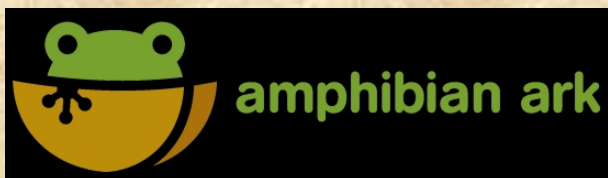


Techniques for gender determination & individual identification in amphibians



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Anurans

Juveniles are often very difficult / impossible to sex using external morphological features.

Adults of many species exhibit sexually dimorphic external morphological characters, **but** these differences often only exist during the breeding season.

External characters that can be used to identify sexually mature individuals include:

- SVL (females usually larger than males, but males larger in some spp.)
- Body shape (females often more rounded than males)
- Relative thickness of forearms (*e.g.* hypertrophied forelimb musculature in males)

Anurans



External characters that can be used to identify sexually mature individuals include (cont'd)

- Relative size of tympanic membranes (larger in males in some spp., e.g. *Rana catesbeiana*, larger in females of some spp., e.g. *Hyla ebracata*)
- Chromatic dimorphism (e.g. *Bufo periglens*, *Hyperolius cinnamomeoventris*)
- Gravid females with visible eggs
- Males with vocal sacs / darker throats
- Males with nuptial pads
- Males with prepollical ('thumb') spines / humeral spines / labial spines
- Males with sharp odontoids or tusks behaviour
- faecal steroids



In some frogs, you can easily see eggs through the ventrum of the female.



In some dendrobatids (especially those in the *tinctorius* tribe), males tend to have larger and wider toe pads than females.



Female *D. auratus*



Male *D. tinctorius*

Usually only found on males.
Can be on digits and/or on forearms.
Usually rough in texture, and dark in color.
Used to grasp slippery females during amplexus.



Note the female's front foot in the left picture has no signs of a darkened or roughened spot. While the male's front foot below has the "typical" nuptial pad.



Vocal sacs come in many sizes, shapes, and colors. Some are single, and some are paired. Used in male calling behavior.



- When the vocal sac is deflated, it often appears darker in color and sometimes more rugose in texture.



Bufo debilis male

- In many anurans, males may be slightly smaller than females.
- Males are often more “straight-sided” when viewed from above.
- Females are more rounded when viewed from above.



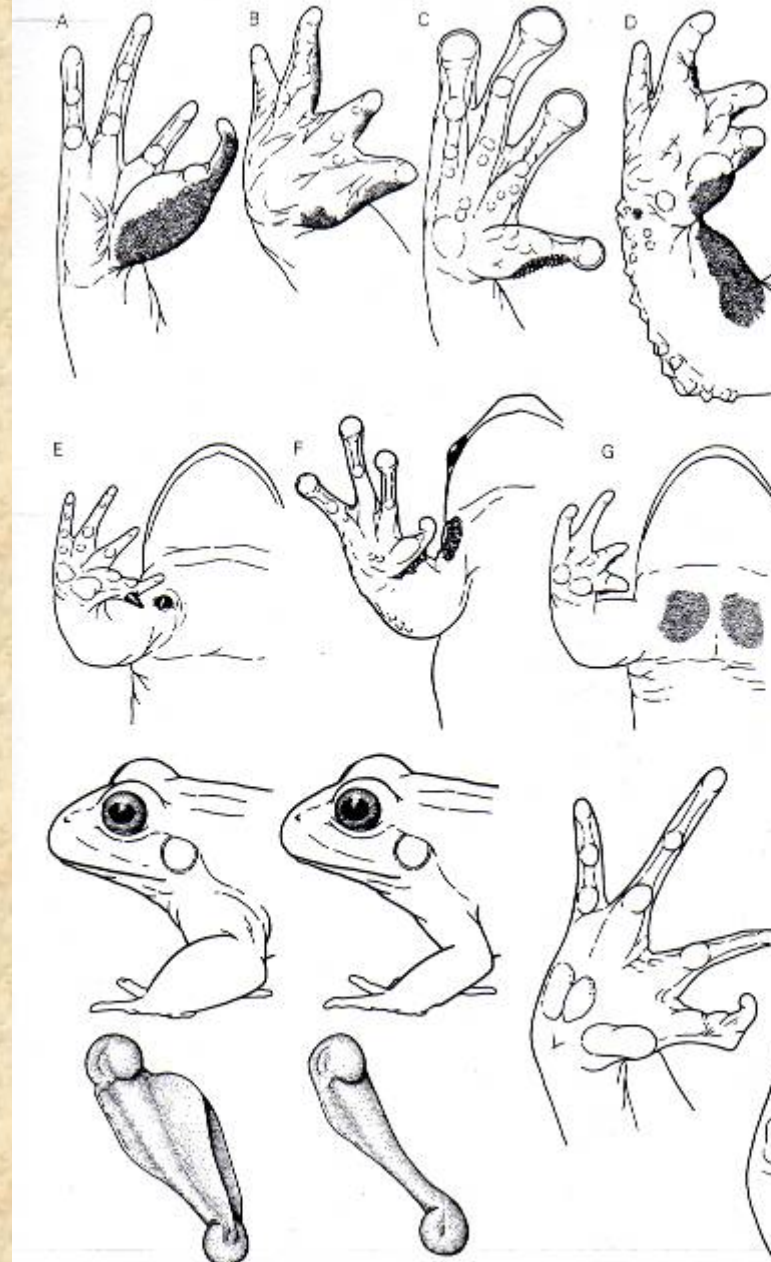


Figure 3-8. Nuptial excrescences in breeding male frogs. A. *Rana maculata*. B. *Bufo bufo*. C. *Pseudohyla spinipollex*. D. *Bombina orientalis*. E. *Leptodactylus pentadactylus*. F. *Hyla armata*. G. *Scutiger odungensis* (modified from Dubois, 1979a). All are ventral views.

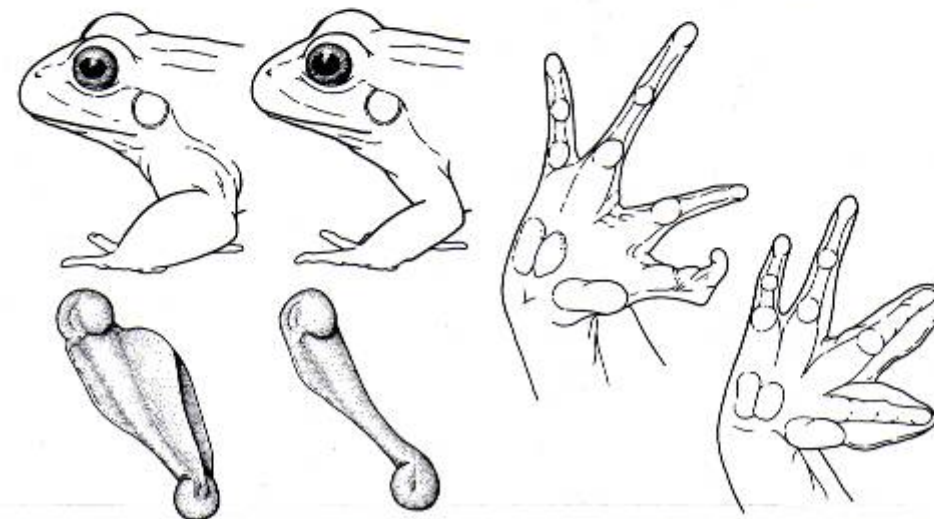
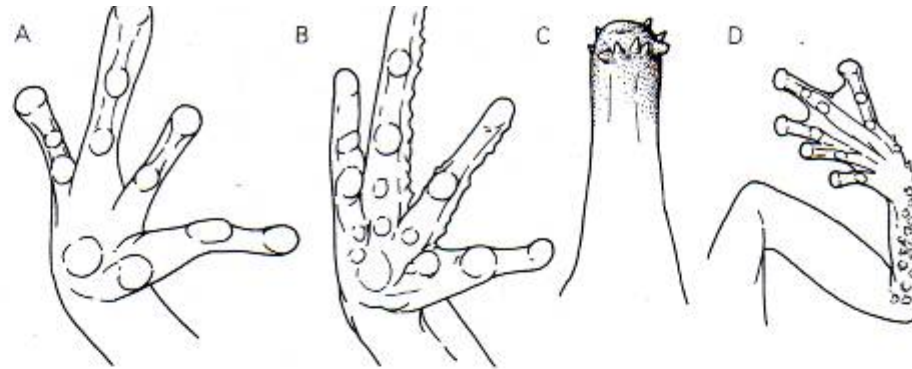


