our surveys include more abnormalities than are reported by most researchers studying abnormal frogs. We developed this abnormal classification standard operating procedure (SOP) to enable a more accurate comparison of our field data with published research. Regional amphibian coordinators will utilize this SOP to further refine our "abnormal" category and ensure consistency in our classification of abnormal frogs. The SOP combines abnormalities into categories and subcategories, which may stem from similar causes, and provides us with more power to detect relationships between environmental variables and certain types of abnormalities. The abnormality classification SOP has its origins in the *Field Guide to Malformations of Frogs and Toads with Radiographic Interpretations* (Meteyer, 2000), which describes malformations in detail with photographs and radiographs, and in the body of literature cited above. Additional support for this SOP comes from knowledge gained from a large subset of newly metamorphosed frogs radiographed and analyzed for parasites and diseases between 2000 and 2005 for the Abnormal Amphibian surveys. Those analyses provided insight into the gross appearance of certain injuries, malformations, infections, and diseases.

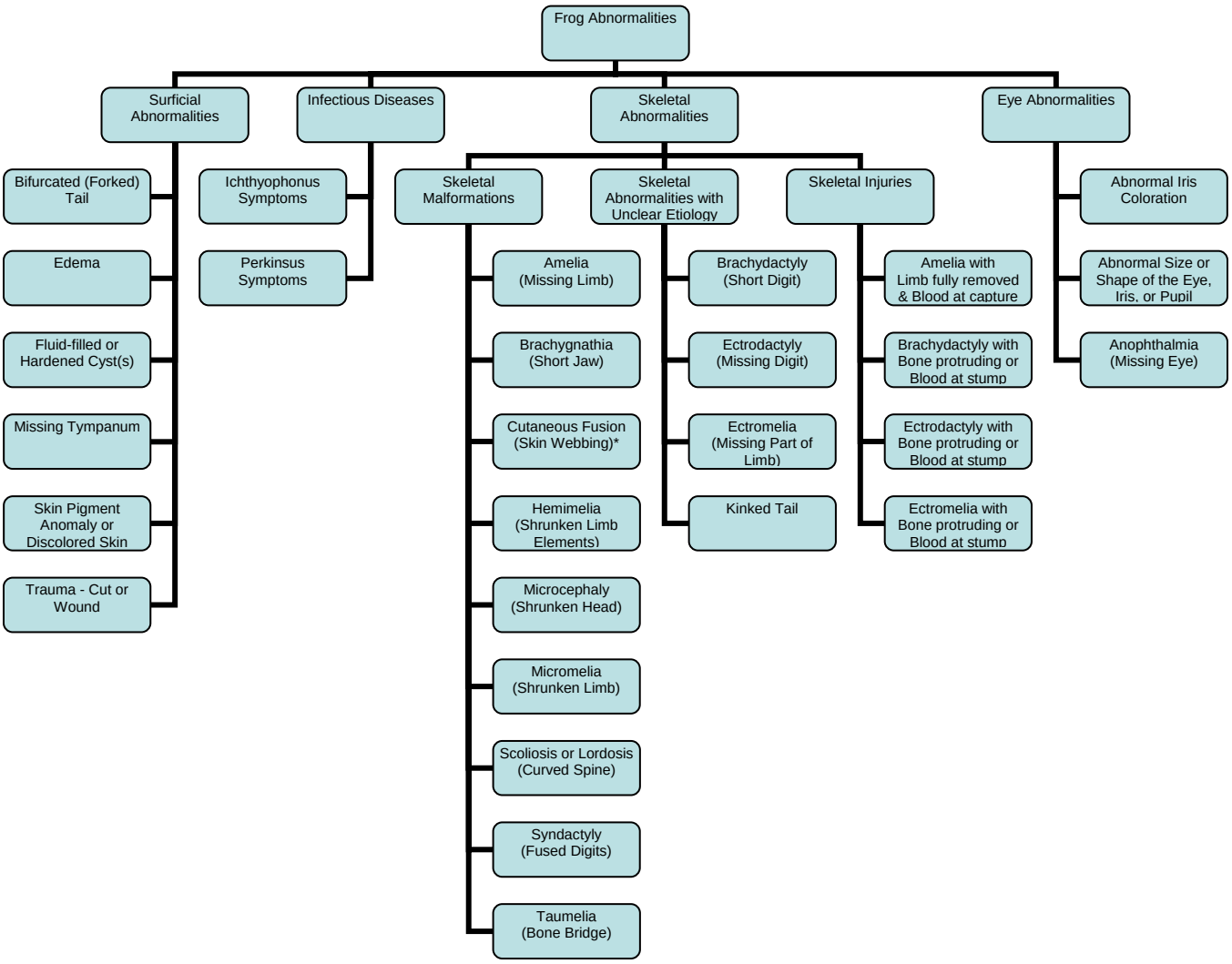
C. Existing Definitions: We recognize there are different definitions and classification schemes that have been used in published literature or by the public when describing the gross physical condition of an amphibian. For our purposes, we will use the following definitions from Johnson et al. (2001) as they are best suited to the goals of the USFWS Abnormal Amphibian surveys:

i. **Abnormality**: Any gross deviation from the normal range in morphological variation, be it traumatic or developmental.
ii. **Malformation:** Permanent structural defect resulting from abnormal development.
iii. **Deformity:** Alteration in an organ or structure that originally formed correctly. Deformities result from mechanical factors, such as amputation.

D. **Existing Resources:** The USGS field guide to common malformations in frogs and toads:

Meteyer, C.U. 2000. *Field Guide to Malformations of Frogs and Toads with Radiographic Interpretations*, 2000. Biological Science Report USGS/BRD/BSR-2000-0005.

E. **Further Classification:** An analysis of project data from 2000-2006, and of the available literature, suggests that the initial USFWS "Abnormal" classification can be further subdivided into the following categories: Surficial Abnormality, Infectious Disease, Skeletal Abnormality, and Eye Abnormality, according to the flow chart below. Skeletal Abnormality is further divided into 3 subcategories: Skeletal Malformation, Skeletal Abnormality with Unclear Etiology, and Skeletal Injury. The flow chart is not exhaustive for all abnormalities, but rather shows abnormalities commonly encountered by USFWS employees. The text following the diagram has a more extensive list of abnormality types in each category, as does Table 1. The terminology for each abnormality is taken from Meteyer (2000). Photographs of some common abnormalities are included in the text. **NOTE: Every USFWS employee using this SOP should <u>first</u> use the Meteyer (2000) guide to identify the type of abnormality (or abnormalities) that a frog has before using this SOP to further classify the abnormal frog**.



*Although skin webbing is not technically a skeletal malformation, we have decided to include it under the skeletal malformation category for several reasons. First, skin webbing, along with other skeletal malformations such as polymelia, polydactyly, and taumelia, may be diagnostic of *Ribeiroia* infections. Second, skin webbing restricts the

movement of the skeletal system and, as such, could be viewed as a type of skeletal abnormality. Finally, skin webbing has been described in Meteyer (2000) as a malformation. For the purposes of this SOP and the USFWS Abnormal Amphibian surveys, skin webbing will be categorized as a skeletal malformation.

F. Surficial Abnormalities: Surficial Abnormalities include abnormal pigmentation, wounds, scars, cysts, infections, subcutaneous hemorrhaging, or edema. Wounds can be caused by predation or other trauma. These may leave hematomas (bruises) or scars, which disrupt the normal skin pigmentation and appear a different color than the rest of the animal (red, brown, black, or even bluish). Split or forked tails may be healed injuries and are therefore considered surficial abnormalities. Edema, or fluid-filled swelling under the skin, is considered a surficial abnormality for this SOP. Small, fluid-filled or hardened cysts not affecting the skeletal system also should be included in this category for the purposes of this SOP. Some species of trematodes and nematodes create rather distinctive cysts on the surface of the skin.

G. Infectious Diseases: Frogs exhibiting disease symptoms that manifest as physical abnormalities should be classified in the disease category. Two diseases encountered and diagnosed between 2000 and 2005 are a Perkinsus-like protozoan organism and Ichthyophonus. Perkinsus symptoms include severely swollen viscera that lead to a bloated body and an infected, swollen (enlarged) heart. The swollen heart is occasionally displaced to the throat area and is visible through the skin. Ichthyophonus symptoms include a swollen tail resorption site that matches the surrounding skin in color and translucency (i.e., not a cyst).

H. Skeletal Abnormalities: The Skeletal Abnormality category comprises three subcategories: Skeletal Malformations, Skeletal Abnormalities with Unclear Etiology, and Skeletal Injuries. If a frog has any of the abnormalities in these three subcategories, it should be placed in the Skeletal Abnormality category, and also in the appropriate subcategory.

i. **Skeletal Malformations:** Frogs with skeletal malformations have skeletal systems that have grown in an abnormal way. Examples of skeletal malformations include:

1. small head or blunt snout (microcephaly),
2. shortened or malformed jaw (brachygnathia),
3. curved spine in a lateral direction (scoliosis),
4. curved spine in a dorsoventral direction (lordosis),
5. completely missing limbs with no stump (amelia),
6. shrunken limb (micromelia)
7. shrunken limb elements, such as shortened tibiae, fibulae, or metatarsals (hemimelia),
8. extra limb or limbs (polymelia),
9. bone bridge (taumelia),
10. extra digits (polydactyly),
11. fused digits (syndactyly),
12. digit bent at right angle to normal trajectory (clinodactyly),
13. extra bones in a digit (polyphalangy),
14. rotated long bones with reversed digit order (anteversion), or
15. skin webbing that restricts skeletal movement (cutaneous fusion).

ii. Skeletal Abnormalities of Unclear Etiology: If a frog is missing part of a limb (ectromelia) or has a shortened or missing digit or digits (brachydactyly or ectrodactyly) yet there is no evidence of trauma (i.e., no blood visible at capture

and the bone is contained within the skin), it should be scored as a skeletal abnormality of unclear origin. If a leg stump is present, even if very small, the abnormality should be classified as ectromelia.

iii. **Skeletal Injuries:** If a frog has a missing or broken limb (or part of a limb) where either blood is noted at capture or bone is protruding through the skin, the abnormality should be classified as a Skeletal Injury. *There must be evidence of trauma (e.g., blood or a broken bone) to qualify for this category*.

I. Eye Abnormalities: Any abnormality of the eye should be classified in this category. The most common eye abnormalities are anophthalmia, abnormal iris coloration, and abnormal eye, iris, or pupil sizes.

i. **Anophthalmia**: The eye is not present, and skin is grown over where the eye should be.

ii. **Abnormal Iris Coloration**: The eye is present, but something is unusual about the coloration. The most common eye color anomalies are heterochromia (the eyes are two different colors), reduced pigment in one or both eyes, and melanistic or "black" eye, where either one or both eyes are all black.

iii. **Abnormal Size or Shape**: One eye, iris, or pupil is a different size or shape than the other, or both eyes deviate from the normal size range of the rest of the population.

Table 1. Abnormality descriptions for different classification categories and subcategories.

CATEGORY and SUBCATEGORY	ABNORMALITY DESCRIPTION
SURFICIAL ABNORMALITY	BLOOD POOLED IN LEGS OR ELSEWHERE
	CYST OR LUMP UNDER SKIN
	DISCOLORED SKIN - INDICATIVE OF SCARRING
	EDEMA
	FLESH CUT OR WOUND WITH BLOOD
	BIFURCATED OR FORKED TAIL
	INTESTINES PROTRUDING
	MISSING TYMPANUM
	OTHER PIGMENT ANOMALY
	SORE OR WOUND BUT NO BROKEN BONES, AND/OR NO DISLOCATIONS
INFECTIOUS DISEASE	ICHTHYOPHONUS SYMPTOMS
	PERKINSUS SYMPTOMS
SKELETAL ABNORMALITY	
Skeletal Malformation	AMELIA - MISSING LIMB
	ANTEVERSION - TOES CURLED INTO FIST OR FINGERS/TOES IN REVERSE ORDER WITH NO BLOOD
	BRACHYGNATHIA - SHORTENED OR MALFORMED JAW
	CLINODACTYLY - DIGIT BENT AT RIGHT ANGLE TO NORMAL TRAJECTORY
	CUTANEOUS FUSION - SKIN WEBBING THAT RESTRICTS SKELETAL MOVEMENT
	HEMIMELIA - SHRUNKEN LIMB ELEMENTS - SOME ELEMENTS ARE SHRUNKEN, BUT NOT ALL
	LORDOSIS - SPINAL CURVATURE IN A DORSOVENTRAL DIRECTION
	MICROCEPHALY - SMALL HEAD OR SHORTENED SNOUT
	MICROMELIA - SHRUNKEN LIMB - ALL LIMB ELEMENTS SHRUNKEN INCLUDING FOOT
	POLYDACTYLY - EXTRA DIGITS
	POLYMELIA - EXTRA LIMB
	POLYPHALANGY - EXTRA BONES IN A DIGIT
	SCOLIOSIS - SPINAL CURVATURE IN A LATERAL DIRECTION
	SYNDACTYLY - DIGITS FUSED TOGETHER
	TAUMELIA - BONE BRIDGES OR BONY TRIANGLES
Unclear Etiology	BRACHYDACTYLY - TOES SHORT OR MISSING PHALANGES WITH NO EVIDENCE OF TRAUMA
	ECTRODACTYLY - DIGIT OR DIGITS MISSING WITH NO EVIDENCE OF TRAUMA
	ECTROMELIA - MISSING PART OF LIMB - NO BLOOD OR BONE PROTRUDING (OR NOT MENTIONED)
	KINKED TAIL
	NON-FLEXIBLE LIMB
	ONE LEG THINNER THAN OTHERS
Skeletal Injury	AMELIA - LIMB FULLY REMOVED WITH BLOOD AT CAPTURE
	APPENDAGE DISLOCATED, DANGLING, OR HANGING OFF
	BRACHYDACTYLY - TOES MISSING PHALANGES WITH PROTRUDING BONES OR FLESH WOUND
	ECTRODACTYLY - DIGIT OR DIGITS MISSING WITH BLOOD OR PROTRUDING BONE
	ECTROMELIA - MISSING PART OF LIMB - WITH BLOOD OR PROTRUDING BONE
	TOES CURLED OR SMASHED WITH BLOOD
EYE ABNORMALITY	ABNORMAL IRIS COLORATION - HETEROCHROMIA (EYES TWO DIFFERENT COLORS), REDUCED PIGMENT IN IRIS, OR UNPIGMENTED IRIS (ONE OR BOTH EYES ALL BLACK)
	ABNORMAL SIZE OR SHAPE OF EYE, IRIS, OR PUPIL
	ANOPTHALMIA - MISSING EYE

References

Dubois A. 1979. Anomalies and mutations of natural populations of the *Rana "esculenta"* complex (Amphibia, Anura). *Mitteilungen aus dem Zoologischen Museum in Berlin* 55(1): 59-87.

Eaton BR, Eaves S, Stevens C, Puchniak A, Paszkowski CA. 2004. Deformity levels in wild populations of the wood frog *Rana sylvatica* in three ecoregions of Western Canada. *Journal of Herpetology* 38: 283-287.

Helgen JC, Gernes MC, Kersten SM, Chirhart JW, Canfield JT, Bowers D, Haferman J, McKinnell G, Hoppe DM. 2000. Field investigations of malformed frogs in Minnesota 1993-1997. *Journal of the Iowa Academy of Science* 107: 96-112.

Hoppe DM. 2000. History of Minnesota frog abnormalities: Do recent findings represent a new phenomenon? *Journal of the Iowa Academy of Science* 107(3): 86-89.

Hoppe DM. 2005. Malformed frogs in Minnesota: History and interspecific differences. In: *Amphibian Declines: The Conservation Status of United States Species*, pp. 103-108. University of California Press, Berkeley, California.

Johnson PTJ, Lunde KB, Ritchie EG, Reaser JK, Launer AE. 2001. Morphological abnormality patterns in a California amphibian community. *Herpetologica* 57(3): 336-352.

Johnson PTJ, Lunde KB, Thurman EM, Ritchier EG, Wray SN, Sutherland DR. 2002. Parasite *Ribeiroia ondatrae* infection linked to amphibian malformations in the western United States. *Ecological Monographs* 72: 151-168.

Lannoo MJ, Sutherland DR, Jones P, Rosenberry D, Klaver RW, Hoppe DM, Johnson PTJ, Lunde KB, Facemire C, Kapfer JM. 2003. Multiple causes for the malformed frog phenomenon. pp. 233–262. In: Linder G, Krest S, Sparling D, and Little, E. (Eds.), *Multiple Stressor Effects in Relation to Declining Amphibian Populations*. American Society for Testing Materials International, West Conshoshocken, Pennsylvania.

Levey R. 2003. Investigations into the causes of amphibian malformations in the Lake Champlain basin of New England. Vermont Department of Environmental Conservation, Waterbury, Vermont.

McCallum ML, Trauth SE. 2003. A forty-three year museum study of northern cricket frog *Acris crepitans* abnormalities in Arkansas: Upward trends and distributions. *Journal of Wildlife Diseases* 39: 522-528.

Meteyer CU. 2000. *Field guide to malformations of frogs and toads with radiographic interpretations.* Biological Science Report USGS/BRD/BSR-2000-0005. U.S. Geological Survey, Madison, Wisconsin.

Meteyer CU, Loeffler IK, Fallon JF, Converse KA, Green E, Helgen JC, Kersten S, Levey R, Eaton-Poole L, Burkhart JG. 2000. Hind limb malformations in free-living northern leopard frogs (*Rana pipiens*) from Maine, Minnesota, and Vermont suggest multiple etiologies. *Teratology* 62: 151-171.

Ouellet M, Bonin J, Rodrigue J, DesGranges J, Lair S. 1997. Hindlimb deformities ectromelia, ectrodactyly in free-living anurans from agricultural habitats. *Journal of Wildlife Diseases* 33: 95-104.

Schoff PK, Johnson CM, Schotthoefer AM, Murphy JE, Lieske C, Cole RA, Johnson LB, Beasley VR. 2003. Prevalence of skeletal and eye malformations in frogs from north-central United States: Estimations based on collections from randomly selected sites. *Journal of Wildlife Diseases* 39: 510-521.

Taylor B, Skelly D, Demarchis LK, Slade MD, Galusha D, Rabinowitz PM. 2005. Proximity to pollution sources and risk of amphibian limb malformation. *Environmental Health Perspectives* 113: 1497-1501.

USFWS. 1999. Standard operating procedures for abnormal amphibian surveys. U.S. Fish and Wildlife Service, Annapolis, Maryland.

GENERAL SOP

A. Objective

1. To determine the prevalence of abnormalities in frog populations on National Wildlife Refuges.

B. Procedures

1. Scout for suitable amphibian breeding areas on refuge. The timing will be region specific, but generally begins in mid-late April.

2. Evaluate sites using the **Site Assessment SOP**.

3. For each suitable site selected, fill out a **Site Characterization Form**:

   - Assign a unique 5 character Site ID to all sites that will be monitored throughout season using the 3 character **Refuge ID Codes**, followed by sequential two digit numbers (ex: FSL01) [Note: the Refuge Code has been changed from a 2 character code to a 3 character code to be consistent with other FWS databases].

   - Take digital photographs of the site that best represents the habitat.
     - Name photos using standard format: FSL01-N-050103

   (Site ID – cardinal direction facing when photo was taken – date photographed without dashes)
     - Take additional photos throughout season to document any significant changes.
       If digital camera is not available, submit to your regional coordinator:
       (in order of preference): prints, 35 mm slides, or film negatives

   - Collect position data using a GPS unit and record on **Site Characterization Form**.
     - Required datum & format: WGS 84 in decimal degrees (hddd.ddddd°)
     - Required metadata: Make/model of GPS unit used.

4. Monitor selected sites for developing amphibians regularly once tadpoles are found. Record the following data on standard **Data Collection Forms** on each and every site visit:
   - Refuge Name, Site ID, Date, Collectors, Start & End time, ambient air and water temp (C°)
   - Note the presence, number and average water depth for any anuran egg masses (ID egg masses to species when possible).
   - If tadpoles are present, determine species & Gosner stage on 10 tadpoles for all species found.
   - Record any incidental species encountered (particularly potential predator species).
   - Record comments regarding when and why (or why not) a site should be revisited.

5. The goal is four full metamorph collections/refuge/season. Depending on the species of frogs in your region, you may or may not attain this goal. If possible, you should make two collections of between 50-100 metamorphs/cohort from a minimum of two sites per refuge each season. Collections can consist preferably of two distinct cohorts of the same species from each site or of two different species through the season from each site. Given the highly unpredictable nature of the weather and frog breeding activity, to ensure the minimum collections are made, it is advisable to aim at collecting multiple cohorts from multiple species from as many sites as possible. Select enough sites to monitor throughout the season to increase your chances of success.

6. Sites must fall within the refuge boundaries and whenever possible should be selected to reflect areas on the refuge with suspected contaminant inputs as well as those without such inputs. Clearly, you will have to collect "where the frogs are" which may or may not coincide with our selection criteria. Metamorphs should be collected using the **Capture SOP** and processed using the **Data Collection SOP.** All data should be recorded using standard **Data Collection Forms**.

7. If abnormal metamorphs are found, they should be documented with digital photographs and an **Abnormal Frog Form** for each individual. Consult your regional coordinator to determine if they need to be sent for diagnostics. If specimens are targeted for diagnostics, then prepare specimens using either the **Specimen Preservation SOP** or ship live according to the appropriate **Shipping SOP**. Track all abnormal specimens carefully on **Specimen Log** and copy your regional coordinator on all documentation that accompanies shipments.

8. Communicate any difficulties you encounter throughout the season to your regional coordinator as soon as possible. A regional teleconference with all project leaders may be scheduled to discuss sampling progress, diagnostic needs for abnormal specimens and any problems the group may have encountered.

9. Reporting requirements will include submission of all of the following to your regional amphibian coordinator by no later than September 30.
   - Site Characterization Forms
   - Sample Site & Specimen Photos (digital preferred)
   - Map (refuge brochure map with sampling sites labeled by hand is sufficient)
   - Data Collection Forms (electronic forms preferred)
   - Abnormal Frog Forms
   - Specimen Log (electronic forms preferred)
   - Preserved voucher specimens
   - Documentation of any animals shipped

NOTE: S*ubmitting your data throughout the season as it is collected or at any point before the deadline would be preferred and appreciated!*

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SITE ASSESSMENT & MONITORING SOP

A. Objective

1. To determine suitability of wetlands for frog sampling and guidance for site visits.

B. Procedures

1. Suitable frog sampling sites will primarily be small, isolated wetlands. When possible, sites should be selected to represent any impacted areas of the refuge as well as areas free from any known impacts. Contaminants of concern and surrounding land use should be determined for every selected site. Consult with refuge personnel who might be familiar with the variety of refuge habitats and current and historic activities on/off refuge.

2. When first scouting for suitable sites, it is also helpful to drive around the refuge on warm, humid evenings to locate wetlands where adult frogs are chorusing. If distant chorusing cannot be pinpointed after dark, take GPS coordinates (**see GPS SOP**) to facilitate finding wetland during the day. Considering that the peak breeding/chorusing seasons will vary between species throughout the season, it is helpful to periodically repeat these scouting trips for breeding choruses. Even if you feel sufficient sites have initially been selected, additional suitable sites may be added at any point during the season and may provide valuable data for another taxa that may not choose to breed at one of the previously selected sites.

3. A **Data Collection Form** should be filled out *every time* you visit a site for monitoring purposes. This will provide you the information needed to schedule when to revisit a site later this season AND will provide valuable information for anyone conducting the subsequent seasons monitoring efforts. Even when no tadpoles or metamorphs are found on a site visit, fill out as much of the form as possible: date, Site ID, collectors, ambient air and water temperature, presence of egg masses, water depth and general weather conditions, etc.

NOTE: It is very important to maintain detailed field records of your efforts, given the very unpredictable nature of this project. We may get to the end the season without encountering metamorphs at a particular site where ample tadpoles were found. There would not only be a record to justify your efforts, but the information needed to guide future investigations may become evident in your field notes. The lack of successful breeding activity at suitable sites, or impairment of proper development and subsequent metamorphosis are of concern and would otherwise be difficult to see.

4. When visiting a wetland, you should walk the perimeter looking for metamorphs that jump into the water or into marginal vegetation. If metamorphs are present, capture them using the **Capture SOP**. If metamorphs are infrequently encountered or appear to be absent, dip-net the edges of the pond (sweep the net along bottom and bring it all the way into the bank).

5. If any tadpoles are encountered, record species (when possible) and stage of development (Gosner 1960) of at least 10 tadpoles for each species encountered. Any individuals with both

hind and forelimbs emerged are considered metamorphs and should be collected according to **Capture SOP** (note maximum size restrictions).

6. Determine when or if the site should be visited again based on the following factors:
   a) Ephemerality of the wetland. Does the wetland appear that it will hold sufficient water for long enough to allow development of resident anurans?
   b) Productivity of the wetland. Consider amount of submerged aquatic vegetation, insect life, # tadpoles present, and amount of cover available for frogs. Metamorphs are more likely to stay in/near a productive wetland longer after transformation than in an unproductive mud hole, thus your chances of intercepting them before they emigrate from the area are greater in productive areas. (CAUTION: more productive areas may also be more difficult to sample).
   c) Developmental stage of any tadpoles present (see Section 2 of Training Manual for guidance on the expected window of development for each species). Consider the amount of tree cover at each site when predicting developmental rates. Sites with full sun will most likely experience faster developmental rates than those in heavily wooded or otherwise shady areas.
   d) Projected weather. Because anurans may aestivate in mud or under live and dead vegetation, some wetlands may seem devoid of amphibians during very hot, dry weather, however, after a heavy rain storm or during a period of cool weather, the frogs may reappear. Also be aware that extreme changes in environmental conditions can drive development of tadpoles more quickly than expected. Don't base your site visit schedule through the entire season on the developmental rates encountered early in season, during cooler weather.
   e) Sample ease. Keep in mind that dense vegetation; steep banks and deep mud will all seriously hamper your ability to capture frogs.

NOTE: You should seriously consider the feasibility of sampling for each site early in the season, so you have time to select alternate sites before the breeding window is over. At MINIMUM, four sites per refuge (preferably half impacted, half un-impacted sites) should be monitored throughout the season to ENSURE at least the minimum required collections are feasible. Don't put all your eggs in one basket – so to speak. The resident frogs may not like that particular basket!

7. Keep in mind that bufonids (toads) can successfully breed in extremely short-lived pools and develop very quickly. Even when permanent water supplies are used, newly metamorphosed toads may range widely from the water's edge (this distance increases steadily with age and size). Treefrog metamorphs may begin climbing soon after transformation, so be sure to inspect the entire height of vegetation near the edge of the wetland.

NOTE: ON MONITORING TRIPS TO YOUR REFUGE, TRY TO VISIT EVERY SITE WHEN FEASIBLE; DO NOT ASSUME DEVELOPMENTAL RATES ARE SIMILAR BETWEEN NEARBY SITES OR YOU MAY MISS VITAL SAMPLING OPPORTUNITIES!

C. Reference

Gosner, K.L. 1960. A simplified table for the staging of anuran embryos and larvae with notes on identification. Herpetologica 16:183-190.

