

Marking techniques: what options are there?

Gerardo Garcia, PhD

Herpetology Department,
Durrell Wildlife Conservation Trust

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Ethics and amphibians

Robert M. May

A statistical study shows convincingly that a technique for marking frogs in ecological field experiments compromises the results. Present practices need a rethink — and not only for practical reasons.

It was 25 years ago, at an ecology seminar at Princeton University, that I first learned of the standard method for ‘marking’ individual newts or other amphibians (Fig. 1) by clipping their toes. In this way, each individual can be identified by the unique combination of digits removed. I remember being impressed by the elegance of the experiments concerned—but even more impressed by the casual barbarity of the toe clipping.

Seeking to avoid a larger ethical minefield, I asked whether such removal of digits would affect survival, particularly in more heavily clipped individuals, thus compromising the conclusions. My question was swept aside as silly (the sort of thing you might expect a theoretician to ask). But it now appears to have been answered. Writing in the *Journal of Applied Ecology*, McCarthy and Parris¹ find that “toe clipping reduces the return rate [recapture of marked individuals] by 4–11%



Figure 1 Toe show. Frogs are often the subject of studies involving toe clipping.

seems to me that all these conclusions apply to all amphibians.

More generally, I see McCarthy and Parris’s paper as a notable addition to a growing literature that raises both practical and larger ethical questions about time-honoured procedures in some ecological field studies. There are obvious parallels with the recent study of long-term effects of flipper tags on penguins, by Gauthier-Clerc *et al.*⁸. This work attracted considerable media attention with its finding, after five years’ work on king penguins implanted with electronic tags (some also with flipper bands and others not), that “banding results in later arrival at the colony for courtship in some years, lower breeding probability and lower



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

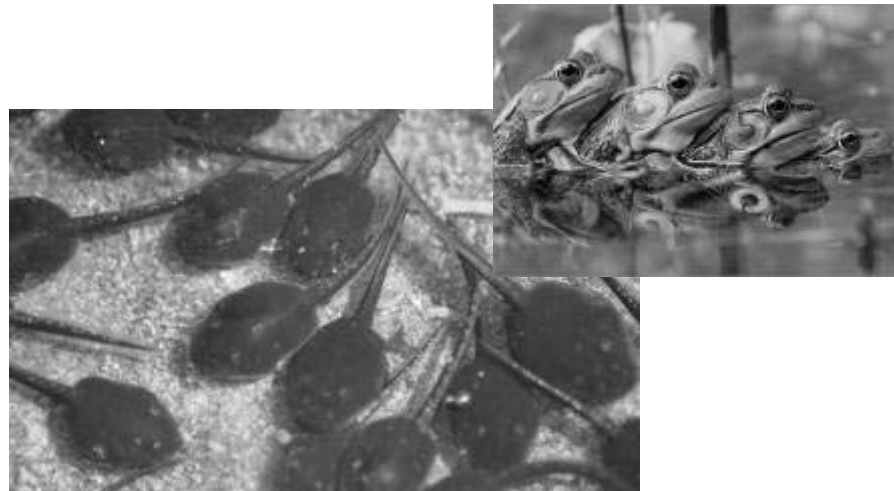
- Non-invasive
- Marks are quick and easy to apply / document
- Once marked, animals do not need to be handled to determine mark status (i.e. marked / unmarked) and individual ID
- Allows the identification of individual animals at all stages of development (e.g. egg through to adult)
- Inexpensive





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



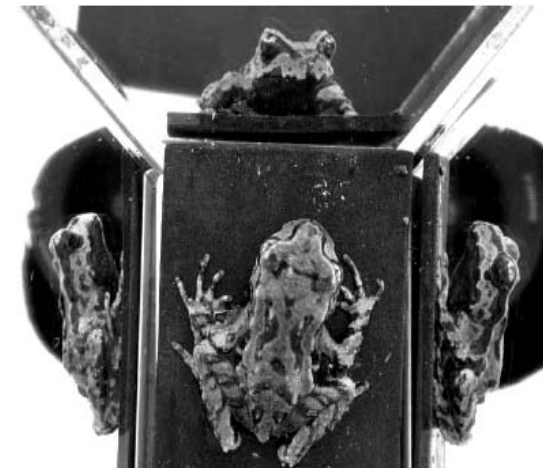


Non invasive techniques



Pattern mapping/digital photos

Using natural markings





A496 Male; Pit Tag: 958000000703842



PARKEN ZOO



A559 Male; Pit Tag: 956000000270067



PARKEN ZOO

NORDENS ARK

A491 Female; Pit Tag: 968000000280287

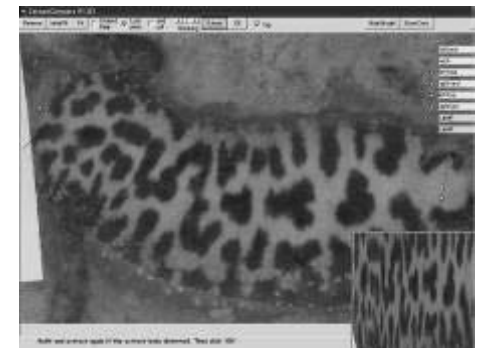
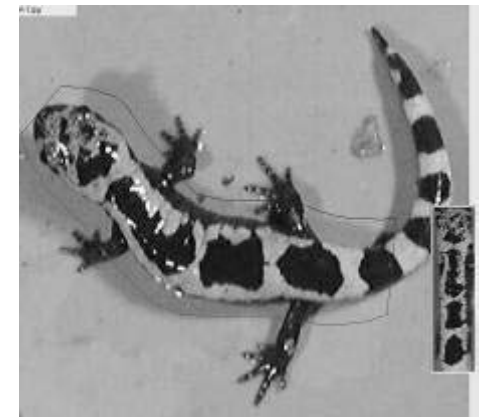
NORDENS ARK