Figure 3-9. Hypertrophied forelimb of male *Leptodactylus pentadactylus* (left) and normal forelimb of female (right) and extensive development of flanges on humerus of male as compared with female.

Figure 3-10. Sexual differences in hands of *Limnodynastes peronii*, showing projection on first finger of male (left) and dermal fringes on fingers of female (right).

Figure 3-11. Secondary sexual characters of hands and feet of male anurans. **A.** Swollen third finger in *Colostethus nubicola*. **B.** Elongate third finger with lateral denticles in *Cardioglossa cyanocephala*. **C.** Elevations on dorsal surfaces of second finger in *Kaloula verrucosa* (redrawn from H. Parker, 1934). **D.** Tarsal spines in *Acanthixalus spinosus* (adapted from Perret, 1966).



regularities on the fingers (H. Parker, 1934; Liu, 1950) (Fig. 3-11).

Glands. Glands develop on the ventral surfaces of breeding males in many kinds of anurans (Figs. 3-12, 3-13). Abdominal glands are present in many microhylids that are excessively rotund-bodied (e.g., *Breviceps*, *Gastrophryne*, *Kaloula*); these glands secrete an adhesive substance that helps the male maintain amplexus (Conaway and Metter, 1967; Jurgens, 1978). At least in *Breviceps gibbosus*, females have similar adhesive glands on the dorsum (Visser et al., 1982). The function of the other ventral glands in male frogs is unknown, but because they are in contact with the female during amplexus, it is assumed that the secretions from these glands have some stimulating effect on ovulation or ovipositional behavior by the female. Mental (gular) glands of various shapes are present in all genera of hyperoliids, except *Leptopelis*, and in members of the Neotropical *Hyla bogotensis* and Australian *Litoria citropa* groups; these glands seem to be present throughout the year in most species. Extensive, thickened, and pigmented ventrolateral glands develop in males of all species of the Middle American *Ptychohyla*. Males of at least some species of *Leptopelis* have a pair of pectoral glands or a single transverse gland in the pectoral region; these glands consist of groups of glandules identical to those forming the nuptial pads (K. Schmidt and Inger, 1959). Round or ovoid "femoral" glands are present on the ventral surfaces of the thighs of some African ranids (*Petropedetes*, *Phrynodon*, and some species of *Phrynobatrachus*) and Madagascan ranids (*Laurentomantis* and some species of *Mantidactylus*). A postaxillary gland is present in breeding males of the pipid genera *Hymenochirus* and *Pseudhymeno-*

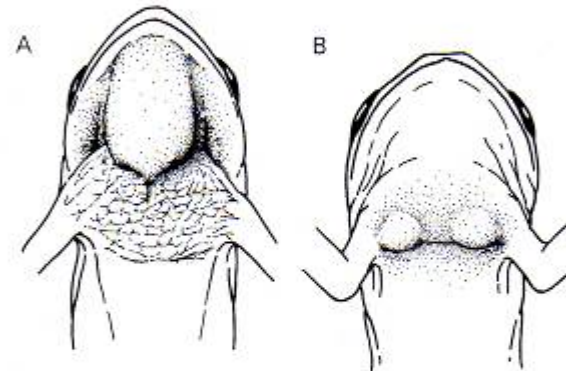


Figure 3-12. Glands of male frogs. **A.** Mental gland of *Kassina senegalensis*. **B.** Pectoral glands of *Leptopelis karissimbensis*.

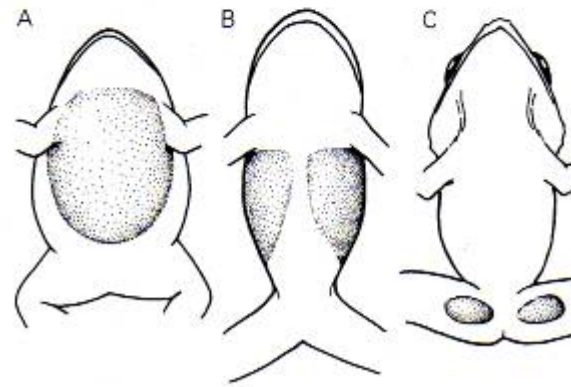


Figure 3-13. Glands of male frogs. **A.** Abdominal gland of *Kaloula verrucosa*. **B.** Ventrolateral glands of *Ptychohyla schmidtorum*. **C.** Femoral glands of *Mantidactylus pseudoasper*.

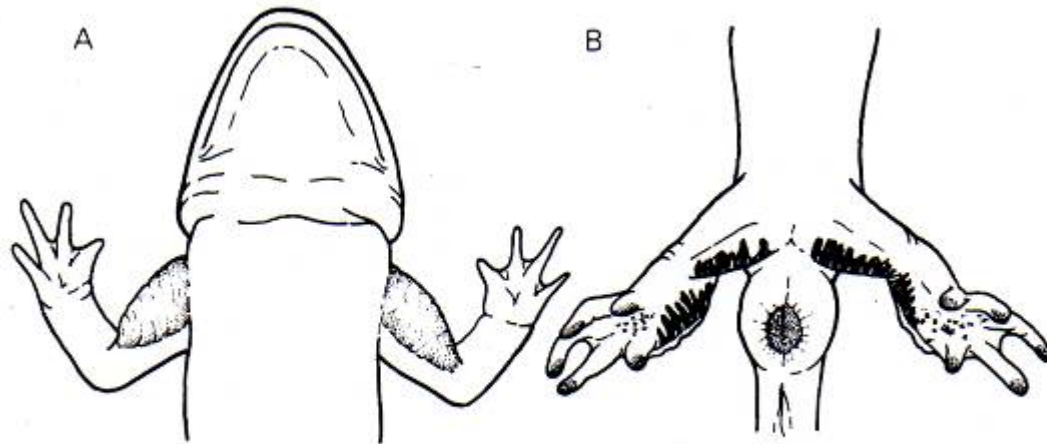


Figure 3-5. Nuptial excrescences in salamanders. **A.** Forelimbs of *Pleurodeles waltl*. **B.** Hindlimbs of *Notophthalmus viridescens*.