CAPTURE SOP

A. Objective

1. To capture 50 to 100 recently metamorphosed frogs. These guidelines will get you started, but once you actually encounter animals, you will likely develop your own technique quickly!

B. Procedure

1. The most efficient time to sample metamorphs is typically when ambient temperatures are mild (i.e. dawn or dusk). Most metamorphosed frogs will inhabit the edges of the wetland, preferentially choosing areas adjacent to shallow water. If there is a high degree of vegetative structure in the wetland, the metamorphs may be found in the interior of the wetland as well.

2. The field team should split into pairs or triplets and begin walking along the edge (or through other promising habitat). Because populations of metamorphs usually exhibit clumped distributions, when a productive area is found, the others should converge on that spot to capture as many frogs as possible before moving the teams elsewhere.

3. Metamorphs vary in size depending on species (consult your regional coordinator for information on species in your area). The listed snout-vent lengths (SVLs) should be used as a general guide. Use your best judgment to determine whether the frogs you have in hand all belong in the same cohort. As the season progresses, it will be very important to note SVL's to ensure you are not resampling the same cohort from the same site.

4. The most effective technique of capturing frog metamorphs is using a dip net . The type of net chosen depends on the habitat. If the edges are fairly open and there is not much debris in the water, a deep, cone shaped net seems to work best. In more dense wetlands, a heavy duty, fairly shallow net should be used. Depending on the characteristics of the site or target species, techniques will vary. Note: there are other acceptable methods for capturing frogs (e.g., sweep nets, traps, electroshocking,e tc.), however, please consult with your regional coordinator prior to using alternative methods. The capture method will depend on the habitat you are sampling

5. You can place the net in front of the selected frog and attempt to "herd" the individual into the net by stepping quickly towards it. This technique can be refined, by having two or more people strategically place their nets to cut off avenues of escape. If working alone, one can also try using a small aquarium net to "herd" the frogs into the larger net. When collecting treefrogs, it is often easier to pick the frogs from the vegetation by hand. However, if they are clinging to sharp edged or other dense vegetation try placing a net on the opposite side of vegetation and coax them to jump off into your net, in order to avoid injury to yourself & to the delicate froglets.

6. Try sweeping your net quickly through the water where a frog is seen (or where one recently jumped into the water). Often this requires the netter to sort through copious amounts of mud and leaf litter before finding the frog; however, this is the most effective technique when sampling densely vegetated wetlands. If frogs are difficult to collect once they have jumped into the water, often they will return to the margins if they are left undisturbed for some time. Make sure to revisit the areas where large numbers of metamorphs were seen (but may have escaped your net).

Spend time working other areas of the wetland or return to site the following day if ambient temperatures are extreme. Once frogs have entered the water during the heat of the day, they tend to stay there, whereas during milder weather, they tend to return to edges more readily if left undisturbed for a short time.

7. Once a frog is captured, it should be placed into a plastic container with a perforated lid, along with a moist paper towel or wet leaves from site. If the individual is a tadpole or still has a tail (>4mm), then it should be placed in a closed lid container or zip-lock bag along with water from the site. In this case, at least half of the container should remain air to prevent the water from becoming anoxic.

ALTERNATIVELY: plastic minnow bags can be used effectively during field capture. They are easily tucked under your belt to free the hands. Be sure to place no more than a few drops of site water to keep bag moist and blow air into bag to provide protective "pillow". Minnow bags should be used to hold no more than 5-10 animals at a time, depending on size/species and should NOT be filled with very much water. Frogs can drown if held in a large volume of sloshing water with no way to take a breath of air!

NOTE - Pickerel frogs (*Rana palustris*) secrete a substance from their skin, which is toxic to other amphibians (Green and Pauley 1987). This species should always be kept in a container separate from the others.

8. Once 50-100 individuals of a single species have been captured, all individuals should be processed. If at least 50 individuals could not be collected at once, animals should be held in a cool place to ensure that none are resampled. The site can be revisited for up to two more days to fulfill the minimum required sample size. Animals should not be held for more than three days before being released back to capture site. It is preferable to process animals on the day of capture for consistency. Do not wait until you get at least 50 to process them, take advantage of the time between visits to process any animals in hand. If even after repeated visits, the minimum of 50 animals could not be collected from a particular site, the **data for any and all metamorphs collected should be recorded.**

C. References

Green, N.B. and T.K. Pauley. 1987. Amphibians and reptiles in West Virginia. Pittsburgh.

DATA COLLECTION SOP

A. Objective

1. To collect the required data and inspect each metamorph for abnormalities.

B. Procedures

1. Once any metamorphs have been collected, the processing may begin. If processing animals that need to be held between additional collection days, make sure to clearly mark which containers have the processed individuals, so that no individuals are resampled.

2. Metamorphs can be held in small groups in the same containers used to collect them if they have sufficient moisture and air. Place the containers in a cooler, layered with blue ice on the bottom, then with a towel or other barrier between the ice & the frog bags. Transfer frogs into marked containers as they are processed. This method is particularly useful if you are working alone. The metamorphs will attempt escape from coolers or other large containers *every time* the lid is opened, which also presents the opportunity for injury to occur.

NOTE: Pickerel frogs *(Rana palustris*) should not be held in same container with any other species. Avoid having too much moisture with *Bufo* (toad) specimens, they can tolerate much drier conditions and are much easier to handle when they are dry. A full collection of toads (50-100) can easily be held in a deep plastic tub during holding or processing, with minimal risk of escape. Just make sure they stay cool & do not desiccate in an air-conditioned building if held overnight.

3. One person should record all data while others measure and inspect each frog. When possible, assign the same duties to the same crewmembers during each collection to keep data consistent throughout the season. If the crew is larger than three, it is helpful to have someone responsible for getting frogs out of holding and to keep track of which ones have/have not been processed. It is also very helpful for handlers to keep their fingers moist (with the exception of handling toads).

4. Identify species as accurately as possible and assign the appropriate code (see **Species Codes Table** in Section 2 of Training Manual). If there is any question on ID, assign the generic code (ex. BUFO for unknown *Bufo* sp.). This should only be the case where similar species coincide.

5. Measure snout to vent length (SVL= from the tip of nose to the cloaca/vent) using a 10 cm ruler. Place ruler on the ventral (belly) side of the selected frog. If there is a tail present, it should be measured separately by placing the edge of the ruler at the base of the tail. Record all information in the appropriate columns on **Data Collection Form**. All collectors and inspectors names should be noted on data sheets.

6. Determine developmental stage for each frog (see **Gosner Stage Chart** in Section 2 of Training Manual). Target stages are 44-46. Any stages after 42 (i.e. all four legs emerged) qualify as metamorphs, however the later staged animals will have more fully calcified bones and will be more

suitable for radiography. If an abnormal animal is found and is still at some stage between 42 and 44, it can be held in a cooler with site water until tail is more fully resorbed, prior to preservation.

CAUTION: Under no circumstances should late stage tadpoles (i.e. animals without hind and/or forelimbs already emerged) be held until their legs emerge! This would create an artificial environment during a critical developmental phase. Any data obtained in this manner must be excluded from the study.

7. Keep in mind, that if disease is evident or if parasitology is warranted, animals should not be held to maximize tail resorption, they should be shipped ASAP (see **Live Specimen Shipping SOP**).

8. After identifying, measuring and staging the frog, the inspector will examine it for any of the abnormalities listed on the standard **Abnormal Frog Forms** (or any others not listed):

a. Hold frog under front legs, with the hind legs dangling down to look for body symmetry. This may take some gentle coaxing of the animal to relax and allow the legs to hang freely.
b. Examine head and jaws for any abnormalities (missing or misplaced eyes, overbite, underbite, unfused, or shortened jaws). Be sure to look at eyes carefully to note the pupil/iris.
c. Examine the front legs, feet, and toes (look for clubbed or missing feet, extra or missing toes, extra or missing limbs, webbing in unusual locations, etc.).
d. Examine the fore and hind legs, feet, and toes.
E. Examine tail. At this point, pay attention to tail length & Gosner stage together. Not abnormalities of the tail [two categories: (1) kinked or bent tail or (2) cysts, lumps, or growths].
F.

NOTE: To ensure all categories are examined, it is advisable to consult an **Abnormal Frog Form** before inspections, particularly if it has been a while since you last inspected frogs.

9. If an abnormality is found, a standard **Abnormal Frog Form** will be completed for each individual abnormal frog. The individual will be placed in a uniquely labeled container or bag and kept moist with site water. See guidance on the form for assigning an appropriate Abnormal Frog ID.

NOTE: DO NOT MAKE ANY FIELD JUDGEMENTS ON CAUSE! A bloody stump that seems apparent to have been caused by a predator (or possibly by your boot or dip-net) may not have looked that way if you had inspected that particular animal one week later. So, in order to avoid bias in our samples, treat ANY animal that is not 100% normal as "ABNORMAL". Just be sure to note any obvious cause of trauma or injury in the comments section of **Abnormal Frog Form**. We don't want to lose any known information about an abnormality and we also don't want to release any injured animals as there may have been another cause which was not evident in the field (i.e. bloody stump of a leg may turn out to be an animal with a malformed pelvis (evident only through radiography), which made that animal more susceptible to being caught and injured by a predator).

10. Normal frogs should always be held until all animals in a collection are processed.

11. Once the entire collection has been processed, consider the following things to determine the fate of your hard-earned collection of metamorphs before releasing any of them at the collection site:

- Do the number and severity of abnormalities fit the criteria for diagnostic tests? (Consult with your regional coordinator for these criteria early in the season. If you are unsure, hold animals until you can contact your coordinator.)
- If parasitology will be required, determine if you need to retain any normal individuals from the collection to complete a total sample of 10 animals.
- Your Regional Coordinator may require you to preserve one normal representative individual for each collection on a refuge (as part of *this* study) as a voucher specimen. If species ID is uncertain, due to the very young age of the animals, consider keeping a few normal animals alive until they develop sufficiently to confirm ID. Keep in mind, this will require some husbandry and possibly several months or longer to exhibit specific diagnostic characteristics, so if you are not so inclined, just preserve one. Please check with your Regional Coordinator for details.
- If your collection consists of any species other than those in genus: *Rana, Bufo,* or *Hyla*, please preserve two normal individuals for comparative radiographic analysis.

12. Once you are certain of the fate of the collection, release any remaining normal frogs by dispersing them along the areas where they were caught. Do not dump them all in one spot because this may facilitate an unnatural predation event. Also, to reduce the possibility of physiological shock, it is preferable not to release chilled animals during the heat of the day. Either hold them until the next morning, or allow them to warm to ambient temperatures before releasing them.

13. Any abnormal animals should then be preserved or prepared for shipping according to the appropriate SOPs.

C. References

Gosner, K.L. 1960. A simplified table for the staging of anuran embryos and larvae with notes on identification. Herpetologica 16:183-190.

SPECIMEN PRESERVATION SOP

A. Objective

1. To preserve frogs and tadpoles so they may be used effectively as diagnostic or voucher specimens.

A. Procedures

1. Prepare a mixture of tricane methanesulfonate (MS-222) with a concentration of 0.5 g/L. This mixture will last for weeks if it is not exposed to sunlight. Store it in a dark bottle and in a cool dark place.

ALTERNATIVELY: A dilute chloretone solution (~10%), which will also keep for weeks if stored in a cool, dark place OR a benzocaine cream can be used to euthanized specimens.

2. Prepare all specimen tags using the standard naming convention (see **Abnormal Frog Form**). Tags should not be affixed to specimens, rather they should be placed inside each frog's individual shipping container. Tags affixed to frogs with wire can impede radiograph quality. And tags affixed with string can become tangled during shipment.

3. Set up a staging area for photo-documentation. Take time to experiment with different backgrounds and camera settings for best focus, lighting, etc. Macro is usually necessary for very small specimens. Note your optimized settings for future photo sessions.

4. Select a container appropriate for the size of specimens you have. Fill with anesthetic, and place one subject in the solution at a time. Cover the jar and allow it to sit until the animal is fully anesthetized (euthanized). Be sure to maintain the identity of each specimen, by keeping the ID tag with the animal. You may choose to set up extra anesthetic chambers, as some individuals may take longer than others to be anesthetized, depending on how fresh the solution is, or number of specimens it has been used on. Specimens can remain in solution temporarily while you photograph/prepare others.

5. Remove euthanized specimen from solution and position in a prone, flattened position, with all toes clearly separated for photography. Lay the appropriately prepared identification tag next to specimen and place a 10 cm ruler in view for scale. Take any additional photographs that might best portray the particular abnormality, by laying specimen on dorsal side or by changing the angle of photography. Photo filenames should be the Abnormal Frog ID (plus sequential numbers at the end for multiple photos of same individual). Often the body is grossly distorted in the preservation process, so whenever possible, take an additional photo of the animal after preservation and note the photo ID with a "P" before Abnormal Frog ID.

TIPS: Use a solid, light colored background. To avoid glare, drain liquid off of each specimen by blotting it on a paper towel just before photography (but be careful that specimens do not dry out completely).

6. If any specimens are larger than one gram, their chest cavity must be opened with a single incision on the ventral side that runs from the cloaca to the sternum. This allows the preservative to quickly reach and preserve internal organs.
7. 75-80% ethyl alcohol (EtOH) should be used as preservative for metamorphs. Do not use formalin unless instructed by your regional coordinator (e.g., a subset of specimens may be prepared for histology).

8. Place specimen in paraffin filled plastic tray for preservation, in prone, flattened position with all toes clearly separated. Using lab tape and dissecting pins to secure specimen in place and carefully fill container with enough preservative to completely cover specimen. If more than one specimen will be prepared, you can tie the identification tag to one of the pins or secure tags to the lid of container in such a way to maintain the ID of each specimen. Be sure to keep specimens from drying out by covering them with a "puddle" of EtOH until all specimens are positioned in tray. Once you fill the tray completely, it is very difficult to properly position any subsequent animals if they are floating.

ALTERNATIVELY: Use several small trays to accommodate specimens separately, but keep in mind this method will require that you use larger volumes of EtOH. Label each container clearly so you know when to remove specimens.

9. After 48-72 hours, carefully remove specimens from fixing chambers. Place specimens and tags into individually labeled jars filled with fresh 70% EtOH for storage. Specimens should be separated by site and should not exceed a 10:1 EtOH:frog volume ratio. Note: larger metamorphs (e.g., leopard frogs) may need to remain in 80% ethanol for a longer period of time, please check with your Regional Coordinator for instructions.

10. If tadpole collections are required, they should be handled differently than metamorphs. Young tadpole specimens will be very fragile, and should be handled delicately and as minimally as possible. Euthanized tadpoles without legs can be fixed by dropping them in a jar of 10% buffered formalin for 24-48 hours. After which, specimens should be moved to fresh formalin for storage. The old formalin can be reused several times, then poured down the sink with copious amounts of tap water to dilute. Late-stage tadpoles with limbs should be preserved the same as metamorphs, with toes separated, etc., if possible. Prop the long tail up at several points using pins, to keep it from twisting or curving unnaturally during fixation.

11. The following information should be noted in Specimen Log for EVERY preserved specimen:
    a. Dates:
        -Field collection date
        -Date specimens fixed
        -Date specimens moved to storage solution
        -Date specimens shipped
    b. Refuge name
    c. Site ID
    d. Abnormal Frog ID (or note as Normal or Voucher specimens)
    e. Species ID Code
    f. Type and concentration of fixative & final preservative used
    g. Name of curator

12. All specimens should be stored in jars, separated by SITE. All jars should be labeled at least with:
    a. Frog ID
    b. Date stored
    c. Type & concentration of solution in jar
    d. Initials of curator

13. All abnormal animals must be documented thoroughly. You will need one photocopy of the **Abnormal Frog Form** for your records, one copy to send to your regional coordinator. **Original abnormal frog forms and a copy of the Site form should accompany specimens when/if shipped for diagnostics.**

Send preserved frogs for radiography to:

Contact Information: Dr. Mike Lannoo Indiana University School of Medicine - TH Holmstedt Hall, Room 135, ISU Terre Haute, IN, 47809 Phone 812-237-2059 Email: mlannoo@iupui.edu	**NOTE: BE SURE TO COPY YOUR REGIONAL COORDINATOR ON ALL DOCUMENTATION & COMMUNICATIONS FOR TRACKING PURPOSES!! SEND THE ORIGINAL ABNORMAL FROG FORM AND A COPY OF THE SITE ID FORM WITH THE FROGS GOING FOR DIAGNOSTICS!**

LIVE SPECIMEN SHIPPING SOP

NOTE: LIVE FROGS SHOULD NOT BE SENT FOR PARASITOLOGY WITHOUT PREVIOUS DISCUSSION/APPROVAL THROUGH REGIONAL COORDINATOR.

A. Objective

1. To pack live frogs and safely ship them for parasitology.

WHICH REFUGES WILL NEED TO SEND FROGS FOR PARASITOLOGY?

Parasitology studies will vary depending on your region so it is important to check with your regional coordinator. These allotted samples are reserved for previously sampled refuges where elevated abnormality rates have been detected. BUT, everyone should keep your coordinator updated on your sampling efforts & results throughout the season.

ONCE APPROVED, HOW DO I SELECT FROGS TO SEND FOR PARASITOLOGY?

Parasitology samples should consist of a total of 10 frogs from one SITE. A SITE is a discrete pool/pond, so sites within a refuge should not be mixed. The sample should be composed of either ALL abnormals from a full collection (50-100 of a single species/cohort). OR, if fewer than 10 abnormals were found in that collection, fill in the sample with normal animals from the same collection to a total of 10. IF >10 abnormals were found in that collection, select the ones with the most severe problems or select individuals that best represent the different types of the most severe abnormalities found. Also, if possible, collect 2-3 adult frogs to send along with the metamorphs. Due to differences in feeding habits, adults often accumulate a different parasite load than metamorphs, and will help provide a more complete picture of the site. NOTE: Toads and Hyla may be sent for parasitological analysis. Non-native species (e.g., bullfrogs in the West) are also acceptable if native species are tough to come by.

B. Procedures

1. **First, call Pieter Johnson to make sure he will be available to receive a shipment.** If frogs will not arrive before Friday, hold frogs in a cool place over the weekend.

2. Place blue ice on the bottom of cooler and cover with several layers of newspaper. Put folded white (not colored or patterned) paper towels on the bottom of small Tupperware/plastic container and saturate with site water or UNCHLORINATED tap water. Place one frog per container. Put perforated lid on container without squishing the frog that will inevitably try to escape. Label the container with the abnormal/normal frog ID#.

3. Include a pre-labeled histology vial for each frog sent. Dr. Johnson has agreed to archive the gonads from each frog, so we need to provide pre-labeled vials to expedite the process.

4. Clearly label the top of container with masking or lab tape with appropriate Abnormal Frog ID or Normal Frog ID. Place the containers on the bed of newspaper in the cooler. Place blue ice on sides of cooler and pack newspaper around the containers and blue ice. **Make sure the blue ice is NOT in contact with the frog containers as the frogs may freeze.** Place several layers of newspaper on top of the containers and then one more layer of blue ice on the top. Fill in with newspapers as needed so that the cargo is snug.

5. Fill out the Parasitology Shipping Form and include a preaddressed FEDEX form for returning your cooler. Also, include the original abnormal frog forms (after making copies for yourself). Note: Dr. Johnson has requested some additional information that is not currently on the abnormal frog or parasitology shipping forms. Thus, please provide him with the full name of the refuge (not just the refuge code) and the GPS

latitude and longitude information for each collection site (this information is on the Site Collection form). Place all documentation in a large Ziploc bag and tape it to the inside lid of the cooler. Secure the closed cooler by taping around the lid several times. Ship via priority overnight.

Contact Information:

Dr. Pieter Johnson
University of Colorado
Ramaley N122
Campus Box 334
Boulder, CO 80309-0334
Phone: 303-492-5623
Email: pieter.johnson@colorado.edu

NOTE: BE SURE TO COPY YOUR REGIONAL COORDINATOR ON ALL DOCUMENTATION & COMMUNICATIONS FOR TRACKING PURPOSES!! SEND THE ORIGINAL ABNORMAL FROG FORM AND COPY OF THE SITE ID FORM ALONG WITH THE FROGS BEING SHIPPED FOR DIAGNOSTICS!!

PRESERVED SPECIMEN SHIPPING SOP

A. Objective

1. To safely ship preserved specimens for radiography or archival purposes.

NOTE: A minimal volume of EtOH is considered a hazardous material when going through US Postal Service or other mail carrier. There is a GREAT deal of liability involved, both for you personally & for the service, so please follow these specific guidelines closely**.**

Alcohol preserved specimens can be shipped non-restricted as long as it conforms to 49 CFR 173.4 - Small quantity exceptions.

B. Procedures (These are the essentials that cover us on frogs from the above mentioned CFR):

1. Select and prepare enough "primary containment vessels" to accommodate all of specimens to be shipped. These primary vessels must be leak proof containers, such as glass, plastic, or metal jars -- as long as it has a thickness of no less than 0.2mm.

NOTE: Ziplocs and seal-a-meal pouches DO NOT qualify for use as primary containment vessels.

2. Specimens should be placed in individual containers and remain separated by collection site.
3. Preserved specimens should be removed from storage EtOH and carefully wrapped in plain white paper towels. When packing the animal in the jar, keep them flat. You may have to have different sized containers for different species of animal. Use the smallest container possible to minimize movement of the frog within the container to minimize damage to fragile extremities.
4. Carefully place the wrapped specimens inside the primary vessel, and saturate completely in 70% EtOH. Once saturated, drain as much liquid from the vessel as possible by inverting the jar for a few minutes, avoid pressing on wrapped specimens. Saturated paper towels in a tightly sealed jar should be sufficient to avoid desiccation of specimens, but make sure the shipment will get to its destination within a few days and that Dr. Lannoo or Dr. Green knows the shipment is coming and that the specimens will need to have ethanol added to the containers upon arrival.
5. The volume of EtOH inside each primary containment vessel must be less than 30mL. (You can ship as many primary vessels together as you need to, but **each** one is allowed only up to 30mL EtOH).
6. The lid of the primary vessels must be secured by "positive means". This means that each sealed jar must be securely taped all they way around the lid.
7. Select an appropriate "secondary containment vessel" that can accommodate all the primary vessels for shipping. It also must be leak proof. You can use either large plastic quart jars to individually hold smaller primary jars, and then fill them with absorbent paper OR use an ice cooler (not styrofoam) that will be shipped back to you (if properly labeled with return address and a pre-labeled FEDEX slip with your account # is included in your shipment).

NOTE: A properly packed, sturdy ice cooler serving as the secondary containment vessel should qualify as the final outer packaging too.

8. Absorbent material must surround the primary vessel(s) in sufficient quantity to absorb the total volume of liquid. Stuffing shredded paper around the jars works well. Shredded paper covers both the requirements for sufficient absorbency and helps to secure the jars from shifting. A layer of bubble wrap between glass jars is a very good idea to prevent breakage, but it is NOT absorbent, so you must add paper too.

9. Again, your secondary container can be the final outer packaging, **if** it is STURDY enough. Officially, it needs to be able to withstand the following:

   - Drop from a height of 6ft, landing at least once flat on bottom, top, long side, short side and once landing on the corner junction of three intersecting edges.

10. Clearly label the outside of the package with the following:

"THIS PACKAGE CONFORMS TO 49 CFR 173.4"

NOTE: It wouldn't hurt to also label the package with FRAGILE / THIS SIDE UP.

11. Make sure to notify the recipient of when to expect your package, include the original abnormal frog form, and copy your regional coordinator on ALL documentation.

** **Alert the recipient that your specimens are being shipped in saturated paper towels, so they will know that they need to open the package immediately and put specimens back in 70% EtOH to avoid desiccation.**

BIOSAFETY DURING AMPHIBIAN HANDLING AND MORTALITY EVENTS

Adapted from: Speare, R., L. Berger, (School of Public Health and Tropical Medicine, James Cook University, Townsville, Australia, 4811); and H. Hines, (Department of Environment, Moggill, Queensland, Australia). 1998. HOW TO REDUCE THE RISKS OF YOU TRANSMITTING AN INFECTIOUS AGENT BETWEEN FROGS AND BETWEEN SITES

A. Objectives
1) Reduce risk of you becoming exposed to a disease agent.
2) Reduce risk of disease transmission between amphibians within infected sites.
3) Reduce spread of disease to amphibians at new sites (long and short distances).

B. Problem
1) Disease agents, pathogenic to frogs, are present in the environment
   - Disease agents include toxins, bacteria, viruses, parasites, fungi
2) Rates of disease transmission within an amphibian population can be increased.
3) Disease can be transmitted to humans or uninfected amphibians at new locations.
4) Potential for long term impact on amphibian populations such as local declines.

C. Background
1) Microorganism
   a) Durable and can survive in a range of environments.
   b) Present in water and substrate as well as infected frogs.
   c) Number of infectious particles available (inoculating dose) can determine outcome.
      - Low numbers of organisms may result in no disease or mild disease
      - High numbers of organisms may result in rapid onset and severe disease.
2) Procedures available to reduce infectivity of durable microorganisms:
   a) Measures may not kill all particles of agent or prevent exposure
   b) Reduction of the number of particles of the agent may improve outcome
   c) Procedures in any disease control are done on a cost-benefit basis:
      - If preventing transmission is critical (i.e. endangered species), expensive and tedious control procedures may be justified to minimize the risk.

D. Reducing risk of transmission between amphibians within infected sites.
1) Do not handle uninfected amphibians during or following contact with sick or dead animals.
2) Invasive procedures, such as toe clipping, on infected amphibians enhance inoculation of disease agent into toes of uninfected amphibians.

E. Handling procedures that reduce disease transmission when handling infected amphibians:
1) Wash and rinse hands well using a disinfectant soap or rinse with an antiseptic
2) Use disposable vinyl gloves for each animal
3) Capture and handle amphibians using new plastic bags for each new animal
4) Prevent contact between amphibian and handler's skin or clothing
   a) Wear protective clothing, chest waders, plastic apron or coveralls that can be rinsed in contact occurs.
   b) Ensure that used gloves and bags do not come in contact with clean ones

5) If invasive procedures are used, decrease the risk of disease transmission:
   a) Use disposable sterile instruments or sterilized previously used instruments
   b) Seal any open cuts (i.e. toe or PIT tag hole) to decrease risk of infection.
      - Use of a cyanoacrylate compound will seal the wound until it heals naturally
   c) Immerse cleaned instruments in a sterilizing solution or boiling water for recommended time (70% methanol for 30 min, 100% methanol and then flamed, 1% glutaraldehyde for 15 minutes or boiling water for 10 minutes).

