A Field Guide to Amphibian Larvae and Eggs of Minnesota, Wisconsin, and Iowa

By

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Apparent worldwide declines in amphibian populations (Pechmann and Wake 1997) have stimulated interest in amphibians as bioindicators of the health of ecosystems. Because we have little information on the population status of many species, there is interest by public and private land management agencies in monitoring amphibian populations. Amphibian egg and larval surveys are established methods of surveying pond-breeding amphibians. Adults may be widely dispersed across the landscape, but eggs and larvae are confined to the breeding site during a specific season of the year. Also, observations of late-stage larvae or metamorphs are evidence of successful reproduction, which is an important indicator of the viability of the population. The goal of this guide is to help students, natural resources personnel, and biologists identify eggs and larval stages of amphibians in the field without the aid of a microscope.

Anyone undertaking field identification of amphibians has a responsibility to avoid harming the amphibians or their habitats. Persons planning to sample amphibians should work in cooperation with state or federal wildlife professionals. Lack of knowledge about sensitive habitats or populations could result in the spread of diseases, damage to breeding habitats, or local reproductive failure of amphibian populations. State and federal laws protect amphibians from exploitation. Collection permits are required from the appropriate state or federal authorities before capturing, handling, or collecting amphibians. Permission for sampling should also be obtained from the landowner.

Research for this publication began with a study of farm ponds in southeastern Minnesota. We quickly recognized the need for a way to identify eggs and larvae of the amphibians encountered. We used our field knowledge of most of the species included here to produce this field guide. Additional information came from published keys for Wisconsin amphibians (Vogt 1981; Watermolen 1995; Watermolen and Gilbertson 1996) and descriptions from a variety of sources (e.g., Johnson 2000; Petranka 1998; Harding 1997; Russell and Bauer 1993; Stebbins 1985; Wright and Wright 1949).

There is no simple way to morphologically distinguish between many amphibian species during the egg and tadpole stage. This might be expected in closely related species such as the gray treefrogs *Hyla chrysoscelis* and *Hyla versicolor*, which vary in chromosome number and in subtle morphological traits. It is surprising that, to our knowledge, no one has reported a way to morphologically distinguish between larvae of several common frog species such as the Crawfish Frog and Southern Leopard Frog, which bear little resemblance to one another as adults. Even with the technical keys available to herpetologists, amphibian eggs and larvae are often difficult to identify to species. An excellent key to all species in the United States (Altig et al. 1998) is available on the internet (http://www.pwrc.usgs.gov/tadpole/). The Altig key (along with earlier versions such as Altig [1970] for tadpoles and Altig and Ireland [1984] for salamander larvae) requires detailed knowledge of the anatomy of amphibian larvae. Altig et al. (1998) requires examination of tadpole mouthparts, which are only observable with dead specimens and the aid of a microscope. Tadpole biology is summarized by McDiarmid and Altig (1999). Additional research is needed to better describe and understand larval amphibians.

Three major factors make amphibian larvae, especially tadpoles, difficult to identify. First, many species are conservative in their anatomy and members of the true frog (Ranidae) and toad (Bufonidae) families are very similar in larval form. Second, morphology among tadpoles of a single species can vary geographically and with developmental stages. Third, larvae reveal differing anatomies depending on the physical and biotic environment where they are found. The proportions, coloration, and anatomy of larvae change as they grow from hatchlings to metamorphs. Larvae in early stages frequently do not show the characteristics necessary for their identification.

In one description, we summarize closely-related species that cannot be distinguished without the aid of a microscope. We believe that the weakness of specific identification is balanced by ease of use in the field. In many instances, identification to species is not necessary to meet land managers' goals. However, professional herpetologists working for universities, museums, and state and federal agencies can assist with identifying larvae to species.