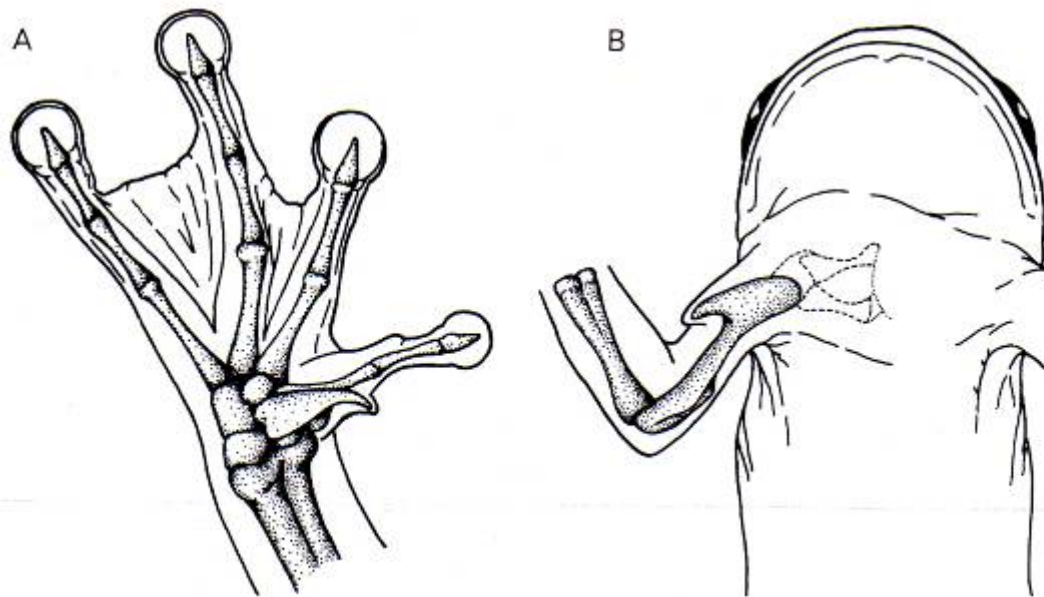


Figure 3-6. Spines on limbs of male anurans. **A.** Prepollical spine of *Hyla rosenbergi*. **B.** Humeral spine of *Centrolenella buckleyi*. Bones are stippled.

Newts and salamanders

Every species has different sex characteristics, so first make sure you know what species you have.

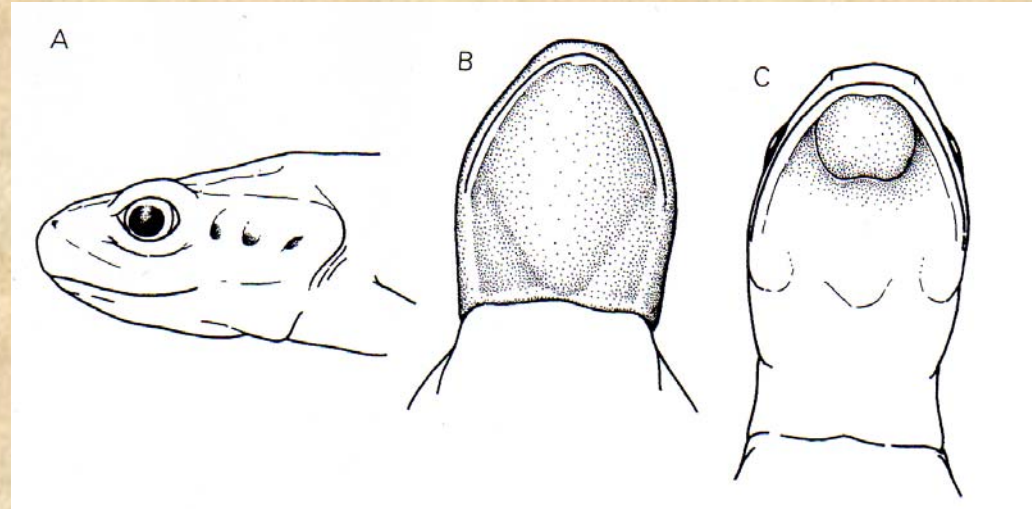
Two general rules:

- (1) Often, the male has a larger cloaca. This rule does ***not*** apply to all species.
- (2) Often, the female is larger and/or more plump. This rule depends on the animals compared being healthy, mature, well-fed, and similar ages.

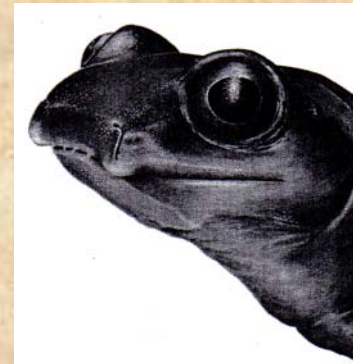
There may be no way to know. Some species cannot be sexed outside of the breeding season. If you don't have both a male and female for comparison, you might not be able to tell. Also, immature animals (larvae, juveniles, and efts) are almost always impossible to sex.

Head glands of male salamander:

- A. Genital glands on side of head of *Notophtalmus viridescens*.
- B. Diffuse submaxilar glands of *Taricha torosa*.
- C. Mental gland of *Pseudoeurycea smithii*.



Adult male *Pseudoeurycea belli* in breeding condition showing enlarged cirri and elongates premaxillary teeth protruding upper lip.



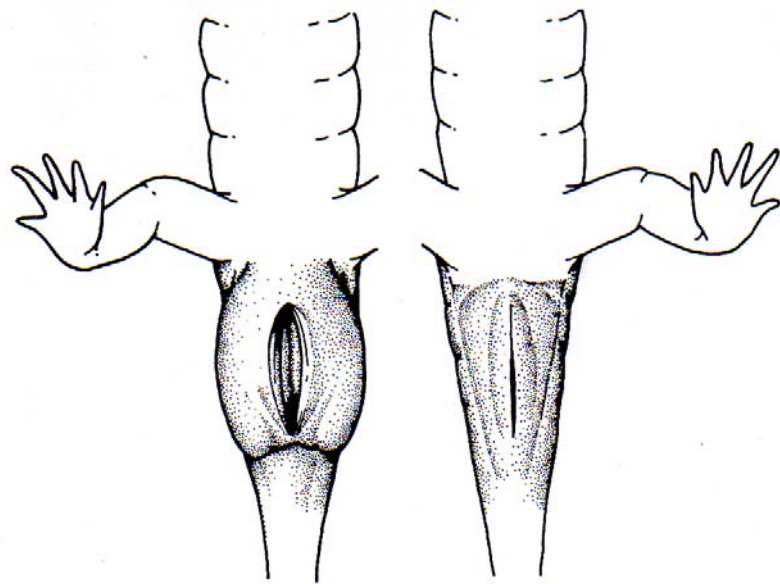


Figure 3-1. Cloacal regions of *Ambystoma jeffersonianum* showing swollen glandular area around vent and cloacal papillae in male (left) and unswollen cloacal area of female (right).

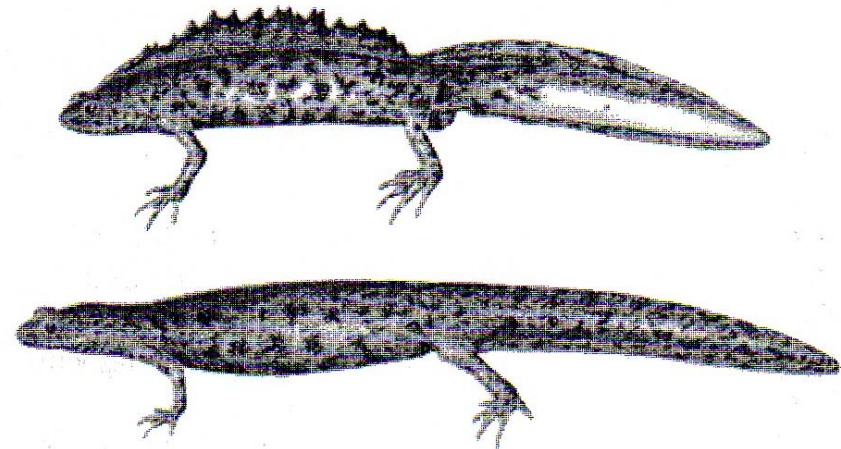


Figure 3-2. Sexual differences in fin structure and coloration in newts, *Triturus cristatus*; male above, female below.