F. Do not let amphibians come in contact with the disinfectants

G. Do not discard contaminated or disinfected solutions in the water
   1) Use disinfectants at least 200m away from water

H. Reducing risks of spread to new areas
   1) Always visit uninfected sites (typically ephemeral sites) before sites suspected to be infected (typically permanent wetlands)
   2) Long distance movements of handlers greatly increase the possibility of introducing new agents
   3) Disinfect skin before leaving site after handling amphibians
   4) Change clothes, bag and wash
   5) Rinse off visible mud and debris from boots, nets and equipment
   6) Scrub with soap & water (Biodegradable soaps are available at most local outdoors shops, this is preferable if you will be cleaning equipment in the field. If you have access to hose & sewer drains, a general detergent is fine. DO NOT use IODINE based soaps. Do not use combination disinfectant/antibiotic solutions)
   7) Disinfect boots, nets and equipment using a 5% Clorox bleach solution by soaking for 10-15 minutes. DO NOT RINSE. Allow equipment to air dry before going to next site. If possible, having a second set of boots/nets may be useful if sampling between distant sites
   8) Clean outside and underneath vehicle. If mud or water was introduced into vehicle, then scrub floor and pedals with disinfectant
   9) Do not translocate adult amphibians or their eggs or tadpoles over long distances
   10) Consider not returning captive held amphibians or tadpoles to the wild, particularly if they have been in contact with other captive amphibians or tadpoles
   11) If amphibians of high value (threatened or endangered) are being returned to the wild, insist on an examination by a disease specialist prior to release

NOTE: IF AT ANY TIME, YOUR CREW HAPPENS UPON **DEAD OR DYING** AMPHIBIANS IN THE FIELD, PLEASE COLLECT THE CASUALTIES, PUT THEM ON ICE (DON'T FREEZE THEM SOLID, BUT KEEP THEM WELL CHILLED BY PACKING THEM ON PLENTY OF ICE FOR SHIPPING). SEND THEM TO THE NATIONAL WILDLIFE HEALTH CENTER ASAP (SEE CONTACT INFO BELOW). INVESTIGATION OF DISEASE OUTBREAKS AND DIE-OFFS IN WILDLIFE IS THE PRIMARY MISSION OF THE NWHC. SO, ALTHOUGH THAT IS NOT PART OF OUR SURVEYS, PER SE, IT IS OF GREAT INTEREST TO THEM AND THEY WILL TAKE A LOOK AT THOSE SPECIMENS FREE OF CHARGE. TO AVOID ANY CONFUSION ON CHARGES THAT MAY COME THROUGH, MAKE SURE YOU IDENTIFY THEM CLEARLY AS DISEASE OR DIE-OFF ANIMALS AND NOT AS ANIMALS COMING FROM THE USFWS FROG SURVEYS!

IF ANY **DEAD OR DYING** ANIMALS ARE FOUND AT ONE OF YOUR SITES, BIOSAFETY PROCEDURES SHOULD BE IMPLEMENTED IMMEDIATELY ON ALL EXPOSED FIELD GEAR BEFORE VISITING ANY OF YOUR OTHER SITES.

AT MINIMUM, BIOSAFETY PROCEDURES SHOULD **ALWAYS** BE CONDUCTED UPON RETURN TO YOUR FIELD STATIONS OR AFTER VISITING ANY SITES OFF REFUGE OR WHERE YOU OTHERWISE SUSPECT A PROBLEM.

CONDUCTING BIOSAFETY PROCEDURES BETWEEN SAMPLING SITES WITHIN A REFUGE IS NOT TYPICALLY FEASIBLE AND WILL BE LEFT TO THE DISCRETION OF THE RESEARCHER. IF YOUR SITES ARE ALL WITHIN A 50 MILE RADIUS, THEY LIKELY HAVE SIMILAR PARASITE OR BACTERIA LOADS, BUT **USE YOUR BEST JUDGEMENT**. IF YOU HAVE A PARTICULARLY NASTY SITE, CONSIDER CARRYING A SEPARATE SET OF NETS/BOOTS RESERVED FOR USE ONLY AT THAT SITE.

THESE BIOSAFETY PROCEDURES WERE DEVELOPED ORIGINALLY FOR THE FOLKS IN R5, WHERE A SINGLE CREW CONDUCTS MOST OF THE SAMPLING ACROSS SEVERAL STATES. OVER SEVERAL SEASONS, RESEARCHERS HAVE DOCUMENTED THE PROGRESSIVE SPREAD OF PATHOGENS TO SITES THEY VISITED, WHERE PREVIOUSLY NO SIGNS OF PATHOGENS WERE EVIDENT.

If shipping animals, first contact the NWHC to alert Dr. David Green to expect your shipment and how many/type/condition of specimens they will receive.

NWHC:
PH (608) 270-2400
FAX (608) 270-2415

If you cannot reach them by phone, FAX them a note or send an e-mail to Dr. Green (**david_green@usgs.gov**)

Shipping Address:

National Wildlife Health Center
ATTN: DIAGNOSTIC SPECIMENS – WILDLIFE
6006 Schroeder Road
Madison, WI 53711-6223

PLEASE COPY ANY CORRESPONDENCE WITH NWHC TO YOUR REGIONAL AMPHIBIAN COORDINATOR, SHOULD THERE BE QUESTIONS!

GLOBAL POSITIONING SYSTEM PROCEDURES

A. Objective: To collect required geospatial data in the correct format.

Datum (Projection): Data should be collected in WGS84 format (sometimes written as WGS1984). If your GPS unit doesn't support WGS84, the contingent Datum is NAD83. If you collect NAD83 (or any other datum), the datum must be converted to WGS84 before being entered in to the database.

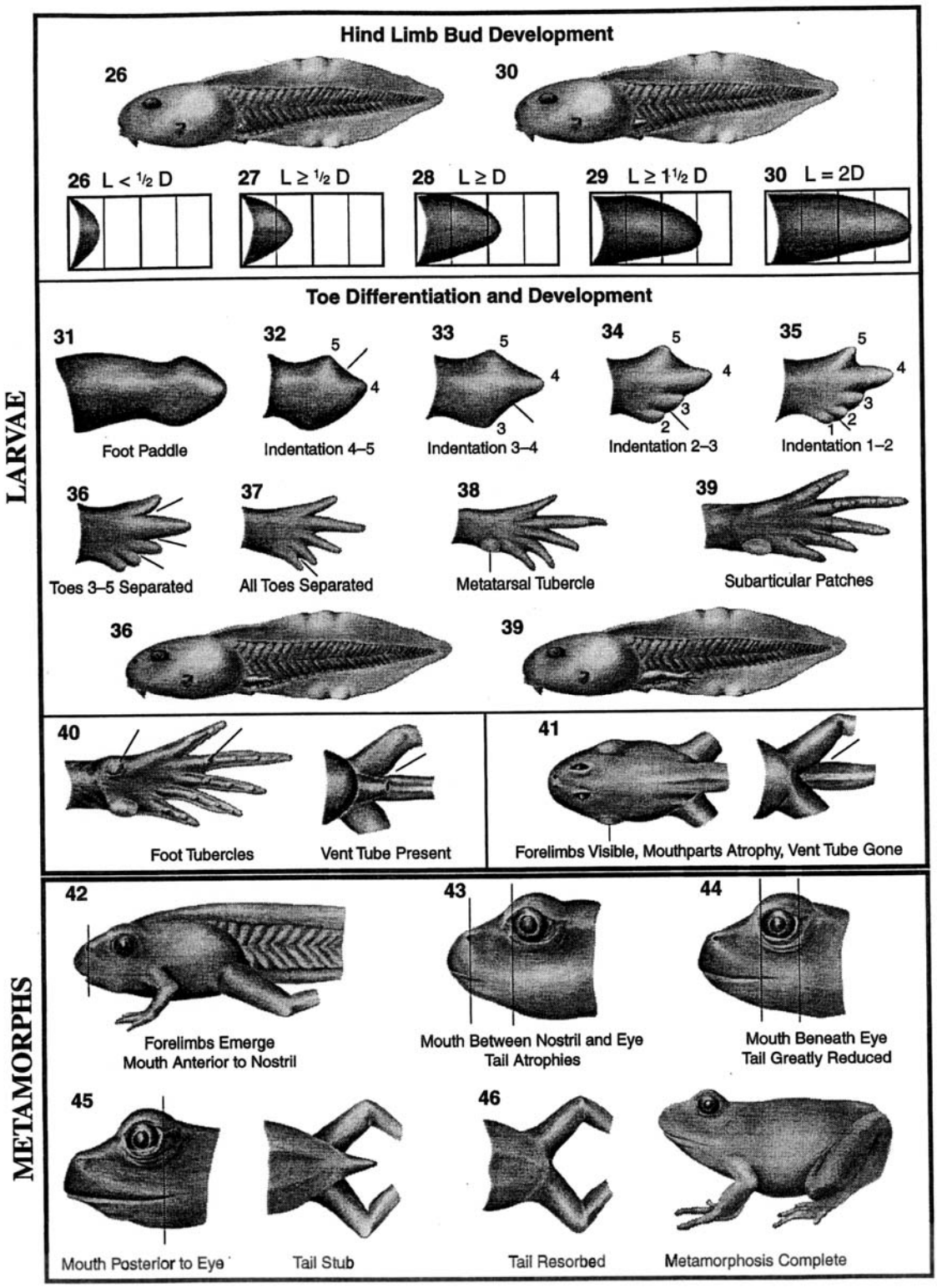
Units (Coordinate System): Points should be collected in Geographic Decimal Degrees (**hddd.ddddd).** If points are collected in Decimal Minutes (hddd.mm.mmmm) or Degrees Minutes Seconds (hddd mm ss.s) then they must be converted to Decimal Degrees before being entered into the database.

NOTE: It is always best to collect the datum and units in the preferred format since there is a loss of accuracy during the conversion process.

Satellites: Ensure that your unit, at the time of collection, is tracking at least 3 satellites (the minimum number required to take an accurate point). Most GPS units will not take a reading unless it has locked on to at least 3 satellites. However there is an override feature on some of the high end units, so it is always prudent to make sure you are tracking a sufficient number of satellites.

WAAS: If your GPS unit is capable of differential correction, make sure it is enabled. Sometimes there will be a small symbol that looks like an antenna on the display, other times you will see the WAAS symbol. Most small units won't have this option, but if you do, turn it on.

Chapter 2



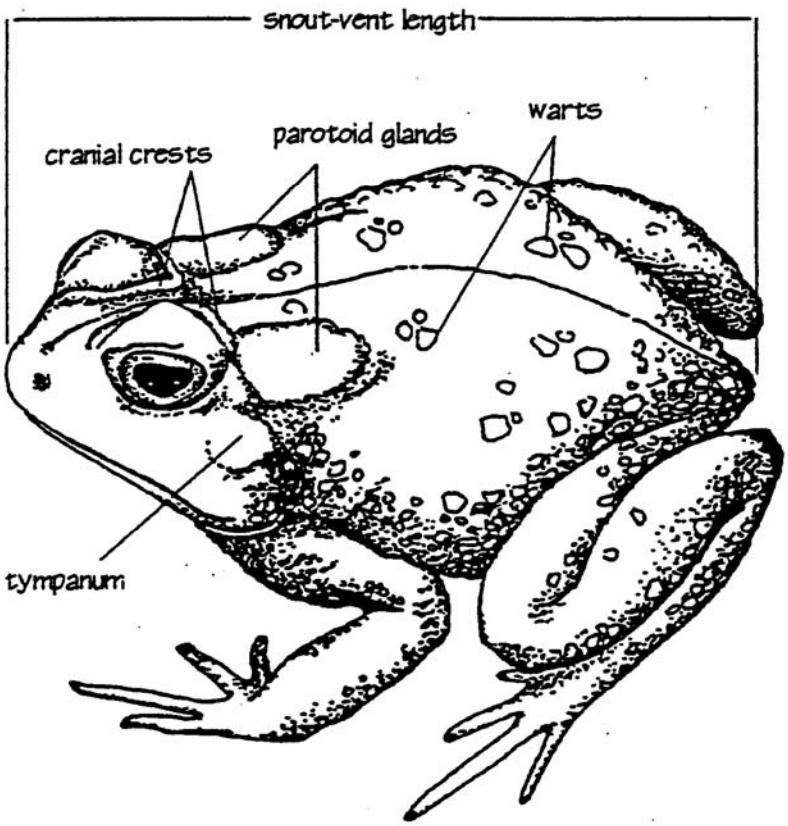
Tadpole Metamorphosis

Based on Gosner staging system (1960). Stages 26-46 were redrawn by Kate Spencer

Published in Tadpoles, R.W. McDiarmid & R. Altis, eds. 1999.

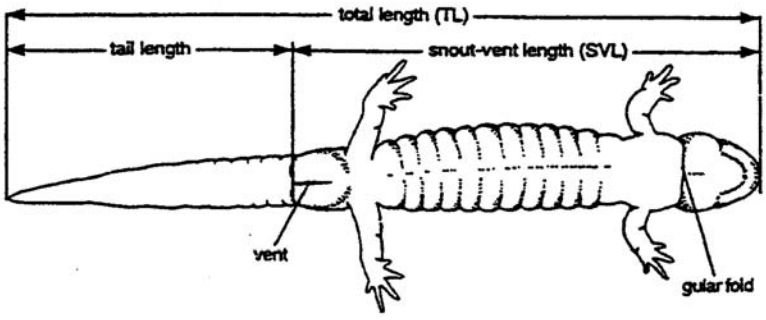
Measurements of frogs and toads

Principal measurement is the **snout to vent length** (SVL)



From: Leonard. Brown. Jones. McAllister & Storm. 1993 (Amphibians of Washington and Oregon)

Measurements of adult salamanders are the same as for larvae and neotenes.



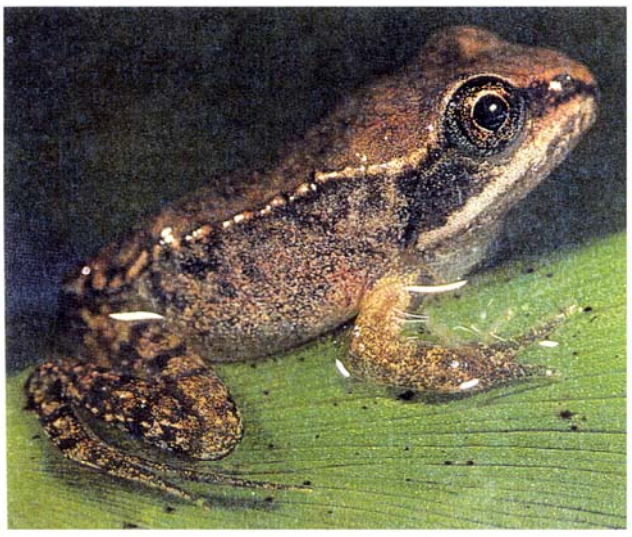
From: Petranka. 1998 (Salamanders of the United States and Canada)

Species	Body description:	Cont'd description:	Breeding season:	Typical breeding habitat:	Egg description:	Tadpole description:	Conservation status in AR
Acris crepitans (Northern Cricket Frog)	small, warty hylid with dark jagged edged stripe on thigh; blunt head with dark V-shaped triangle between eyes	upper jaw with alternating light/dark vertical lines	April-July	streams pond and lakes, mudflats, barren shorelines and stream edges	eggs laid singly or in clusters, attached to debris in water column or floating; hatch in several days	*(Blanchard's: metamorphose in 5-10 weeks; 36mm max)*	
Bufo americanus (American Toad)	small, red-brown toad with small dorsal spots; one or two warts in each of large spots, belly whitish to cream with or without faint spots; narrow light stripe MAY extend down dorsum	kidney shaped parotids, cranial ridges connected or separated by bony spur	mid Mar - July (peaks Apr); males will have enlarged horny pad on inside of each forelimb & dark grey throat	ditches, small temp ponds, shallow slow streams	long tubular, gelatinous strings released free to pool bottom or onto submerged veg	black, distinct bicolored tail, 27mm max	
Bufo fowleri (Fowler's Toad)	medium, warty toad with numerous irregular dark brown or black dorsal spots; one-six warts/spot; pupil is horizontally elliptical; variable body color (gray to brick red);	oblong parotids connected by shallow bony crest; belly white with only occasional single breast spot. Light stripe down length of back.	late Mar-May (peak mid-May); males have dark throat area & horny pads on inside forelegs	permanent water; lakes, farm ponds, creeks rivers & drainage ditches	very LARGE clutches, long tubular strands; ONE week to development; June-mid July to metamorphose		
Bufo nebulifer (Coastal Plain Toad)	flat, med sized black w/ orange spots or yellow/brown w/ white spots; lateral broad, dark stripe bordered by broad middorsal yellow stripe; prominent yellow/cream middorsal stripe;	males have clear yellow/green throat; triangular shaped parotids behind eyes. Strongly dev., sharp ridged crests, forming distinct "Y" ends	Mar - Sept	shallow pools	long gelatinous strings, often double rows; 20-30 d development	dark with dark tail musculature, light dorsal saddles, 25 mm max	
Gastrophryne carolinensis (Eastern Narrowmouth Toad)	small, squat-bodied, short legged burrowing frog with a pointed snout, tiny head and distinctive fold of skin across the back of the head; tympanum is concealed, skin is smooth and no webbing present on toes	body color tan to reddish/brown with plain or irregularly blotched pattern; dark stripe extends from snout to side of hind legs; belly mottled with dark gray	April-August (peak calling May/June)	breeds in roadside ditches, ponds, sloughs and lakes; found mainly burrowing in moist soil conditions, under rocks, logs, stumps etc.	up to 850 eggs in small groups as a 1-2 egg deep surface layer; eggs have flat surface rather than spherical; hatching in less than 2 days; metamorphosis occurs in 20-30 days	unique with a sucking disc rather than labial teeth, mandibles and oral papillae	
Gastrophryne olivacea (Great Plains Narrowmouth Toad)							
Hyla avivoca (Bird-voiced Treefrog)	small hylid with expanded, adhesive toe discs, gray to green dorsum with irregularly shaped large dark blotch; young lack blotch & body green;	inside of legs have green to yellow/green color with conspicuou white spot below eyes; subarticular tubercle on outeer figer of front foot often divided	spring-summer	temp & perm woodland and floodplain pools with lots of low, woody veg; often perched high off ground while calling	eggs laid in submerged pockets; 29 d to dev	distintive with series of red saddles on dorsum	Spp of special concern due to low pop density & spotty occurrence

Hyla chrysoscelis (Cope's Gray Treefrog)	large warty or roughly granular hylid; large conspicuous adhesive discs; large, prominent squarish white spot beneath eyes; body color highly variable, lt gray to brown/greenish;	dark stripe slants from rear of eye to front leg, belly white, inner surface of hind leg & groin bright yellow or orange with dark spots/reticulations	Apr-Aug (mainly during warm rainy periods, but have been heard in Mar)	semi-perm pools or ponds, flooded barrow pits or ditches, males perch up to 60ft up in trees	up to 2K eggs laid in small packets ~45; either free-floating or loosely attached to veg; few days to hatch	boldly marked with large, dark blotches on long, red/black-finned tails; 50mm max; metamorphosis approx. 45-65 days	statewide dist; pesticide app of trees & shrubs near wetlands will impact these frogs
Hyla cinerea (Green Treefrog)	slender green hylid with expanded toe tips; dorsum bright green, with occasional conspicuous white or yellow lateral stripe running from upper lip over front leg & back to groin	dorsum often has small yellow spots while venter is white or yellow	late April-Aug; active egg laying peaks Jun-early July	vegetated areas near perm water; cypress swamps, floodplains, marshes, sloughs, ponds, lakes & streams; some reports habitat preference in soybean, cotton and grain sorghum fields ; males call from 1-7 ft above water	large clutches deposited in several small floating packets		
Hyla versicolor (Gray Treefrog)	large warty or roughly granular hylid; large conspicuous adhesive discs; large, prominent squarish white spot beneath eyes; body color highly variable, lt gray to brown/greenish; dark stripe slants from rear of eye to front leg	belly white; inner surface of hind leg & groin bright yellow or orange with dark spots/reticulations	Apr-Aug (mainly during warm rainy periods; but have been heard in Mar)	semi-perm pools or ponds, flooded barrow pits or ditches; males call more frequently from ground; differntiated from *chrysocelis* only by call & chrom number; *versicolor* - 35 trill per min. rate; *chrysocelis* - 69 tpm rate	up to 2K eggs laid in small packets ~45; either free-floating or loosely attached to veg; few days to hatch	boldly marked with large, dark blotches on long, red/black-finned tails; 50mm max; metamorphosis approx. 45-65 days	statewide dist; pesticide app of trees & shrubs near wetlands will impact these frogs
Pseudacris crucifer (Northern Spring Peeper)	small hylid with conspicuous black X-shaped mark on dorsum; head lacks light spot beneath eye, but dark line runs across top of head between eyes;	body from pink to brownis/gray-olive; belly unmarked and usually light cream color; tips of digits expanded	early March-May (calling may begin as early as mid-Jan)	semiperm, fishless, woodland pools; males smaller & darker than females & during breeding season have dark throats	eggs deposited individually to submerged sticks, twigs or floating veg; hatching occurs in 3-4 days	brownish-green with metallic gold flecks on upper surface; belly lighter & iridescent, caudal fin clear or orange-tipped with black/purple blotches at outer edges	drainage of wetlands will cont. to reduce populations
Pseudacris feriarum (Upland Chorus Frog)							
Pseudacris illinoensis (Illinois Chorus Frog)							

Pseudacris streckeri (Strecker's Chorus Frog)							
Pseudacris triseriata (Western Chorus Frog)	small hylid with variable ground color (gray to brown); dorsal markings typically with three dark, narrow, longitudinal stripes, broken into three rows of spots; toes without webbing; tips slightly reduced discs;	dark triangle between eyes with white stripe running along upper lip, bordered by dark stripe extending through eye to groin; belly cream or white; skin only slightly bumpy	calls as early as late Dec, early Jan; breeding occurs late Feb-lat Apr;	swamps, edges of marshes, temp rain-filled pools in mowed fields and roadside ditches	clusters of up to 100 eggs laid adhering to grass and debris in water; hatch in a few days; metamorphose 1-2 months later	plump, rounded gray or brown bodies, sometimes mottled with brassy color in older ones; caudal fin is clear with dark flecks and intestinal coil seen through body wall	quite tolerant of human activities - common near firing range of LRAFB
Rana areolata (Crawfish Frog)	med sized frog with ground color varying from tan to light gray with many closely set dark spots enriched by light borders;	prominent dorsolateral fold present from eye to thigh; belly while; males smaller than females	late Jan-late Feb (dep on precip & temp	secretive; use crawfish and other burrows; move to breeding ponds after heavy rains for 2 wks before females arrive;	up to 7K eggs laid; 3-4 days to hatch; metamorphose in early Jun-early July		uncommon; scarcely seen other than during prime breeding season
Rana blairi (Plains Leopard Frog)							
Rana catesbeiana (Bullfrog)	large, olive-green ranid with well-developed tympanic membranes and NO dorsolateral folds;	extensive webbing b/w toes extends to tips; venter light with some yellow pigment on throat	calling begins early April; peak breeding activity late June, early July (in MO)	large lakes, reservoirs to small farm ponds, backwater swamps, marshes, and sloughs	up to 20K eggs in mass; 4-5 days to hatch; metamorphose from 11-14 months		
Rana clamitans (Green Frog)	medium sized ranid with well-developed dorsolateral ridges; hind feet have extensive webbing; edge of jaw has alternating lt/dark spots	body color basically brown, bronze or green without disjunct spots; undersurface of body has light worm-like dark markings;	April - Aug/Sep (in AL); peak breeding probably June	swamps, floodplain pools and small streams, ponds & river banks; breeding habitat varies, but typically permanent water bodies used;	up to 4K eggs in floating mass on surface; hatching at 3-4 days; metamorphosis occurs during the FOLLOWING SUMMER	plump with long tails with olive to brown/green color above; varying amounts of dark flecks over back, upper and lower tail fins; belly irridescent white or cream, often mottled with gray, cannot see intestines through body wall	
Rana palustris (Pickerel Frog)	medium sized ranid with prominent rectangular or square black/brown blotches arranged in two parallel rows extending down back between well-developed dorsolateral folds	body color gray or tan, hind legs have black/brown cross bars; venter is white with hind legs and groin having bright yellow to orange pigmentation	early Feb-early May (in other states near AR)	occupies floodplain swamps, or cool, clear water at mouths of caves; breeding occurs in woodland pools, streams; males have enlarged thumbs (with inner pads) during breeding season	up to 3K eggs laid adhering to twigs and submerged debris; 10 days to hatch; metamorphosis in 3.5 months	range from green to gray/brown often with scattered black/yellow spots; irridescent white/cream bellies; tail fins heavily pigmented with dark spots or blotches; max 7cm	

Rana sphenocephala (Southern Leopard Frog)	medium sized spotted ranid, typically green, green/brown or brown with numerous rounded/oblong dark spots on dorsum; well developed dorsolateral folds present	belly white, back of thigh has interrupted bars, extensive webbing on hind feet, long head, pointed snout with distinctive whitel line present on upper jaw, tympanum usually has distinct light spot in center	typically mid-Mar-early May, however, can breed at all times of the year!	males call from farm ponds, ditches, backwater areas of streams, margins of cultivated fields	communal egglaying; up to 75 globular egg masses attached to submergent and emergent veg or laid unattached in under 3m square area; up to 5K eggs/female; hatching in 2 weeks; metamorphosis in mid-June-July		
Rana sylvatica (Wood Frog)							
Scaphiopus holbrooki (Eastern Spadefoot)	med sized burrower; dorsal color varies from greyish, blackish to greenish-brown; two major lyre-shaped lines run from each eye down back, additional broken light line may occur on sides of body	interorbital boss is absent; belly white to pale gray; spade elongate & sickle shaped; pupil vertical; skin relatively smooth	mid-Feb through June; coincides with heavy rains	small shallow temp pools in open areas	short, stringy egg masses attached to grasses and twigs; hatch in one to several days; metamorphose 14-60 days; both depend on temperature	tadpoles aggregate into large isolated social groups; may become cannibalistic; males grow faster post-metamorphosis	ANHC ranked as rare, susceptible to extirpation. Skin secretions particularly irritating!!
Scaphiopus hurteri (Hurter's Spadefoot)	med sized burrower; dorsal color varies from greyish, blackish to greenish-brown; two major lyre-shaped lines run from each eye down back, additional broken light line may occur on sides of body	interorbital boss is present; belly white to pale gray; spade elongate & sickle shaped; pupil vertical; skin relatively smooth	mid-Feb through June; coincides with heavy rains	small shallow temp pools in open areas	short, stringy egg masses attached to grasses and twigs; hatch in one to several days; metamorphose 14-60 days; both depend on temperature	tadpoles aggregate into large isolated social groups; may become cannibalistic; males grow faster post-metamorphosis	ANHC ranked as rare, susceptible to extirpation. Skin secretions particularly irritating!!
Spea bombifrons (Plains Spadefoot)							



Chapter 3

Field Equipment List

- ☐ Hip boots and waders (have both)
- ☐ Long-handled 24" deep mesh net
- ☐ Plastic containers with tight fitting lids and holes punched for frog collection (Rubbermaid brand or disposable type)
- ☐ Clear plastic jar or ziplock bags for tadpole collection
- ☐ Data sheets (to record measurements and NARCAM malformation forms)
- ☐ Ruler (metric)
- ☐ Field Guide to Malformations for Frogs and Toads
- ☐ Species keys or guides
- ☐ Hand lens or magnifying glass
- ☐ Field vest for holding plastic containers
- ☐ 2-foot deep plastic container with lid for holding frogs (with 1" site water in the bottom)
- ☐ (OR) 5-gallon bucket for holding frogs
- ☐ Cold packs or blue ice (for holding container)
- ☐ Camera (digital)
- ☐ GPS recorder
- ☐ Hydro lab or other water quality meter

RECOMMENDED MALPHIBIAN SUPPLIES & SUPPLIERS:

FIELD EQUIPMENT:

PURPOSE FOR ITEM:	Comments:	Supplier Name	Catalog Edition	Supplier Contact Info:	Catalog Page #	Item Name	Catalog #	Item Description	Price	Unit:	Qty	Sub-total
Taking air & water temperatures	Data must be in o Celcius. These are heavy-duty field worthy thermometers. It is very helpful to lanyard with bright colored line so you don't lose it in the field! Getting a backup is highly recommended.	Carolina Biologicals	Science & Math 2003-2004 Catalog	PH 800-334-5551 FAX 800-222-7112 www.carolina.com	(p 1130)	Enviro-Safe Pocket Thermometers (°C)	ER-74-5405	Open metal case 6" thermometer (-5°C to 50°C in 0.5° graduations)	$13.15	each	2	$26.30
Collecting tadpoles & metamorphs	Very lightweight & durable. Unfortunately, they are often out of stock - high demand for this item.	Carolina Biologicals	Science & Math 2003-2004 Catalog	PH 800-334-5551 FAX 800-222-7112 www.carolina.com	(p 643)	Aluminum dip net	RG-65-1320	(D-shaped hoop; 14 x 14; 42" handle, ¼" mesh)	$16.95	each	3	$50.85
Close inspection of tadpoles and metamorphs in the field	Especially helpful for tadpole ID and looking at toes on tiny toad & treefrog metamorphs!	Forestry Suppliers, Inc.	Catalog 54 (2003)	PH 800-647-5368 FAX 800-543-4203	(p 364)	Economical Folding Pocket Magnifiers	61122	(D. 2.5 cm lens - 5X-10X-15X)	$3.95	each	1	$3.95

SPECIMEN PRESERVATION:

Frog euthanasia	This amount should last all season if kept refrigerated in an amber bottle.	Carolina Biologicals	Science & Math 2003-2004 Catalog	PH 800-334-5551 FAX 800-222-7112 www.carolina.com	(p 784)	Tricaine Methane Sulfonate (MS-222)	89-6720	(reagent grade - 5g)	$17.50	5 gram	1	$17.50
Frog euthanasia	Store solution in fridge or keep in cooler when taking to the field to keep it "fresh".	Carolina Biologicals	Science & Math 2003-2004 Catalog	PH 800-334-5551 FAX 800-222-7112 www.carolina.com	(p 1081)	Amber Polyethylene Widemouth Bottles	ER-71-6255	(1,000 mL)	$6.95	each	1	$6.95
Specimen handling during preservation	This kit has a good assortment of the minimum styles of tools needed. Any dissection kit should suffice.	Carolina Biologicals	Science & Math 2003-2004 Catalog	PH 800-334-5551 FAX 800-222-7112 www.carolina.com	(p 395)	Dissecting set	RG-62-1430	(11 piece Teacher's dissecting set)	$19.25	set	1	$19.25
Preserved Specimen ID	These tags are much bigger than needed for our tiny frogs, so you can trim them down after labeling just to keep them from tangling too much in the jars. Any museum tags should do, just make sure they are big enough for the Abnormal Frog ID (ex:R4-OVF02-RASP-060303-013)	Carolina Biologicals	Science & Math 2003-2004 Catalog	PH 800-334-5551 FAX 800-222-7112 www.carolina.com	(p 646)	Tyvek Tags	RG-65-7557	(Size: 3 1/4 x 1 5/8"; 100/pack)	$9.55	pack	1	$9.55
Specimen fixative	Fixative to be used in FY03 for metamorphs. 10% buffered formalin should be used for TADPOLES only.	Carolina Biologicals	Science & Math 2003-2004 Catalog	PH 800-334-5551 FAX 800-222-7112 www.carolina.com	(p 766)	Ethanol	RG-86-1283	(95% lab grade EtOH)	$18.75	4 L	1	$18.75
Specimen Preservative	Preservative to be used for metamorphs in FY03	Carolina Biologicals	Science & Math 2003-2004 Catalog	PH 800-334-5551 FAX 800-222-7112 www.carolina.com	(p 766)	Ethanol	RG-86-1263	(70% lab grade EtOH)	$18.75	4L	1	$18.75
Preserved Specimen Storage	Remember to keep frogs separated by SITE. These jars will suffice for storing up to 15 frogs from a site, depending on size, maybe more. Should have a minimum 10:1 volume ratio.	Carolina Biologicals	Science & Math 2003-2004 Catalog	PH 800-334-5551 FAX 800-222-7112 www.carolina.com	(p 1122)	Glass Screw-cap specimen jars, low form	RG-71-5605	(16oz.)	$11.50	12 pack	1	$11.50
Preserved Specimen Storage	The lids need to be chemically resistant, given the expected contents. These were the cheapest we could find from any of the scientific suppliers.	Carolina Biologicals	Science & Math 2003-2004 Catalog	PH 800-334-5551 FAX 800-222-7112 www.carolina.com	(p 1123)	Black Molded screw caps	RG-71-5698	(to fit 16oz jars above) (89 mm diameter)	$6.10	12 pack	1	$6.10
Labeling Specimen Storage Jars (and other equipment)	Masking tape works just as well. If you are working on more than one refuge, colored tape helps to distinguish between them. Can also be used for securing specimens during fixing, but transparent tape preferred.	Carolina Biologicals	Science & Math 2003-2004 Catalog	PH 800-334-5551 FAX 800-222-7112 www.carolina.com	(p 331)	Labeling Tape	ER-21-5620	(1/2 x 500", 10 assorted colors)	$34.50	10 pack	1	$34.50

OR labeling tape can be purchased by the roll at:

Labeling Specimen Storage Jars (and other equipment)	Masking tape works just as well. If you are working on more than one refuge, colored tape helps to distinguish between them. Can also be used for securing specimens during fixing, but transparent tape preferred.	Fisher Scientific	2000/2001	PH 800-766-7000 FAX 800-926-1166	(p 938)	Lab tape	11-880B	(1/2 inch wide yellow)	$3.99	each	1	$3.99

OTHER FIELD/LAB EQUIPMENT:

PURPOSE FOR ITEM:	Comments:	Supplier Name	Catalog Edition	Supplier Contact Info:	Catalog Page #	Item Name	Catalog #	Item Description	Price	Unit:	Qty	Sub-total
Field collecting & holding bags for metamorphs	We never leave home without 'em!! Tuck 2-3 in your pocket when sampling, pull them through your beltloop to free the hands once animals are found, then just tie them up for short term storage of animals in cooler. A big time saver since it avoids transferring animals around too much and keeps them contained in small groups during processing.	Memphis Net & Twine Co., Inc.	1999A	PH 800-238-6380 FAX 901-458-1601	(p 44)	Polyethylene bags	PB1020A	(10" x 20" - 2 mm thickness) Comes in a box of 500 bags	$32.29	box	1	$32.29
Lightweight seine for sampling tadpoles or collecting metamorphs	These are VERY lightweight and do not come with poles. Two cheap broom handles do a great job in most of the types of habitat we encounter (roadside puddles, ditches). But they won't likely survive sampling at sites with heavy vegetation or other obstructions.	Memphis Net & Twine Co., Inc.	1999A	PH 800-238-6380 FAX 901-458-1601	(p 32)	Common Sense Minnow Seine	CSS1	(1/8" sq. mesh size, 10 ft. length)	$11.76	each	1	$11.76
Cleaning field gear.	Remember, disinfection requires FIRST removing any visible dirt. Look fo a VERY heavy duty scrubber with long handle.	Walmart				Large scrubbing brush		approximately	$5.00	each	2	$10.00
Cleaning field gear.	Use plain liquid soap. No added fragrance, antibiotics or antiseptics! No iodine based soaps. Better alternative - use a biodegradable soap, which can be purchased at outdoor shops.	Walmart				Ivory liquid soap		approximately	$1.00	each	1	$1.00
Decontaminating field gear.	Make sure one is wide/deep enough to dip field boots & nets into for cleaning/disinfecting. Use it to store bleach/soap/brushes, etc. Do not use the DECON tub to hold frogs. Use the other for hauling field gear, etc. or holding frogs temporarily.	Walmart				Large, deep plastic bins with lids		approximately	$3.00	each	2	$6.00
Decontaminating field gear.	Used at ~10% to disinfect pre-cleaned field gear by soaking 15 minutes, then allowing to air dry completely.	Walmart				Clorox bleach		approximately	$2.00	each	1	$2.00
Holding animals.	For both temporary & overnight holding. Make sure the cooler has a tight fitting lid. Having at least two comes in very handy, particularly when processing animals, but also for being prepared for successful collecting at multiple sites in a single day	Walmart				48-quart ice cooler		approximately	$15.00	each	2	$30.00
Holding animals.	To keep animals cool during holding periods. It is recommended to have two full sets, so you can be refreezing one set overnight if holding animals with another set, particularly if heading back out to field the next day. If shipping any live animals, you'll need more.	Walmart				Blue Ice packs		Buy enough to make a full layer on the bottom of your cooler	$0.99	each	10	$9.90
Restraining animals during fixation	Trasparent medical adhesive tape is very useful in getting a good view of the toes on specimens going into fixative. So the tape doesn't stick to specimen, cut short strips and tape them sticky side together. Use pins to secure each foot with the toes splayed apart underneath the taped strips.	Walmart				Medical Adhesive Tape		One roll is plenty if you reuse the taped strips	$5.00	roll	1	$5.00

Items needed for Shipping Live Frogs

- ☐ Live animal specimens
- ☐ Shipping cooler addressed to NWHC
- ☐ Small plastic jars with holes punched and unique number on lid
- ☐ Marking pen (use lab grade pen like Fisherbrand Marking Pen)
- ☐ Unbleached, untreated, moist paper towels or moist site vegetation
- ☐ Declorinated water or site water
- ☐ Packing slip inside a ziplock bag
- ☐ Ice packs or blue ice
- ☐ Packing material or shredded newspaper

Items needed for Preservation and Shipping of Dead Frogs

- ☐ Euthanized frogs
- ☐ 90% ethanol (for 2 weeks to fix) 70% ethanol (for long-term storage)
- ☐ Wax-bottomed container (like tupperware or glass pan)
- ☐ Pins (Dissection T-pins, map pins, sewing straight pins, etc.)
- ☐ Surgical scissors
- ☐ Surgical tape
- ☐ Marking pen (use lab grade pen like Fisherbrand Marking Pen)
- ☐ I-Chem glass jars to transfer prepared frog for shipping.
- ☐ Personal Protective Equipment (chemically resistant gloves and goggles)
- ☐ Chemical collection container (like Lab safety disposal container)

Shipping Label:

[Make sure you have an established project with the Health Center and they know when your specimens are expected to arrive.]

Diagnostic Specimens – WILDLIFE National Wildlife Health Center 6006 Schroeder Rd. Madison, WI 53711 (Phone: 608-270-2400)

Decontamination Equipment List

For cleaning waders, nets and buckets between sampling sites

- ☐ Garden hose or fresh water supply (field decon. is accomplished with spray bottles over a protective ground cover)
- ☐ 5% bleach solution (soak equipment for 15 minutes)
- ☐ Brushes (large plastic equipment brushes)
- ☐ Soaking bucket (plastic garbage can or 5-gallon bucket in field situation)

Chapter 4

Health and Safety for Animal Preservation Chemicals

Information from: Jewel Bennett, NCTC Science Lab Director
Phone: 304-876-7469, E-mail: Jewel_Bennett@fws.gov

This session will provide students with an overview of how to work safely and responsibly with the chemicals used in the preservation of amphibians.

Specific learning objectives are:

1. Know about the chemicals you maybe handling.

2. Select the proper personal protective equipment (PPE) for the job.

3. How to deal with chemical waste produced in your lab or field activities.

4. How to store and dispose of your chemicals.

5. How to plan for a chemical spill.

6. Where to obtain items needed for your health and safety.

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1. Know About the Chemicals You Are Handling

A. You may be working with ethyl alcohol (ethanol) and possibly formalin (10% formaldehyde).

B. In order to work safety and responsibly with chemicals you must have a working knowledge about them (see also Box 1.1 for components of an overall safety program).

1. Maintain Material Safety Data Sheets (MSDS) on each chemical (see Box 1.2)

2. Know how to find information on the sheets, and keep them filed in a location useful to lab workers.

2. Select Proper Personal Protective Equipment (PPE)

A. Eye Protection

1. Safety glasses - large, curved plastic glasses provide more protection from the top and side, and are good for most general uses of planned activities.

B. Gloves - gloves should be used routinely to limit your dermal exposure.

1. Chose the most protective glove material for the chemical you will be handling (see write-up and Chemical Resistance Guide tables in Box 1.3).

2. Get gloves that fit well, and have enough so that you can throw them out and use a new pair if they break, tear, or get so full of sweat you are tempted to work without them.

C. Respiratory Protection (see Formaldehyde Standard in Box 1.4).

3. How to Deal with Chemical Waste Produced in your Lab or Field Activities

A. Use the following principles:

1. Use the smallest volume of chemical possible that can still do the job for you.

2. Capture the waste streams in proper containers.

3. Return the waste to you facility for proper disposal or storage until you can dispose it properly.

4. Work diligently to limit the amount of chemical that is accidently released to the environment.

4. How to Store and Dispose of your Chemicals.

A. Storage chemicals in cabinets that provide certain safety features

1. Lockable

2. Outside warning labels

3. Separate areas for flammables and corrosive chemicals

Rule of Thumb: *Keep volumes of chemical stored to a minimum. It's safer and cheaper!!*

B. How to Dispose of your Chemicals

1. Determine what things can be disposed of via your sanitary sewer. Drain disposal should only be used for "safe" chemicals, and that must be determined by environmental officers at your water treatment facility. You can obtain that information by contacting them, and obtaining a "safe" list with quantity and dilution guidelines.

2. In general, the only types of chemicals or chemical solutions that may be permitted into water treatment systems are soapy water solutions, dilute buffer solutions, and dilute solutions of inorganic salts.

3. Do not dispose of any chemicals into any drain that does not go to a sanitary sewer. For example, storm drains go directly to surface water.

4. Any chemicals or chemical solutions that cannot be disposed of via your sanitary sewer system will need to be held for disposal by a waste handler.

5. If your chemical waste stream is relatively small, you may be able to add your waste to that of a cooperative larger generator in your locale, such as a research lab, college science lab, or hospital. Be prepared however, to maintain records of what you've transferred to them, and you may still incur costs.

6. Some waste handling companies provide services for small generators, and will pick up relatively small quantities on a frequent basis. A responsible vendor will work with you to obtain a Generator Identification Number from EPA, and will help you maintain the chemical manifests, and final disposal records. Do not obtain the services of a vendor that does not provide you with such information, as your facility retains legal responsibility for the handling of the chemical through it's final disposal.

5. How to Plan for a Chemical Spill

A. Chemical releases frequently occur when:

- Containers break or rupture
- Shelf space is too crowded
- Shelves break, lean or don't have spill lips
- Chemicals or containers are handled inappropriately (use a funnel, pump and spill trays)

How to Respond to Various Types of Spills - a quick reference

Spill Response for Chemicals used in Preserving Amphibians			
Chemical	**Type of Chemical**	**Primary Response Action**	**Type of Cleanup Supplies**
formaldehyde	volatile poison	convert to a gel, transfer to waste drum	formaldehyde polymerizing granules
ethyl alcohol	flammable	remove ignition sources, use absorbents, ventilate area	absorbents, waste drum for flammable solids

6. Where to Get More Information and to Obtain Items for your Health and Safety

References:

Furr, A.K. 1995. CRC Handbook of Laboratory Safety. 4th Ed. CRC Press, Boca Raton. 783 pp.

Merck Index: an Encyclopedia of Chemicals, Drugs, and Biologicals. 1989. 11th Ed. Merck and Co., Inc., Rahway, N.J. (there are more recent editions).

Pipitone, D.A. 1991. Safe Storage of Laboratory Chemicals, 2nd ed. Wiley Interscience. John Wiley and Sons. New York. 297 pp.

Sittig, M. 1991. Handbook of Toxic and hazardous Chemicals and Carcinogens. 2 Volumes. 3rd Ed. Noyes Pubications, Westwood ,NJ.

Vendors:

Gempler's, Belleville, WI. 800-382-8473. www.gemplers.com.
Lab Safety Supply. Janesville, WI. www.labsafety.com. (800.356.0783)
Fisher Scientific. 800-766-7000. www.fishersci.com.

Box 1.1

The Hazard Communication (Right-to-Know) Standard 29 CFR 1910.1200

Introduction

The Hazard Communication Standard, also known as the Right-to-Know Law, was first enacted on November 25, 1983, by the Occupational Safety and Health Administration (OSHA). It was later modified with minor changes and technical amendments to take effect March 11, 1994. The standard is referenced by Title 29, *Code of Federal Regulations* (CFR) 1910.1200 and amended in the February 9, 1994, *Federal Register.*

The purpose of the standard is to ensure that chemical hazards in the workplace are identified and evaluated, and that information concerning these hazards is communicated to employers and employees. This transfer of information is to be accomplished by means of a comprehensive hazard communication program, which includes container labeling and other forms of warning, Material Safety Data Sheets (MSDS) and employee training.

Categories of the Right-to-Know Law The standard is comprised of six major categories: **Hazard Determination, Material Safety Data Sheets, Chemical Labeling, Employee Training, The Written Program** and **Trade Secrets.**

The first category, **Hazard Determination** (29 CFR 1910.1200 (d)) requires employers to identify and evaluate all chemicals used in the workplace. This evaluation is based on two hazard categories: listed and defined. **Listed hazards** are those included in one of the following references: OSHA 29 CFR 1910.1000 Z tables; American Conference of Governmental Industrial Hygienists (ACGIH) Threshold Limit Values (TLV); the National Toxicology Program; or the International Agency for Research on Cancer. **Defined hazards** are those specified by OSHA as physical or health hazards, such as combustible liquids, oxidizers, corrosives, reproductive toxins and non-toxins.

Chemicals exempt from the standard include: wood and wood products (except wood dust), regulated hazardous waste, tobacco products, food, drugs, cosmetics, alcoholic beverages, agricultural or vegetable seed treated with pesticides, various types of pesticides, nuisance particulate, and articles. These are exempt because they are all regulated by separate government standards.

The second major category of the standard is the **Material Safety Data Sheet** (29 CFR 1910.1200(g)). Once you have evaluated and identified all of the hazardous chemicals in your workplace, you must document them and obtain an MSDS for each item. MSDS are available from the chemical supplier or manufacturer. These sheets contain specific chemical hazard information such as: physical hazards, health hazards, routes of entry, exposure limits (if any), precautions for safe handling and use (if known), spill clean-up procedures, personal protective equipment to be used, emergency and first aid procedures, and the name, address and telephone number of the chemical manufacturer. All of the information on the MSDS must be in English and be available to employees working with or near the hazardous chemical.

The third category, **Chemical Labeling**, (29 CFR 1910.1200(f)) requires labels on all chemicals in the workplace. The label should contain the identity of the material, appropriate hazard warnings and the name and address of the manufacturer, importer or other responsible party. Other appropriate warning information (such as pictures and symbols) may be used in conjunction with the hazard

information. Labels must be legible and in English. Labels in a second language may be added as long as the English label is present. [For more information on labeling, please refer to EZ Facts document number 200, "Chemical Labeling Requirements."]

The fourth category, **Employee Training** (29 CFR 1910.1200(h)) requires employers to provide employees with effective information and training on hazardous chemicals in their work area at the time of their initial assignment and whenever a new physical or health hazard is introduced into the area. The training shall include: methods and observations used to detect the presence or release of the chemical, physical and health hazards, protective measures, labeling and explanation of the MSDS.

The fifth category is **The Written Program** (29 CFR 1910.1200(e)). It requires employers to fully document the actions taken to comply with all of the provision of the standard and to list the responsible person(s) for each area of the program. A copy of the written program must be made available, upon request, to all employees and OSHA officials.

The sixth and final category of Hazard Communication involves manufacturer **Trade Secrets** (29 CFR 1910.1200(i)). The chemical manufacturer may withhold the chemical identity, including the chemical name and other specific information, from the MSDS. However, under special conditions, this secret information may be obtained by health care professionals.

Sources for More Information

29 CFR 1910.1200, Hazard Communication.

Federal Register, February 9, 1994.

ACGIH 6500 Glenway Ave Bldg D-7 Cincinnati, OH 45211

National Toxicology Program Contact: National Technical Information Services 5285 Port Royal Rd Springfield, VA 22161

International Agency for Cancer Research
Contact: World Health Organization
49 Sheridan Street
Albany, NY 12210

Box 1.2

What is a Material Safety Data Sheet (MSDS)?

A Material Safety Data Sheet (MSDS) is designed to provide both workers and emergency personnel with the proper procedures for handling or working with a particular substance. MSDS's include information such as physical data (melting point, boiling point, flash point etc.), toxicity, health effects, first aid, reactivity, storage, disposal, protective equipment, and spill/leak procedures. These are of particular use if a spill or other accident occurs.

Who are MSDS's for?

MSDS's are meant for:

Employees who may be occupationally exposed to a hazard at work.

Employers who need to know the proper methods for storage etc.

Emergency responders such as fire fighters, hazardous material crews, emergency medical technicians, and emergency room personnel.

MSDS's are not meant for consumers. An MSDS reflects the hazards of working with the material in an occupational fashion. For example, an MSDS for paint is not highly pertinent to someone who uses a can of paint once a year, but is extremely important to someone who does this in a confined space 40 hours a week.

See also: Manufacturer and employer responsibilities when providing MSDSs electronically for more about employer and manufacturer responsibilities.

What does an MSDS look like?

The formats of MSDS's tend to vary, but they usually convey the same basic kinds of information.

The American National Standards Institute (ANSI) developed a new standard (Z400.1-1993) to assist with the format and preparation of Material Safety Data Sheets (MSDS). The purpose of this *voluntary* standard is to provide information in a consistent manner and to make it easier to find information regardless of the supplier of the MSDS. The following list indicates the 16 sections of the new MSDS standardized format.

Section 1: Chemical Product and Company Identification This section links the chemical name on the label to the MSDS. The MSDS also lists the name, address and the phone number of the company, manufacturer or distributor who provides the chemical.

Section 2: Composition, Information or Ingredients This section must identify all the hazardous ingredients of the material. This section may also include OSHA Permissible Exposure Limits

(PELs) and ACGIH (American Conference of Governmental Industrial Hygienists) Threshold Limit Values (TLVs).

Section 3: Hazard Identification This section discusses the health effects one may encounter when exposed to the material. The section will describe the appearance of the material, the potential health effects and symptoms associated with exposure, routes of entry, target organs that could be affected, and so on.

Section 4: First Aid Measures This section will describe possible first aid procedures for each route of entry. The procedures will be written so that untrained individuals can understand the information.

Section 5: Fire-Fighting Measures This section will describe information on the fire and explosive properties of the material, extinguishing items, and general fire-fighting instructions.

Section 6: Accidental Release Measures This section gives information on how to respond when a material spills, leaks or is released into the air. This information may include how to contain a spill or the types of equipment that may be needed for protection.

Section 7: Handling and Storage This section discusses information on handling and storage of the material. Topics that could be described are: general warnings to prevent overexposure, handling procedures, and hygiene instructions to prevent continued exposure.

Section 8: Exposure Controls and Personal Protection This section discusses engineering controls and personal protective equipment that would help reduce exposure to the material. The necessary personal protective equipment should be considered for eye/face protection, skin protection and respiratory protection.

Section 9: Physical and Chemical Properties This section will include information about the physical and chemical properties of the material. The following characteristics should be detailed: appearance, odor, physical state, pH, vapor pressure, vapor density, boiling point, freezing/melting point, solubility in water and specific gravity or density. Indicate if these characteristics do not apply to your material.

Section 10: Stability and Reactivity This section requires that potentially hazardous chemical reactions be identified. It addresses chemical stability, conditions to avoid, incompatibility with other materials, hazardous decomposition and hazardous polymerization.

Section 11: Toxicological Information This section discusses data used to determine the hazards that are given in Section 3, "Hazard Identification." The following information can be addressed: acute data, carcinogenicity, reproductive effects, target organ effects, etc.

Section 12: Ecological Information This section will help determine the environmental impact should the material ever be released into the environment.

Section 13: Disposal Considerations This section gives important information that may be helpful in the proper disposal of the material. The information can cover disposal, recycling and reclamation.

Section 14: Transport Information This section is designed to give basic shipping information. The basic shipping information could include: the hazardous materials description, hazard class and the identification number (UN or NA numbers).

Section 15: Regulatory Information This section discusses information on the regulations under which the material falls. Examples of a few regulatory agencies are: OSHA, TSCA (Toxic Substance Control Act), CERCLA (Comprehensive Environmental Response, Compensation, and Liability Act), SARA Title III (Superfund Amendments and Reauthorization Act).

Section 16: Other Information This section should include any other important information concerning the material. This information can include: hazard ratings, preparation and revisions of the MSDS, and label information.

This is a general overview of the ANSI Z400.1-1993 standard. The standard is intended to help develop consistent, understandable MSDSs that will provide useful information to a cross-section of education levels, from the average person to the chemist. The MSDS sections were prioritized according to the usefulness of the information. If you wish to obtain a copy of this standard, please contact:

American National Standards Institute 11 West 42nd Street New York, NY 10036

Where can I get MSDS's?

There are several places you can get them.

Your laboratory or workplace should have a collection of MSDS that came with the hazardous chemicals you have ordered (don't throw them away!)

Most universities and businesses have a collection somewhere on site. Check with your Environmental or Occupational Health Office or science librarian.

Some organizations use commercial services to obtain printed, FAX or on-line copies of MSDS's.

You can get them from the distributor that sold you the material. If you can't find them then contact the manufacturer's customer service department.

The Internet has a wide range of **FREE** resources.

You can purchase software or internet subsciption services.

Box 1.3

Chemical Protective Gloves

Introduction

On October 5, 1994, OSHA's personal protective equipment standards for general industry (29 CFR 1910.132-1910.138) went into effect. One of these new standards (29 CFR 1910.138) specifically addresses the need for hand protection:

(a) Appropriate hand protection must be worn when hands are exposed to hazards such as skin absorption of harmful substances, severe cuts, lacerations or abrasions, punctures, chemical or thermal burns, and harmful temperature extremes.

(b) Employers shall base selection of appropriate hand protection on an evaluation of the performance characteristics of the hand protection relative to the task(s) to be performed, conditions present, duration of use, and the hazards and potential hazards identified.

This rule makes it mandatory to match the right glove material with each application or task. This includes assessing the job for chemical exposures, and then selecting the appropriate glove based on material, thickness, length and other traits.

Hazard Assessment

NOTE: *An industrial hygienist or other safety professional familiar with the task can perform a hazard assessment.* The hazard assessment begins with knowing what chemicals or combination of chemicals the task or job requires. Determine the chemicals' toxic properties by looking up the actual chemical names on the **Material Safety Data Sheets** (MSDSs). Look for whether the chemicals can cause local effects on the skin, or if they are readily absorbed by the skin and cause systemic effects.

When reviewing the job requirements, take into account the degree of dexterity needed for the task. Tasks that require fine motor skills, such as laboratory work, may require a thinner glove material, while operations such as industrial parts cleaning may not.

Also, consider the length of exposure to the chemical. Some tasks may require only splash protection or include intermittent contact, while others may involve complete immersion or continual contact with the chemical.

