PURDUE EXTENSION

FNR-496

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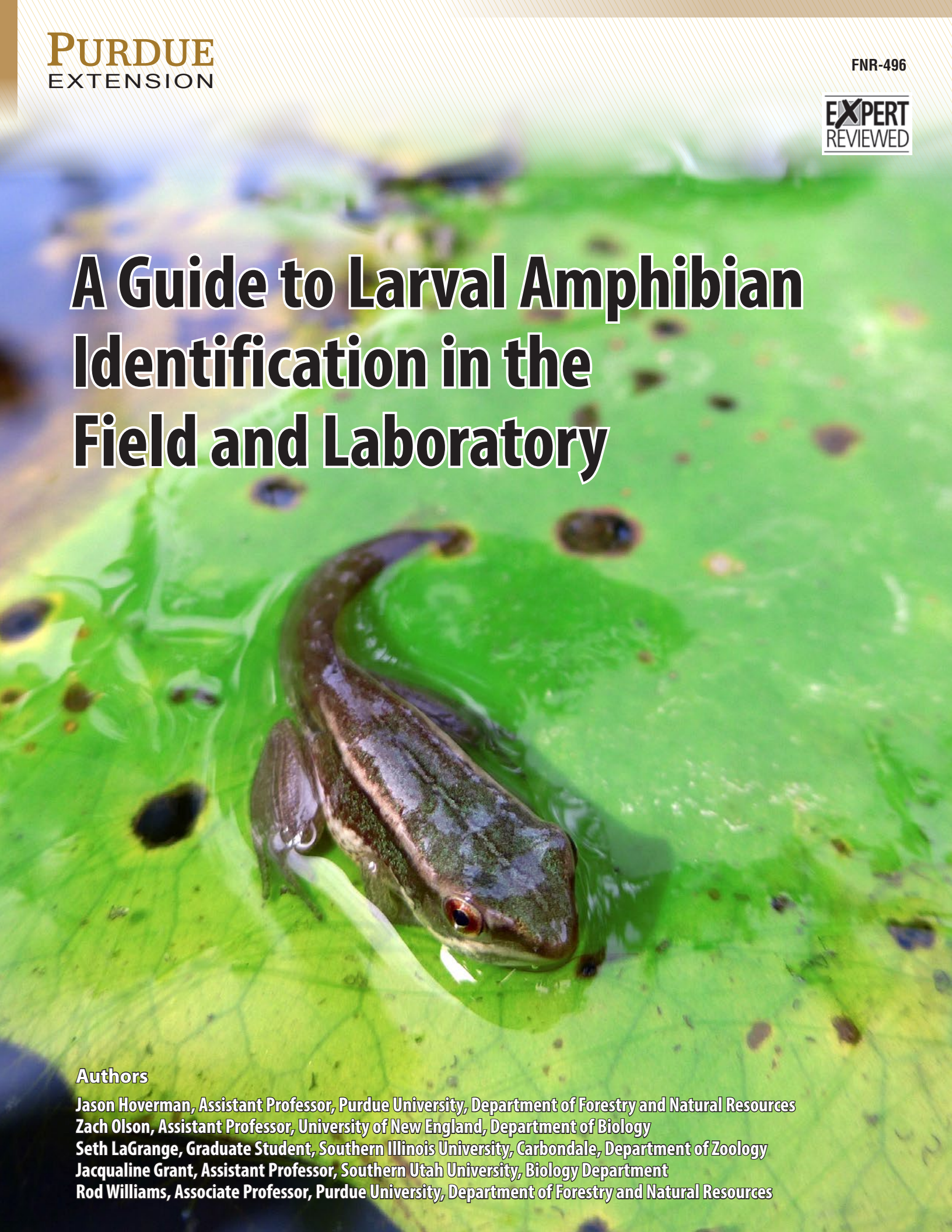
A Guide to Larval Amphibian Identification in the Field and Laboratory



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A Guide to Larval Amphibian Identification in the Field and Laboratory

Introduction

Amphibians are an amazingly diverse group of vertebrates with approximately 7,000 species described worldwide. This ancient lineage, which arose nearly 370 million years ago, has colonized nearly every freshwater and terrestrial biome on the planet. One of the key features of amphibians is their biphasic life history; they spend part of their lives as larvae before transforming into adults. For many amphibian species, the larval stage occurs within an aquatic environment (e.g., pond, lake, stream) while the adult stage occurs in the terrestrial environment. Because most amphibians require both aquatic and terrestrial environments to complete their life cycles, their populations are increasingly threatened by the destruction, degradation, and fragmentation of natural habitats. Globally, 43% of amphibian species are experiencing population declines with habitat loss listed as the major contributor. Given the imperiled status of amphibians, there is a need for research that monitors amphibians to assess population trends. Importantly, the first step in amphibian research is to learn how to identify species; it is impossible to study a species if it cannot be identified.

Currently, 39 salamander, toad and frog species call Indiana home. There are many identification keys available for adult amphibians at local and regional levels. However, a persistent challenge for biologists, especially beginners, is identifying larval amphibians. Larval amphibians are small and often lack many of the obvious and distinguishing characteristics (e.g., color, patterns) that allow us to identify adults of the same species. Despite these difficulties, it is possible to identify larval amphibians down to the species level.

Here we provide a larval amphibian identification guide for the species found in Indiana. Titles of suggested guides for other states can be found in the reference section. This guide is intended for use by biologists, seasoned amateur herpetologists, and secondary educators for classroom activities. Information for this key was gathered from multiple sources as well as personal experience. The identification guide is in the form of a dichotomous key that leads the user to the correct species identification based on a series of two-part questions. Users of the key will need a basic understanding of the external anatomy, developmental stages, and mouthparts of amphibian larvae (http://www.pwrc.usgs.gov/tadpole/tutorial.htm, http://www.virginiaherpetologicalsociety.com/amphibians/amphibian-development/amphibian-development.htm); access to a magnifying glass or dissecting scope; and a ruler. Several online tutorials listed in the reference section can help you become familiar with larval amphibian anatomy. Users of this key should be aware that a couple of species complexes cannot be reliably keyed to the species level with larvae. These include the Jefferson and Blue-spotted Salamander species complex and the Leopard Frog species complex. These complexes have been noted in the dichotomous key. Users should also note that we have excluded salamander species that possess *direct development* (i.e., do not have a larval stage).

In addition to the dichotomous key, we provide a less formal guide that can be used in the field for the identification of *anuran larvae* (i.e., tadpoles) in Indiana. In the laboratory, identifying tadpoles is relatively easy, because you have a detailed guide, a dissecting scope, and time at your disposal. However, this is not typically the case when identifying tadpoles in the field. Moreover, biologists often capture hundreds of tadpoles in dipnets and seines at a site, which underscores the need for a quick identification guide that can be applied rapidly when sampling in the field.