Newts

Japanese Firebelly newts (*Cynops pyrrhogaster*).

Males often have longer toes. During the breeding season, the males often get gray or blue highlights on their skin, particularly on the tail.



Newts

Eastern, red-spotted, or broken-striped newts (*Notophthalmus viridescens*).

Outside of breeding season, eastern newts have no consistent difference in the size of the cloaca. The sexes are distinguished by the width of the back legs.

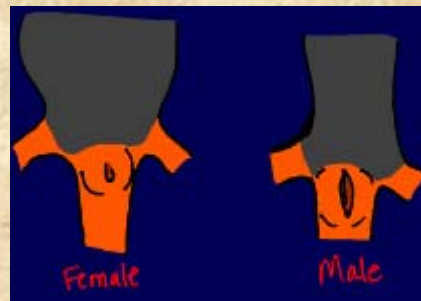
During the breeding season, the males may exhibit a larger cloaca and nuptial pads on the underside of the back legs and back toes.



Newts

Mandarin, crocodile, Kweichow newts (*Tylototriton spp.*).

The cloaca sizes are not very different, but the male's may be slightly larger in proportion to his body size. The male's cloaca will have a longer slit length. Males may also have slightly wider front legs. Females are often both longer and fatter.



Salamanders

Axolotls (*Ambystoma mexicanum*). The male has a larger cloaca.



Non-random sex ratio

Some factors may cause you to get a non-random sex ratio:

If you get only the largest or smallest of the juveniles, you may tend to get mostly one sex.

If you choose a group with one particular color pattern, or the most aquatic individuals, you may be predisposed to getting all one sex.

Some species have a phenomenon of sex reversal when the offspring are raised at unusually high or low temperatures. In these cases, the offspring may be predominantly of a single sex.

Random sex ratio

Since juveniles cannot be sexed, how many do you need to have in order to have a good chance of having at least one pair? If you take a truly random group of juveniles, your odds are as shown below.

There are some factors that may cause you to get non-random distribution of genders. These factors are listed below.

Number of juveniles	Chance of having at least one pair
1	0%
2	50%
3	75%
4	88%
5	94%
6	97%
8	99%

**Identifying individual
amphibians:
Natural & Artificial
Marks**

Why do we need to be able to identify individuals?

In-situ biology & conservation

- Demographic studies

- Behavioural studies

- Capture-recapture population monitoring

Ex-situ biology & conservation

- Managing collection / experimental animals

 - Genetics

 - Breeding

 - Medical treatment of particular individuals

 - Observing the behaviour of particular individuals

The ‘ideal’ marking technique

Non-invasive

Marks are quick and easy to apply / document

Once marked, animals do not need to be handled to determine mark status (i.e. marked / unmarked) and individual ID

Allows the identification of individual animals at all stages of development (e.g. egg through to adult)

Inexpensive

Important considerations

All techniques require some degree of handling → risk of transmitting diseases amongst individuals

Recognise risks and take measures to minimize them

Unique individual marks vs. batch marks

Batch marks can be used for capture-recapture Studies and to follow cohorts, but not individuals

Techniques for identifying post- metamorphic anurans

Non-Invasive methods

Pattern mapping/digital photos



Bufo baxteri Record

BUFO HEMIOPHRYS BAXTERI

WYOMING TOAD
SR 5
WYOMING TOAD

Sire SB 45 Dam SB 4

SMTWTFS SMTWTFS
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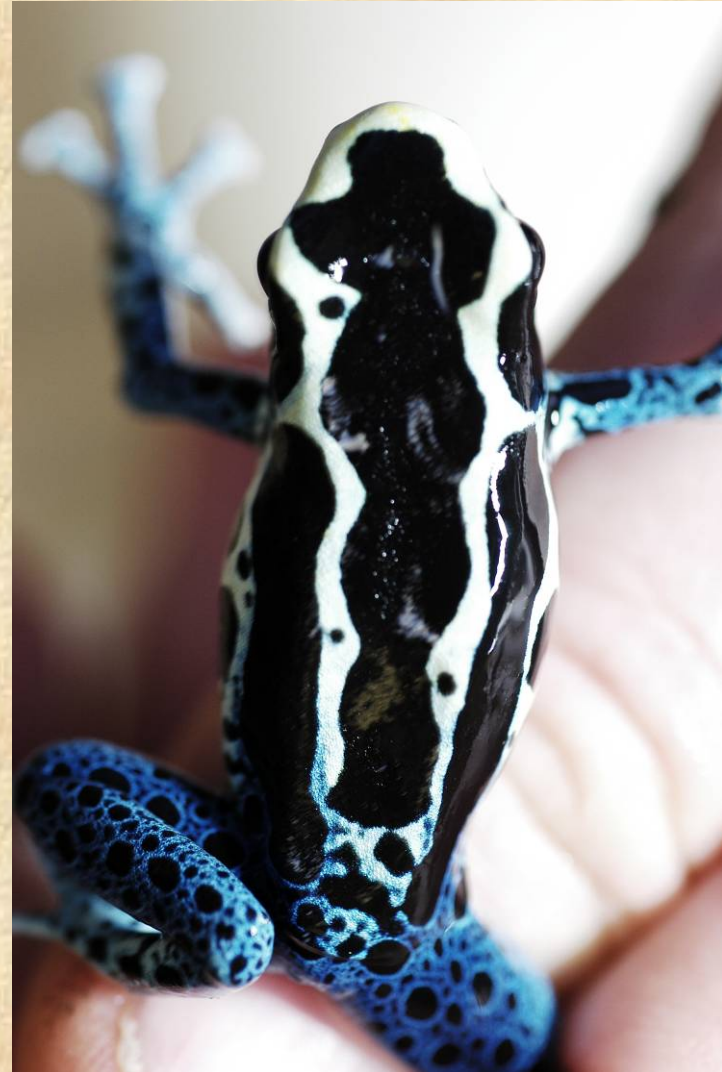
13 JAN: 25.6g, 23 JAN MOVED INTO HIBERNATION CAN.
24 JAN: MOVED INTO TOAD Q-PODGE (44°F) 19 JAN:
METRONIDAZOLE 100mg/kg
15 MAY: OUT OF HIBERNATION, INTO TUA-2.
WEIGHT: 18.9g
31 MAY: WT. 17.3g (SEEN SHEDDING)
5 JUNE: WT. 18.8g: MOVED TO TOAD-PODGE #3 FOR BREEDING

17 JUNE: VERIFIED CHRH @ 1mg/kg @ 0804h, @ 2059h
EGG LAYING IN PROGRESS
9 JUNE: 0800h: RETURNED TO TUA-2
19 JUNE: 19.5g; MOVED TO TOAD-PODGE #1 FOR
BREEDING w/ ♀ 953/85. Given 0.02g of
HRA @ 1mg/kg @ 17:32h
20 JUNE: REPEAT LHA @ 08:20 & 18:30h.
21 JUNE: 20.66g; RETURNED TO TUA-2.

Dendrobates sp.



Female



Male

Separation

- Individuals in enclosure
- Pairs in enclosure

Toe-clipping

Between one & eight toes are removed to create a unique code

Advantages:

- cheap
- quick
- easy
- provides material for skeletochronology, histology, DNA, etc.