Another factor to consider is chemical concentration. The higher the concentration of a chemical, the shorter the breakthrough time. The hazard assessment will also take into account other hazards of the job, such as cut or abrasion hazards. It's important to remember that although the number of glove choices can be staggering, no one glove can possibly address all types of hand hazards. Gloves are never a substitute for safe work practices.

Choosing a Glove Material

Because different glove materials resist different chemicals, no one glove is suited for all chemical exposures. A glove that is suited well for one application may prove dangerous for another. Base your glove material selection on the manufacturer’s chemical resistance guide. From this guide, choose a glove that is most resistant to the chemicals being used. Remember, the actual chemical compatibility of a given glove material can vary from manufacturer to manufacturer. Make sure your

selection is based on that particular manufacturer's test data.

A standard released in March of 2000 from the American National Standards Institute (ANSI) titled, American National Standard for Hand Protection Selection Criteria (ANSI/ISEA 105-2000), may prove helpful to both users and manufacturers of chemical protective gloves. This standard establishes rating levels and identifies acceptable test protocols for a variety of glove characteristics, of which chemical protection is one element. This standard should some day make it easier for glove consumers to accurately compare the performance of gloves between different manufacturers. However, until this standard is widely adopted, chemical protective glove users will have to closely analyze each manufacturers testing data to ensure they're comparing apples to apples and getting the proper information.

Thickness and Length

Other choices you must make in selecting chemical protective gloves include thickness and length. Thicker gauge gloves are heavier and have better chemical resistance than thinner gauge gloves. Thinner, lighter gloves offer better touch sensitivity and flexibility, but in doing so sacrifice some of their chemical resistance. The manufacturers generally state that doubling the thickness of a glove quadruples the breakthrough time of the chemical.

Glove thickness is stated in either mils or gauge. A 10-gauge glove equals 10 mils, or 0.010 inches. When choosing your glove, look for the stated thickness on the manufacturer's test data.

When assessing the job, also take into account the length of glove needed. Extra splash or immersion protection is provided by gloves longer than 14 inches. For deep tank cleaning or glove box applications, gloves can be as long as 31 inches. Remember that longer gloves are also made with thicker materials.

Finishes and Linings

Gloves also have a variety of finishes and linings. Textured finishes are applied to gloves to give you a better grip on a wet object. Flock and knit linings are offered on many styles of gloves. Cotton flock linings are applied for basic perspiration absorption. Knit linings do this too, as well as offering a small amount of temperature protection.

Inspection and Care

Even the best chemically resistant glove will break down after repeated chemical exposures. Before each use, inspect all gloves for signs of chemical degradation such as swelling, cracking, shrinking or discoloration of the material. When you detect any of these, it means the glove material has undergone a physical change due to chemical contact and will no longer provide chemical protection. Take the gloves out of service and request a new pair from your supervisor. Also during inspection, look for any signs of holes or punctures. Again, if you find any, take these gloves out of service and notify your supervisor or on-site safety professional. For complete care and maintenance instructions, refer to the glove manufacturer's information.

Chemical Compatibility

Glove Material: NITRILE

Challenge Chemical	Degradation	Breakthrough Time	Permeation Rate
Isopropanol	E	>480 min.	.001

Breakthrough Time: The elapsed time between initial contact of the chemical on the glove surface and the analytical detection on the inside of the glove. Typically expressed as a greater than symbol (>), the example shows the test was run for 480 minutes and then stopped. Also may be expressed as "ND" for none detected

Degradation: A change in one or more of the physical properties of a glove due to contact with a chemical. Can appear as a swelling, softening, shrinkage or cracking of the material. Rating example is "E" for excellent, meaning the glove has little or no signs of degradation when exposed to the challenge chemical. A good degradation rating does not guarantee an acceptable breakthrough time.

Permeation Rate: The rate at which a chemical passes through a glove material. This process involves absorption on the glove surface, the diffusion of the chemical through the material, and the desorption on the glove's inside surface. This is a complex measurement: µg/cm2/MIN (micrograms per square centimeter per minute). This measurement is also limited to the "LDL" or Lower Detection Limit of the equipment used. The example given is .001, but is sometimes expressed as "E" or "P" for excellent or poor.

Glove Material Applications

Butyl: A synthetic rubber material that offers the highest permeation resistance to gas and water vapors. Especially suited for use with esters and ketones.

Neoprene A synthetic rubber material that provides excellent tensile strength and heat resistance. Neoprene is compatible with some acids and caustics. It has moderate abrasion resistance.

Nitrile A synthetic rubber material that offers chemical and abrasion resistance-a very good general-duty glove. Nitrile also provides protection from oils, greases, petroleum products and some acids and caustics.

PVC (polyvinyl chloride) A synthetic thermoplastic polymer that provides excellent resistance to most acids, fats and petroleum hydrocarbons. Good abrasion resistance.

PVA™ (polyvinyl alcohol) A water-soluble synthetic material that is highly impermeable to gases. Excellent chemical resistance to aromatic and chlorinated solvents. This glove cannot be used in water or water-based solutions.

Viton® A fluoroelastomer material that provides exceptional chemical resistance to chlorinated and aromatic solvents. Viton is very flexible, but has minimal resistance to cuts and abrasions.

SilverShield® A lightweight, flexible laminated material that resists permeation from a wide range of toxic and hazardous chemicals. It offers the highest level of overall chemical resistance, but has virtually on cut resistance.

4H A lightweight, patented plastic laminate that protects against many chemicals. Good dexterity.

Chemical Degradation, Breakthrough Time, and Permeation Rate for
Ethyl Alcohol and Formaldehyde (37% in Water)

(See next page for term definitions)

Each glove type by brand is rated by three attributes for both chemicals.
The attributes are Degradation (on top), Breakthrough Time (middle), and Permutation Rate (bottom).

	Vitron (North, 10 mil)	Vitron (Best, 30 mil)	Silvershield (North, 4 mil)	4H (Safety 4, 3 mil)	PVA (Ansell Edmont)	Butyl (Lab Safety Supply, 25 mil)	Butyl (North, 17 mil)	Neoprene (Ansell Edmont, 15 mil, unsupported)	Neoprene (Best Ultraflex)	Neoprene (Best Neoprene)	PVC (Best Hustler)	Nitrile (Lab Safety Supply, 22 mil)	Nitrile (Ansell Edmont, 22 mil)
Ethyl Alcohol	NT NT NT	E ND ND	NT NT NT	NT >480 minutes NT	NR NT NT	NT ND NT	NT NT NT	E 90 minutes VG	E 71 minutes 23	E ND ND	E 66 minutes 4	E >480 minutes ND	E 4 hours VG
Formaldehyde (37% in water)	E > 16 hours ND	E ND ND	E > 6 hours ND	NT >240 minutes NT	P NT NT	NT NT NT	E 16 hours ND	E 2 hours E	E ND ND	E ND ND	E ND ND	E >480 minutes ND	E ND E

The previous chart will help you select the proper glove for handling ethyl alcohol and formaldehyde. Choose the most appropriate glove by comparing the degradation, breakthrough time and permeation rate. This chart is a collection of data provided by the individual glove manufacturers. Remember that these tests were conducted on specific gloves under laboratory conditions.

DEGRADATION (D) is a reduction in physical properties of the glove material. Common effects include swelling, wrinkling, stiffness, change in color or other deterioration. The degradation ratings indicate how well a glove will hold up when working with a specific chemical. Degradation tests vary by manufacturer - there's no standardized test that's used by everyone in the industry. However, the glove material is usually exposed to the test chemical and the percent weight change is then determined at four time intervals: 5, 30, 60 and 240 minutes. *In our chart, we report results for the 30 minute test.*

DEGRADATION KEY

RATING	**% WEIGHT CHANGE**
E = Excellent	0-10%
G = Good	11-20%
F = Fair	21-30%
P = Poor	over 30%
NR = Not Recommended	
NT = Not Tested	

Degradation resistance is essential to worker safety but should not be the sole determining factor. The chemical's *breakthrough* time and permeation rate are the two other important factors that must be considered.

BREAKTHROUGH TIME (BT) is the elapsed time between initial contact of the chemical on the glove material and the analytical detection if the chemical passes through the glove material. This test is conducted per ASTM F739 standard test method for *Resistance of Protective Clothing Materials to Permeation by Hazardous Liquid Chemicals*. The higher the result, the longer it takes for chemical to pass though the glove material. The actual time is reported on the chemical compatibility chart. If breakthrough did not occur, the data reported is ND *(none detected)* or > (*greater than*) the indicated test period.

BREAKTHROUGH TIME KEY

ND = None Detected

> = Greater than

NR = Not Recommended

< = Less Than

NT = Not Tested

PERMEATION RATE (PR) is a measurement which describes the rate of chemical breakthrough that has occurred. Manufacturers report permeation rate in different ways. Some report in micrograms of chemical per square centimeter of glove material per minute. The higher the result, the more chemical passing through the glove material. Other manufacturers rate the permeation similar to that done for degradation - *Excellent* (E), *Good* (G), *Fair* (F), *Poor* (P) and *Not Recommended* (NR). If chemical breakthrough does not occur, then permeation is not measured. This is reported as NC (*none detected*) or NT (*not tested*), depending upon the manufacturer. This test is also conducted per ASTM F739.

PERMEATION RATE

E = Excellent

G = Good

F = Fair

P = Poor

ID = Insufficient Data

VG = Very Good

NR = Not Recommended

ND = None Detected

NT = Not Tested

NOTE: This chemical compatibility information shows how each specific glove material performed in degradation, breakthrough and permeation tests against ethyl alcohol (ethanol) and formaldehyde. These tests were performed under laboratory test conditions which can vary from their end use. Therefore, this information must be used only as a guide. The suppliers of this chemical data (North, Best, Safety4, Mapa Professional and Ansell Edmont) encourage all users to conduct their own backup tests to determine the suitability of a glove for a specific application.

Box 1.4

Formaldehyde Standard, 1910.1048

Introduction

The Formaldehyde Standard (29 CFR 1910.1048) was designed to protect workers from occupational exposures to any form of formaldehyde including its solutions, gas, or any materials that release it into the workplace.

Exposure Limits

• Action Level: Airborne concentration of 0.5ppm formaldehyde. If this level is exceeded, the employer must perform periodic air monitoring until levels can be reduced below this point (29 CFR 1910.1048(b)).

• Permissible Exposure Limit(PEL): Airborne concentration of 0.75ppm formaldehyde as an 8-hour time-weighted average (29 CFR 1910.1048(c)(1)).

• Short-Term Exposure Limit(STEL): Airborne concentration of 2ppm formaldehyde over a 15 minute time interval (29 CFR 1910.1048(c)(2)).

Exposure Monitoring

All employers who have any form of formaldehyde in the workplace must monitor employee exposure unless they can objectively document that the presence of airborne formaldehyde will not exceed the action level or STEL under foreseeable conditions (29 CFR 1910.1048(d)(1)). If this cannot be done the employer must begin initial monitoring.

Initial monitoring is accomplished by identifying all employees who potentially have an exposure at or above the action level or STEL. Each potentially exposed employee may be monitored or a representative sampling plan implemented for each job classification and work shift. Monitoring will occur each time a change in equipment, process, production, personnel, or control measures is instituted (29 CFR 1910.1048(d)(2)). If monitoring reveals formaldehyde concentrations at or in excess of the action level, monitoring will be repeated every six months, or if at or above the STEL, annual monitoring is required (29 CFR 1910.1048(d)(3)).

Monitoring can be discontinued if after two consecutive sampling periods taken at least seven days apart, if airborne concentrations are below the action level or STEL (29 CFR 1910.1048(d)(4)).

REGULATED AREAS

In areas that exceed the PEL or STEL for formaldehyde, all entrances and accessways must post warning signs as shown below. These areas must have access limited to employees who have been trained to recognize the hazards of formaldehyde (29 CFR 1910.1048(e)).

Methods of Compliance

Employers must implement engineering or work practice controls to decrease employee exposure to formaldehyde below the PEL and the STEL (29 CFR 1910.1048(f)). If feasible engineering or work practice controls cannot decrease the airborne exposure below the PEL or STEL, the controls must be applied and supplemented with respirators. Following are some of the various protection methods that can be used.

Respiratory Protection

In areas that require respiratory protection, respirators must be provided at no cost to employees and employers must assure they are properly used to reduce exposure below the PEL and STEL. Respirators should only be used in the interval necessary to install engineering or work practice controls, or operations in which controls are not feasible such as maintenance, repair or emergencies.

Employers must use NIOSH/MSHA-approved respirators and establish a written respiratory protection program in accordance to 29 CFR 1910.134. Minimum requirements for respiratory protection are shown in the chart below, which illustrates the type of protection required under specific concentrations of formaldehyde. Note: Half-mask respirators with formaldehyde cartridges are permitted if gas-proof goggles are used with the mask.

Formaldehyde Concentration	Minimum respirator concentration required
Up to 7.5 ppm:	Full facepiece with cartridges or canisters specific for formaldehyde.
Up to 75 ppm:	Full facepiece with chin, back, or chest style industrial sized canisters specific for formaldehyde. Type C supplied-air respirator (continuous flow or pressure-demand) with hood, helmet, or full facepiece.
Above 75 ppm:	Self-contained breathing apparatus with positive- or unknown pressure full facepiece. Combination supplied-air, full facepiece, positive-pressure, with auxiliary self-contained air supply.
Firefighting:	SCBA, positive-pressure, full-facepiece
Escape:	SCBA, demand or pressure-demand mode. Full face mask with chin, back, or chest style industrial sized canisters specific for formaldehyde.

When air-purifying cartridge respirators are used, the cartridge must be replaced after three hours of use or at the end of the workshift, whichever is shorter, unless the cartridge contains a NIOSH approved end-of-service life indicator to show when breakthrough occurs. Similarly, if a canister does not have an end of service life indicator it must be replaced after four hours while industrial-sized canisters must be replaced every two hours or the end of the workshift, whichever is shorter (29 CFR 1910.1048(g)).

Personal Protective Equipment

Employers should select protective clothing and equipment based on the form of formaldehyde, conditions of use, and any hazards to be prevented (29CFR1910.1048(h)). For additional information on choosing chemical protective clothing and gloves, see EZ Facts Document Nos. 190 and 191.

Hygiene Protection

Employers are required to provide drench showers for employees skin splashes of solutions containing 1% or more of formaldehyde. If an employees eyes could be splashed with a 0.1% or greater formaldehyde solution the employer must provide acceptable eyewash facilities (29 CFR 1910.1048(i)). For additional information on Eyewash and Emergency Showers, see EZ Facts Document No. 120.

Housekeeping and Emergencies

Operations involving any form of formaldehyde require employers to conduct visual inspections to detect leaks and spills, provide preventative maintenance of equipment at regular intervals, and assure leaks are properly repaired(29 CFR 1910.1048(j)). Emergency procedures are required to be implemented to minimize injury and loss of life (29CFR1910.1048(k)).

Medical Surveillance

Employers must implement a medical surveillance program for all employees exposed to formaldehyde at concentrations at or above the action level or exceeding the STEL (29 CFR 1910.1048(l)). This program will consists of medical and physical examinations performed by a physician to detect any symptoms of overexposure and prescribe necessary remedies. A medical disease questionnaire must be completed by employees prior to assignment to work areas that could expose them to formaldehyde. Respirator fit testing to verify an employees ability to wear a respirator in the work environment must also be completed.

Chapter 5

USFWS National Abnormal Amphibian Database
DATA FORMATTING REQUIREMENTS

NOTE: The following tables detail the formatting requirements for all data fields required in FY04. These guidelines must be followed closely so that our data can readily be incorporated into the database, which is currently under development and will likely be implemented by the end of this season. Adhering closely to these guidelines will help avoid the need to reformat your data later in the season and will reduce the potential for introducing error when repeating data entry.

SITE CHARACTERIZATION FORM:

This form should be filled out for every site that has been selected for monitoring.

Field Name	Format	Description
Refuge	Text	Refuge Name
Site ID	LLL##	3 letter Refuge ID & 2 digits (See Refuge ID Codes Table)
Date Selected	##/ ##/ ####	MM/DD/YYYY
Collectors	Text	List full names of all collectors present
GPS Coord (Latitude)	### . #######	WGS 84 (decimal degrees)
GPS Coord (Longitude)	### . #######	WGS 84 (decimal degrees)
GPS Model	Text	Make, Model
Elevation	####	Meters
Area	###	square meters
Water levels	select from list	**deep** (> 3ft in some areas) **shallow** (< 3ft throughout) **drying** (between 3 ft and dry) **dried up** (no water)
% Canopy Cover	### %	Estimate % canopy cover over pool
Habitat Type	select from list	(See Habitat Types on Look Up Table)
Contaminants of Concern	select from list	(See Contaminants of Concern on Look Up Table)
SLU-North	select from list	(See Surrounding Land Uses on Look Up Table)
SLU-East	select from list	(See Surrounding Land Uses on Look Up Table)
SLU-South	select from list	(See Surrounding Land Uses on Look Up Table)
SLU-West	select from list	(See Surrounding Land Uses on Look Up Table)
Site Photo Filename	LLL##-LL-######	Site ID - direction facing - date w/o dashes (resolution minimum: 640 x 480, preferred file format - .jpg) If no digital camera is available, submit one of the following, in order of preference: original photo, 35mm slide, film negative
Comments	Text	200 characters of relevant comments

Note: "L"=Letter & "#"=Integer

DATA FORMATTING REQUIREMENTS CONTINUED

STANDARD DATA COLLECTION FORM:

This form should be filled out during EVERY site visit.

Field Name	Format	Description
Monitoring/Collection Date	##-##-####	MM/DD/YYYY
Site ID	LLL##	3 letter Refuge ID & 2 digits (See Refuge ID Codes Table)
Collectors	Text	List full names of all collectors present
Start Time	##: ##	Military format. Record time arrived at site.
End Time	##: ##	Military format. Record time leaving site.
Ambient Temp	##.#	Celsius with one decimal place
Water Temp	##.#	Celsius with one decimal place
Water levels	select from list	**deep** (> 3ft in some areas) **shallow** (< 3ft throughout) **drying** (between 3 ft and dry) **dried up** (no water)
Weather	Text	200 characters. General description of weather (ex: cloudy, rainy, overcast, sunny)
# Egg Masses Present	##	Estimate # egg masses present
Species of Egg Masses	LLLL	Use four letter Species Codes (note with ? if Species ID is questionable)
Egg Mass Water Depth	##.#	Centimeters with one decimal place. Estimate the average water depth where masses are located
Gosner Stage of Tadpoles	##	Determine Gosner stage for 8-10 tadpole /species using Gosner Stage Chart
Species of tadpoles staged	LLLL	Use four letter Species Codes
Species of Metamorphs	LLLL	Use four letter Species Codes
Gosner Stage	##	(See Gosner Stage Chart)
Snout-Vent Length	##.#	Millimeters with one decimal place
Tail Length	##.#	Millimeters with one decimal place
Abnormal Frog ID	L# - LLL## - ###### -LLLL - ####	Region - SiteID – CollectionDate – SpeciesCode – frog # (Frog # is from the sequentially numbered frogs in the collection it came from - 3 digits, up to 100) ex: R4-OVF01-060603-RASP-001
Incidental Species	Text	200 characters (use Species Codes for any anurans)
Comments	Text	200 characters. Note any decisions made regarding when site will be revisited or if not, why.

Note: "L"=Letter & "#"=Integer

USFWS AMPHIBIAN DATABASE CODES - LOOKUP TABLE DEFINITIONS

Habitat Types:	Definition:
Natural Lake	(= a naturally formed inland body of water, of considerable size)
Man-made Lake	(= artificially confined body of water, resembling a natural lake, attributed to human activity)
Oxbow/Slough	(= a body of water that is out of the main current of a larger body of water)
Backwater/Floodplain	(= level land that may be submerged by floodwaters)
Natural pond	(= a naturally formed body of water, smaller than a lake)
Beaver Pond	(= artificially confined body of water, attributed to beaver activity)
Man-made Pond	(= artificially confined body of water, resembling a natural pond, attributed to human activity)
Impoundment	(= artificially confined body of water, primary function is water reservoir)
River	(= a natural stream of water, usually of considerable volume)
Creek/Stream	(= a natural stream of water, normally smaller than and often tributary to a river)
Forested wetland	(= a wetland with saturated soils that is dominated by woody plants taller than 20ft)
Emergent wetland	(= a wetland dominated by emergent plants, includes wet meadows & marshes)
Bog/Fen	(= a poorly drained area of wet spongy ground, often with: sedges, heaths, and sphagnum)
Spring/Seep	(= a source of water issuing from the ground)
Cave	(= a natural underground chamber or series of chambers open to the surface)
Roadside Ditch	(= shallow man-made ditch, primary function drainage)
Irrigation Ditch	(= deep man-made ditch, primary function provide irrigation water for ag fields)
Barrow Pit	(= man-made entrenchment that fills with water, primarily created during road building)
Roadside Puddle	(= shallow "natural" low spot along roadside that fills with water)
Tire Ruts	(= ruts that fill with water, primarily created by vehicle tires)
Cultivated Field	(= includes active or recently cultivated row crop fields)
Rangeland/Pasture	(= includes grazed lands or old fields where cultivation may no longer be apparent)
Tundra	(= treeless plain of the arctic, where mosses, lichens, etc. are dominant vegetation)
Prairie	(= treeless plain where grasses are dominant vegetation)
Savannah	(= an open canopy area, characterized by scattered trees and drought-resistant undergrowth)
Other (describe in comments)	(= describe & define new category in comments)

Contaminants of Concern:	Definitions
Pesticides (current)	(= potentially receives inputs from active ag in distant past & active THIS season)
Pesticides (recent)	(= potentially receives inputs from active ag in distant past through previous 1-2 yrs)
Pesticides (historic)	(= potentially receives inputs from active ag in distant past)
Highway runoff	(= potentially receives runoff from paved road)
Gravel road runoff	(= potentially receives runoff from gravel road)
Sewage	(= potentially receives effluents from raw or treated sewage)
Nutrients	(= potentially receives effluents rich in nutrients)
Sediments	(= potentially receives sediment rich effluents)
Petroleum (current)	(= potentially has persistent compounds present & receives inputs from current oil industry activities)
Petroleum (historic)	(= potentially has persistent compounds present from historic oil industry activities)
Fertilizers	(= potentially receives fertilizer ammendments through agricultural practices)
Heavy Metals	(= potentially receives effluents or aerial drift containing heavy metals)
Other	(= describe in comments)
None Suspected	(= reference site)

Surrounding Land Use:	Definitions:
Agriculture (current)	(= active ag in past & active THIS season)
Agriculture (recent)	(= active ag in past through previous 1-2 yrs)
Agriculture (historic)	(= active ag in distant past)
Aquaculture	(= any aquaculture facility: fish, turtle, alligator, crayfish, etc.)
CAFO-Cattle	(= Confined Animal Feeding Operation - cattle)
CAFO-Swine	(= Confined Animal Feeding Operation - swine)
CAFO-Poultry	(= Confined Animal Feeding Operation - poultry)
Industrial Forest	(= managed primarily for production of wood products)
Natural Forest	(= managed primarily for something other than largescale harvest)
Industry	(= all types of industry, including wood product processing)
Mining	(= includes gravel, metals, gypsum, kaolin operations, etc.)
Oil Production	(= includes any petroleum industry activity, specify in comments)
Urban	(= residential or commercial development)
Waterbody / Riparian Corridor	(= adjacent to waterbody with riparian buffer: river, oxbow, lake, ocean, etc.)
Waterbody / NO Riparian Corridor	(= adjacent to waterbody without riparian buffer: river, oxbow, lake, ocean, etc.)
Other (describe in comments)	(= specify crops, waterbody or other land uses not covered in above categories)

Try to select the single best habitat & surrounding land use category for each site. If none of the above are well-suited, add a category and define it in comments. Also note whether each site is: Permanent or Non-Permanent. For contaminants of concern, up to three categories can be selected for each site, any additional concerns should be noted in comments.