No key by itself can help identify every specimen; there will always be variant individuals. In identifying anurans, for example, knowledge of which species were calling at the pond may help sort out identification questions. It also is helpful to note the geographic distribution of species; you can reasonably rule out Canadian Toads if the site is in southern Iowa. Ruling out a species by geography is not easy or reliable, however, if your survey site is near the edge of a species' range. You could also return several times to the breeding site to observe late-stage metamorphs. Amphibians often are easier to identify once they develop mature traits. If a specimen cannot be identified using this key, preserve the specimen and attempt identification using a more detailed key such as Altig et al. (1998). If still not confident in the identification, contact the senior author of this key or another herpetologist in your area for assistance. Another approach, if adequate time and facilities are available, is to raise a few eggs or larvae to metamorphosis and identify the late-stage metamorphs or adults from their mature characteristics. We anticipate that rapid molecular DNA techniques suitable for field identification will be available within the next few decades. Until then, keys will be needed.

How to use the keys

Most amphibians lay their eggs in clusters or strings. Four general types of amphibian egg masses are illustrated (Figure 1); globular clusters are large and easier to see than eggs in long strings, whereas eggs laid singly or in small clusters are difficult to find, especially in dense vegetation.

To use the circular keys (Figures 2–4), start in the center and choose among the available options, ending in the outer circle with the identification of a species or group of species. In some instances, dashed lines are used if a species exhibits multiple traits. These traits are described to the left and right of the section enclosed by the dashed line. After making an identification, use the species description to confirm the identification. If the identification seems in error (e.g., the collection location is geographically distant from the range of that species, the specimen looks nothing like the illustration, or the description doesn't match the specimen), return to the key and work backwards to find another species that more closely fits your specimen.

The jellies of most amphibian eggs are very small when first laid, but quickly swell with water. All amphibian eggs in this key are pigmented and have one or several gelatinous envelopes surrounding them (Figure 2). The gelatinous capsules are difficult to see, but it helps to use a magnifying lens and adjust the lighting. The egg key groups most ranids together, and mole salamanders also have similar eggs.

Salamanders (Figure 3) have four limbs and feathery external gills, whereas tadpoles (Figure 4) have four limbs only when they are close to metamorphosis and their gills are internal. The location of the eyes on the tadpole is an important character separating the treefrogs (hylids) from the true frogs (ranids). Eyes dorsal means the eyes do not interrupt the lateral margin of the head when viewed from above (superior view; Figure 4a). Another early characteristic in the key is whether the vent is medial or dextral (Figure 4b). This is difficult to see on a squirming live tadpole; however, hold it with the bottom (ventral) side up, straighten the tail fin, and look carefully at the location of the opening of the vent. A magnifying lens will help.

The scale bar by each drawing in the species description indicates 1 cm. Drawings of typical individuals are presented, but all specimens will not look exactly like the drawings because of natural variation. The size ranges (TL = total length from tip of snout to tip of tail, SVL = snout to vent length) may help rule out some larvae. The range maps were produced from information at Minnesota and Wisconsin DNR web sites (http://www.dnr.state.mn.us; http://www.dnr.state.wi.us) as well as from Christoffel et al. (2001) and Casper (1996) for Wisconsin, Oldfield and Moriarty (1994) and J. Moriarity (personal communication) for Minnesota, and Christiansen and Bailey (1991) and J. L.

Christiansen (personal communication) for Iowa. The range maps are color-coded as follows: dark green indicates the current range of the species and red indicates the former range of the species (range contraction). Common and scientific names follow Crother (2000).

Preserving amphibian eggs and larvae

Samples of amphibian eggs and larvae can easily be preserved to make a voucher or reference collection or to send to a specialist for positive identification. Most states require collection permits issued by the state Department of Natural Resources or other similar agency. Remember to observe all wildlife laws and only collect where it is legal and where the collection of a few individuals will not affect the population.

Larvae should be anesthetized according to the procedure recommended by Green (2001). There is no perfect preservative and techniques for preserving specimens are still debated (McDiarmid and Altig 1999). We recommend preserving amphibian eggs and larvae by placing them in a small vial filled with a 10% formalin solution. Alcohol is more pleasant to work with and safer than formaldehyde, but tends to dehydrate specimens. Whatever preservative you use, read the relevant Material Safety Data Sheets (MSDS) to learn how to safely handle and store that chemical. Larvae can be placed individually, or as a lot of 5-20 individuals, in screw top vials. Do not place too many individuals in one container. Immediate labeling is a must; use pencil or indelible ink on all submerged tags. Field tags should be linked to corresponding field notes; labels with detailed information must be kept with the specimens. Do not rely on memory as a record of locality, date, and habitat information. The minimum information includes date, locality (kilometers from a crossroad or other landmark, or GPS coordinates), habitat description, and name of the collector. We recommend maintaining a numbered log that links to tags on the vials. Other important information includes notes on live coloration (specimens quickly lose color in preservative). Specimens should be deposited in a museum or university collection where they can be appropriately cataloged, maintained, and available for researchers worldwide.