A490 Female; Pit Tag: 968000000709717



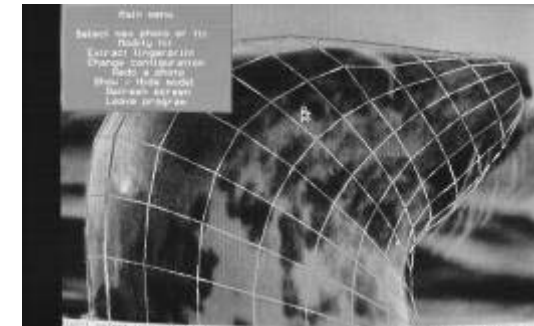
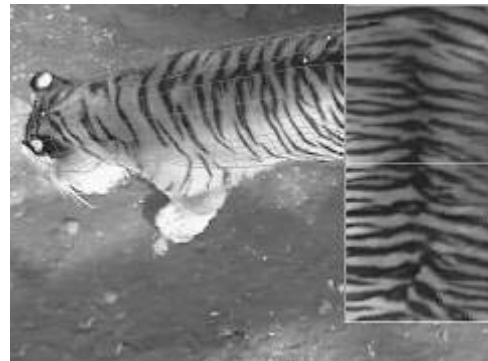
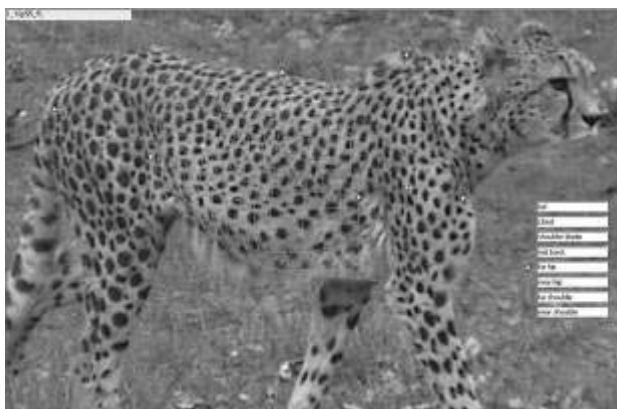
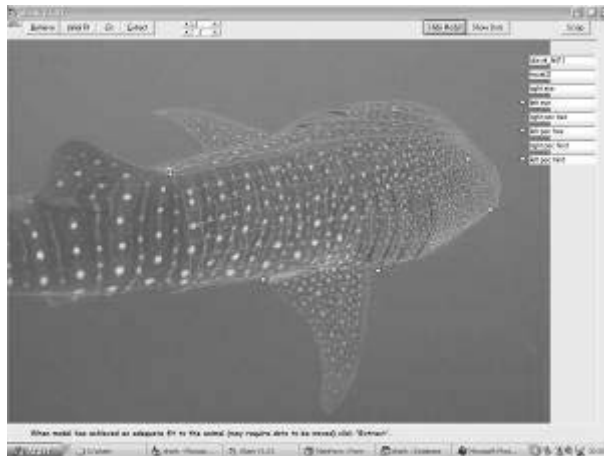
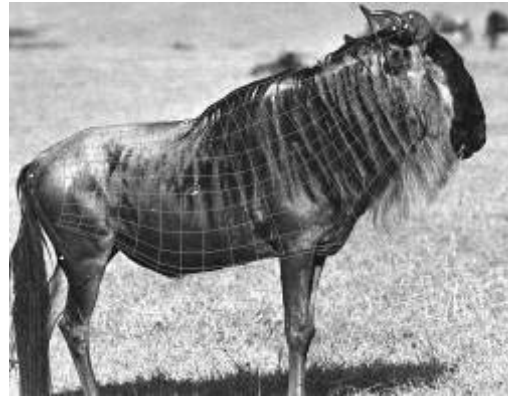
Automated photo-id catalogue

- Scanning patterns from dorsal surface photos of salamanders, newts, frogs or toads
- Fitting a 3D surface model to the individual
- Programs capture a pattern that is unaffected by the camera angle or the animal's posture
- Program then compares the new pattern with previous patterns stored in a library and displays the most likely matches
- The final match decision is left to the user
- Mark/recapture studies such as the monitoring of population size and other parameters