R4 NATIONAL WILDLIFE REFUGE ID CODES

State	Refuge Name	Ref ID
AL		
1	Bon Secour NWR	BON
2	Cahaba NWR	CAH
3	Choctaw NWR	CHC
4	Eufaula NWR	EFL
5	Fern Cave NWR	FEC
6	Grand Bay NWR	GRA
7	Key Cave NWR	KEC
8	Sauta Cave NWR	BWC
9	Watercress Darter NWR	WAT
10	Wheeler NWR	WLR
AR		
1	Bald Knob NWR	BLD
2	Big Lake NWR	BGL
3	Cache River NWR	CCH
4	Felsenthal NWR	FSL
5	Holla Bend NWR	HLB
6	Logan Cave NWR	LOG
7	Overflow NWR	OVF
8	Oakwood NWR	
9	Pond Creek NWR	PNC
10	Wapanocca NWR	WPN
11	White River NWR	WHR
GA		
1	Banks Lake NWR	BLM
2	Blackbeard Island NWR	BLB
3	Bond Swamp NWR	BND
4	Harris Neck NWR	HSN
5	Okefenokee NWR	OKE
6	Piedmont NWR	PDM
7	Savannah NWR	SAV
8	Tybee NWR	TYB
9	Wassaw NWR	WSW
10	Wolf Island NWR	WLF
FL		
1	A.R.M. Loxahatchee NWR	LXH
2	Archie Carr NWR	ACR
3	Caloosahatchee NWR	CAL
4	Cedar Keys NWR	CKS
5	Chassahowitza NWR	CHS
6	Crocodile Lake NWR	CLA
7	Crystal River NWR	CRY
8	Egmont Key NWR	EGK
9	Florida Panther NWR	FLP
10	Great White Heron NWR	GWH
11	Hobe Sound NWR	HBS

State	Refuge Name	Ref ID
FL	**Cont.**	
12	Island Bay NWR	ISB
13	J.N. "Ding" Darling NWR	JND
14	Key West NWR	KEW
15	Lake Wales Ridge NWR	LKW
16	Lake Woodruff NWR	LWD
17	Lower Suwannee NWR	SWE
18	Matlacha Pass NWR	MAP
19	Merritt Island NWR	MRT
20	National Key Deer NWR	NKD
21	Passage Key NWR	PAK
22	Pelican Island NWR	PLC
23	Pine Island NWR	PII
24	Pinellas NWR	PIN
25	St Johns NWR	SJH
26	St Marks NWR	SMK
27	St Vincent NWR	SVN
28	Ten Thousand Islands NWR	TTH
KY		
1	Clarks River NWR	CLK
2	Green River NWR	
LA		
1	Atchafalaya NWR	ATC
2	Bayou Cocodrie NWR	BYC
3	Bayou Savage NWR	BSU
4	Bayou Teche NWR	BYT
5	Big Branch Marsh NWR	BBM
6	Black Bayou Lake NWR	BBL
7	Bogue Chitto NWR	BCT
8	Breton NWR	BTN
9	Cameron Prairie NWR	CAM
10	Cat Island NWR	CAT
11	Catahoula NWR	CTH
12	D'Arbonne NWR	DRB
13	Delta NWR	DLT
14	Grand Cote NWR	GDC
15	Handy Brake NWR	HND
16	Lacassine NWR	LCS
17	Lake Ophelia NWR	LOP
18	Mandalay NWR	MNL
19	Red River NWR	RER
20	Sabine NWR	SBN
21	Shell Keys NWR	SHK
22	Tensas River NWR	TNR
23	Upper Ouachita NWR	UOC

State	Refuge Name	Ref ID
MS		
1	Coldwater NWR	CLD
2	Dahomey NWR	DHM
3	Hillside NWR	HLD
4	Mathews Brake NWR	MBR
5	Mississippi Sandhill Crane NWR	MIS
6	Morgan Brake NWR	MBK
7	Noxubee NWR	NXB
8	Panther Swamp NWR	PNS
9	Tallahatchie NWR	TLH
10	Yazoo NWR	YZO
11	St. Catherine Creek NWR	SCC
NC		
1	Alligator River/Pea Island NWR	ALL
2	Cedar Island NWR	CDR
3	Currituck NWR	CRT
4	Mackay Island NWR	MCI
5	Mattamuskeete NWR	MTK
6	Pea Island NWR	PLD
7	Pee Dee NWR	PED
8	Pocosin Lakes NWR	POC
9	Roanoke River NWR	RRV
10	Swanquarter NWR	SWQ
TN		
1	Chickasaw NWR	CHK
2	Cross Creeks NWR	CRK
3	Hatchie NWR	HTC
4	Lake Isom NWR	LIS
5	Lower Hatchie NWR	LHA
6	Reelfoot NWR	REL
7	Tennessee NWR	TNS
SC		
1	Ace Basin NWR	ABS
2	Cape Romain NWR	CRM
3	Carolina Sandhills NWR	CRS
4	Pinckney Island NWR	PKY
5	Santee NWR	SNT
6	Waccamaw NWR	WAW
P.R.		
1	Buck Island NWR	BIS
2	Cabo Rojo NWR	CAB
3	Culebra NWR	CBR
4	DeSecheo NWR	DES
5	Green Cay NWR	GRC
6	Laguna Cartagena NWR	LGC
7	Sandy Point NWR	SDP
8	Vieques NWR	VEQ

R4 SPECIES CODES / DISTRIBUTIONS

COMMON NAME	SCIENTIFIC NAME	SPECIES CODE	NC Alligator River	AR Bald Knob	MS Dahomey	AR Felsenthal	TN Hatchie	FL Lower Suwannee	AR Overflow	NC Pocosin Lakes	MS Sandhill Crane	GA Savannah	MS Tallahatchie
Unknown Acris species	***Acris* sp.**	ACRI	X	X	X	X	X	X	X	X	X	X	X
northern cricket frog	***Acris crepitans***	ACCR	X	X	X	X	X		X	X		X	X
southern cricket frog	***Acris gryllus***	ACGR	X		X			X		X	X	X	X
Unknown toad species	***Bufo* sp.**	BUFO	X	X	X	X	X	X	X	X	X	X	X
American toad	***Bufo americanus***	BUAM		X	X	X	X		X				X
Fowler's toad	***Bufo fowleri***	BFOW	X	X	X	X	X		X	X	X	?	X
coastal plain toad	***Bufo nebulifer***	BUNE				X			X				
oak toad	***Bufo quercicus***	BUQU	X					X		X	X	X	
southern toad	***Bufo terrestris***	BUTE	X					X		X	X	X	
eastern narrowmouth toad	***Gastrophryne carolinensis***	GACA	X	X	X	X	X	X	X	X	X	X	X
Unknown introduced tropical	***Eleutherodactylus* sp.**	ELEU											
greenhouse frog	***Eleutherodactylus planirostris***	ELPL						X				X	
Unknown treefrog species	***Hyla* sp.**	HYLA	X	X	X	X	X	X	X	X	X	X	X
bird voice treefrog	***Hyla avivoca***	HYAV			X	X	X				X	X	X
gray **OR** Cope's gray treefrog	***Hyla chrysocelis* OR *versicolor***	HYCH	X	X	X	X	X	X	X	X		X	X
gree treefrog	***Hyla cinerea***	HYCI	X	X	X	X	X	X	X	X	X	X	X
pine woods treefrog	***Hyla femoralis***	HYFE	X					X		X		X	
barking treefrog	***Hyla gratiosa***	HYGR	X				X	X		X	X	X	
squirrel treefrog	***Hyla squirella***	HYSQ	X					X		X	X	X	
Unknown Pseudacris species	***Pseudacris* sp.**	PSEU	X	X	X	X	X	X	X	X	X	X	X
Brimley's chorus frog	***Pseudacris brimleyi***	PBRI	X							X		X	
spring peeper	***Pseudacris crucifer***	PSCR	X	X	X	X	?	X	X	X	X	X	X
upland chorus frog	***Pseudacris feriarum***	PSFE		X	X	X	X						X
southern chorus frog	***Pseudacris nigrita***	PSNI	?		?			X		?	X	X	?
little grass frog	***Pseudacris ocularis***	PSOC	X					X		X		X	
ornate chorus frog	***Pseudacris ornata***	PSOR						X			X	X	
Strecker's chorus frog	***Pseudacris streckeri***	PSST		X									
western chorus frog	***Pseudacris triseriata***	PSTR		X					X				
Unknown Rana species	***Rana* sp.**	RANA	X	X	X	X	X	X	X	X	X	X	X
crawfish frog	***Rana areolata***	RAAR		X		X	X		X				
gopher frog	***Rana capito***	RACP						X				X	
bullfrog	***Rana catesbeiana***	RACA	X	X	X	X	X	X	X	X	X	X	X
green **OR** bronze frog	***Rana melanota* OR *clamitans***	RACL		X	X	X	X	X	X	X	X	X	X
pig frog	***Rana grylio***	RAGR						X			X	X	
river frog	***Rana heckscheri***	RAHE						X				X	
pickerel frog	***Rana palustris***	RAPA	X	X	X	X	X		X	X		X	X
southern leopard frog	***Rana sphenocephala* OR *utricularia***	RASP	X	X	X	X	X	X	X	X	X	X	X
carpenter frog	***Rana virgatipes***	RAVI	X							X		X	
Unknown spadefoot species	***Scaphiopus* sp.**	SCAP	X	X	X	X	X	X	X	X		X	X
eastern spadefoot	***Scaphiopus holbrooki***	SCHO	X	X	X	X	X	X	X	X		X	X
Hurter's spadefoot	***Scaphiopus hurteri***	SCHU				X							

R4 SPECIES CODES / DISTRIBUTIONS

X = recorded in refuge county ? = recorded in adjacent county

Sources: Conant & Collins, 1998. Peterson Field Guide: Reptiles & Amphibians, Eastern/Central North America;
USGS - ARMI National Atlas for Amphibian Distributions http://www.mp2-pwrc.usgs.gov/armiatlas/

Species	Body description:	Cont'd description:	Breeding season:	Typical breeding habitat:	Egg description:	Tadpole description:	Conservation status in AR
Acris crepitans (Northern Cricket Frog)	small, warty hylid with dark jagged edged stripe on thigh; blunt head with dark V-shaped triangle between eyes	upper jaw with alternating light/dark vertical lines	April-July	streams pond and lakes, mudflats, barren shorelines and stream edges	eggs laid singly or in clusters, attached to debris in water column or floating; hatch in several days	*(Blanchard's: metamorphose in 5-10 weeks; 36mm max)*	
Bufo americanus (American Toad)	small, red-brown toad with small dorsal spots; one or two warts in each of large spots; belly whitish to cream with or without faint spots; narrow ligh stripe MAY extend down dorsum	kidney shaped parotids; cranial ridges connected or separated by bony spur	mid Mar - July (peaks Apr); males will have enlarged horny pad on inside of each forelimb & dark grey throat	ditches, small temp ponds, shallow slow streams	long tubular, gelatinous strings released free to pool bottom or onto submerged veg	black, distinct bicolored tail, 27mm max	
Bufo fowleri (Fowler's Toad)	medium, warty toad with numerous irregular dark brown or black dorsal spots; one-six warts/spot; pupil is horizontally elliptical; variable body color (gray to brick red);	oblong parotids connected by shallow bony crest; belly white with only occasional single breast spot. Light stripe down length of back.	late Mar-May (peak mid-May); males have dark throat area & horny pads on inside forelegs	permanent water; lakes, farm ponds, creeks rivers & drainage ditches	very LARGE clutches, long tubular strands; ONE week to development; June-mid July to metamorphose		
Bufo nebulifer (Coastal Plain Toad)	flat, med sized black w/ orange spots or yellow/brown w/ white spots; lateral broad, dark stripe bordered by broad middorsal yellow stripe; prominent yellow/cream middorsal stripe;	males have clear yellow/green throat; triangular shaped parotids behind eyes. Strongly dev., sharp ridged crests, forming distinct "Y" ends	Mar - Sept	shallow pools	long gelatinous strings, often double rows; 20-30 d development	dark with dark tail musculature, light dorsal saddles, 25 mm max	
Gastrophryne carolinensis (Eastern Narrowmouth Toad)	small, squat-bodied, short legged burrowing frog with a pointed snout, tiny head and distinctive fold of skin across the back of the head; tympanum is concealed, skin is smooth and no webbing present on toes	body color tan to reddish/brown with plain or irregularly blotched pattern; dark stripe extends from snout to side of hind legs; belly mottled with dark gray	April-August (peak calling May/June)	breeds in roadside ditches, ponds, sloughs and lakes; found mainly burrowing in moist soil conditions, under rocks, logs, stumps etc.	up to 850 eggs in small groups as a 1-2 egg deep surface layer; eggs have flat surface rather than spherical; hatching in less than 2 days; metamorphosis occurs in 20-30 days	unique with a sucking disc rather than labial teeth, mandibles and oral papillae	
Gastrophryne olivacea (Great Plains Narrowmouth Toad)							
Hyla avivoca (Bird-voiced Treefrog)	small hylid with expanded, adhesive toe discs, gray to green dorsum with irregularly shaped large dark blotch; young lack blotch & body green;	inside of legs have green to yellow/green color with conspicuou white spot below eyes; subarticular tubercle on outeer figer of front foot often divided	spring-summer	temp & perm woodland and floodplain pools with lots of low, woody veg; often perched high off ground while calling	eggs laid in submerged pockets; 29 d to dev	distintive with series of red saddles on dorsum	Spp of special concern due to low pop density & spotty occurrence
Hyla chrysoscelis (Cope's Gray Treefrog)	large warty or roughly granular hylid; large conspicuous adhesive discs; large, prominen squarish white spot beneath eyes; body color highly variable, lt gray to brown/greenish;	dark stripe slants from rear of eye to front leg; belly white; inner surface of hind leg & groin bright yellow or orange with dark spots/reticulations	Apr-Aug (mainly during warm rainy periods; but have been heard in Mar)	semi-perm pools or ponds, flooded barrow pits or ditches; males perch up to 60ft up in trees	up to 2K eggs laid in small packets ~45; either free-floating or loosely attached to veg; few days to hatch	boldly marked with large, dark blotches on long, red/black-finned tails; 50mm max; metamorphosis approx. 45-65 days	statewide dist; pesticide app of trees & shrubs near wetlands will impact these frogs
Hyla cinerea (Green Treefrog)	slender green hylid with expanded toe tips; dorsum bright green, with occasional conspicuous white or yellow lateral stripe running from upper lip over front leg & back to groin	dorsum often has small yellow spots while venter is white or yellow	late April-Aug; active egg laying peaks Jun-early July	vegetated areas near perm water; cypress swamps, floodplains, marshes, sloughs, ponds, lakes & streams; some reports habitat preference in soybean, cotton and grain sorghum fields ; males call from 1-7 ft above water	large clutches deposited in several small floating packets		

Species	Body description:	Cont'd description:	Breeding season:	Typical breeding habitat:	Egg description:	Tadpole description:	Conservation status in AR
Hyla versicolor (Gray Treefrog)	large warty or roughly granular hylid; large conspicuous adhesive discs; large, prominen squarish white spot beneath eyes; body color highly variable, lt gray to brown/greenish; dark stripe slants from rear of eye to front leg	belly white; inner surface of hind leg & groin bright yellow or orange with dark spots/reticulations	Apr-Aug (mainly during warm rainy periods; but have been heard in Mar)	semi-perm pools or ponds, flooded barrow pits or ditches; males call more frequently from ground; differntiated from *chrysocelis* only by call & chrom number; *versicolor* - 35 trill per min. rate; *chrysocelis* - 69 tpm rate	up to 2K eggs laid in small packets ~45; either free-floating or loosely attached to veg; few days to hatch	boldly marked with large, dark blotches on long, red/black-finned tails; 50mm max; metamorphosis approx. 45-65 days	statewide dist; pesticide app of trees & shrubs near wetlands will impact these frogs
Pseudacris crucifer (Northern Spring Peeper)	small hylid with conspicuous black X-shaped mark on dorsum; head lacks light spot beneath eye, but dark line runs across top of head between eyes;	body from pink to brownis/gray-olive; belly unmarked and usually light cream color; tips of digits expanded	early March-May (calling may begin as early as mid-Jan)	semiperm, fishless, woodland pools; males smaller & darker than females & during breeding season have dark throats	eggs deposited individually to submerged sticks, twigs or floating veg; hatching occurs in 3-4 days	brownish-green with metallic gold flecks on upper surface; belly lighter & iridescent, caudal fin clear or orange-tipped with black/purple blotches at outer edges	drainage of wetlands will cont. to reduce populations
Pseudacris feriarum (Upland Chorus Frog)							
Pseudacris illinoensis (Illinois Chorus Frog)							
Pseudacris streckeri (Strecker's Chorus Frog)							
Pseudacris triseriata (Western Chorus Frog)	small hylid with variable ground color (gray to brown); dorsal markings typically with three dark, narrow, longitudinal stripes, broken into three rows of spots; toes without webbing; tips slightly reduced discs;	dark triangle between eyes with white stripe running along upper lip, bordered by dark stripe extending through eye to groin; belly cream or white; skin only slightly bumpy	calls as early as late Dec, early Jan; breeding occurs late Feb-lat Apr;	swamps, edges of marshes, temp rain-filled pools in mowed fields and roadside ditches	clusters of up to 100 eggs laid adhering to grass and debris in water; hatch in a few days; metamorphose 1-2 months later	plump, rounded gray or brown bodies, sometimes mottled with brassy color in older ones; caudal fin is clear with dark flecks and intestinal coil seen through body wall	quite tolerant of human activities - common near firing range of LRAFB
Rana areolata (Crawfish Frog)	med sized frog with ground color varying from tan to light gray with many closely set dark spots enriched by light borders;	prominent dorsolateral fold present from eye to thigh; belly while; males smaller than females	late Jan-late Feb (dep on precip & temp	secretive; use crawfish and other burrows; move to breeding ponds after heavy rains for 2 wks before females arrive;	up to 7K eggs laid; 3-4 days to hatch; metamorphose in early Jun-early July		uncommon; scarcely seen other than during prime breeding season
Rana blairi (Plains Leopard Frog)							
Rana catesbeiana (Bullfrog)	large, olive-green ranid with well-developed tympanic membranes and NO dorsolateral folds;	extensive webbing b/w toes extends to tips; venter light with some yellow pigment on throat	calling begins early April; peak breeding activity late June, early July (in MO)	large lakes, reservoirs to small farm ponds, backwater swamps, marshes, and sloughs	up to 20K eggs in mass; 4-5 days to hatch; metamorphose from 11-14 months		

Species	Body description:	Cont'd description:	Breeding season:	Typical breeding habitat:	Egg description:	Tadpole description:	Conservation status in AR
Rana clamitans (Green Frog)	medium sized ranid with well-developed dorsolateral ridges; hind feet have extensive webbing; edge of jaw has alternating lt/dark spots	body color basically brown, bronze or green without disjunct spots; undersurface of body has light worm-like dark markings;	April - Aug/Sep (in AL); peak breeding probably June	swamps, floodplain pools and small streams, ponds & river banks; breeding habitat varies, but typically permanent water bodies used;	up to 4K eggs in floating mass on surface; hatching at 3-4 days; metamorphosis occurs during the FOLLOWING SUMMER	plump with long tails with olive to brown/green color above; varying amounts of dark flecks over back, upper and lower tail fins; belly irridescent white or cream, often mottled with gray, cannot see intestines through body wall	
Rana palustris (Pickerel Frog)	medium sized ranid with prominent rectangular or square black/brown blotches arranged in two parallel rows extending down back between well-developed dorsolateral folds	body color gray or tan, hind legs have black/brown cross bars; venter is white with hind legs and groin having bright yellow to orange pigmentation	early Feb-early May (in other states near AR)	occupies floodplain swamps, or cool, clear water at mouths of caves; breeding occurs in woodland pools, streams; males have enlarged thumbs (with inner pads) during breeding season	up to 3K eggs laid adhering to twigs and submerged debris; 10 days to hatch; metamorphosis in 3.5 months	range from green to gray/brown often with scattered black/yellow spots; irridescent white/cream bellies; tail fins heavily pigmented with dark spots or blotches; max 7cm	
Rana sphenocephala (Southern Leopard Frog)	medium sized spotted ranid; typically green, green/brown or brown with numerous rounded/oblong dark spots on dorsum; well developed dorsolateral folds present	belly white; back of thigh has interrupted bars; extensive webbing on hind feet; long head, pointed snout with distinctive whitel line present on upper jaw; tympanum usually has distinct light spot in center	typically mid-Mar-early May; however, can breed at all times of the year!	males call from farm ponds, ditches, backwater areas of streams, margins of cultivated fields	communal egglaying; up to 75 globular egg masses attached to submergent and emergent veg or laid unattached in under 3m square area; up to 5K eggs/female; hatching in 2 weeks; metamorphosis in mid-June-July		
Rana sylvatica (Wood Frog)							
Scaphiopus holbrooki (Eastern Spadefoot)	med sized burrower; dorsal color varies from greyish, blackish to greenish-brown; two major lyre-shaped lines run from each eye down back, additional broken light line may occur on sides of body	interorbital boss is absent; belly white to pale gray; spade elongate & sickle shaped; pupil vertical; skin relatively smooth	mid-Feb through June; coincides with heavy rains	small shallow temp pools in open areas	short, stringy egg masses attached to grasses and twigs; hatch in one to several days; metamorphose 14-60 days; both depend on temperature	tadpoles aggregate into large isolated social groups; may become cannibalistic; males grow faster post-metamorphosis	ANHC ranked as rare, susceptible to extirpation. Skin secretions particularly irritating!!
Scaphiopus hurteri (Hurter's Spadefoot)	med sized burrower; dorsal color varies from greyish, blackish to greenish-brown; two major lyre-shaped lines run from each eye down back, additional broken light line may occur on sides of body	interorbital boss is present; belly white to pale gray; spade elongate & sickle shaped; pupil vertical; skin relatively smooth	mid-Feb through June; coincides with heavy rains	small shallow temp pools in open areas	short, stringy egg masses attached to grasses and twigs; hatch in one to several days; metamorphose 14-60 days; both depend on temperature	tadpoles aggregate into large isolated social groups; may become cannibalistic; males grow faster post-metamorphosis	ANHC ranked as rare, susceptible to extirpation. Skin secretions particularly irritating!!
Spea bombifrons (Plains Spadefoot)							

R4 ANURAN BREEDING INFORMATION

Note: This table provides general guidelines for the expected window of breeding & development for each species. These may vary greatly between years or in different areas of the region or between. Environmental conditions will be the primary driving factor. This information is based on breeding data for Louisiana, so refuges in other areas of the country may expect the window of breeding activity to be slightly earlier or later. The size range of metamorphs and adults may also differ by region. Please check with your regional coordinator regarding size limits for metamorphs, and document what ranges you used for each species.

COMMON NAME	SCIENTIFIC NAME	CODE	Meta SVL limit (mm)	*Range Adult SVL (mm)	Jan			Feb			Mar			Apr			May			Jun			Jul			Aug			Sep			Oct			Nov			Dec		
					early	mid	late	early	mid	late	early	mid	late	early	mid	late	early	mid	late	early	mid	late	early	mid	late	early	mid	late	early	mid	late	early	mid	late	early	mid	late	early	mid	late
northern cricket frog	*Acris crepitans*	ACCR	15	16-35																																			
southern cricket frog	*Acris gryllus*	ACGR	15	16-32																																			
American toad	*Bufo americanus*	BUAM	15	51-90																																			
Fowler's toad*	*Bufo fowleri*	BFOW	15	51-75																																			
coastal plain toad	*Bufo nebulifer*	BUNE	15	51-100																																			
oak toad	*Bufo quercicus*	BUQU	10	19-33																																			
southern toad	*Bufo terrestris*	BUTE	15	41-75																																			
Puerto Rican coqui (introduced)*	*Eleutherodactylus coqui*	ELCO	15	25-58																																			
greenhouse frog (introduced)*	*Eleutherodactylus planirostris*	ELPL	13	16-32																																			
eastern narrowmouth toad	*Gastrophryne carolinensis*	GACA	15	22-32																																			
bird voice treefrog	*Hyla avivoca*	HYAV	15	28-44																																			
gray treefrog	*Hyla chrysocelis/versicolor*	HYCH	15	32-51																																			
green treefrog	*Hyla cinerea*	HYCI	25	32-57																																			
pine woods treefrog	*Hyla femoralis*	HYFE	15	25-38																																			
barking treefrog	*Hyla gratiosa*	HYGR	40	51-67																																			
squirrel treefrog	*Hyla squirella*	HYSQ	13	22-41																																			
Brimley's chorus frog*	*Pseudacris brimleyi*	PBRI	15	25-32																																			
spring peeper	*Pseudacris crucifer*	PSCR	15	19-32																																			
upland chorus frog	*Pseudacris feriarum*	PSFE	15	19-39																																			
southern chorus frog*	*Pseudacris nigrita*	PSNI	15	19-32																																			
little grass frog*	*Pseudacris ocularis*	PSOC	10	11-16																																			
ornate chorus frog	*Pseudacris ornata*	PSOR	15	25-32																																			
Strecker's chorus frog	*Pseudacris streckeri*	PSST	15	25-41																																			
western chorus frog	*Pseudacris triseriata*	PSTR	15	19-35																																			
crawfish frog	*Rana areolata*	RAAR	40	57-75																																			
gopher frog*	*Rana capito*	RACP	40	64-90																																			
bullfrog	*Rana catesbeiana*	RACA	60	90-152																																			
green/bronze frog	*Rana melanota/clamitans*	RACL	40	54-75																																			
pig frog	*Rana grylio*	RAGR	50	83-140																																			
river frog*	*Rana heckscheri*	RAHE	50	83-117																																			
pickerel frog	*Rana palustris*	RAPA	40	44-75																																			
southern leopard frog	*Rana sphenocephala OR utricularia*	RASP	40	51-90																																			
carpenter frog*	*Rana virgatipes*	RAVI	35	41-67																																			
eastern spadefoot	*Scaphiopus holbrooki*	SCHO	35	44-57																																			
Hurter's spadefoot*	*Scaphiopus hurteri*	SCHU	35	44-57																																			

= Chorusing. Eggs/larvae may be present = Eggs/larvae to be expected

R4 ANURAN BREEDING INFORMATION

Breeding information adapted mostly from: Dundee & Rossman, 1989. The Amphibians & Reptiles of Louisiana. Louisiana State University Press, Baton Rouge.
*** Information taken primarily from: Conant & Collins, 1998. Peterson Field Guides: Reptiles & Amphibians, Eastern/Central North America.**

Anuran Species Codes for USFWS Abnormal Amphibian Database

Source: Adapted from Sept 2002 printing of "Standard Common & Current Scientific Names for North American Amphibians, Turtles, Reptiles & Crocodilians." J.T.Collins & T.W. Taggart

NOTE: Green shading indicates that the code designated for that species does not follow the standard coding convention, which is the first two letters of the genus & species (or subspecies), due to overlap with a code assigned to a more common species or to a generic code for an unknown/ unidentifiable species in a particular group. Instead, it consists of the first letter of the genus & first three letters of species (or subspecies).

COMMON NAME	SCIENTIFIC NAME	CODE
Unknown cricket frog species	***Acris* sp.**	**ACRI**
Blanchard's cricket frog	*Acris c. blanchardi*	**ACBL**
coastal cricket frog	*Acris c. paludicola*	**ACPA**
northern cricket frog	*Acris c. crepitans*	**ACCR**
southern cricket frog	*Acris c. gryllus*	**ACGR**
Unknown tailed frog species	***Ascaphus* sp.**	**ASCA**
eastern tailed frog	*Ascaphus montanus*	**ASMO**
western tailed frog	*Ascaphus truei*	**ASTR**
Unknown toad species	***Bufo* sp.**	**BUFO**
Amargosa toad	*Bufo nelsoni*	**BNEL**
American toad	*Bufo a. americanus*	**BUAM**
Arizona toad	*Bufo microschapus*	**BUMI**
Arroyo toad	*Bufo californicus*	**BCAL**
black toad	*Bufo exsul*	**BUEX**
California toad	*Bufo b. halophilus*	**BUHA**
Canadian toad	*Bufo hemiophrys*	**BUHE**
cane toad	*Bufo marinus*	**BUMA**
coastal plain toad	*Bufo nebulifer*	**BUNE**
Colorado river toad	*Bufo alvarius*	**BUAL**
dwarf American toad	*Bufo a. charlesmithi*	**BUCH**
east Texas toad	*Bufo w. velatus*	**BUVE**
eastern green toad	*Bufo d. debilis*	**BUDE**
Fowler's toad	*Bufo fowleri*	**BFOW**
great plains toad	*Bufo cognatus*	**BUCO**
Houston toad	*Bufo houstonensis*	**BUHO**
oak toad	*Bufo quercicus*	**BUQU**
red spotted toad	*Bufo punctatus*	**BUPU**
Sonoran green toad	*Bufo retiformis*	**BURE**
southern toad	*Bufo terrestris*	**BUTE**
southwestern Woodhouse's toad	*Bufo w. australis*	**BUAU**
Texas toad	*Bufo speciosus*	**BUSP**
western (boreal) toad	*Bufo b. boreas*	**BUBO**
western green toad	*Bufo d. insidior*	**BUIN**
Woodhouse's toad	*Bufo woodhousii*	**BUWO**
Wyoming toad	*Bufo baxteri*	**BUBA**
Yosemite toad	*Bufo canorus*	**BCAN**
Unknown tropical frog species	***Eleutherodactylus* sp.**	**ELEU**
barking frog	*Eleutherodactylus augusti*	**EUAU**

Anuran Species Codes for USFWS Abnormal Amphibian Database

Source: Adapted from Sept 2002 printing of "Standard Common & Current Scientific Names for North American Amphibians, Turtles, Reptiles & Crocodilians." J.T.Collins & T.W. Taggart

NOTE: Green shading indicates that the code designated for that species does not follow the standard coding convention, which is the first two letters of the genus & species (or subspecies), due to overlap with a code assigned to a more common species or to a generic code for an unknown/ unidentifiable species in a particular group. Instead, it consists of the first letter of the genus & first three letters of species (or subspecies).