Raising Larvae

If a specific identification cannot be accomplished in the field, raising amphibian eggs or larvae to metamorphosis is one alternative. To appropriately provide for the needs of captive larvae, consult Mattison (1993). We provide a general description of larval care here to familiarize the reader with the procedures involved.

Raising amphibian larvae in an aquarium is not difficult, but avoid overcrowding (raise only one or two animals per ~4 liters [~1 gallon] of water). Water quality is the most important factor. Treat the water as you would for aquarium fish or collect natural pond water or rain water. Tap water contains chlorine, which will kill larvae as well as many fish. Water can be effectively de-chlorinated by letting it stand uncovered for several days. Change the water every few days or as needed. An aquarium or large, shallow container is preferred; the larger the surface area the more oxygen will be available. An airstone is usually not necessary. Do not clean the tank with soap or other cleansers because residues may kill the animals.

Salamander larvae are carnivorous and must be fed an animal diet. Smaller salamanders can be fed “zooplankton” (small invertebrates) netted out of streams or ponds. Larger salamander larvae can be fed small worms (earthworms or tubifex), tadpoles, feeder fish, and even pieces of liver if you wriggle it in front of their mouths. If you feed them liver, remember to not leave any in the container, as it will quickly foul the water.

Frog and toad tadpoles can be raised on a diet of fish flakes or rabbit pellets. Boiled lettuce or spinach, naturally collected algae (be careful not to introduce predaceous invertebrates such as dragonfly larvae or diving beetles), and small amounts of cooked egg yolk can also be fed to tadpoles. Offer these foods daily, but be sure to replace the aquarium water frequently.

The length of time required for metamorphosis varies from several years for American Bullfrogs to a few weeks in the Plains Spadefoot. Some Tiger Salamanders never metamorphose and remain aquatic. Most treefrogs and toads take about two months to develop from eggs to metamorphs. Salamander metamorphosis is less dramatic than frog metamorphosis. Salamanders absorb their gills and any tail fins when they metamorphose. Because amphibians are ectothermic, the temperature of the environment affects their development time. Raise larvae at room temperature or slightly warmer. Light will help maintain the algae that tadpoles feed on; direct sunlight may cause overheating.

If your tadpoles are healthy and developing as expected, you will notice the appearance of hind limbs, then a reshaping of the body, and finally, front limb emergence. At this stage, the tadpole will begin to absorb its tail and many changes will occur both internally and externally as it develops into a juvenile frog. Feeding will cease at some point; the metamorphosing frog is quite vulnerable at this stage. It can no longer swim well, but is not yet able to survive on land. Provide a solid surface area for the metamorphosing animals to climb onto and cover the container to prevent escape. Tilting the container is an easy way to offer both land and water. You may now be able to identify the frog from the developing adult characteristics. If you need to continue to raise your larvae for identification or other purposes, you should feed the froglet or newly-terrestrial salamander tiny insects such as pinhead crickets, wingless fruit flies, or bloodworms, which can be purchased in pet stores.

To prevent the spread of disease to native populations, any frogs or salamanders you raise should not be released back into the environment. Lab-raised amphibians can be anesthetized and euthanized with benzocaine or tricaine methanesulfonate (MS 222, Green 2001). If you anticipate difficulty complying with this guidance, you should not undertake raising larvae in captivity.