Considerations Prior to Sampling

Spend time in the field. The amphibian community at a site will vary from year to year and throughout the season. Consequently, spending time in the field exploring your sites for adult amphibians is a valuable approach to identifying larvae before you actually begin sampling. At the start of the breeding season, the perimeter of a wetland can be searched for migrating adults. Also, the calls of adults can be identified to provide a list of potential species that could be encountered during larval sampling. This is particularly useful when the ranges of hard-to-differentiate species overlap. For instance, consider a pond that is within the range of both Northern Leopard Frogs (*Lithobates pipiens*) and Pickerel Frogs (*L. palustris*). If you find and hear only adult Pickerel Frogs at the site, it is unlikely that you will find Northern Leopard Frog tadpoles while sampling. Thus, the identification of larval amphibians can be greatly simplified by obtaining detailed information on the natural history of sites and their species composition.

Know your ecology. Before heading into the field, it is important to understand the ecology of the species you are about to encounter. Like all species, amphibians have habitat preferences and times of the year that they are active (e.g., breeding season). Using these two factors, you can effectively narrow the list of possible species at a site.

The breeding habitats of amphibian species are aligned along a *hydroperiod gradient* (i.e., the amount of time the pond holds water). At one end of the gradient are ephemeral ponds that fill in the winter and spring, but dry early in the summer. At the other end of the gradient are permanent ponds that hold water year-round. Permanent ponds also tend to have fish, which strongly influence the amphibian community present. The middle portion of the hydroperiod gradient consists of semi-permanent ponds. These habitats generally hold water throughout the season, especially in wet years, but can dry completely during dry years. Fish are not able to persist in these habitats because they occasionally dry out. Differences in hydroperiod and the presence/absence of predatory fish influence the composition of the larval amphibian community and can be used to help identify the species. For example, Wood Frogs (*L. sylvaticus*) are largely restricted to temporary ponds while American Bullfrogs (*L. catesbeianus*) are generally limited to permanent ponds.

Each amphibian species has a characteristic time of year that it will start breeding. This is known as its *breeding phenology*. The species in Indiana can be broadly divided into early-spring, late-spring, and summer breeders. Although factors such as temperature and precipitation will influence the timing of breeding within the season, breeding phenology can be used to narrow the list of species possible at a site, especially when coupled with habitat usage (Table 2 and 3). For example, if you are sampling a temporary pond in the spring, you are not likely to encounter individuals of summer-breeding species such as American Bullfrog. In addition to influencing the composition of the amphibian community at a site, breeding phenology can also be used to understand differences in the development of tadpoles. At a particular time of the year, tadpoles of early breeding species will tend to be more developed than tadpoles of late-breeding species. For example, Spring Peepers (*Pseudacris crucifer*) breed earlier than Gray Treefrogs (*Hyla versicolor*) and transform at a smaller size. Thus, Spring Peepers will be more developed when Gray Treefrogs begin to breed. Although Gray Treefrogs can grow rapidly to be similar in size to Spring Peepers, Spring Peepers will generally have well developed hind limbs when Gray Treefrogs will not.

Photo by: Greg Stephens

Photo by: Nathan Haislip

Larval Amphibian ID Guide

1. Gills external; body not abruptly wider than tail; forelimbs visible externally
   (Fig. 1a—salamander larvae) 2
   Gills internal; body abruptly wider than tail; forelimbs not visible externally
   (Fig. 1b—anuran larvae)..... 15

Figure 1a

Generalized salamander larvae

Figure 1b

Generalized anuran larvae

2. Hind limbs never present; specimens of 40 mm SVL (snout-vent length)
   or less with orange markings on head and dorsal fin..... *Siren intermedia* (Fig. 2)
   Hind limbs present in specimens of 40 mm SVL or more; no orange markings on head 3

Figure 2

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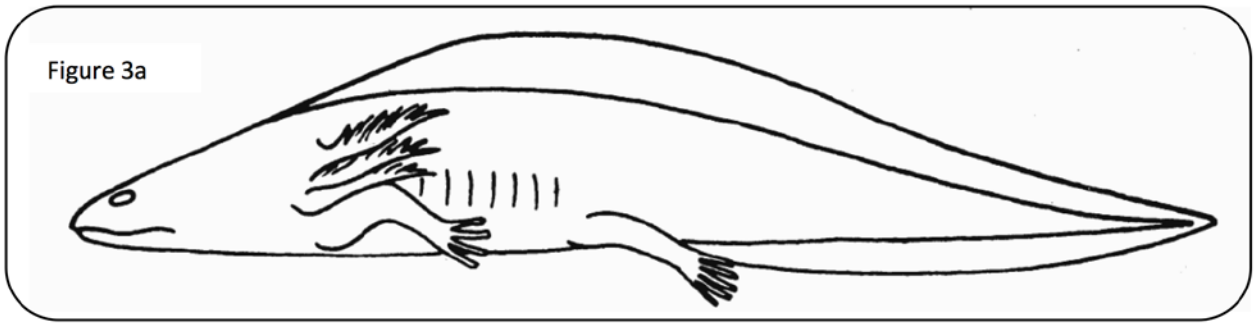
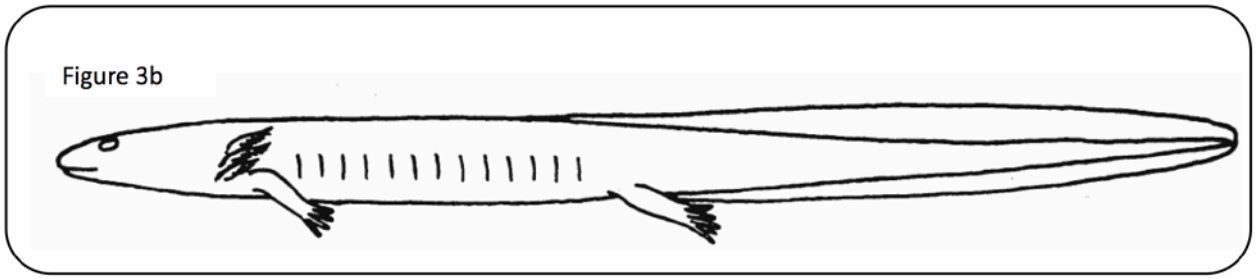
Western Lesser Siren (*Siren intermedia*)

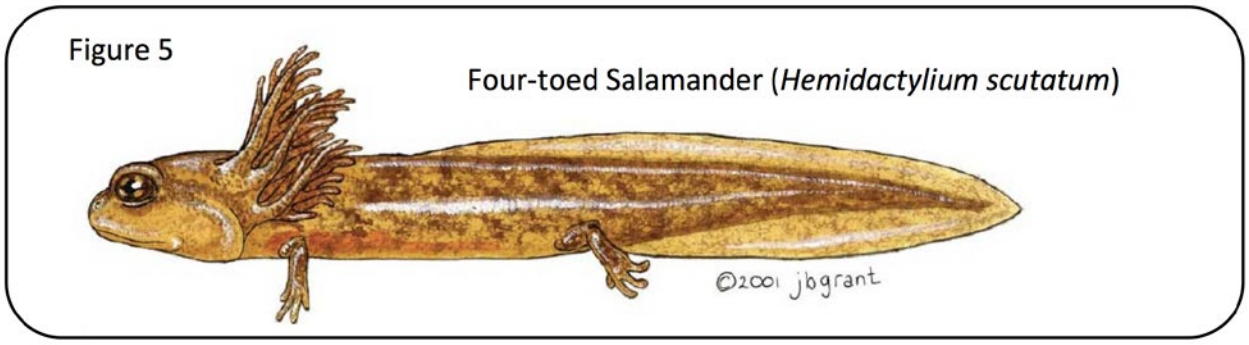
3. Dorsal fin extends nearly to head; gills long (Fig. 3a) 4
   Dorsal fin terminating approximately above vent; gills short (Fig. 3b) 1