Disadvantages:

- invasive
 - potential to affect survival rates & behaviour (which violates an assumption underlying most c-r methods) → conflicting evidence from studies on effects of toe-clipping
- some spp. regenerate toes → short-term mark only

Toe-clipping

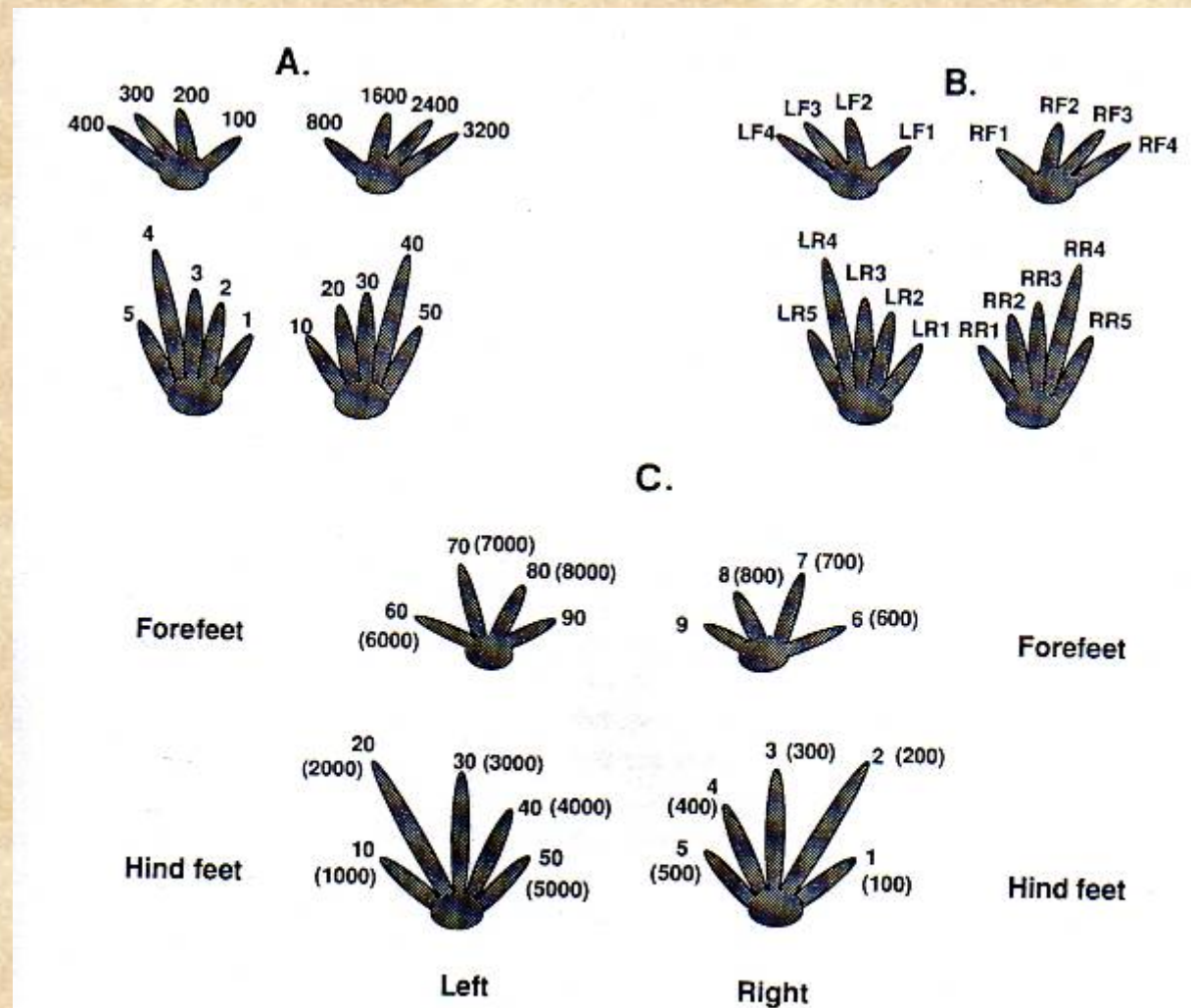


Figure 29. Clip-code schemes for marking frogs. A. Martof (1953) system. B. Donnelly (1989) system. C. Hero (1989) system. Using the Martof system, code 4967 would require clipping two toes on the right forefoot (3200 and 1600), one toe on the left forefoot (100), two toes on the right hind foot (40 and 20), and two toes on the left hind foot (2 and 5). See text for explanation of the Donnelly scheme. See text and Table 28 for explanation of the Hero scheme.

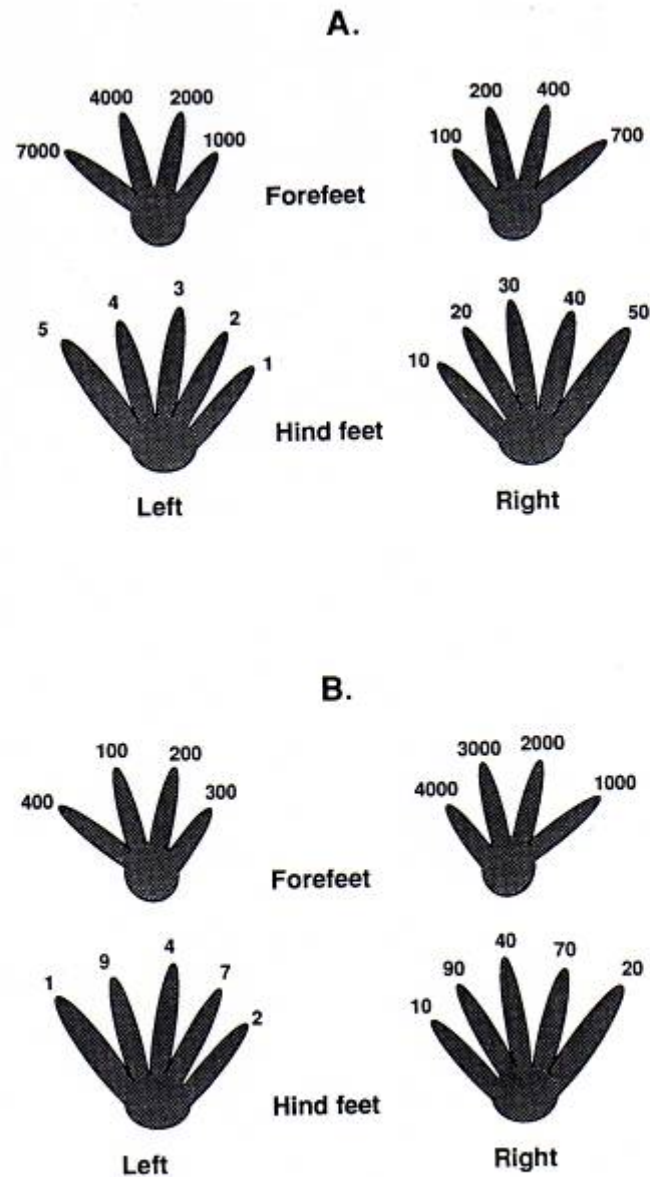


Figure 28. Clip-code schemes for marking salamanders. A. Twitty (1966) scheme. B. Scheme used by David B. Wake (pers. comm.). Using the Twitty scheme, code 4967 would require clipping one toe on the left forefoot (4000), two toes on the right forefoot (200 and 700), two toes on the right hind foot (20 and 40), and two toes on the left hind foot (5 and 2). Using the Wake scheme, code 4967 would require clipping one toe on the right forefoot (4000), three toes on the left forefoot (400, 200, and 300), two toes on the right hind foot (40 and 20), and one digit on the left hind foot (7).