Post-metamorphic anurans: Invasive Techniques

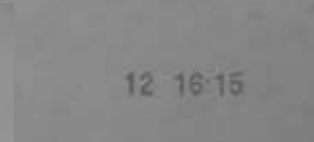
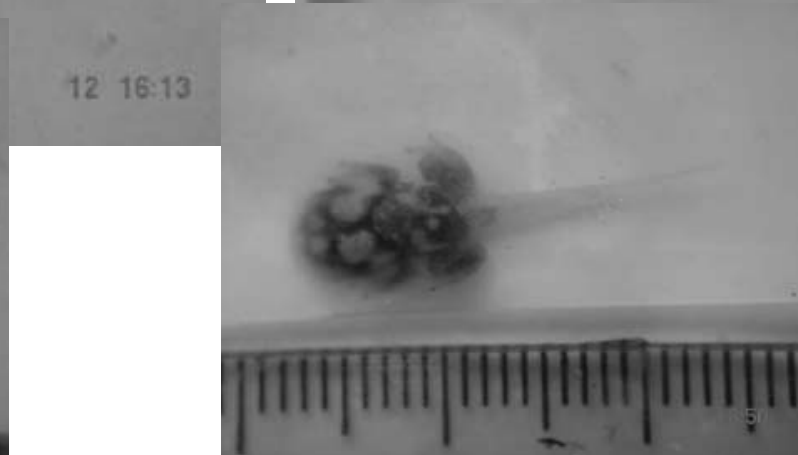
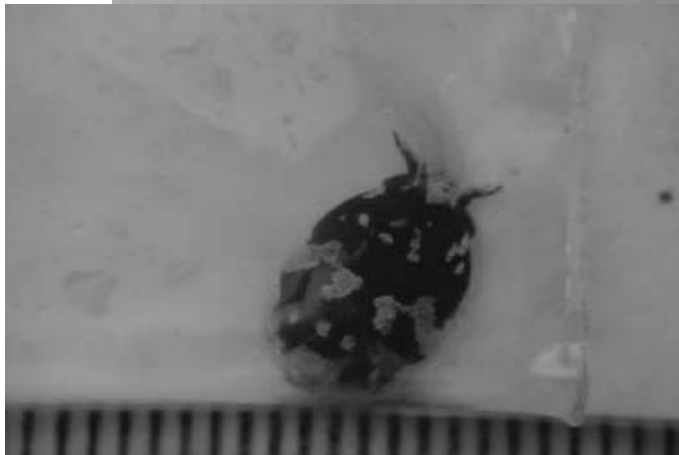
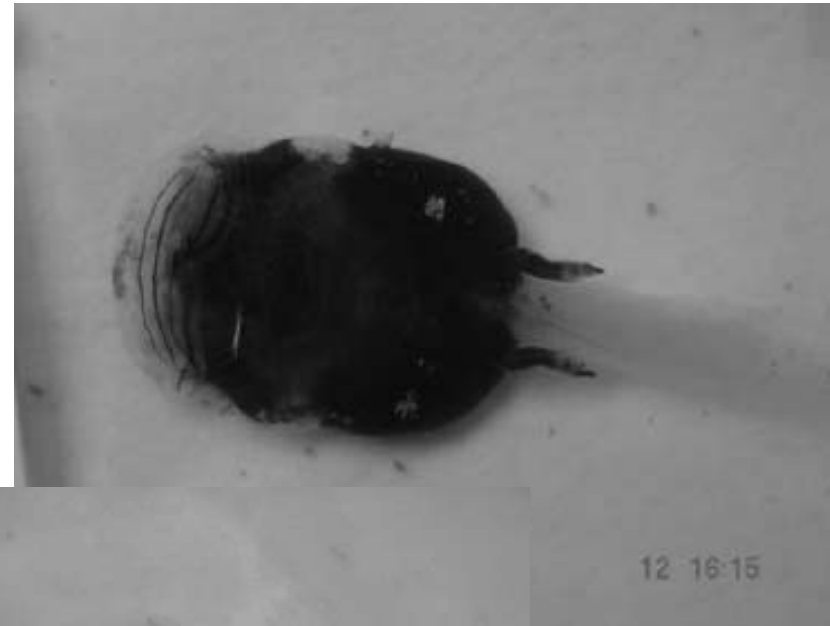




Larval stages: Non-intrusive technique

Non invasive technique

Using natural markings





Invasive techniques



Invasive Techniques I

- Pressurised fluorescent colorant powder hind legs (e.g. *Eleutherodactylus*); Negative: difficulty, harmful, expensive compared with toe clipping
- Jaw tagging. Negative: high loss, considerable irritation. Not longer used
- Aluminium toe bands (butt-end bird band, # 1242, size 2). Not restriction circulation but pierced the webbing of the foot



Invasive Techniques II

- Glass bead tags (e.g. *Xenopus*) left fore limb, lateral to the humerus or (hind limb medial to the femur) pierced 21 gauge hypodermic needle
- Sequence of up to 4 coloured glass beads. 9999 combinations; leg retention up to 3 years in the lab
- Not recommended for field (snagging on substrates)



Toe-clipping (I)

- Between one and eight toes are removed to create a unique code
- Advantages:
 - cheap
 - quick
 - easy
 - provides material for chytrid, skeletochronology, histology, DNA, etc
- Disadvantages:
 - Invasive: Potential to affect survival rates & behaviour (which violates an assumption underlying most m-rc methods) → conflicting evidence from studies on effects of toe-clipping
 - some spp. regenerate toes → short-term mark only
 - Negative impact in amplexus

Toe-clipping (II)

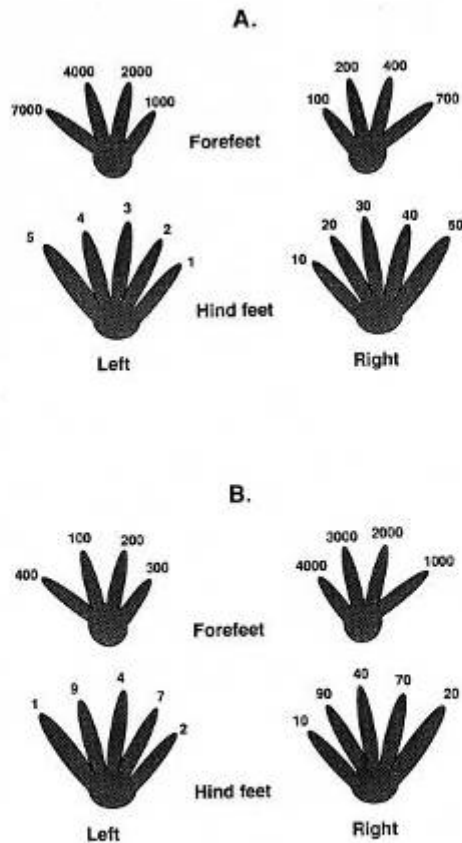


Figure 28. Clip-code schemes for marking salamanders. A. Twitty (1966) scheme. B. Scheme used by David B. Wake (pers. comm.). Using the Twitty scheme, code 4967 would require clipping one toe on the left forefoot (4000), two toes on the right forefoot (200 and 100), two toes on the right hind foot (20 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).