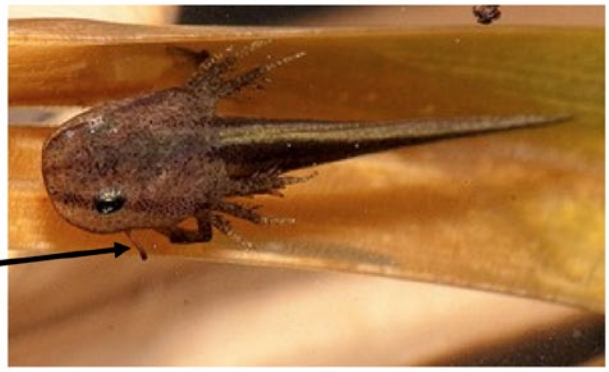
4. Head small with more or less pointed snout; dark stripe through eye;
   body gray or yellowish sometimes with reddish spots ..... *Notophthalmus viridescens* (Fig. 4)
   Head proportionally larger and snout blunt; no dark stripe through eye; reddish spots never present 5

5. Hind foot with 4 toes; body length not greatly exceeding 20 mm ..... *Hemidactylium scutatum*
   Hind foot with 5 toes; length often exceeding 20 mm 6



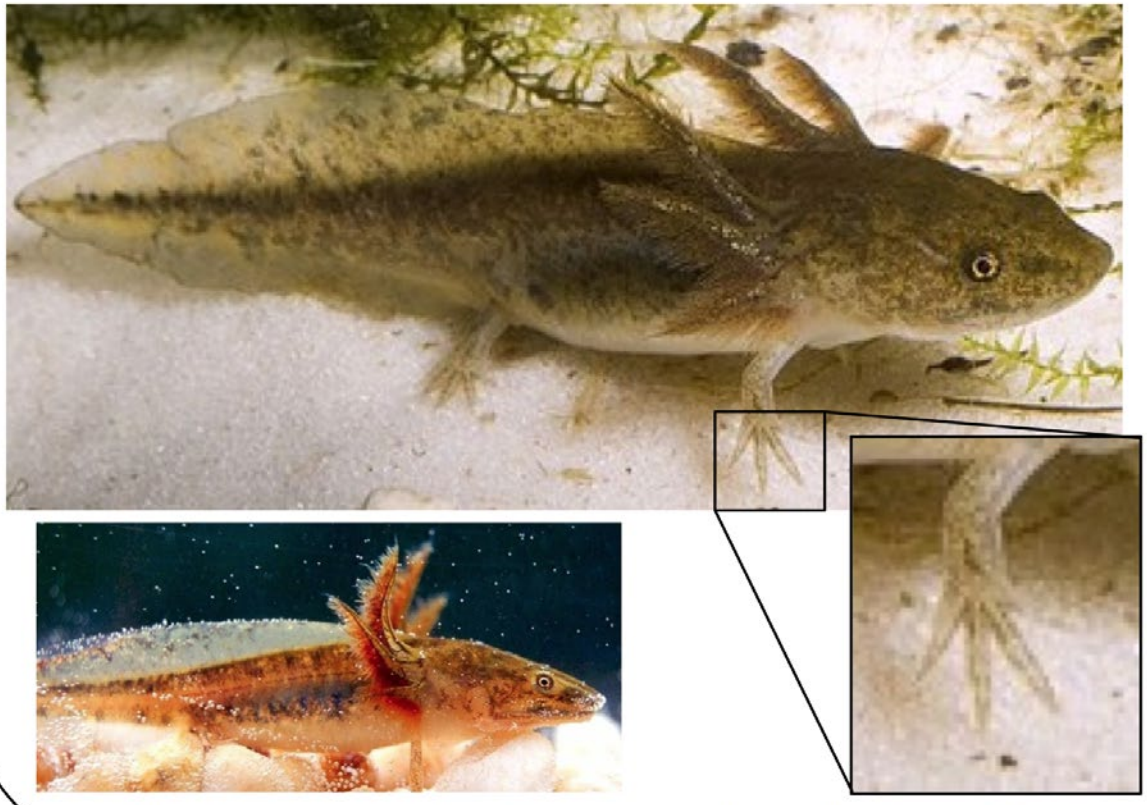
6. Hatchling larvae with balancers (Fig. 6a); toes of older larvae not flattened, rounded at tips; maximum length 50–70 mm 7
   Hatchling larvae without balancers; toes of older larvae flattened and with pointed tips; length often exceeding 70 mm ..... *Ambystoma tigrinum* (Fig. 6b)

Figure 6a



The left balancer is clearly visible

Figure 6b Eastern Tiger Salamander (*Ambystoma tigrinum*)



Note: Toes have pointed tips. This is unique to Eastern Tiger Salamanders

7. Trunk and tail fin with uniform dark pigmentation; small light lateral spots;
   larvae occur in late fall and winter ..... *Ambystoma opacum* (Fig. 7)
   Trunk with dark pattern; tail fin spotted or mottled; occur in late winter or spring 8

Figure 7

Marbled Salamander (*Ambystoma opacum*)

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Look for light spots on the side

8. Trunk with dark reticulation or spots; tail fin lightly pigmented ..... *Ambystoma maculatum* (Fig. 8)
   Trunk with stripes or dark blotches or saddles; tail fin heavily pigmented 9

Figure 8

Spotted Salamander (*Ambystoma maculatum*)

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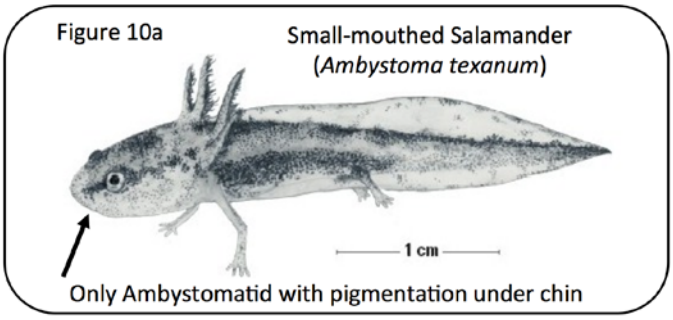
9. 10–11 *costal grooves* (parallel grooves on the side, between front and hind limbs);
   trunk with two conspicuous yellow stripes on each side ..... *Ambystoma talpoideum* (Fig. 9)
   > 11 costal grooves; trunk with dark blotches or saddles..... 10