PIT (Passive Integrated Transponder) Tags

Radio frequency ID uses a signal transmitted between an electronic device (*e.g.* a tag, transponder or microchip) and a reading device (*e.g.* a scanner, reader or transceiver)

Passive integrated transponders have no battery – a scanner is used to read the unique code in each one

Usually injected subcutaneously using a 12-gauge hypodermic needle and syringe; can also be externally attached with adhesive

Designed to last the life of the animal



PIT Tags (cont'd)

Advantages

reliable, long term identification method

rapid, accurate ID

Disadvantages

unsuitable for small species / individuals

expensive (~ \$3 / tag)

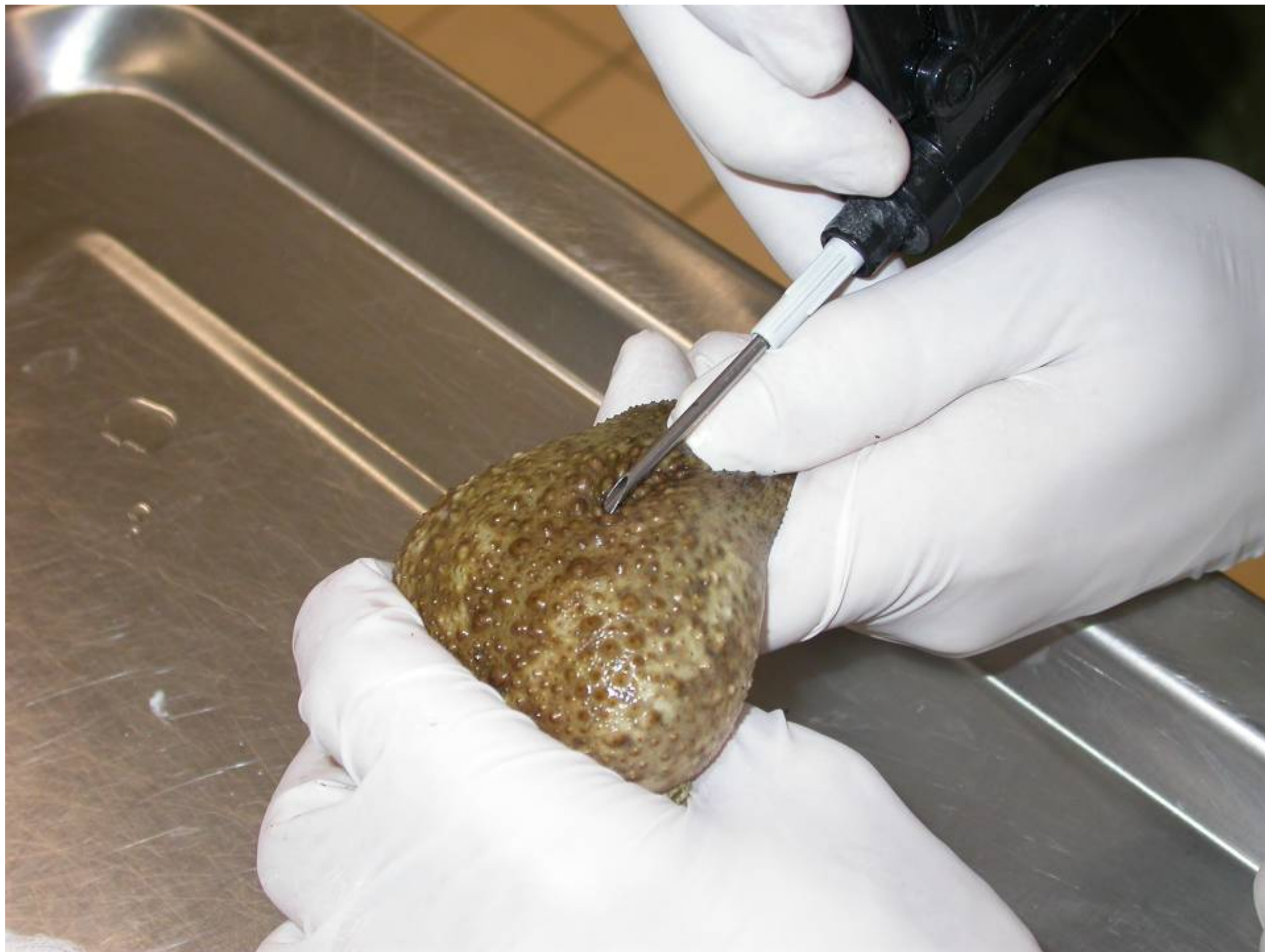
























VIE (Visible Implant Elastomer)

A medical grade, two-part silicone-based material that is mixed immediately before use

Tags are injected as a liquid that soon cures into a pliable solid

Tags are implanted beneath transparent or translucent tissue, so are externally visible

VIE is available in six fluorescent (red, pink, orange, yellow, green, blue) and four non-fluorescent colors (white, black, brown, purple) – detection of fluorescent tags is greatly enhanced when the VI Light is used

VIE (cont'd)

Ideal for batch marking, but can be used to ID individuals by combining different colors, multiple tags per animal, and multiple tag injection sites

Advantages

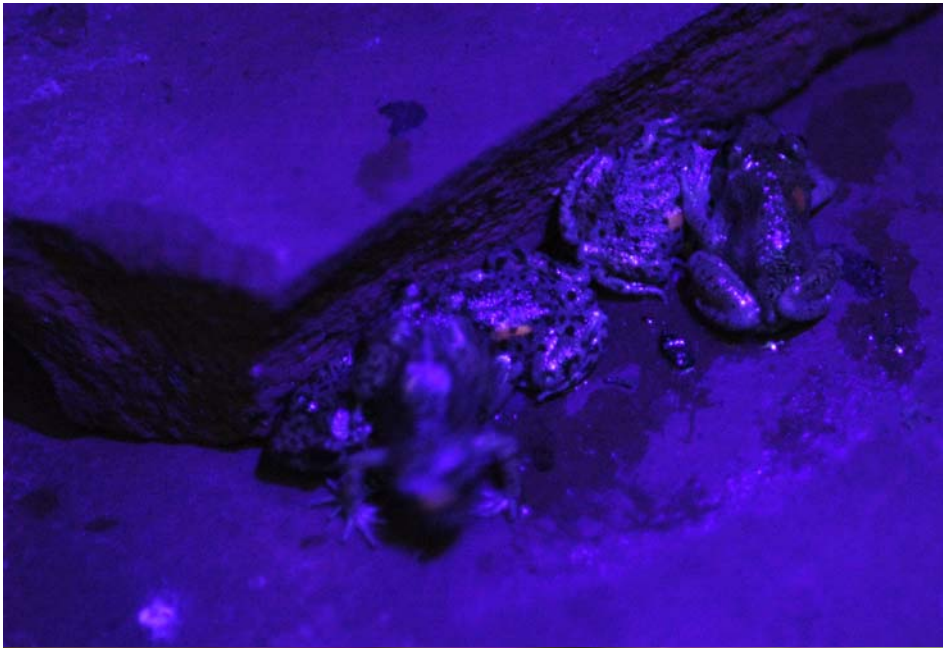
- only a small volume of material is necessary for a visible tag
- can be used in smaller animals than many other marking techniques