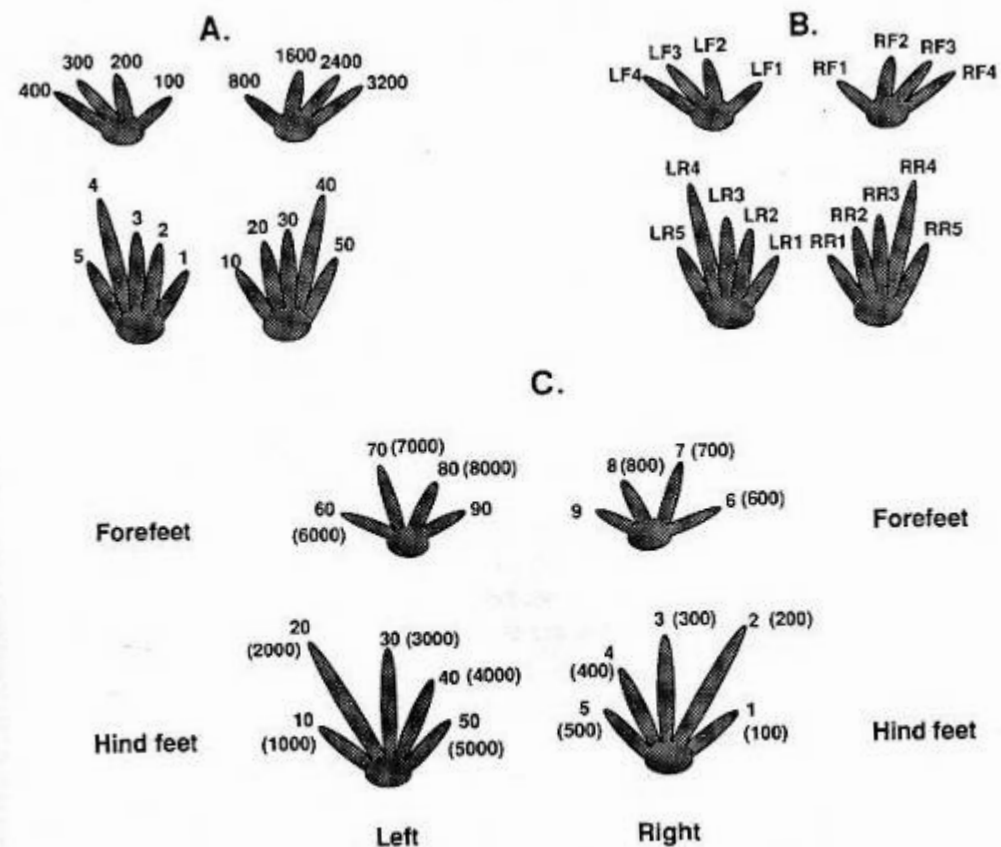


Figure 29. Clip-code schemes for marking frogs. A. Martof (1953) system. B. Donnelly (1989) system. C. Hero (1989) system. Using the Martof system, code 4967 would require clipping two toes on the right forefoot (3200 and 1600), one toe on the left forefoot (100), two toes on the right hind foot (40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



Toe-clipping (III)

Hygienic, sterile techniques to minimise the risk of infection/mortality

Still the most common marking technique for anurans

Ethical issues



Clarifying the effect of toe clipping on frogs with Bayesian statistics. **MICHAEL A. MCCARTHY** and **KIRSTEN M. PARRIS**. *Journal of Applied Ecology* (2004) **41**, 780–786

Alternative views of amphibian toe-clipping; W. Chris Funk, Maureen A. Donnelly, Karen R. Lips. *NATURE* | VOL 433 | 20 JANUARY 2005



Invasive Techniques I

- Injecting / staining with dye (e.g. Neutral Red dye)
Shallow pans 0.05% solution for 30' (Herreid and Kinney 1966)
Rana sylvatica and *R. calamitans*: 1/25-50,000 parts of pond water: high mortality, 8.7% and retain 10 days.
Hyla grayiosa: slow growth

Staining methods: time limited

- Oxytetracycline on metamorphosed (e.g. *Bufo boreas*)
Recapture to detect any microscopic fluorescence from tetracycline (toe clip)



Invasive Techniques II

- Fluorescent pigment (e.g. *Rana calamitans*)
Using compressor air spray gun; minimal mortality (3%) over a month after marking)
- 24-sodium (*Ambystoma* larvae): short term retention
- Acrylic polymers
Ventral or dorsal tail fins (e.g. *Rana catesbiana*) tadpoles. Retained 5-6 months/2 years. Reabsorbed at metamorphosis without known impact
- Clipping notches out of tail fins (Turner 1960): High mortality
- Tail tags
- Radio-active tags



VIE (Visible Implant Elastomer)



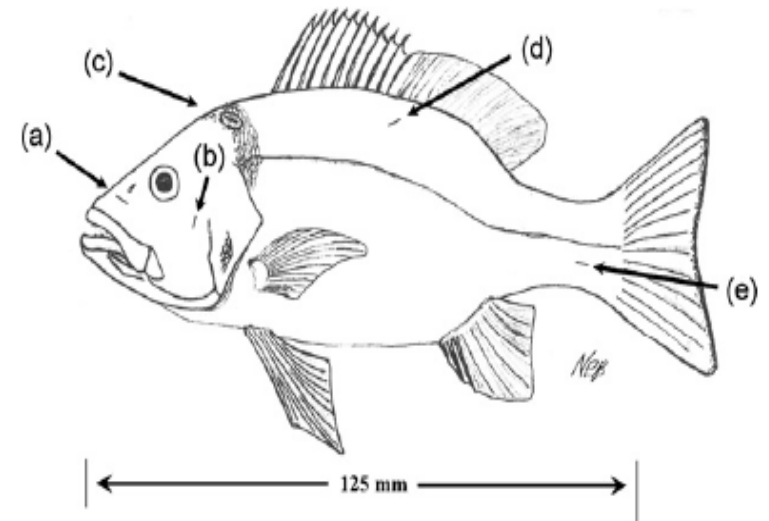


Fig. 1. Schematic of coded-wire tag implant locations and injection angles (indicated by arrows): (a) nose cartilage (b) left cheek, (c) nape, (d) dorsal muscle, and (e) caudal peduncle. Coded-wire tags (dashes) not to scale.