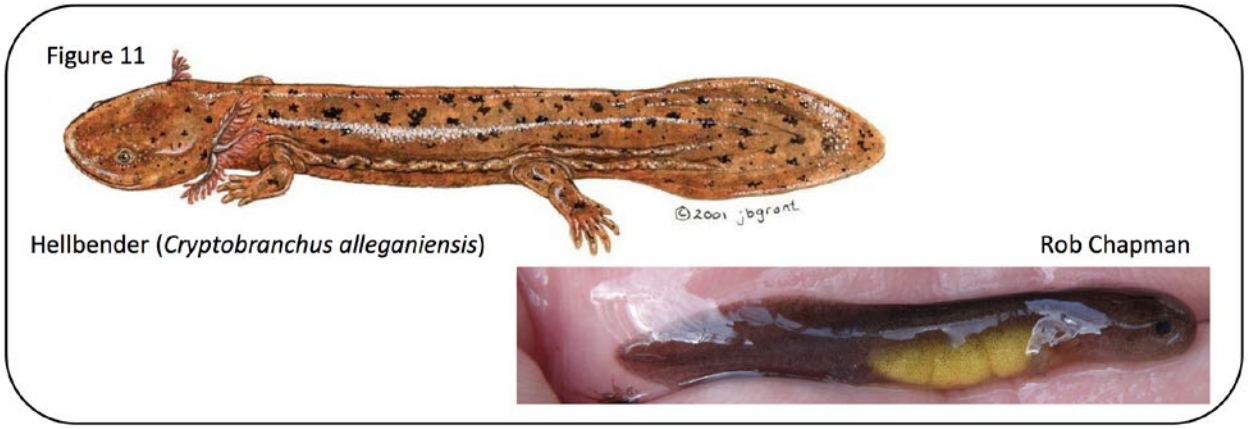
Figure 9

Mole Salamander (*Ambystoma talpodium*)

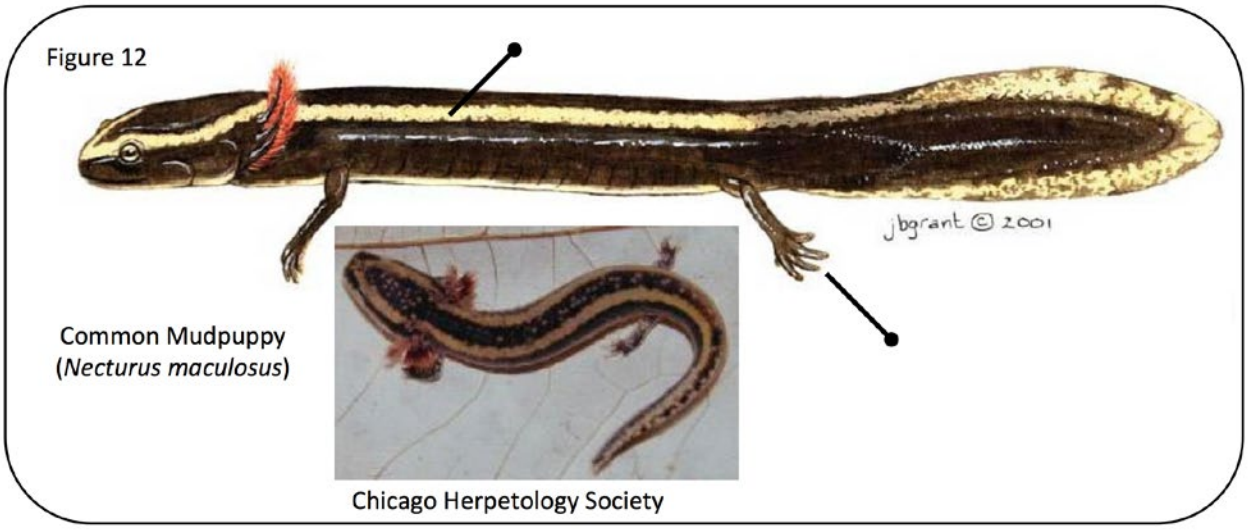
10. Trunk with dark transverse bands; throat with dark pigmentation;
    14 or 15 costal grooves ..... *Ambystoma texanum* (pond) or *A. barbouri* (stream) (Fig. 10a)
    Trunk with dark saddles; throat with little or no dark pigmentation;
    12 or 13 costal grooves..... *Ambystoma jeffersonianum* or *A. laterale* (Fig. 10b)



11. Fold on posterior edge of forelimb ..... *Cryptobranchus alleganiensis* (Fig. 11)
    No fold on posterior edge of forelimb 12



12. Four toes on hind foot; prominent dorsolateral light stripes ..... *Necturus maculosus* (Fig. 12)
    Five toes on hind foot; no dorsolateral light stripes 13



13. Dorsum with paired light spots ..... *Eurycea cirrigera* (Fig. 13)
    Dorsum with light midline stripe or uniformly dark 14

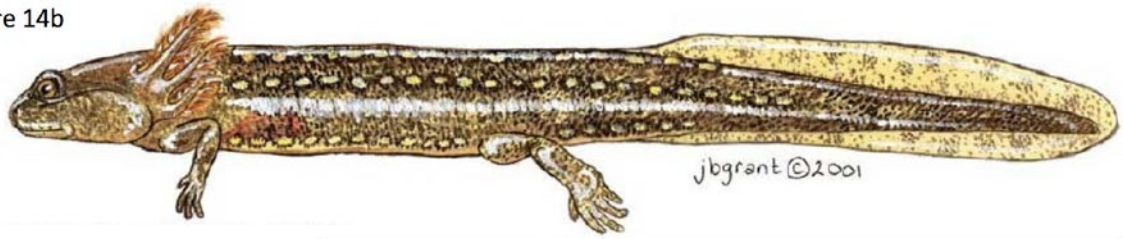
Figure 13 Southern Two-lined Salamander (*Eurycea cirrigera*)

14. Dorsum with light midline stripe; throat sparsely pigmented ..... *Eurycea longicauda* (Fig. 14a)
    Dorsum without light midline stripe; throat heavily pigmented anteriorly ..... *Eurycea lucifuga* (Fig. 14b)

Figure 14a

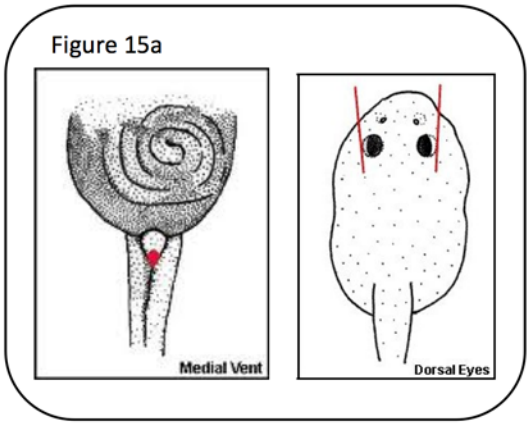
Long-tailed Salamander
(*Eurycea longicauda*)