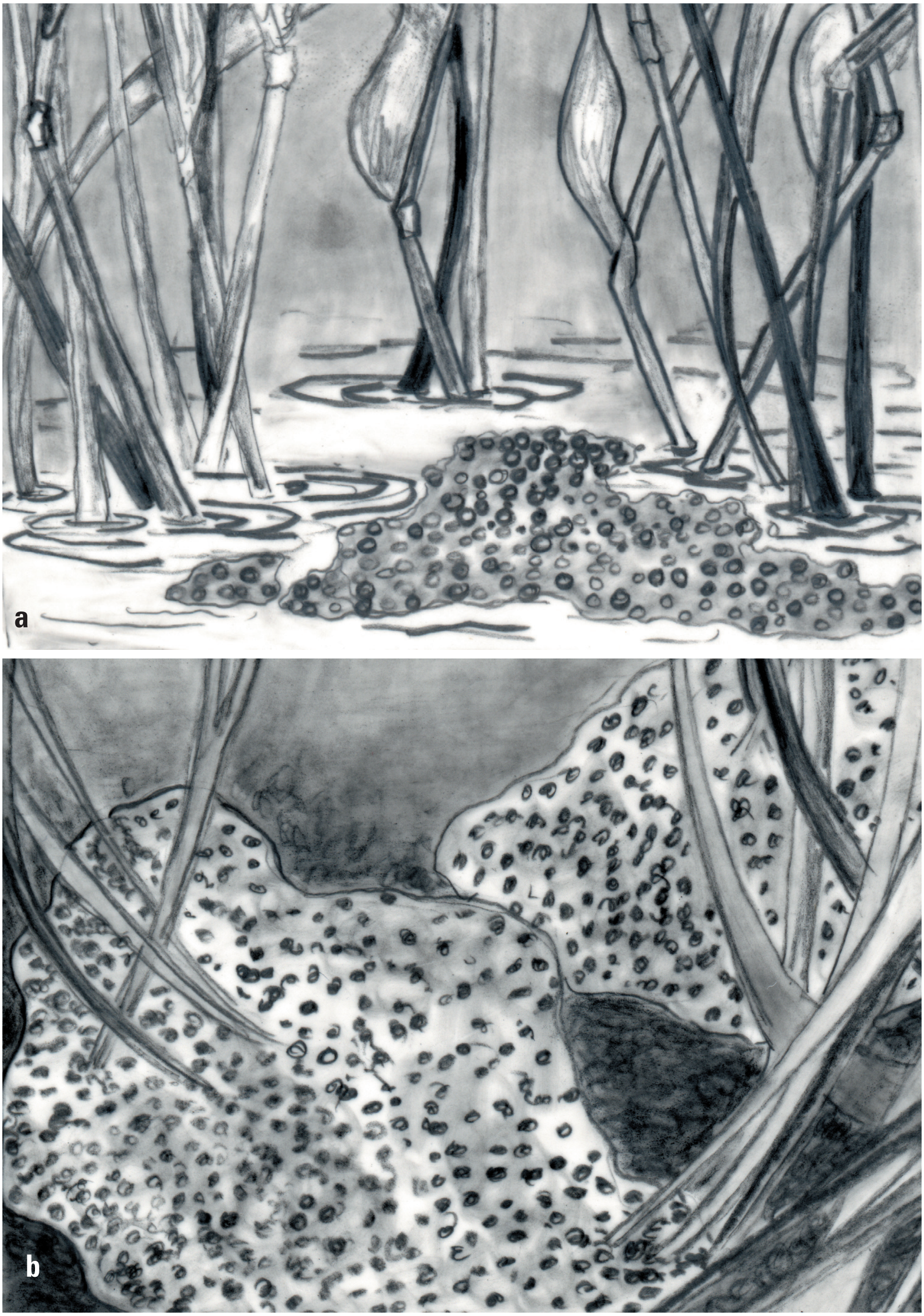
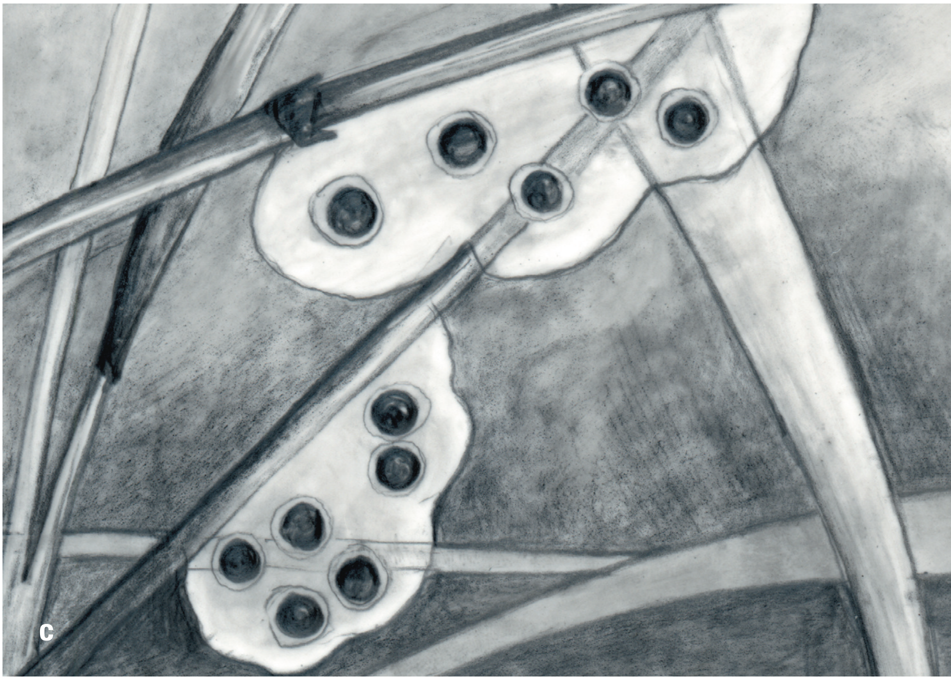
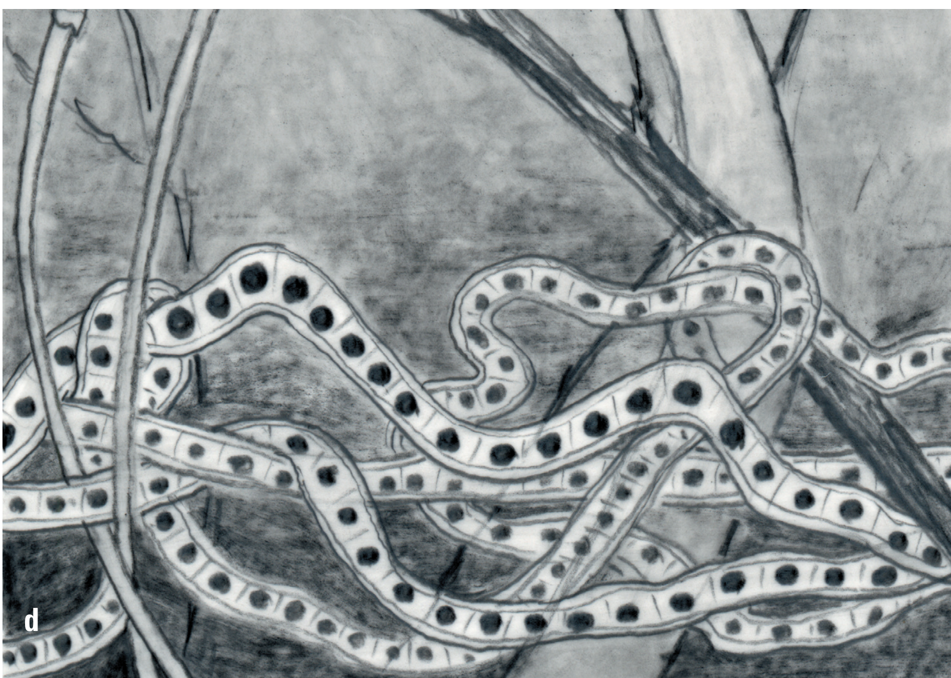


Figure 1. Egg masses of amphibians; (a) a thin raft of eggs (e.g., Green Frog), (b) a globular cluster of eggs (e.g., Leopard Frog), (c) eggs laid singly or in small clusters (e.g., Chorus Frog), (d) eggs laid

in strings (e.g., toads).





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