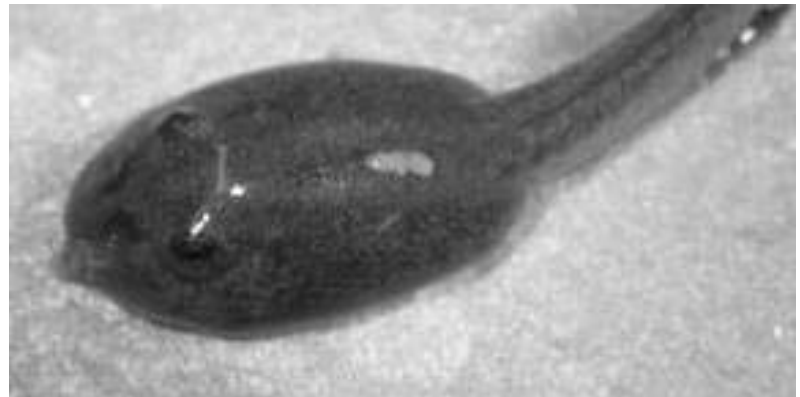
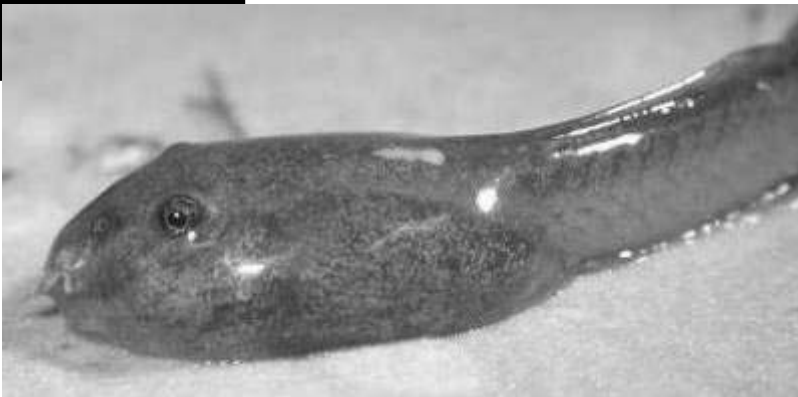
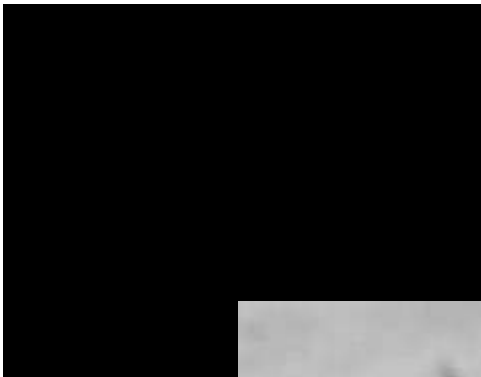
VIE (Visible Implant Elastomer)

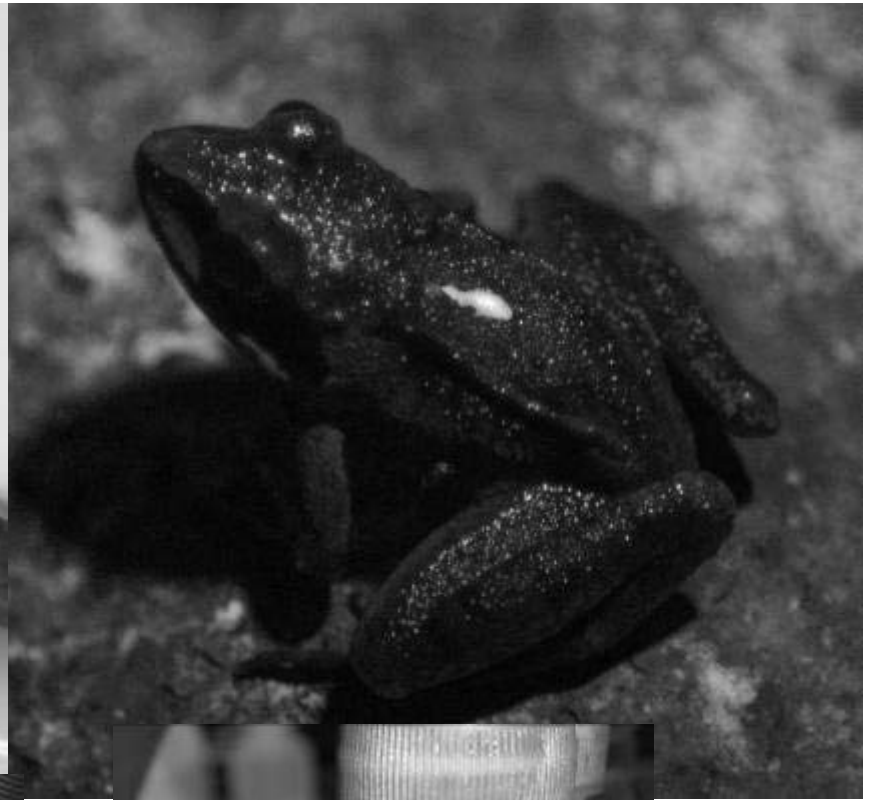
- A medical grade, two-part silicone-based material that is mixed immediately before use
- Tags are injected as a liquid that soon cures into a pliable solid
- Tags are implanted beneath transparent or translucent tissue, so are externally visible
- VIE is available in six fluorescent (red, pink, orange, 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



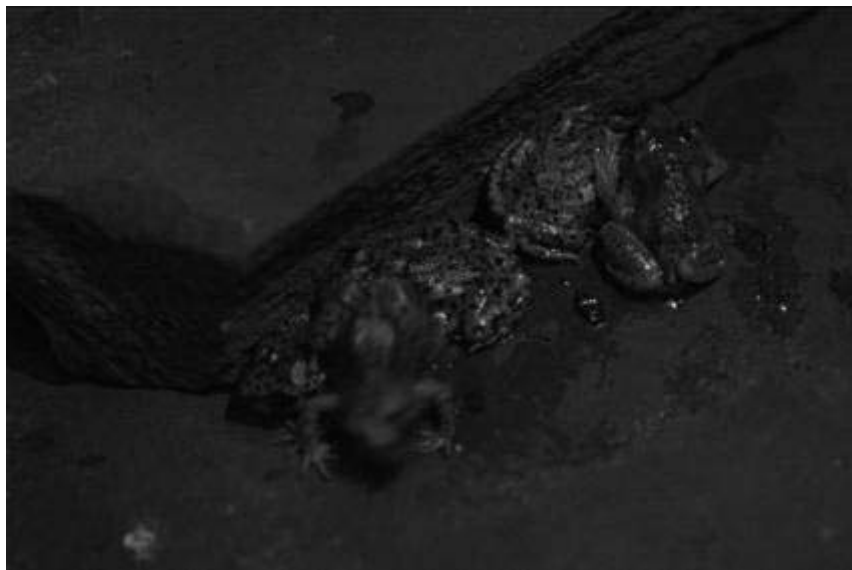
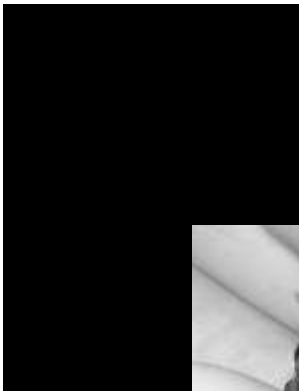
VIE (cont'd)

- 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
 - Initially relative expensive (\$490 US for a 4-colour kit - marks up to 5000 individuals, but elastomer needs to be used within 1 year)
 - Cross contamination?