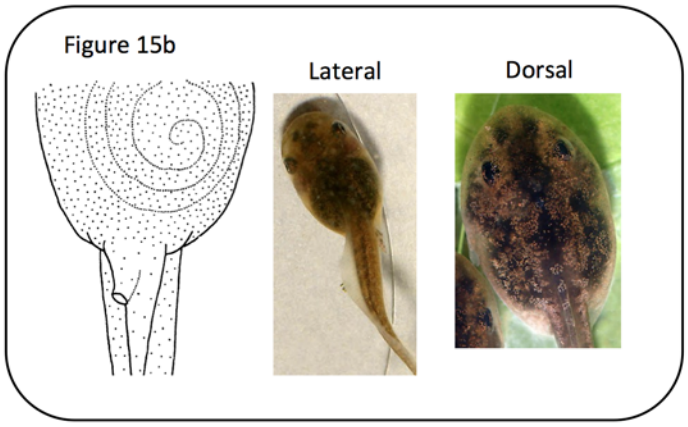
Figure 14b



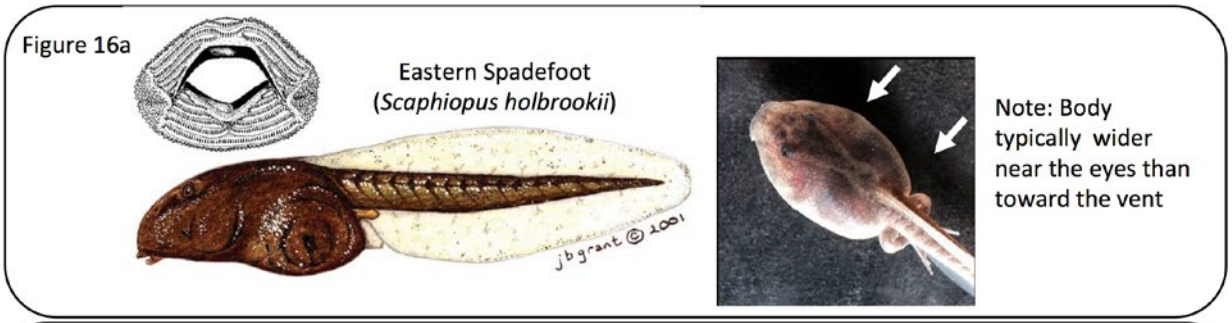
Cave Salamander
(*Eurycea lucifuga*)

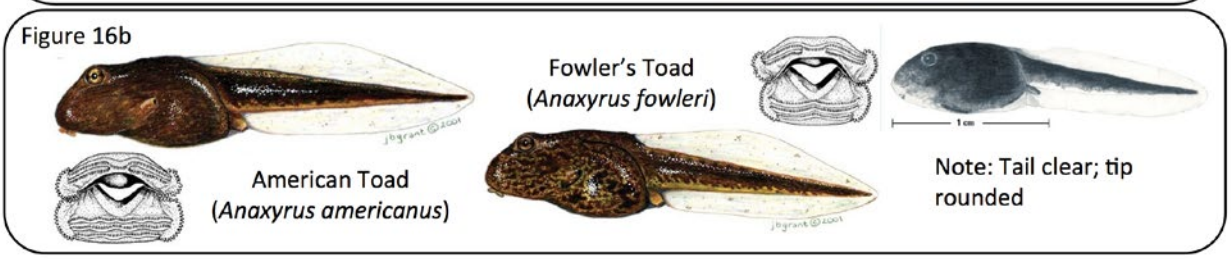
15. Anus in midline at base of ventral fin; eyes dorsal (Fig. 15a) 16
    Anus to right of ventral fin (i.e., dextral); eyes lateral or dorsal (Fig. 15b)..... 17



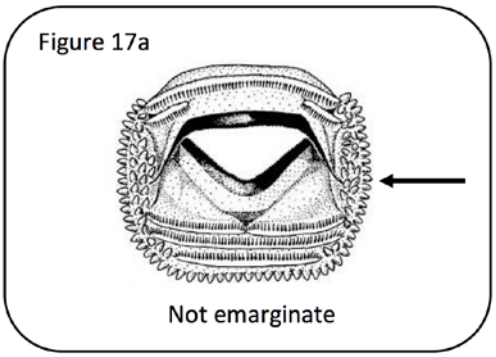


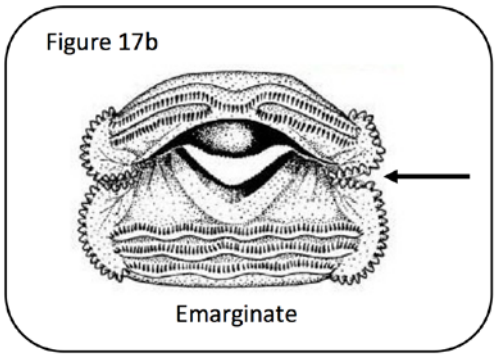
16. Labial tooth rows 10 or more.....*Scaphiopus holbrookii* (Fig. 16a)
    Labial tooth rows fewer than 10.....*Anaxyrus americanus or A. fowleri* (cannot differentiate; Fig. 16b)





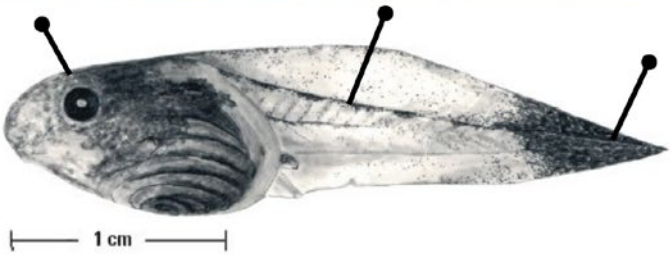
17. Lateral margins of papillary border of mouthparts not folded inward toward corners of mouth (not *emarginate*, Fig. 17a); eyes lateral or dorsolateral; length not more than about 50 mm 18
    Lateral margins of papillary border of mouthparts folded inward towards corners of mouth (emarginate, Fig. 17b); eyes dorsolateral; length often more than 50 mm..... 21



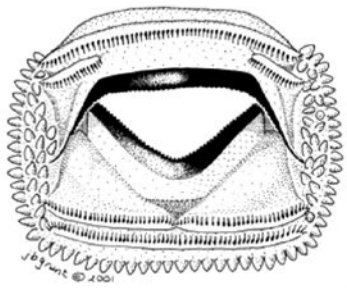


18. Usually 2 lower labial tooth rows; tail with black tip ..... *Acris crepitans* (Fig. 18)
    Usually 3 lower labial tooth rows; tail without black tip 19

Figure 18 Northern Cricket Frog (*Acris crepitans*)



Note: The Northern Cricket Frog is notable for its black tail tip, dark band of pigment on the dorsal edge of the tail musculature, and dorsolateral eye position



19. Upper half of tail musculature distinctly darker than lower half; first posterior labial tooth row (P-1) without a gap; second anterior labial tooth row (A-2) gap ratio 2.0 or more; tail length to height ratio of 2.5 or more ..... *Pseudacris triseriata* (Fig. 19)
    Tail musculature uniformly mottled 20

Figure 19 Western Chorus Frog (*Pseudacris triseriata*)

Note: Western Chorus Frog tadpoles are brownish, black, or gray above and bronze or silvery below, and usually have clear fins with a bicolored tail musculature (dark on the dorsal half, light on the ventral half).