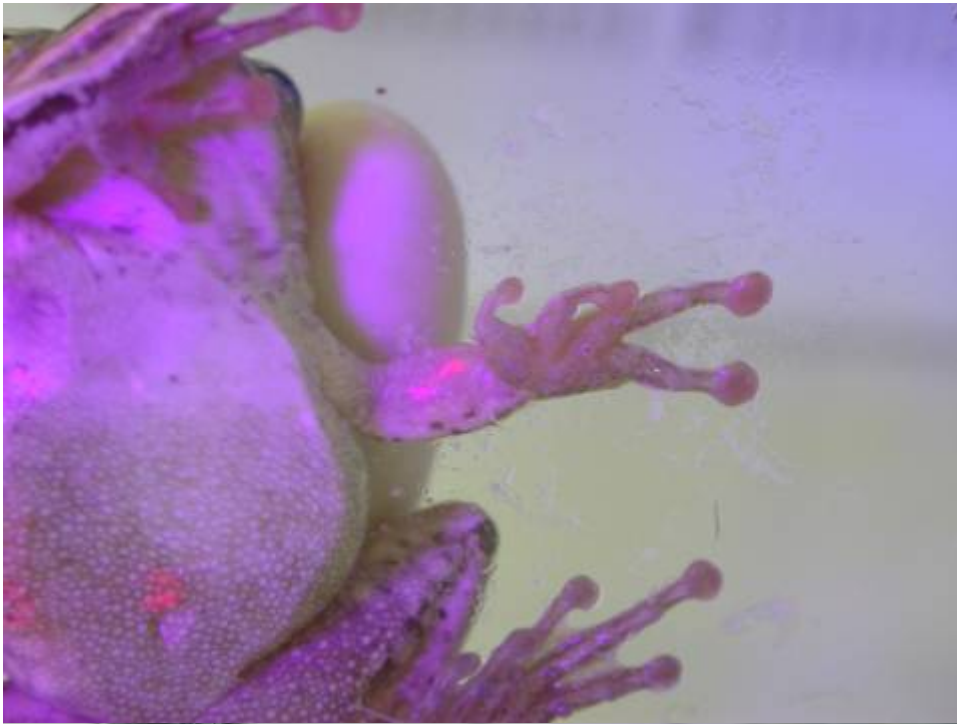
Disadvantages

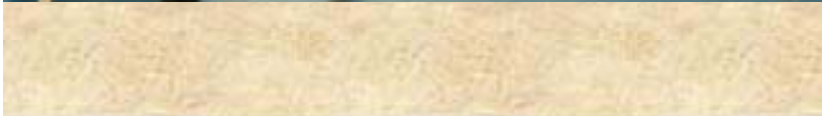
- marks migrate and can be lost
- low visibility of marks due to skin pigmentation in some spp.
- VIE needs to be kept cold until immediately prior to injection
- relatively expensive (\$490 US for a 4-colour kit - marks up to 5000 individuals, but elastomer needs to be used within 1 year)