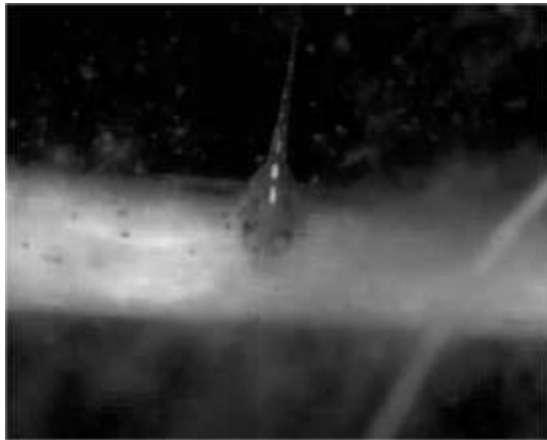
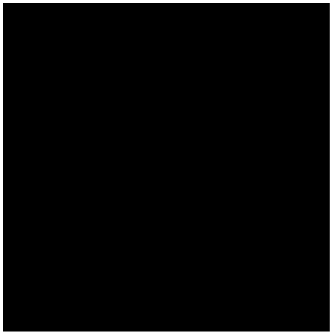








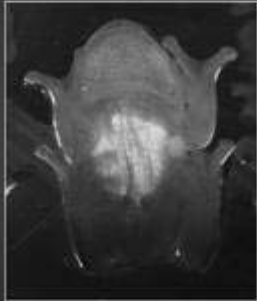





Marking method

Marked juvenile

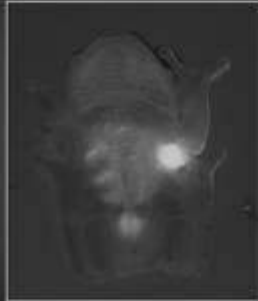
looked at



Plain



UV light



UV light and special filter

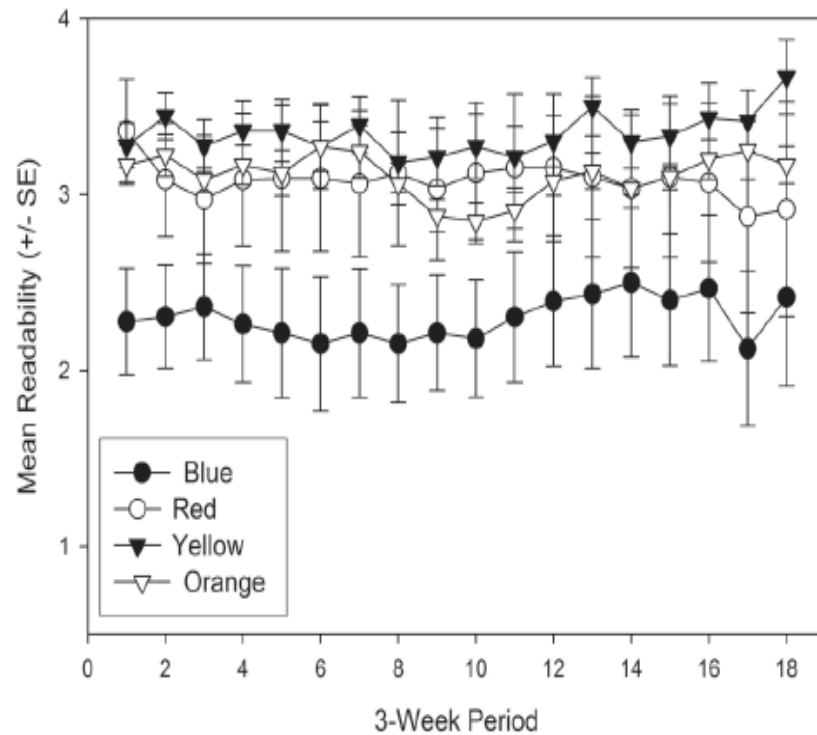
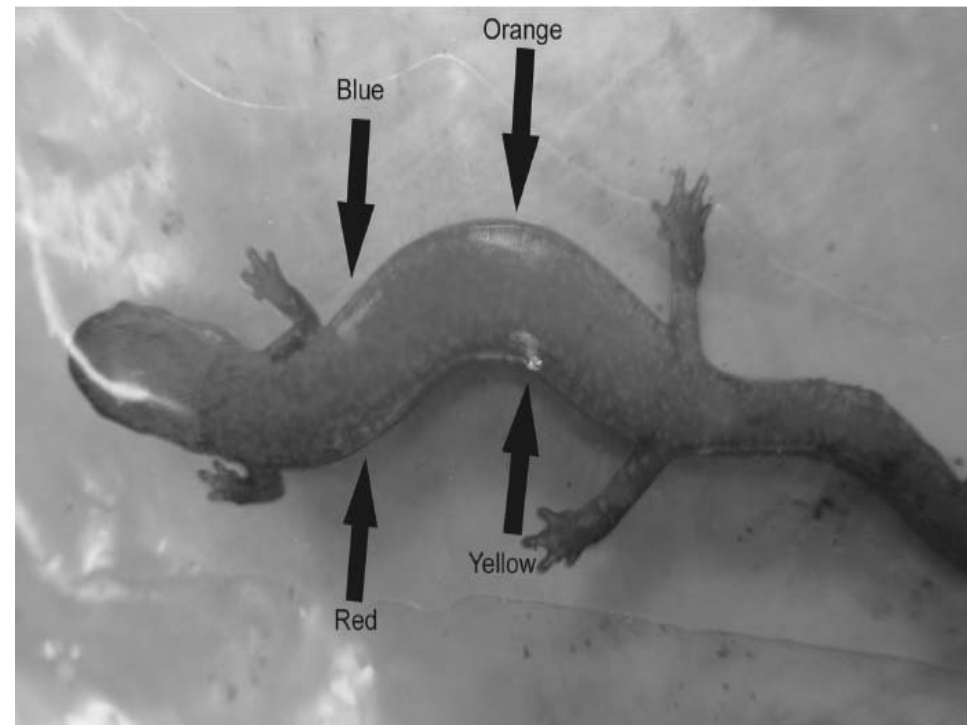