20. Length of second lower labial tooth row (P-2) 1.0 to 1.3x the length of the third labial tooth row (P-3); tail fin reddish..... *Hyla versicolor* or *H. chrysoscelis* (cannot differentiate; Fig. 20)
Length of P-2 greater than 1.3x the length of P-3 21

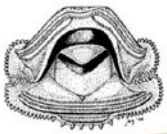
Figure 20 Cope's Gray Treefrog (*Hyla chrysoscelis*)

Note: Red tail pigmentation is diagnostic of Gray Treefrogs

21. Length of P-2 3.0x or greater than the length of P-3 ..... *Pseudacris crucifer* (Fig. 21a)
Length of P-2 less than 3.0x the length of P-3 row; pale yellow stripe often present on either side of the head running from the nostril to the eye ..... *Hyla cinerea* (Fig. 21b)

Figure 21a

Spring Peeper (*Pseudacris crucifer*)



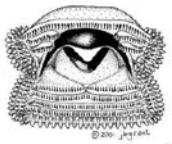
1 cm

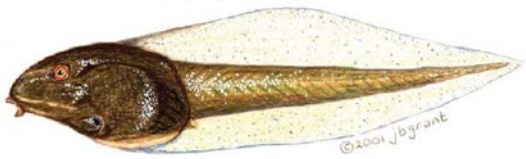
Figure 21b

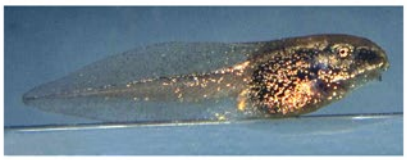
Green Treefrog (*Hyla cinerea*)

22. Papillary border emarginate; 4 lower labial tooth rows ..... *Lithobates sylvaticus* (Fig. 22)
Papillary border emarginate; 3 lower labial tooth rows 23

Figure 22 Wood Frog (*Lithobates sylvaticus*)







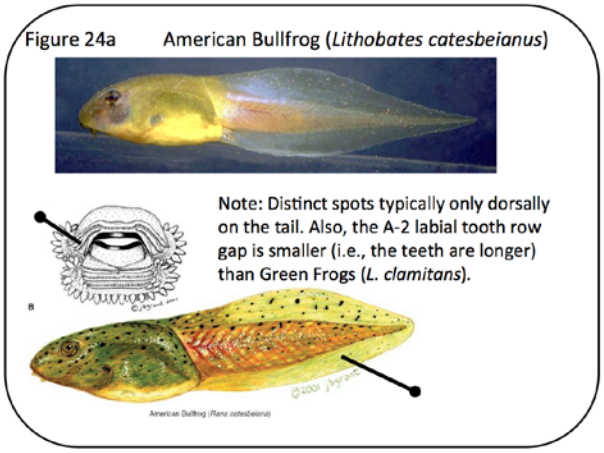
23. Ventral skin opaque (Fig. 23a) 24
Gut usually visible through ventral skin (Fig. 23b) 25

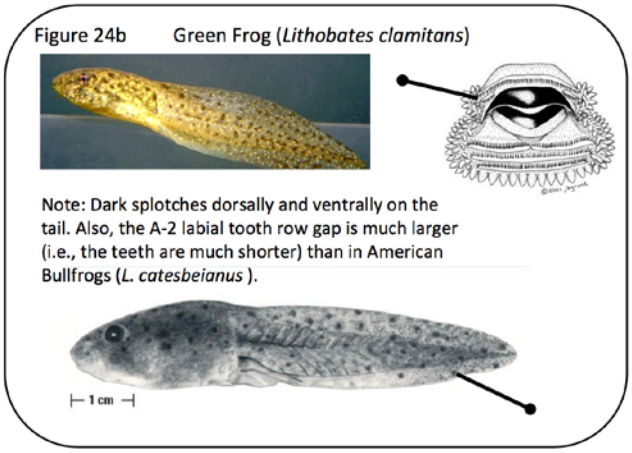
Figure 23a

Figure 23b

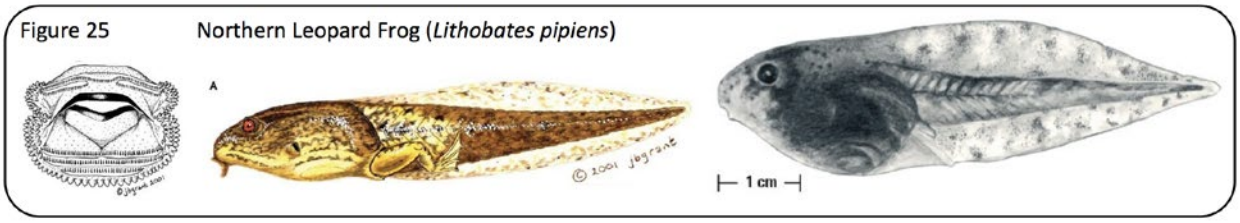
1 cm

24. Body and tail greenish; black dots with distinct borders often more numerous on dorsal than ventral half; tail fin deep and generally exceeding the depth of the body; second anterior labial tooth row (A-2) gap ratio < 2.0 .....*Lithobates catesbeianus* (Fig. 24a)
Body, but especially tail, has numerous dark blotches or fuzzy dots dorsally and ventrally, but not distinct round dots; tail fin shallow and generally not exceeding the depth of the body; second anterior labial tooth row (A-2) gap ratio ≥ 5.0 ……..... *Lithobates clamitans* (Fig. 24b)

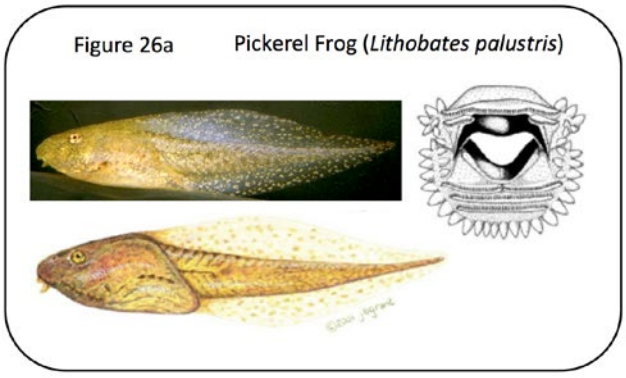


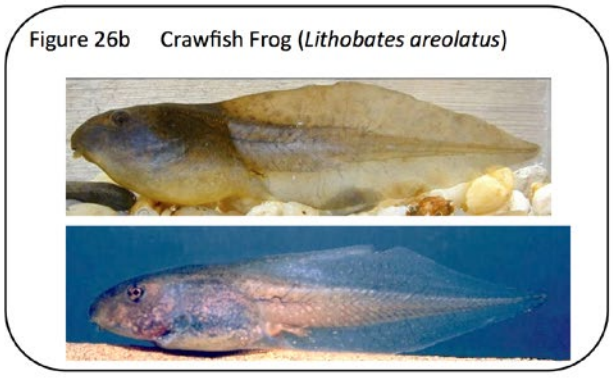


25. Tail fins clear or with light markings; second anterior labial tooth row (A-2) gap < 2.0; no gap in first posterior labial tooth row (P-1) ..... *Lithobates pipiens*, *L. sphenocephalus, L. blairi* complex (Fig. 25)
Tail fins with large spots or dark suffusion..... 26



26. Papillae below mouth large; dorsal fin rounded; no gap in first posterior labial tooth row (P-1) ..... *Lithobates palustris* (Fig. 26a)
Papillae below mouth small; dorsal fin triangular; gap in first posterior labial tooth row (P-1)..... *Lithobates areolatus* (Fig. 26b)





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Acknowledgements

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Photo by: Earl Werner

Table 1. List of Indiana amphibian species covered in the dichotomous key.