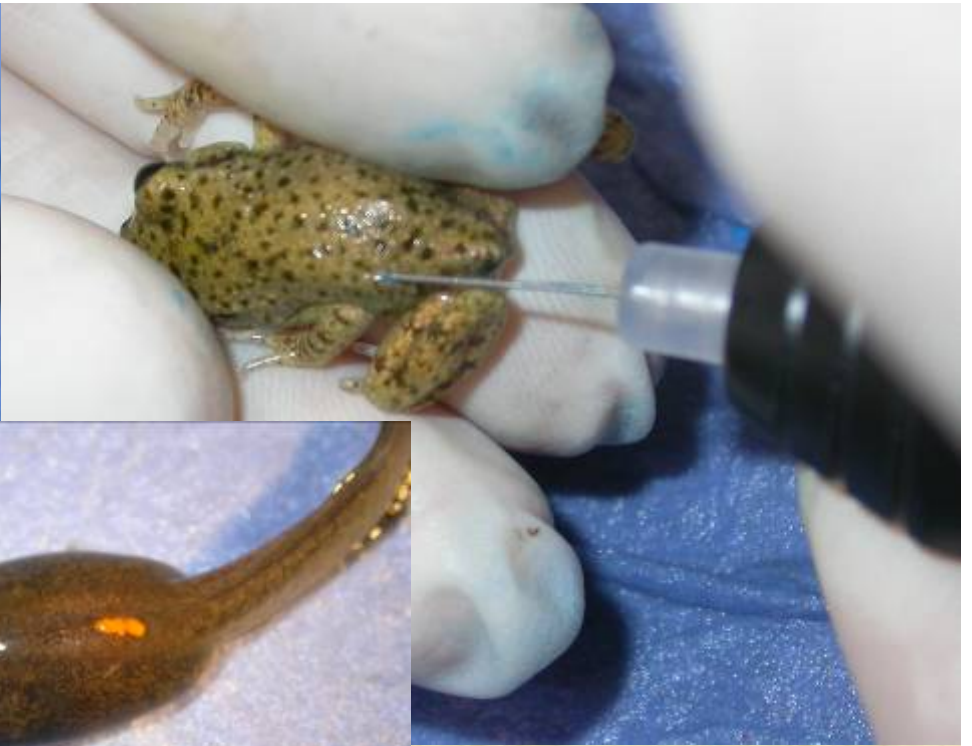


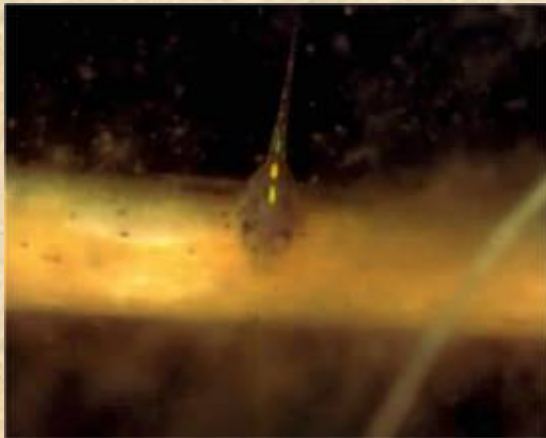












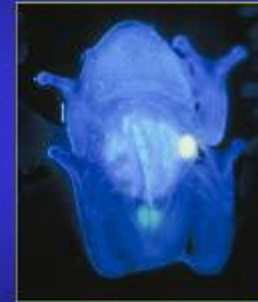
Marking method

Marked juvenile

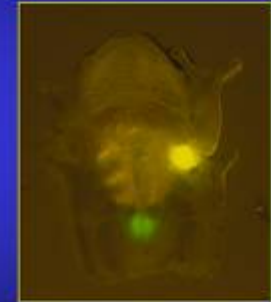
looked at



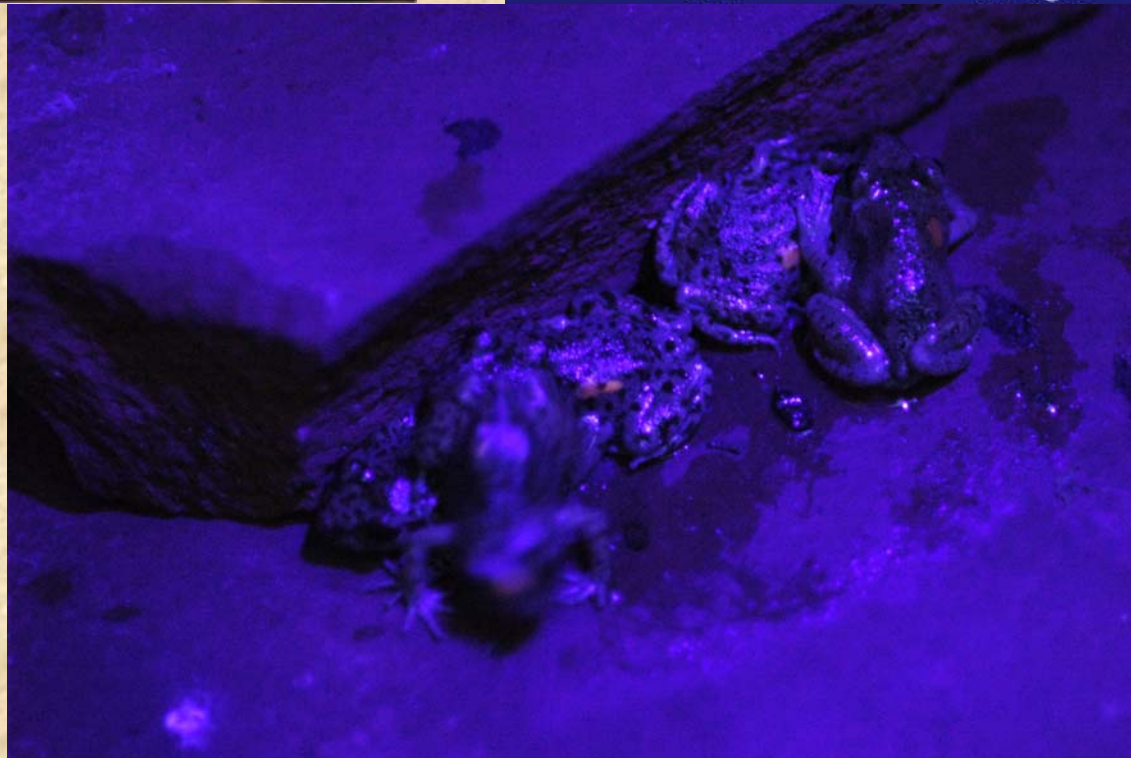
Plain



UV light



UV light and special filter



Other marking techniques

Radio-transmitters

also provide detailed information on individual movements
implantation vs. attachment with a 'waistband'
expensive

VI (Visible Implant) Alpha Tags

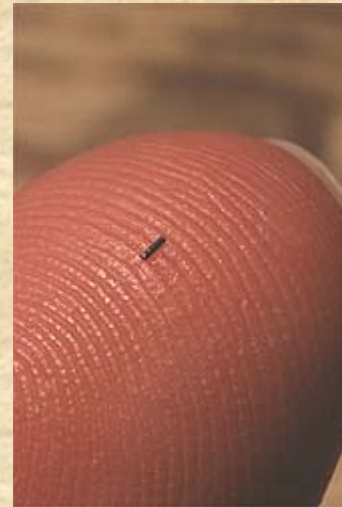
made of the same material as VIE tags, but pre-cured with individual alphanumeric codes on one side
Injected under the skin (in areas of little / no pigmentation)

DCWT (Decimal Coded Wire Tags)

magnetised stainless steel wire marked with rows of numbers that need to be read under magnification
tags are cut from the roll and injected hypodermically
batch or individual codes
4 sizes: 1.1 mm long x 0.25 mm diameter (standard), half standard, 1.5 x standard, 2 x standard



VI Alpha Tags



Decimal Coded Wire Tags™ (CWT)

Other Marking Techniques

Injecting powdered dye with a Panjet Inoculator / tattooing

Freeze or Chemical Branding

Knee Tags

plastic, numerically-coded fingerling tags are tied to the knee

Radio-active Tags

e.g. Cobalt-60

batch marking only

Techniques for identifying larval stages

Invasive Techniques

V.I.E

Injecting / staining with dye (*e.g.* Neutral Red dye)

Clipping notches out of tail fins

Tail tags

Radio-active tags

MARKING CAUDATES

- **natural markings** (via photographs or drawings)
- **toe-clipping** (disadvantages → regenerative abilities mean it is a short-term mark only; invasive, with the potential for sublethal / lethal effects (and increased mortality as a result of marking violates one of the key assumptions of mark-recapture); negative public image)
- **VIE** (problems / considerations associated w/ this technique include mark migration & / or loss, low visibility of marks due to dark skin pigmentation, and the need to keep the elastomer cold until immediately prior to injection)
- **PIT tags**
- **dye** (visibility?)
- **stable isotopes** (*e.g.* ^{15}N , ^{13}C)

MARKING CAECILIANS

- **Panjet tattoos**
- **soft visible implant alphanumeric tags**
- **VIE**
- **freeze-branding**

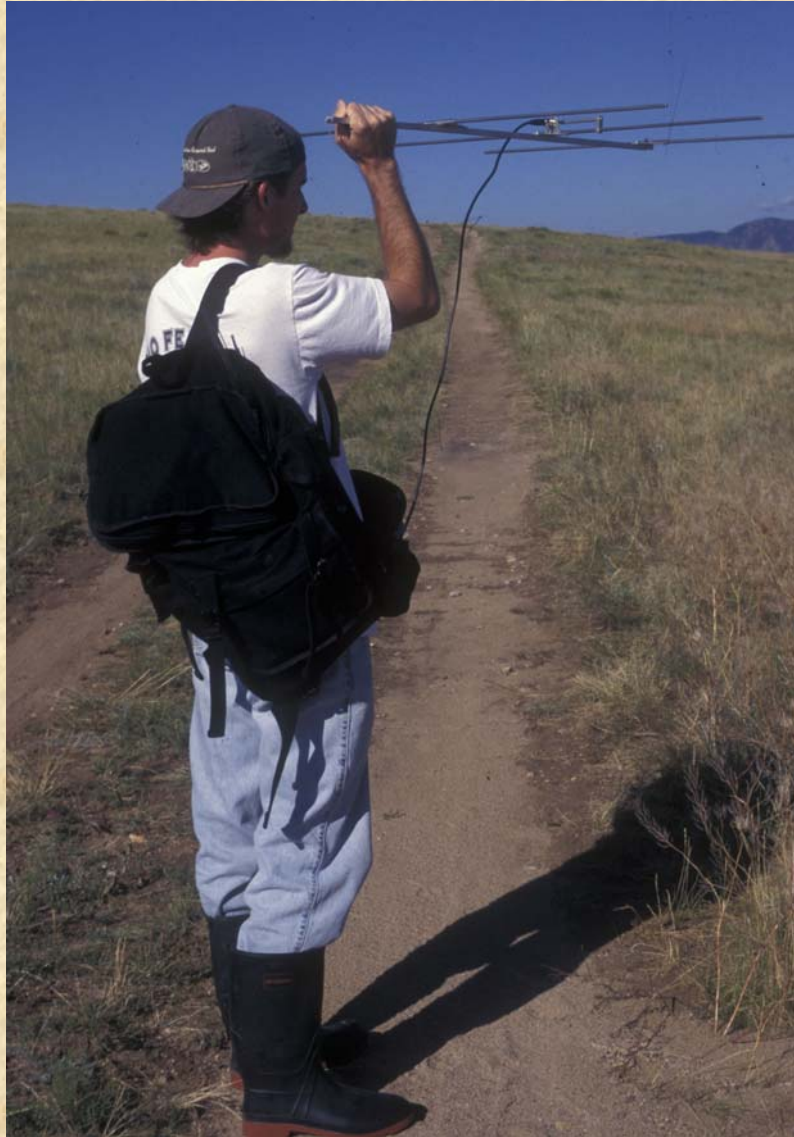
Radiotelemetry







Radio tracking, Wyoming



Bufo baxteri