COMMON NAME	SCIENTIFIC NAME	CODE
cliff chirping frog	*Eleutherodactylus marnockii*	**EUMA**
eastern barking frog	*Eleutherodactylus a. latrans*	**EULA**
Rio Grande chirping frog	*Eleutherodactylus cystignathoides*	**EUCY**
spotted chirping frog	*Eleutherodactylus guttilatus*	**EUGU**
western barking frog	*Eleutherodactylus a. cactorum*	**EUCA**
Unknown narrowmouth toad species	***Gastrophryne* sp.**	**GAST**
eastern narrowmouth toad	*Gastrophryne carolinensis*	**GACA**
great plains narrowmouth toad	*Gastrophryne olivacea*	**GAOL**
Unknown treefrog species	***Hyla* sp.**	**HYLA**
barking treefrog	*Hyla gratiosa*	**HYGR**
bird voice treefrog	*Hyla avivoca*	**HYAV**
canyon treefrog	*Hyla arenicolor*	**HYAR**
Cope's gray treefrog	*Hyla chrysocelis*	**HYCH**
gray treefrog	*Hyla versicolor*	**HYVE**
gree treefrog	*Hyla cinerea*	**HYCI**
mountain treefrog	*Hyla eximia*	**HYEX**
pine barrens treefrog	*Hyla andersonii*	**HYAN**
pine woods treefrog	*Hyla femoralis*	**HYFE**
squirrel treefrog	*Hyla squirella*	**HYSQ**
sheep frog	*Hypopachus variolosus*	**HYVA**
white lipped frog	*Leptodactylus labialis*	**LELA**
Cuban treefrog	*Ostiopilus septentrionalis*	**OSSE**
Unknown chorus frog species	***Pseudacris* sp.**	**PSEU**
boreal chorus frog	*Pseudacris maculata*	**PSMA**
Brimley's chorus frog	*Pseudacris brimleyi*	**PBRI**
California chorus frog	*Pseudacris cadaverina*	**PSCA**
Florida chorus frog	*Pseudacris n. verrucosa*	**PSVE**
Illinois chorus frog	*Pseudacris illinoensis*	**PSIL**
little grass frog	*Pseudacris ocularis*	**PSOC**
mountain chorus frog	*Pseudacris brachyophona*	**PBRA**
New Jersey chorus frog	*Pseudacris kalmi*	**PSKA**
ornate chorus frog	*Pseudacris ornata*	**PSOR**
Pacific chorus frog	*Pseudacris regilla*	**PSRE**
southern chorus frog	*Pseudacris n. nigrita*	**PSNI**
southern spring peeper	*Pseudacris c. bartramiana*	**PBAR**

Anuran Species Codes for USFWS Abnormal Amphibian Database

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COMMON NAME	SCIENTIFIC NAME	CODE
spotted chorus frog	*Pseudacris clarkii*	**PSCL**
northern spring peeper	*Pseudacris c. crucifer*	**PSCR**
Strecker's chorus frog	*Pseudacris streckeri*	**PSST**
upland chorus frog	*Pseudacris feriarum*	**PSFE**
western chorus frog	*Pseudacris triseriata*	**PSTR**
lowland burrowing treefrog	*Pternohyla fodiens*	**PTFO**
Unknown true frog species	***Rana* sp.**	**RANA**
bronze frog	*Rana c. melanota*	**RAME**
bullfrog	*Rana catesbeiana*	**RACA**
California red legged frog	*Rana a. draytonii*	**RADR**
carpenter frog	*Rana virgatipes*	**RAVI**
Cascades frog	*Rana cascadae*	**RCAS**
Chiricahua leopard frog	*Rana chiricahuensis*	**RACH**
Columbia spotted frog	*Rana luteiventris*	**RALU**
dusky gopher frog	*Rana sevosa*	**RASE**
Florida bog frog	*Rana okaloosae*	**RAOK**
Florida leopard frog	*Rana s. sphenocephala*	**RASP**
Foothill yellow legged frog	*Rana boylii*	**RABO**
gopher frog	*Rana capito*	**RACP**
green frog	*Rana c. clamitans*	**RACL**
lowland leopard frog	*Rana yavapaiensis*	**RAYA**
mountain yellow legged frog	*Rana mucosa*	**RAMU**
northern crawfish frog	*Rana a. circulosa*	**RACI**
northern leopard frog	*Rana pipiens*	**RAPI**
northern red legged frog	*Rana a. aurora*	**RAAU**
Oregon spotted frog	*Rana pretiosa*	**RAPR**
pickerel frog	*Rana palustris*	**RAPA**
pig frog	*Rana grylio*	**RAGR**
plains leopard frog	*Rana blairi*	**RABL**
Ramsey Canyon leopard frog	*Rana subaquavocalis*	**RASU**
relict leopard frog	*Rana onca*	**RAON**
Rio Grande leopard frog	*Rana berlandieri*	**RABE**
river frog	*Rana heckscheri*	**RAHE**
southern crawfish frog	*Rana a. areolata*	**RAAR**
southern leopard frog	*Rana sphenocephala* OR *utricularius*	**RASP**
Tarahumara frog	*Rana tarahumarae*	**RATA**
wood frog	*Rana sylvatica*	**RASY**
Mexican burrowing toad	*Rhinophrynus dorsalis*	**RHDO**

Anuran Species Codes for USFWS Abnormal Amphibian Database

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COMMON NAME	SCIENTIFIC NAME	CODE
Unknown southern spadefoot species	***Scaphiopus* sp.**	**SCAP**
Couch's spadefoot	*Scaphiopus couchii*	**SCCO**
eastern spadefoot	*Scaphiopus holbrooki*	**SCHO**
Hurter's spadefoot	*Scaphiopus hurteri*	**SCHU**
Mexican treefrog	*Smilisca baudinii*	**SMBA**
Unknown western spadefoot species	***Spea* sp.**	**SPEA**
Great Basin spadefoot	*Spea intermontana*	**SPIN**
New Mexico spadefoot	*Spea muliplicata*	**SPMU**
plains spadefoot	*Spea bombifrons*	**SPBO**
western spadefoot	*Spea hammondii*	**SPHA**

Chapter 6

USFWS National Abnormal Amphibian Program

SITE CHARACTERIZATION FORM

Refuge : _____ **Collectors :** _____

Date : _____ _____

Site ID : _____ _____

Start Time : _____ **Air (^{o}C) :** _____ **Weather :** _____

End Time : _____ **H_2O (^{o}C) :** _____ _____

Datum : WGS 84 decimal degrees (hddd.ddddd°) **Elevation (+/- m) :** _____

Latitude : _____ **Make of GPS unit :** _____

Longitude : _____ **Model of GPS unit :** _____

Describe where coordinates were taken in relation to wetland :

_____

Area (m^2) : _____ **Water level (*select from*) :** **deep** (> 3ft in some areas) **shallow** (< 3ft throughout) **drying** (between 3 ft and dry) **dried up** (no water)

% Canopy Cover: _____

Habitat type (*Select from list*) : _____

Contaminants of Concern (*Select from list*) :

_____

_____

_____

_____

Surrounding Land Use (*Select from list*) :

North : _____

East : _____

South : _____

West : _____

Site Photo Filenames (*SiteID-direction facing-DATE*) :

_____ _____

_____ _____

Comments :

Sheet _____ of _____

U.S. Fish and Wildlife Service - National Abnormal Amphibian Initiative

Refuge: _____ Date: _____

Collectors: _____ Site/Location: _____

Abnormal Frog ID Number: _____

Check boxes that best describe the abnormalities. Note descriptions should be with dorsal side up

EYES		
Left	Right	
		Smaller than normal
		Pupil abnormally shaped
		Unusual position *(describe)*
		Missing iris, "Hollow Eye"
		Extra eye(s) *(describe)*
		Other *(describe)*

FRONT LIMBS		
Left	Right	
		Partial limb *(describe)*
		Foot missing
		Digits missing, fused or clubbed *(describe)*
		Complete calf present, abnormal musculature (enlarged or atrophied)
		Other *(describe)*

HIND LIMBS		
Left	Right	
		Entire limb present, unusual angle (twisted, rotated, etc. –*describe*)
		Entire limb present, abnormal size (atrophied or enlarged)
		Digits missing from foot (*specify digits*)
		Digits shortened, fused or clubbed
		Digits in abnormal location (*describe*)
		Extra digits (*describe*)
		Foot missing (tarsal bones)
		Complete calf (tibiofibula) present, abnormal musculature (enlarged or atrophied)
		Portion of calf missing (estimate length of calf present)
		Entire calf (tibiofibula) missing
		Complete thigh (femur) present, abnormal musculature (enlarged or atrophied)
		Portion of thigh (femur) missing, (estimate length of thigh present)
		Entire limb missing
		Other (*describe*)

JAWS		
Left	Right	
		Lower jaw shortened
		Upper jaw shortened
		Other *(describe)*

TAIL (NOTE: *<u>damaged</u> tails, such as missing or ragged tipped tails should be noted in comments section on data sheet, but not counted as abnormal)*	
	kinked or bent tail
	cysts, lumps or growths
	other (*describe*)

TORSO	
	lumps / bumps
	abrasions
	bloated or swelling
	bruising or other discoloration
	other (*describe*)

COMMENTS:

OTHER ABNORMALITIES		
Left	Right	
		SPINE - curved to left or right *(describe)*
		WEBBING - fusion between thigh and calf *(describe)*
		Extra Limbs- how many, location (*describe*)
		Fresh Bleeding or Injuries (*describe)*
		Other *(describe)*

Left Right

Draw abnormalities found on diagram with dorsal side up (frog lying on its stomach)

USFWS National Abnormal Amphibian Program

DATA COLLECTION FORM

Date: _____ **Collectors:** _____

Refuge: _____ _____

Site ID: _____ _____

Air Temp (C^o) **Water Temp (C^o)**

Start time: _____ **# Egg Masses Present:** _____

Water level (*select from the following*):

End time: _____ **Egg Mass Water Depth (cm):** _____

Weather: _____ **Egg Mass Species ID Code(s):** _____

deep	**shallow**	**drying**	**dried up**
(> 3ft in some areas)	(< 3ft througout)	(between 3 ft and dry)	(no water)

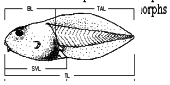
Frog#	Species Code	Length (mm)		Gosner Stage	Abnormal? (mark if Yes)	Preserved? (mark if Yes)	Abnormal Frog ID	Comments
		SVL	TAL					

1. Frog #: Number frogs 1,2,3,… for each species.
2. Process each frog.
3. If abnormal, assign a unique Abnormal Frog ID:
   Region Number (R4) - 5 character SiteID -
   6 digit collection date - Species Code -
   3 digit Frog # from this collection.

Example Abnormal Frog ID: R4-OVF02-060403-RASP-002 WC
Region 4, Overflow NWR, site 02, June 4, 2003, Rana sphenocephala, frog #002

4. Fill out an Abnormal Frog Sheet for each abnormal frog found.

Key Measurements of Tadpoles/Metamorphs



BL = Body Length TAL = Tail Length SVL = Snout-Vent Length TL = Total Length

BL - Tip of the snout to the point where the lateral midline of the tail muscle joins the body

TAL - Point where the lateral midline of the tail muscle joins the body to the tip of the tail.

TAL - Point where the lateral midline of the tail muscle joins the body to the tip of the tail.

Incidental Species Encountered:

Comments:

Please note: The CODE column will be assigned as the database develops. In the meantime, please use the desription for habitat type, land use, etc. as it appears in column 2 on your field notes. That way there will be no confusion later on when codes are assigned. Thanks!

USFWS AMPHIBIAN DATABASE CODES - LOOKUP TABLE DEFINITIONS

Code:	Habitat Types:	Definition:
	Natural Lake	(= a naturally formed inland body of water, of considerable size)
	Man-made Lake	(= artificially confined body of water, resembling a natural lake, attributed to human activity)
	Oxbow/Slough	(= a body of water that is out of the main current of a larger body of water)
	Backwater/Floodplain	(= level land that may be submerged by floodwaters)
	Natural pond	(= a naturally formed body of water, smaller than a lake)
	Beaver Pond	(= artificially confined body of water, attributed to beaver activity)
	Man-made Pond	(= artificially confined body of water, resembling a natural pond, attributed to human activity)
	Impoundment	(= artificially confined body of water, primary function is water reservoir)
	River	(= a natural stream of water, usually of considerable volume)
	Creek/Stream	(= a natural stream of water, normally smaller than and often tributary to a river)
	Forested wetland	(= a wetland with saturated soils that is dominated by woody plants taller than 20ft)
	Emergent wetland	(= a wetland dominated by emergent plants, includes wet meadows & marshes)
	Bog/Fen	(= a poorly drained area of wet spongy ground, often with: sedges, heaths, and sphagnum)
	Spring/Seep	(= a source of water issuing from the ground)
	Cave	(= a natural underground chamber or series of chambers open to the surface)
	Roadside Ditch	(= shallow man-made ditch, primary function drainage)
	Irrigation Ditch	(= deep man-made ditch, primary function provide irrigation water for ag fields)
	Barrow Pit	(= man-made entrenchment that fills with water, primarily created during road building)
	Roadside Puddle	(= shallow "natural" low spot along roadside that fills with water)
	Tire Ruts	(= ruts that fill with water, primarily created by vehicle tires)
	Cultivated Field	(= includes active or recently cultivated row crop fields)
	Pasture / Rangeland	(= includes grazed lands or old fields where cultivation may no longer be apparent)
	Tundra	(= treeless plain of the arctic, where mosses, lichens, etc. are dominant vegetation)
	Prairie	(= treeless plain where grasses are dominant vegetation)
	Savannah	(= an open canopy area, characterized by scattered trees and drought-resistant undergrowth)
	Other (describe in comments)	(= describe & define new category in comments)
	Contaminants of Concern:	**Definitions**
	Pesticides (current)	(= potentially receives inputs from active ag in distant past & active THIS season)
	Pesticides (recent)	(= potentially receives inputs from active ag in distant past through previous 1-2 yrs)
	Pesticides (historic)	(= potentially receives inputs from active ag in distant past)
	Highway runoff	(= potentially receives runoff from paved road)
	Gravel road runoff	(= potentially receives runoff from gravel road)
	Sewage	(= potentially receives effluents from raw or treated sewage)
	Nutrients	(= potentially receives effluents rich in nutrients)
	Sediments	(= potentially receives sediment rich effluents)
	Petroleum (current)	(= potentially has persistent compounds present & receives inputs from current oil industry activities)
	Petroleum (historic)	(= potentially has persistent compounds present from historic oil industry activities)
	Fertilizers	(= potentially receives fertilizer ammendments through agricultural practices)
	Heavy Metals	(= potentially receives effluents or aerial drift containing heavy metals)
	Other	(= describe in comments)
	None Suspected	(= reference site)
	Surrounding Land Use:	**Definitions:**
	Agriculture (current)	(= active ag in past & active THIS season)
	Agriculture (recent)	(= active ag in past through previous 1-2 yrs)
	Agriculture (historic)	(= active ag in distant past)
	Aquaculture	(= any aquaculture facility: fish, turtle, alligator, crayfish, etc.)
	CAFO-Cattle	(= Confined Animal Feeding Operation - cattle)
	CAFO-Swine	(= Confined Animal Feeding Operation - swine)
	CAFO-Poultry	(= Confined Animal Feeding Operation - poultry)
	Industrial Forest	(= managed primarily for production of wood products)
	Natural Forest	(= managed primarily for something other than largescale harvest)
	Industry	(= all types of industry, including wood product processing)
	Mining	(= includes gravel, metals, gypsum, kaolin operations, etc.)
	Oil Production	(= includes any petroleum industry activity, specify in comments)
	Urban	(= commercial development)
	Residential	(= residential development)
	Waterbody / Riparian Corridor	(= adjacent to waterbody with riparian buffer: river, oxbow, lake, ocean, etc.)
	Waterbody / NO Riparian Corridor	(= adjacent to waterbody without riparian buffer: river, oxbow, lake, ocean, etc.)

	Other (describe in comments)	(= specify crops, waterbody or other land uses not covered in above categories)

Try to select the single best habitat & surrounding land use category for each site. If none of the above are well-suited, add a category and define it in comments. For contaminants of concern, up to three categories can be selected for each site, any additional concerns should be noted in comments.

ABNORMAL AMPHIBIAN SHIPPING FORM

Refuge: _____ **Shipped to:** _____

Site ID: _____ **Shipped for:** _____

Date Collected: _____ **Species:** _____

Date Shipped: _____ **# of Frogs in Shipment:** _____

Total # of Frogs Collected at the Site_____ **Shipped by:**_____

Phone #: _____ **E-mail:** _____

Condition of specimens at shipping: **Live**_____ **Preserved**_____ **Frozen** _____

Concentration/Type: Fixative _____ **Preservative** _____

Frog # (Label clearly on container or tag)	**Field Description of Abnormalities:**

NOTE: If you want your shipping cooler returned, include a self-addressed FEDEX form. Copy your Regional Amphibian Coordinator on all documentation at the time of shipment, for tracking purposes. Decontaminate all shipping containers before and after shipping.

- SAMPLE -

ABNORMAL AMPHIBIAN SHIPPING FORM

Refuge: Patuxent NWR **Shipped to:** Dan Sutherland
U. Wisconsin - La Crosse

Site ID: PWR02 **Shipped for:** Parasitology

Date Collected: 8/3/04 **Species:** Pickerel frog (Rana palustris)

Date Shipped: 8/10/04 **# of Frogs in Shipment:** 12

Total # of Frogs Collected at the Site 60 **Shipped by:** Shemy Krest

Phone #: 410-573-4525 **E-mail:** Shemy_Krest@fws.gov

Condition of specimens at shipping: **Live** ✓ **Preserved** _____ **Frozen** _____

Concentration/Type: Fixative _____ **Preservative** _____

Frog # (Label clearly on container or tag)	Field Description of Abnormalities:
R5-PWR02-080304-RAPA-0001	missing both hind limbs
R5-PWR02-080304-RAPA-0002	Right eye all black and enlarged
R5-PWR02-080304-RAPA-0003	Left hind limb is 2 hind limbs
R5-RWR02-080304-RAPA-0010N	Normal

NOTE: If you want your shipping cooler returned, include a self-addressed FEDEX form. Copy your Regional Amphibian Coordinator on all documentation at the time of shipment, for tracking purposes. Decontaminate all shipping containers before and after shipping.

FY___ FROG SPECIMEN TRACKING LOG

										Specimen Preparation								
Date Collect	Date Shipped	Shipped To	Shipped For	Abnormal Frog ID	Spp Code	Ref Code	State	Site ID	Collectors	Fixative Used	Date Fixed	Preserv. Soln Used	Date Preserved	Ship Live?	Re-leased on-site?	Reason this abnormal frog was NOT submitted for diagnostics?	Date Diagnosis Rec'd	Comments

Chapter 7

Reference List

1. Allran, J.W. & Karasov, W.H. (2000) *Effects of Atrazine and Nitrate on Northern Leopard Frog (Rana pipiens) larvae exposed in the laboratory from posthatch through metamorphosis. Environmental Toxicology and Chemistry*, **19**, 2850-2855.Notes: Found atrazine bioconcentrated in tads. Atrazine, nitrate, and interaction had no sig effect on development rate, percent metamorphosis, time to metamorphosis, percent survival, mass at metamorphosis, or hematocrit. Nitrate slowed the growth of larvae. No gonads examined.

2. Alvarez, R., Honrubia, P. & Herraez, M.P. (1995) *Skeletal Malformations Induced by teh Insecticides ZZ-Aphox and Folidol during Larval Development of Rana perezi. Arch. Env. Cont. Tox.*, **28**, 349-356.Notes: carbamate and organophosphate caused scoliosis and short and thick longbones with twisted epiphyses. The authors attribute the malformations to changes in the connective tissue that bound up the bones.

3. Ankley, G.T., Diamond, S.E., Tietge, J.E., Holcombe, G.W., Jensen, K.M., Defoe, D.L. & Peterson, R. (2002) *Assessment of the Risk of Solar Ultraviolet Radiation to Amphibians. I. Dose-dependent induction of Hindlimb Malformations inthe Notthern Leopard Frog (Rana pipiens). Environ. Sci. Technol.*, **36**, 2853-2858.Notes: Exposed R. pipiens to natural sunlight and documented induction of hindlimb malformations. Malformations included missing or truncated digits, generally bilateral and symmetrical.

4. Berrill, M., Bertram, S., Pauli, B., Coulson, D., Kolohon, M. & Ostrander, D. (1995) *Comparative Sensitivity of Amphibian Tadpoles to Single and Pulsed Exposures of the Forest-Use Insecticide Fenitrothion. Environmental Toxicology and Chemistry*, **14**, 1011-1018.Notes: Exposed 1 day and 8 day old tads to pesticide. Both ages were equally sensitive. Different sensitivities among spp. Fenitrothion caused dose-sependent paralysis and mortality (up to 9 ppm) and the carrier compounds exhibited toxicity as well.

5. Berrill, M., Bertram, S., Wilson, A., Louis, S., Brigham, D. & Stromberg, C. (1993) *Lethal and Sublethal Impacts of Pyrethroid Insecticides on Amphibian Embryos and Tadpoles . Environmental Toxicology and Chemistry*, **12**, 525-539.Notes: Exposed 5 amphibians to permethrin and fenvalerate (0.01 - 2 ppm). No significant mortality. Delayed growth. Response to prodding by abnormal twisting, not darting away.

6. Berven, K.A. (1990) *Factors affecting population fluctuations in larval and adult stages of the wood frog (Rana sylvatica). Ecology*, **71**, 1599-1608.Notes: Most variation in survival was due to larval survival. Conducted a 7 year pop'n study on the wood frog. Breeding pop'n size fluctuated by a factor of 10. Pre-metamorphic survival and size at metamorphosis were negatively correlated with #eggs deposited.

7. Betts, K. More evidence that atrazine may affect frog sexuality. Environmental Science and Technology , 444-445A. December 1, 2002. Notes: Another article about the T. Hayes group.Talks about atrazine having effects at concentrations below a threshold that would trigger the animal's defense mechanisms.

8. Blaustein, A.R. & Johnson, P.T.J. (2003) *The complexity of deformed amphibians. Frontiers in Ecology and the Environment*, **1**, 87-94.Notes: Discuss different theories for abnormal amphibians in the context of conservation biology and loss of species. Review evidence for UV, Chemical, and parasite exposure as causes for abnormalities in amphibians. Conclude that multiple stressors are often acting in concert to produce abnormalities and species decline and more complex studies need to be done to evaluate effects of multiple stressors.

9. Blaustein, A.R. & Kiesecker, J.M. (2002) *Complexity in Conservation: lessons from the global decline of amphibian populations. Ecology Letters*, **5**, 597-608.Notes: Thorough review of different and multiple causes of amphibian declines. Argue that amphibian population declines are caused by different abiotic and biotic factors acting together in a context-dependent fashion.

10. Blaustein, A.R., Wake, D.B. & Sousa, W.P. (1994) *Amphibian Declines: Judging Stability, Persistence, and Susceptibility of Populations to Local and Global Extinctions. Conservation Biology*, **8**, 60-71.Notes: reviews populations that are extinct and studies that have been done and are more long term, in relation to the lifetime length. Documents declines and uncertainties. Recommends longer term studies bc of stochasticity in population sizes from year to year.

11. Bridges, C.M. (2000) *Long-term Effects of Pesticide Exposure at Various Life Stages of the*

Southern Leopard Frog (Rana sphenocephala). Arch. Env. Contam. Toxicol. **39**, 91-96.Notes: Exposed eggs, embryos, and tads to low levels of carbaryl. Found mortality at higher doses. At lower doses, all frogs exposed as eggs were smaller at metamorphosis. Frogs exposed only as eggs or embryos were larger at metamorphosis than controls. Found deformities including bent tails, missing lhind imb, and extra front limbs.

12. Brodie, E.D.Jr. & D.R. Formanowicz Jr. (1983) *Prey size preference of predators: differential vulnerability of larval anuran. Herpetologica* , 67-75.

13. Carey, C. & Bryant, C. (1995) *Possible Interrelations among Environmental Toxicants, Amphibian Development, and Decline of Amphibian Populations. Environmental Health Perspectives*, **103**, 13-17.Notes: Review of literature relating contaminants to amphibian declines, Focus on effects on growth and development of the young affecting populations. Immunosuppression, decreased or retarded growth or development, reducing larval ability to avoid predation, altered reproductive potential, and lethal agents. Provides good references.

14. Clark, K. & Hall, R. (1985) *Effects of elevated hydrogen ion and aluminum concentrations on the survival of amphibian embryos and larvae. Can. J. Zool.*, **63**, 116-123.Notes: Found effects on survival of eggs and lower pH (4.3) and higher Al concentrations.

15. Cooke, A.S. (1981) *Tadpoles as indicators of harmful levels of pollution in the field. Environmental Pollution (Series A)*, **25**, 123-133.Notes: Used caged tadpoles to evaluate effects on tadpoles in mainly agricultural habitats. Found deformities including kinked tails and scoliosis. Includes effects of DDT and Oxamyl. Also describes technique for caged tadpoles. Rana temporaria.

16. Cupp, P.V.Jr. (1980) *Thermal Tolerance of Five Salientian Amphibians During Development and Metamorphosis. Herpetologica*, **36**, 234-244.Notes: Evaluated thermal tolerances of 5 different amphib spp. Thermal tolerance decreased right before metamorphosis in all spp. Wood frogs had lowest thermal tolerance.

17. Dawson, D.A. (1994) *Joint actions of Carboxylic Acid Binary Mixtures on Xenopus Embryo Development: Comparison of Joint Actions for Malformation Types..Arch. Environ. Contam. Toxicol.* **27**, 243-249.Notes: Found microcephaly and abnormal gut coiling and eye defects. 96 h static renewal tests.

18. Demaynadier, P.G. & Hunter, M.L.Jr. (1998) *Effects of Silvicultural Edges on the Distribution and Abundance of Amphibians in Maine. Conservation Biology*, **12**, 340-352.Notes: estimated depth of edge effects at 25-35 m for P. cinereus, A. maculatum, A. laterale, and R. sylvatica.

19. Diamond, S.A., Peterson, G.S., Tietge, J.E. & Ankley, G.T. (2002) *Assessment of the Risk of Solar Ultraviolet Radiation to Amphibians. III. Prediction of Impacts in Selected Northern Midwestern Wetlands. Environmental Science and Technology.*, **36**, 2866-2874.Notes: The risk of mortality and malformations due to UV exposure is low for the majority of wetlands evaluated. UV dose exceeded effects doses for mortality for all three spp. in 2 of 26 wetlands evaluated and for one sp in an additional wetland. Didn't find the UV needed to kill or malform amphibs at most of the wetlands evaluated.

20. Formanowicz, D.R.Jr. (1982) *Foraging tactics of larvae of Dytiscus vaticalis (Coleoptera: Dytiscidae) the assessment of prey density. J. Anim. Ecol.*, **51**, 757-768.

21. Formanowicz, D.R.Jr. (1986) *Anuran tadpole / aquatic insect predator-prey interactions: tadpole size and predator capture success. Herpetologica* , **42**, 367-373.