FIG. 3. Readability of Visible Implant Elastomer marks in *P. cinereus* that were ventrally marked and observed to 53 weeks post-marking was consistently less for the color blue than for red, orange, and yellow. Readability ranged from 1 (mark absent) to 4 (mark easily visible under fluorescent light).





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 II

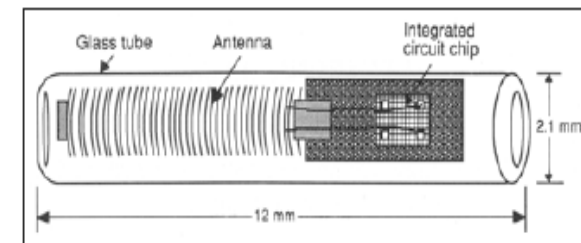
•Advantages

- reliable, long term identification method
- rapid, accurate ID
- Diameter of 2 mm and length of 12 mm
- They do not require a continuous power source (e.g. battery); when the tag is held in an electromagnetic field the microchip transmits its own unique identification code to an electronic reader

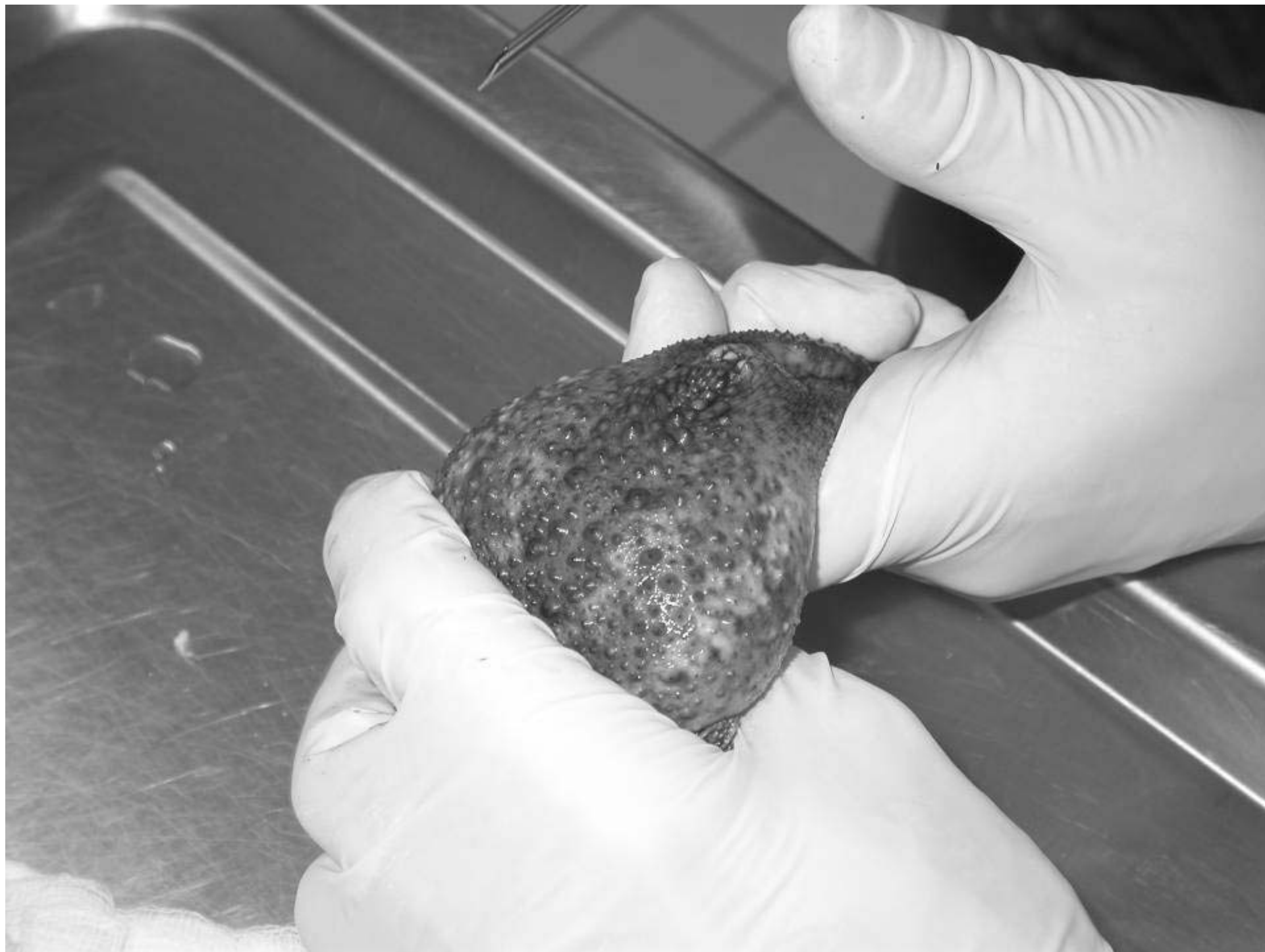


•Disadvantages

- unsuitable for small species / individuals
- expensive (~ \$3 / tag)