Family	Common name	Scientific name
Ranidae	Crawfish Frog	*Lithobates areolatus*
Ranidae	Plains Leopard Frog	*Lithobates blairi*
Ranidae	American Bullfrog	*Lithobates catesbeianus*
Ranidae	Green Frog	*Lithobates clamitans*
Ranidae	Pickerel Frog	*Lithobates palustris*
Ranidae	Northern Leopard Frog	*Lithobates pipiens*
Ranidae	Southern Leopard Frog	*Lithobates sphenocephalus*
Ranidae	Wood Frog	*Lithobates sylvaticus*
Hylidae	Northern Cricket Frog	*Acris crepitans*
Hylidae	Cope's Gray Treefrog	*Hyla chrysoscelis*
Hylidae	Green Treefrog	*Hyla cinerea*
Hylidae	Gray Treefrog	*Hyla versicolor*
Hylidae	Spring Peeper	*Pseudacris crucifer*
Hylidae	Western Chorus Frog	*Pseudacris triseriata*
Bufonidae	American Toad	*Anaxyrus americanus*
Bufonidae	Fowler's Toad	*Anaxyrus fowleri*
Scaphiopodidae	Eastern Spadefoot	*Scaphiopus holbrookii*
Sirenidae	Western Lesser Siren	*Siren intermedia nettingi*
Cryptobranchidae	Eastern Hellbender	*Cryptobranchus alleganiensis alleganiensis*
Proteidae	Common Mudpuppy	*Necturus maculosus maculosus*
Plethodontidae	Southern Two-Lined Salamander	*Eurycea cirrigera*
Plethodontidae	Long-tailed Salamander	*Eurycea longicauda longicauda*
Plethodontidae	Cave Salamander	*Eurycea lucifuga*
Plethodontidae	Four-toed Salamander	*Hemidactylium scutatum*
Salamandridae	Eastern Newt	*Notophthalmus viridescens*
Ambystomatidae	Streamside Salamander	*Ambystoma barbouri*
Ambystomatidae	Jefferson Salamander	*Ambystoma jeffersonianum*
Ambystomatidae	Blue-spotted Salamander	*Ambystoma laterale*
Ambystomatidae	Spotted Salamander	*Ambystoma maculatum*
Ambystomatidae	Marbled Salamander	*Ambystoma opacum*
Ambystomatidae	Mole Salamander	*Ambystoma talpoideum*
Ambystomatidae	Small-mouthed Salamander	*Ambystoma texanum*
Ambystomatidae	Eastern Tiger Salamander	*Ambystoma tigrinum*

Table 2. Breeding phenology of Indiana anuran species and their associated breeding habitats.

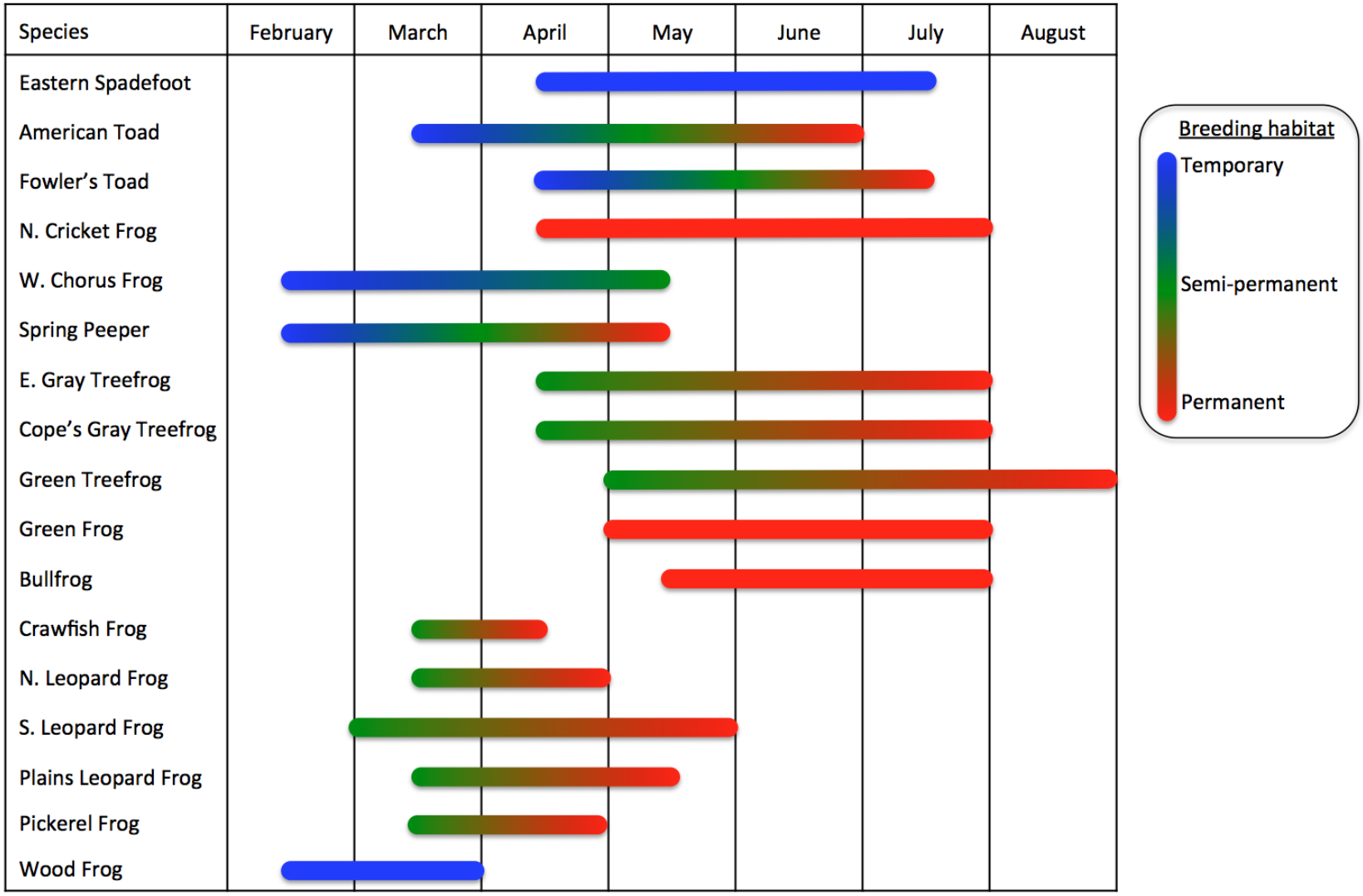
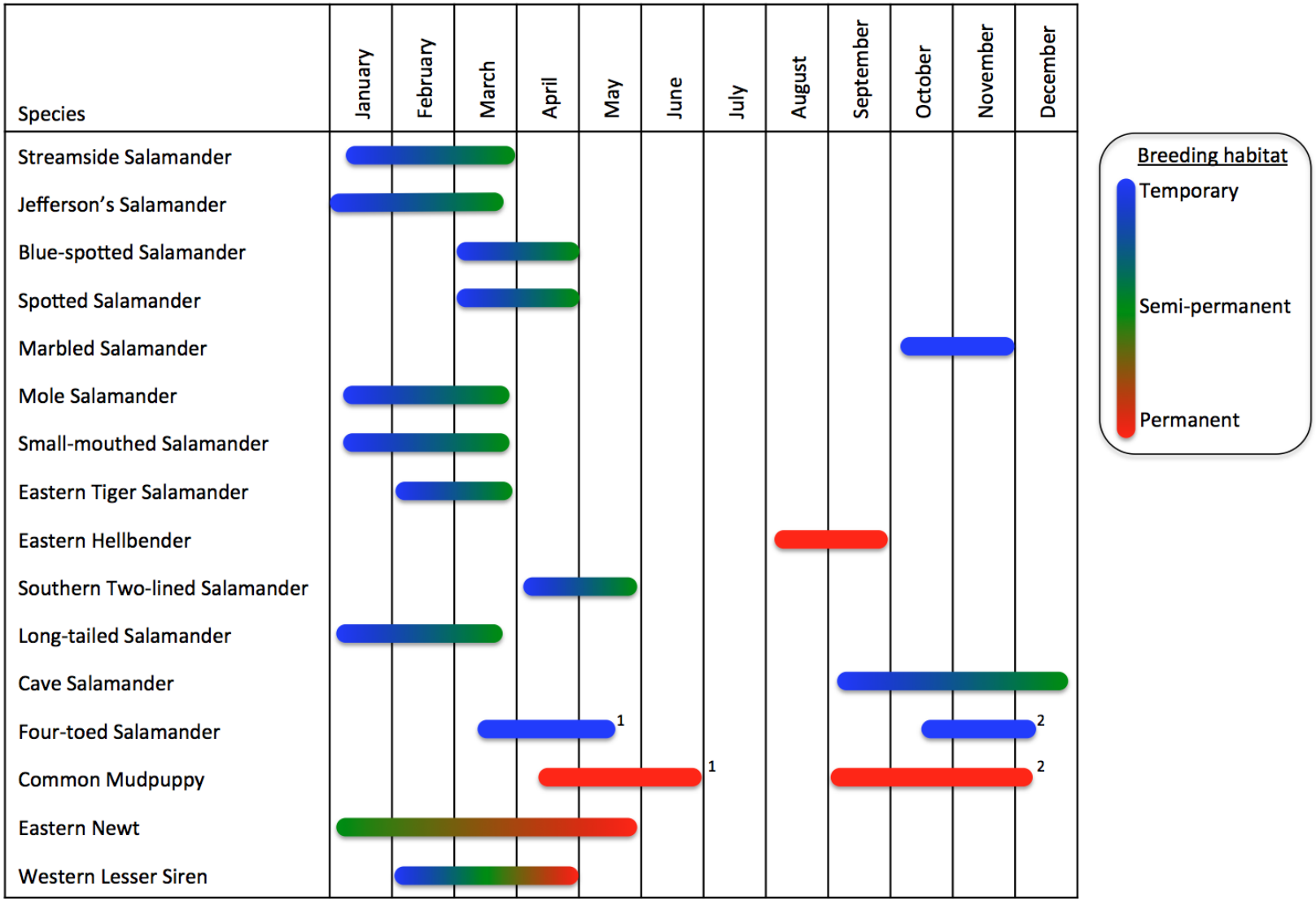
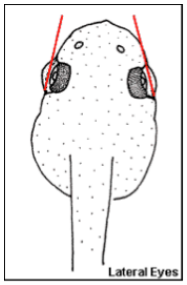