22. Fort, D.J., Propst, T.L., Stover, E.L., Helgen, J.C., Levey, R.B., Gallagher, K. & Burkhart, J.G. (1999) *Effects of Pond Water, Sediment, and Sediment Extracts from Minnesota and Vermont, USA, on Early Development and Metamorphosis of Xenopus. Environmental Toxicology and Chemistry*, **18**, 2305-2315.Notes: Did FETAX and studies of hind limb formation and tail regression. Found malformations, gut miscoiling, craniofacial and mouth abnormalities, hypognathia, stunting, maldevelopment of the lens of eyes, and kinked tails in the 4-d FETAX. Found high malformation rates in the hind limbs, mainly random skeletal reduction deficiencies and incomplete maturation of soft tissue in 30-d exposures= grossly underdeveloped limbs. Also lateral flexures.Also found thyroid disruption. Low hardness and alkalinity. Abnormalities found in the field included reduction defecits, missing limbs, limbs reduced in size, and webbed hind limbs with excessive connective tissue. Did some water analysis, but not specific compounds.

23. Gascon, C. & Planas, D. (1986) *Spring pond water chemistry and the reproduction of the wood frog, Rana sylvatica. Can. J. Zool.*, **64**, 543-550.Notes: Hatching success was reduced and mould increased in low pH ponds. Study of 15 breeding ponds with spring pH between 3.4 and 6.7. Acidity and TOC were correlated with density of egg masses.

24. Gosner, K.L. & Black, I.H. (1957) *The effects of acidity on the development and hatching of New*

Jersey frogs. Ecology, **38**, 256-262.Notes: At low pH, egg membranes failed to expand prior to hatching or failed to hatch. These tads were deformed. Embryos were restricted in movement and tended to curl inside membranes. Observed similar effects from salty solutions. pH varied from 3.6-5.2

25. Grant, K.P. & Licht, L.E. (1995) *Effects of ultraviolet radiation on life-history stages of anurans from Ontario, Canada. Can. J. Zool.*, **73**, 2292-2301.Notes: Ecologically relevant doses of uv-b had no effect on wood frog tadpole s developmental period, duration of metamorphic climax, or mass at metamorphosis. Artificially high uv killed all tads, except for some B. americanus.

26. Gucciardo, L.S. & Farrar, E.S. (19??) *Chronic exposure of anuran larvae to three levels of atrazine dows not affect growth and metamorphosis. Society for Integrative and Comparative Biology Abstracts*, 122 A.Notes: Exposed wood frogs to 30, 300, and 600 ppb atrazine in culture water from stage 25-metamorphosis. No significant differenced were detected among treatments.

27. Harris, M.L., Chora, L., Bishop, C.A. & Bogart, J.P. (2000) *Species- and Age-Related Differences in Susceptibility to Pesticide Exposure for Two Amphibians, Rana pipiens, and Bufo americanus. Bull. Environ. Contam. Toxicol.*, **64**, 263-270.Notes: Found eye and limb abnormalities. Exposed frogs to endosulfan, azinphosmethyl, and mancozeb. 1 egg mass each treated for 96 hour.

28. Hayes, T., Haston, K., Tsu, M., Hoang, A., Haeffele, C. & Vonk, A. (2002) *Atarazine-Induced Hermaphroditism at 0.1 ppb in American Leopard Frogs (Rana pipiens): Laboratory and Field Evidence. Enviornmental Health Perspectives Online.*Notes: Low levels of atrazine resulted in gonadal dysgenesis and hermaphroditism (testicular oogenesis) in R. pipiens.Also observed this in animals from the field in Atrazine contaminated areas.

29. Heatwole, H. (1961) *Habitat selection and activity of the Wood Frog, Rana sylvatica Le Conte. American Midland Naturalist*, **66**, 301-313.Notes: Discusses the habitat of wood frogs during the non-breeding season.

30. Hecnar, S.J. & M'Closkey, R.T. (1996a) *Amphibian species richness and distribution in relation to pond water chemistry in south-western Ontario, Canada. Freshwater Biology*, **36**, 7-15.Notes: Assessed patterns of amphib spp. richness in relation to water chem from 1992-1994. 13 spp. observed at 180 ponds. Used PCA to determine correlations between water qual parameters and spp. richness.

31. Hecnar, S.J. & M'Closkey, R.T. (1996b) *Regional Dynamics and the status of Amphibians. Ecology*, **77**, 2091-2097.Notes: Assessed 11 amphibian spp. in 97 ponds from 1992 to 1994. Detected loss of richness in one of 3 regions and high turnover of spp. in ponds.

32. Henrikson, B.I. (1990) *Predation on amphibian eggs and tadpoles by common predators in acidified lakes. Holarctic Ecol*, **13**, 201-206.

33. Herreid, C.F. & Kinney, S. (1966) *Survival of Alaskan wood frog (Rana sylvatica) larvae. Ecology*, **47**, 1039-1041.Notes: Documents number of eggs per mass and percent of eggs fertilized, and documents survival to metamorphosis at 4%. Avg # eggs per mass is 778 (sd=257, n=52 masses) in an area in Fairbanks.

34. Horne, M.T. & Dunson, W.A. (1995) *The interactive effects of low pH, Toxic Metals, and DOC on a simulated temporary pond community. Environmental Pollution*, **89**, 155-161.Notes: Wood frogs were negatively affected by high DOC and low pH. Effects included increased time to metamorphosis and decreased survival and wet body mass at metamorphosis.Wood frogs were more tolerant of metals (Al, Cu, Fe, Pb, Zn) and low pH than salamanders. Wood frogs were not sensitive to metals. DOC alone delayed development in wood frogs.

35. Howard, R. (1980) *Mating Behaviour and mating success in woodfrogs, Rana sylvatica. Animal Behavior*, **28**, 705-716.Notes: Studied breeding behaviour of wood frogs in a pond in SE Michigan. Males bred at 1 yr, Females at 2. The presence of eggs stimulated egg deposition. Speculates about why they lay eggs communally. Males rarely lost their female once in amplexus. some females died due to males fighting during amplexus.

36. Johnson, P.T., Lunde, K.B., Ritchie, E.G. & Launer, A.E. (1999) *The Effect of Trematode Infection on Amphibian Limb Development and Survivorship. Science*, **284**, 802-804.Notes: Experimentally induced hindlimb malformations by exposing pacific treefrog tadpoles to ribieroia. Most malformations were extra limbs, but missing limbs also occurred.

37. Kiesecker, J.M. (2002) *Synergism between trematode infection and pesticide exposure: A link to amphibian limb deformities in nature? Proceedings of the National Academy of Sciences*, **99**, 9900-9904.Notes: Found increased incidence of trematode infection when R. sylvatica were exposed to pesticides and Ribieroia.

38. LeClair, R.Jr., Alarie, Y. & Bourassa J.P. (1986) *Prey choice in larval Dytiscus harrisii Kirby and D.*

verticalis Say (Coleoptera: Dytiscidae). Ent. Basiliensia, **11**, 337-342.

39. Licht, L.E. (1975) *Time course of uptake, elimination, and tissue levels of [^{14}C] DDT in wood-frog tadpoles. Can. J. Zool.*, **54** , 355-360.Notes: Exposed tadpoles to sub-lethal concentrations of DDT radiolabeled. Tadpoles treated at lower temps got rid of DDT slower. Stored it in fat and liver.

40. Long, L.E., Saylor, L.S. & Soule, M.E. (1995) *A pH/UV-B Synergism in Amphibians. Conservation Biology*, **9**, 1301-1303.Notes: Found most late embryotic failure due to curling bc. membranes did not expand.Fond synergism between pH and UV-B at levels at which neither one individually affected survival or development.

41. Lowcock, L.A., Sharbel, T.F., Bonin, J., Ouellet, M., Rodrigue, J. & DesGranges, J.-L. (1997) *Flow cytometric assay for in vivo genotoxic effects of pesticides in Green frogs (Rana clamitans). Aquatic Toxicology*, **38**, 241-255.Notes: Found increase in abnormal DNA profiles in individuals from corn fields relative to control sites. Deformed juveniles had mroe damage than normal ones.Found DNA damage in both sick and normal individuals.

42. Marian, M.P., Arul, V. & Pandian, T.J. (1983) *Acute and Chronic Effects of Carbaryl on Survival, Growth, and Metamorphosis in the Bullfrog (Rana tigrina). Arch. Environm. Contam. Toxicol.*, **12**, 271-275.Notes: Found rate of feeding and defecation to increase with carbaryl exposures. Reduced size at metamorphosis.

43. Ouellet, M., Bonin, J., Rodrigue, J., DesGranges, J. & Lair, S. (1997) *Hindlimb Deformities (Ectromelia, Ectrodactyly) in Free-living Anurans from Agricultural Habitats. Journal of Wildlife Diseases*, **33**, 95-104.Notes: Found high prevalence of hindlimb abnormalities in wild frogs (Rana and Bufo). Clinical signs varied and were characterized by segmental hypoplasia or agenesis of the affected limbs. More abnormalities were found in agricultural areas than in non-ag areas.

44. Parson, T. A. S. Kenai National Wildlife Refuge Contaminant Assessment. January 2001. Anchorage, AK.

45. Peterson, G.S., Johnson, L.B., Axler, R.P. & Diamond, S.A. (2002) *Assessment of the Risk of Solar Ultraviolet Radiation to Amphibians. II. In Situ Characterization of Exposure in Amphibian Habitats. Environmental Science and Technology*, **36**, 2859-2865.Notes: Mentions need for assessment of organism behavior. mid-season DOC is higher than that of late summer and fall DOC, suggesting increased protection during the potentially sensitive stages of amphib development.

46. Prussian, A. M., Rinella, D. J., and Major, E. Invertebrate Survey of Selected Ponds Along the Road System of the Kenai National Wildlife Refuge. December 2001. Environment and Natural Resources Institute.

47. Reed, J.M. & Blaustein, A.R. (1995) *Assessment of "Nondeclining" Amphibian Populations Using Power Analysis. Conservation Biology*, **9**, 1299-1300.Notes: Used power analysis to evaluate several short term studies that concluded that amphib populations were not in decline. Concluded that none of the studies had the power to protect against type II errors, ie. "all studies had at least a 55% or greater chance of making a Type II error (not rejecting the H0 when it was false)". Population variability due to env. stochasticity made detection of trends difficult.

48. Reeder, A.L., Foley, G.L., Nichols, D.K., Hansen L.G., Wikoff, B., Faeh, S., Eisold, J., Wheeler, M.B., Warner, R., Murphy, J.E. & Beasley, V.R. (1998) *Forms and Prevalence of Intersexuality and Effects of Environmental Contaminants on Sexuality in Cricket Frogs (Acris crepitans). Environmental Health Perspectives*, **106**, 261-266.Notes: Examined 341 frogs for intersex. Found trend towards increase in intersex with Atrazine exposure. Found reversal of sex ratio in PCB and PCDF contaminated sites in Illinois.

49. Renner, R. Pesticide mixture enhances frog abnormalities. Environ. Sci. Tech. February 1, 2003, 52 A. 2003. Notes: Reports on work of T. Hayes lab at Berkeley. They are finding hindlimb defects when 2 or 3 ag pesticides are mixed.

50. Renner, R. More evidence that herbicides feminize amphibians. Environmental Science and Technology , 46 A. February 1, 2003. Notes: Descrives work by Tim Gross and Krista McCoy in cane fields in Florida where atrazine use is heavy. All cane toads examined in the fields had female coloration, and 30% of male toads found at contaminated sites were hermaphrodites. Also found normal plasma steroid concentrations but vitellogenin levels comparable to females.

51. Riley, E.E. & Weil, M.R. (1987) *The Effects of Thiosemicarbazide on Development in teh Wood Frog, Rana sylvatica. Ecotoxicology and Environmental Safety*, **13**, 202-207.Notes: Confirmed results of Reiley and Weil, 1986. Found severity of limb malformations depended on time of exposure (days 24-30, when limbs are developing) no gosner stage reported

52. Roberts, W. & Lewin, V. (1979) *Habitat Utilization and Population Densities of the Amphibians of Northeastern Alberta. Canadian Field-Naturalist*, **93**, 144-154.Notes: Evaluated spawning sites and pop'n densities of Rana sylvatica, Pseudacris, and Bufo and correlated with habitat variables. Also discusses size of animals and dates of metamorphosis.
53. Rosenshield, M.L., Jofre, M.B. & Karasov, W.H. (1999) *Effects of Polychlorinated biphenyl 126 on Green Frog (Rana clamitans) and Leopard Frog (Rana pipiens) hatching success, development, and metamorphosis. Env. Tox. Chem.*, **18**, 2478-2486.Notes: Exposed embryos thru metamorphosis to 0.005 to 50 ppb. Animals accumulated up to 9600 ng/g ww. Few deformities. Survival was affected, especially in the highest treatments. Swimming speed and growth of tadpoles was reduced.
54. Rowe, C.L., Kinney, O.M. & Congdon, J.D. (1998) *Oral Deformities in Tadpoles of the Bullfrog (Rana catesbeiana) Caused by Conditions in a Polluted Habitat. Copeia*, **1**, 244-246.Notes: Found greater incidence of oral deformities in tadpoles raised in a pond containing coal combustion wastes. and lower survival.
55. Russell, R.W., Gillan, K.A. & Haffner, G.D. (1997) *Polychlorinated Biphenyls and Chlorinated Pesticides in Southern Ontario, Canada, Green Frogs. Environmental Toxicology and Chemistry*, **16**, 2258-2263.Notes: measured DDT and PCB concentrations in frogs. Found localized elevated exposures. Used PCA to look at differences among areas.
56. Scadding, S.R. (1990) *Effects of Tributyltin Oxide on the Skeletal Structures of Developing and Regenerating Limbs of the Axolotl Larvae, Ambystoma mexicanum. Bull. Environ. Contam. Toxicol.*, **45**, 574-581.Notes: Found limb defecit reductions in developing limbs but not regenerating limbs of salamanders exposed to tributyl tin.
57. Schuytema, G.S. & Nebeker, A.V. (1998) *Comparative Toxicity of Diuron on Survival and Growth of Pacific Treefrog, Bullfrog, Red-Legged Frog, and African Clawed Frog Embryos and Tadpoles. Arch. Env. Contam. Toxicol.*, **34**, 370-376.Notes: Tests were conducted for 14 d. Diuron affected survival and growth in amphibs at concentrations higher than observed in the field. Observes high deformity rates, but does not describe all deformities observed. Describes edema.
58. Schuytema, G.S., Nebeker, A.V. & Griffis, W.L. (1994) *Toxicity of Guthion and Guthion 2S to Xenopus laevis embryos. Arch. Environ. Contam. Tox.*, **27**, 250-255.Notes: Documents a density dependent relationship between toxic effects and pesticide exposure. Documents a high abnormality rate, but never describes deformities. Commercial formulation was more toxic.
59. Schuytema, G.S., Nebeker, A.V., Griffis, W.L. & Wilson, K.N. (1991) *Teratogenesis, Toxicity, and Bioconcentration in Frogs Exposed to Dieldrin. Archives of Environmental Contamination and Toxicology*, **21**. 332-350.Notes: Gross spinal deformities in embryo-larval tests were observed at technical grade dieldrin concentrations as low as 1.3 ug/L after 10 days of exposure for Xenopus. Tests X. laevis, R. catesbeiana, and R. pipiens. Teratogenic effects were primarily lordosis, kyphosis, and scoliosis.
60. Sessions, S.K. & Ruth, S.B. (1990) *Explanation for Naturally Occurring Supernumerary Limbs in Amphibians. J. Exp. Zool.*, **254**, 38-47.Notes: Document the association between Ribeiroia infection and extra limbs.
61. Snawder, J.E. & Chambers, J.E. (1989) *Toxic and Developmental Effects of Organophosphorus Insecticides in Embryos of the South African Clawed Frog. J. Environ. Sci. Health*, **B24**, 205-218.Notes: All compounds caused dose-dependent developmental defects, such as abnormal pigmentation, abnormal gut development, notochordal defects and reduced growth. 10/16 embryos with notochordal defects that survived to metamorphosis had abnormal or missing limbs, bent spines and acetabular joints, and were slower to develop than controls. This group was exposed for 96 h as blastulae to Dichrotophos (10 mg/l) or Malaoxon (1 mg/l).
62. Sower, S.A., Reed, K.L. & Babbitt, K.J. (2000) *Limb Malformations and Abnormal Sex Hormone Concentrations in Frogs. Environmental Health Perspectives*, **108**, 1085-1090.Notes: Compared brain Gn-RH and in-vitro produced androgen concentrations in normal frogs and frogs with limb malformations. Gn-RH and androgens were nearly 3x higher in normal frogs than in frogs with limb abnormalities. Also found hermaphroditic frogs and lack of development in the testes of abnormal frogs.
63. Tavera-Mendoza, L., Ruby, S., Brousseau, P., Fournier, M., Cyr, D. & Marcogliese, D. (2001) *Response of the Amphibian Tadpole (Xenopus laevis) to Atrazine during Sexual Differentiation of the Testis. Environmental Toxicology and Chemistry*, **21**, 527-531.Notes: Exposed xenopus to atrazine for 48 hours during sexual differentiation to atrazine at 21 ug/l. Found a 57% reduction in testicular volume, 74% reduction in nurse cells, and testicular resorption among 70%.
64. Wake, D.B. (1990) *Declining Amphibian Populations. Science*, **253**, 860.Notes: Highland and more

northern frogs seem more severely affected. Local extinctions have profound implications where spp are specialists for habatitats that are localized or fragmented.

65. Wilbur, H.M. (1971) *Competition, predation and the structure of the Ambystoma-Rana sylvatica community. Ecology*, **53**, 3-20.Notes: salamanders eat wood frog tadpoles.

66. Young, A.M. (1967) *Predation in the larvae of Dytiscus marginalis Linnaeus. Pan-Pacif. Ent.*, **43**, 113-117.Notes: invertebrates

Amphibian Websites:

USFWS Amphibian Website:
http://www.fws.gov/contaminants/Issues/Amphibians.cfm

Northeast ARMI on amphibian diseases
http://www.pwrc.usgs.gov/nearmi/disease/

Arizona State University: Emerging Wildlife Diseases and Threats to Amphibian Biodiversity
http://lifesciences.asu.edu/irceb/amphibians/

Amphibian Research and Monitoring Initiative (ARMI)
http://armi.usgs.gov/

National Wildlife Health Center
http://www.nwhc.usgs.gov/

USGS Amphibian Malformations and Declines (see Field Guide to Malformations of Frogs and Toads in .pdf on right side of page)
http://www.nwhc.usgs.gov/disease_information/amphibian_malformation_and_decline/index.jsp

Tadpoles of the U.S. and Canada: A Tutorial and Key
http://www.pwrc.usgs.gov/tadpole/

Declining Amphibian Populations Task Force (DAPTF)
http://www.open.ac.uk/daptf/index.htm

Amphibiaweb
http://amphibiaweb.org/

Bibliomania: Herplit searchable database
http://herplit.com/contents/

Frogweb
http://www.frogweb.gov/index.html

Savannah River Ecology Lab
http://www.uga.edu/srelherp/

Herptox Page (Affects of environmental contaminants on reptiles and amphibians)
http://www.on.ec.gc.ca/herptox/

The Center for North American Herpetology
http://www.cnah.org/

The Center for North American Herpetology Library
http://www.cnah.org/cnah_pdf.asp

What's Happening to the Frogs?

Amphibian abnormalities have been addressed in scientific literature for some time, but it was only when middle school students in Minnesota discovered large numbers of abnormal frogs that the general public and the Congress began to notice. That was in 1995 and researchers have been investigating the problem at many levels ever since – including research on national wildlife refuges.

Environmental stressors may cause such abnormalities as missing, extra or unusual body parts. In fact, scientists believe frog abnormalities could be caused by multiple factors that may differ from one site to another. These factors may include changes in climate, predators, parasites, bacteria, fungi and viruses or pollution and contaminants such as pesticides, metals and fertilizer, among others.

Roxanna Hinzman, who until recently was the national amphibian coordinator for the U.S. Fish and Wildlife Service, says that since 2000, the Service has had an annual $500,000 Congressional appropriation to research abnormal frogs. With the help of refuge staff, volunteers, Friends organizations and at least one student group, simple first-tier assessments of frog abnormalities have been conducted in ponds, wetlands, puddles, and other water bodies on 131 refuges in 47 states. This effort represents the first nationwide survey of abnormal amphibians that uses standardized collection and evaluation methods.

During the initial assessment, researchers try to collect 50-100 newly metamorphosed frogs of one species from a single pond and document visible abnormalities. Abnormal frogs are sent to a parasitologist who looks for parasites that cause abnormal limb development. Then the frogs are sent for radiography so that any bone abnormalities can be examined and documented. A report released in May 2006 presents the results of radiographic analyses for more than 650 abnormal frogs from refuges across the country.

Frog abnormalities have been observed and recorded in ponds, wetlands, puddles, and other waterbodies on 131 refuges in 47 states. The Service has undertaken the first nationwide survey of abnormal amphibians using standardized collection and evaluation. (Dan Sutherland)

The report concluded that abnormalities were "remarkably similar across all regions," and more research is needed to identify cause and effect relationships.

Analyzing the Data

Researchers are beginning to mine five years worth of data. With the help of Kevin Nguyen, a Service computer technician fascinated by frogs, an online database was created to store the information.

Researchers hope to find trends that will help focus additional studies. "With the tremendous amount of data that has been collected so far, I hope we can get a better understanding about what is happening and what we can do to help," said Kelly Geer, the new national amphibian coordinator.

As currently planned, a second phase of research will identify stressors in ponds; a third stage will work to identify the actual causes of the abnormalities. "There doesn't seem to be one 'smoking gun' or one stressor," says Hinzman. "There may be multiple stressors, or the effects of two or more stressors may be cumulative."

Second stage research is underway at Great Bay (New Hampshire) and Kenai National Wildlife Refuges (Alaska). The discovery of significant numbers of abnormal frogs at Kenai was a surprise. Now a study is underway to identify specific abnormalities and stressors.

Hinzman believes that frogs are good indicators of habitat health because they can be exposed to so many different contaminants throughout their lives and they are found in so many places, from ponds to tire ruts to agricultural ditches. Eventually, she said "we hope to reach site-specific conclusions about the cause or causes of frog abnormalities." She is eager for additional refuges to gather data.

"We've come a long way with the quantity and quality of data collected. We still have a long way to go, and soon we'll be coming to a pond near you," notes Hinzman. ◆

U.S. Fish & Wildlife Service

Evaluating the Health of Our National Wildlife Refuges

Amphibian Abnormalities

Refuge Wildlife Biologist, Randy Hill, Collecting Frogs at Columbia National Wildlife Refuge

Issue

National attention was focused on the issue of amphibian abnormalities in 1995, when a group of middle school students from Minnesota discovered large numbers of frogs with misshapen, extra, or missing limbs. In recent years, an increasing number of frogs and toads with severe abnormalities have been observed throughout the United States, as well as in other parts of the world. Researchers have been investigating the problem at many levels, including conducting surveys, laboratory studies, and developing the North American Reporting Center for Amphibian Malformations, a clearinghouse for the collection and dissemination of information. Although several Federal agencies and other researchers are involved in the amphibian decline and abnormality issue, the U.S. Fish and Wildlife Service (Service), with the expertise provided by its Division of Environmental Quality, is uniquely suited to determine the role of contaminants in amphibian abnormalities.

Reason for Concern

The Service helps conserve habitat through the National Wildlife Refuge System (Refuges), the world's largest and most diverse collection of lands set aside specifically for the conservation of wildlife. To ensure the health of this habitat, Service environmental contaminant specialists monitor the effects of contamination on fish and wildlife. Many amphibian species are sensitive to a variety of environmental stresses and may be good early indicators of the health of their environment. Therefore, the Service is interested in determining whether abnormal frogs occur on Refuges, and if so, investigating potential causes.

What is the Difference Between Malformation and Deformity?

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.

What is Causing Amphibian Abnormalities?

Potential causes include:

- Changes in climate (increased UV-B light due to ozone depletion, acid rain, drought, etc.);
- predatory species (e.g. fish, bullfrogs, invertebrates);
- parasites, bacteria, fungus, and viruses; and,
- pollution and contaminants (pesticides, metals, fertilizer, etc.).

Scientists believe frog abnormalities have a number of different possible causes. In some areas, multiple factors may be causing the abnormalities. It is also possible that the cause(s) may differ from one site to another.

Have Abnormalities Been Found On Any Refuges?

Due to the especially high incidences of frog abnormalities reported in Minnesota and Vermont, the Service's Northeast (Region 5) and Midwest (Region 3) Regions began assessments in 1997 to document the extent of abnormal frogs on Refuges.

A frog with an abnormal leg

Frog with extra fore limb

Abnormal frogs were found on several Refuges in both Regions. In 1999, the Northeast Region conducted a second set of assessments. Abnormal frogs were discovered in nine Refuges. In 2000, the Service expanded its efforts to Refuges nation-wide, using standard procedures developed by Region 5. The objectives of this program are to: 1) determine if Refuges have sites where frogs with abnormalities are frequently observed; 2) evaluate whether the prevalence of abnormalities at a site is consistent within a season and among years; and 3) investigate possible causes. As of December 2002, 85 Refuges in 40 states have been monitored at least once for abnormal frogs, and many Refuges have been visited more than once. Although our assessment of all Refuges is not complete, abnormal frogs occur at frequencies greater than would be expected at some sample sites. Scientific literature suggests that abnormalities in amphibians occur normally at low frequencies in wild populations (0-2%), therefore, the Service set ≥3% abnormalities as a level at which additional sampling would be considered for this project. The presence of abnormal frogs on Refuges varies over time and space; collections vary between and within years, between Refuges, within ponds on individual Refuges, and even within a single pond over the course of one sampling season. These differences may be due to normal fluctuations in amphibian populations, changing levels of environmental stressors, or some combination of the two. Some abnormal frogs were sent to the U.S. Geological Survey's National Wildlife Health Center for confirmatory diagnoses (deformity vs. malformation) using radiographs, dissections, and other tools. Some frogs were sent to the University of Wisconsin-La Crosse for parasitological examinations.

Outcomes

So far, abnormal frogs have been found on Refuges from all regions. 30 Refuges found ≥3% abnormal frogs in at least one pond during at least one sampling period. Several Refuges have found ≥3% abnormal frogs for at least two sampling seasons. These Refuges are being considered for intensive sampling. Predator surveys and toxicity tests were conducted at a Refuge in Alaska, and additional funding was secured to conduct special studies at Refuges in New Hampshire and New Jersey. Laboratory and field studies are also being conducted at other government, educational, and private institutions around the U.S. and abroad. As cause-effect linkages are made, Refuge Managers will take action to mitigate the effects of management practices on amphibians and other wildlife. If the Service determines that land use practices on private property adjacent to Refuges are the likely cause of amphibian abnormalities, the Service will work closely with the landowners to help determine whether there are other cost-effective and efficient management methods available to them.

Green frogs

How do the Service's and USGS's Efforts Complement Each Other?

The U.S. Geological Survey (USGS) is coordinating a cooperative national effort to determine the scope and severity of amphibian population declines and to investigate causes. Scientists from USGS and other agencies have been studying amphibian populations and life history traits, measuring and monitoring environmental

Frog with missing hind legs

characteristics, and conducting research into potential causes of decline. As a result, the USGS formed the National Amphibian Research and Monitoring Initiative. By directly focusing efforts on Refuge lands, the Service can share information with the USGS and avoid duplication of effort, maximize the information generated by the available funding, and implement actions that will maintain the integrity and health of the Refuge system.

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CHAPTER 8

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, there by 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 is 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 sphenocephala; 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 catebeiana; 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)