Table 3. Breeding phenology of Indiana salamander species and their associated breeding habitats.



1 – Period of oviposition
2 – Period of breeding



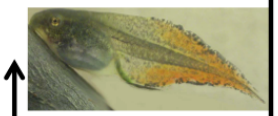
Possible species
Spring Peeper (*Pseudacris crucifer*)
Gray Treefrog (*Hyla versicolor* or *H. chrysoscelis*)
Green Treefrog (*Hyla cinera*)
Western Chorus Frog (*Pseudacris triseriata*)

Quick Reference Field Key for Larval Anurans

Gray Treefrog

Yes

No



Red tail coloration

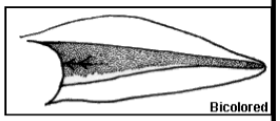
Yes

Green Treefrog

No

White stripe from eye to nostril

No



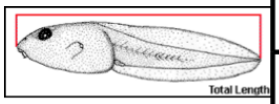
Tail muscle bicolored

Yes

Western Chorus Frog

Spring Peeper
Gray Treefrog

<2 cm



Total length

2 to 3 cm

Gosner stage

>3 cm

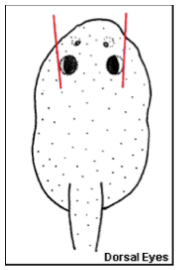
Gray Treefrog

Spring Peeper

>30

<30

Gray Treefrog



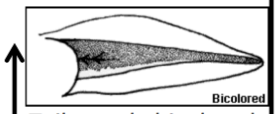
Possible species
Northern cricket Frog (*Acris crepitans*)
American Toad (*Anaxyrus americanus*)
Fowler's Toad (*A. folweri*)
Eastern Spadefoot (*Scaphiopus couchii*)
Wood Frog (*Lithobates sylvaticus*)
Pickerel Frog (*L. palustris*)
Northern Leopard Frog (*L. pipiens*)
Plain's Leopard Frog (*L. blairi*)
Southern Leopard Frog (*L. sphenocephalus*)
Crawfish Frog (*L. areolatus*)
Green Frog (*L. clamitans*)
American Bullfrog (*L. catesbieanus*)

Quick Reference Field Key for Larval Anurans

Wood Frog

Yes

No



Tail muscle bicolored

<10

Black or mottled black body

No

Labial tooth rows

No

Black tail tip and saddle-like markings on tail muscle

Yes

Northern Cricket Frog

10+

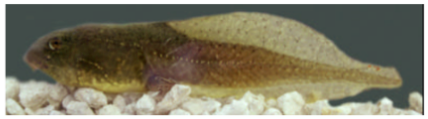
Eastern Spadefoot

Yes

American Toad
Fowler's Toad

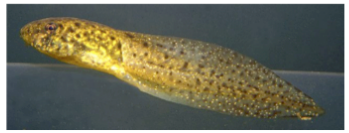
Shape and coloration

American Bullfrog



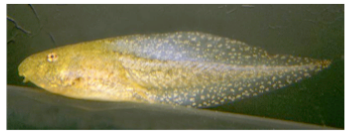
- Relatively deep tail fin with distinct small black spots
- Yellow belly
- Hatchlings – black line through tail muscle

Green Frog



- Relatively shallow tail fin with rectangular black markings
- Compressed body
- White belly
- Hatchlings – no black line through tail muscle

Leopard Frog complex
Pickerel Frog
Crawfish Frog



- Relatively short tails
- Heart-shaped body
- Belly opaque, intestinal coil visible

Jan 2015

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