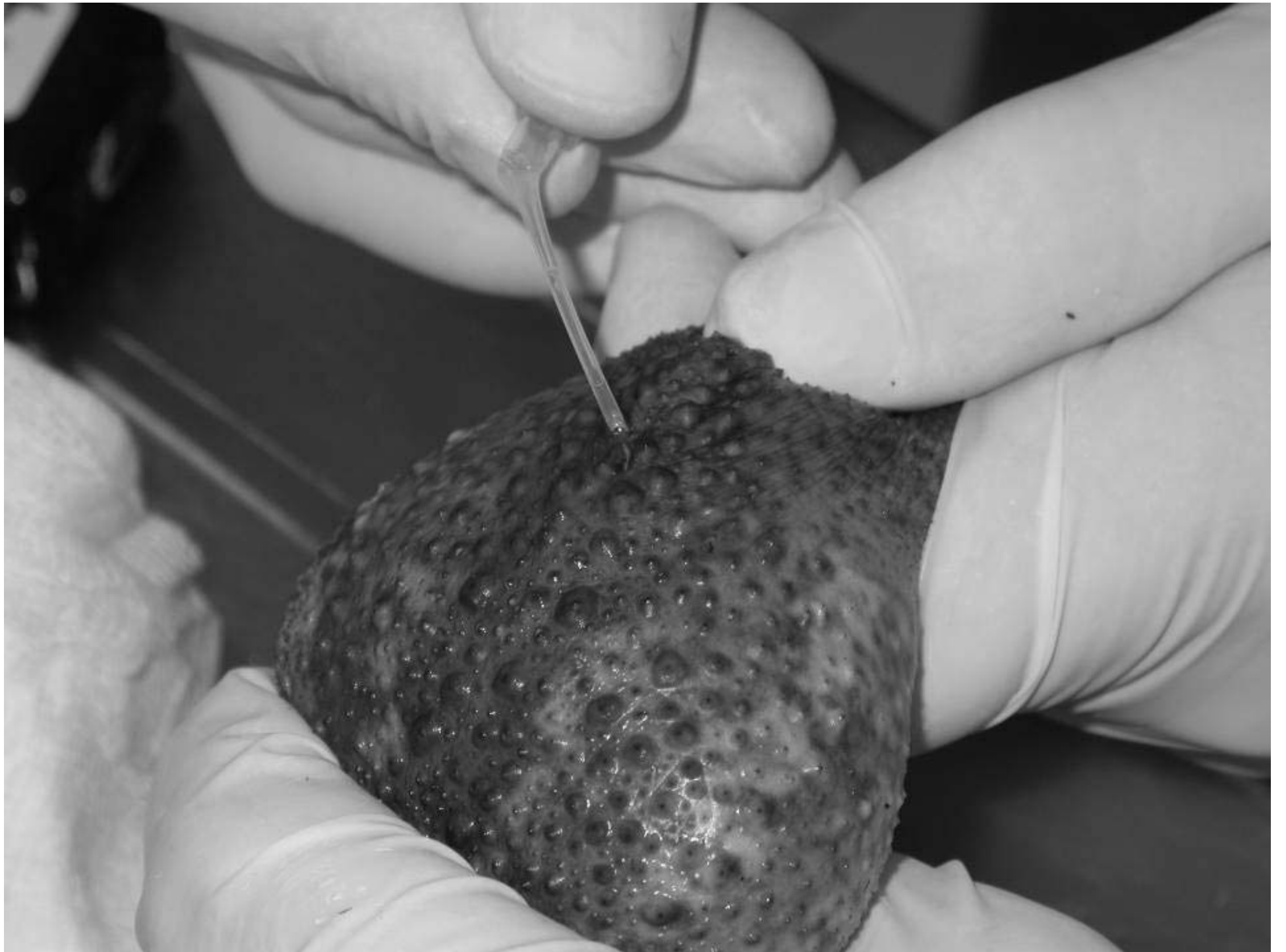




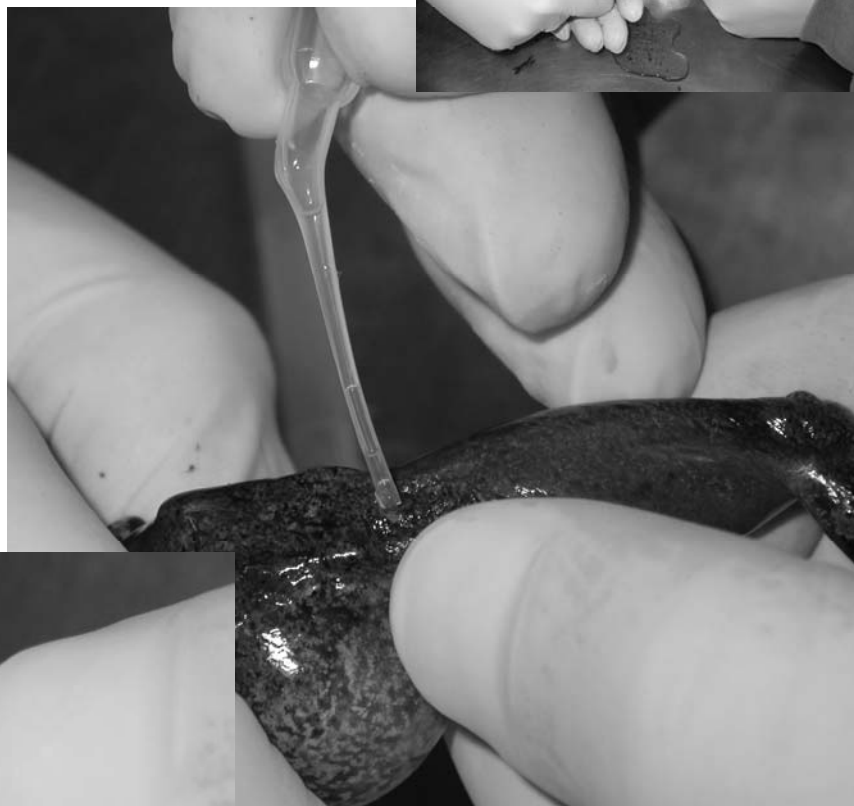




























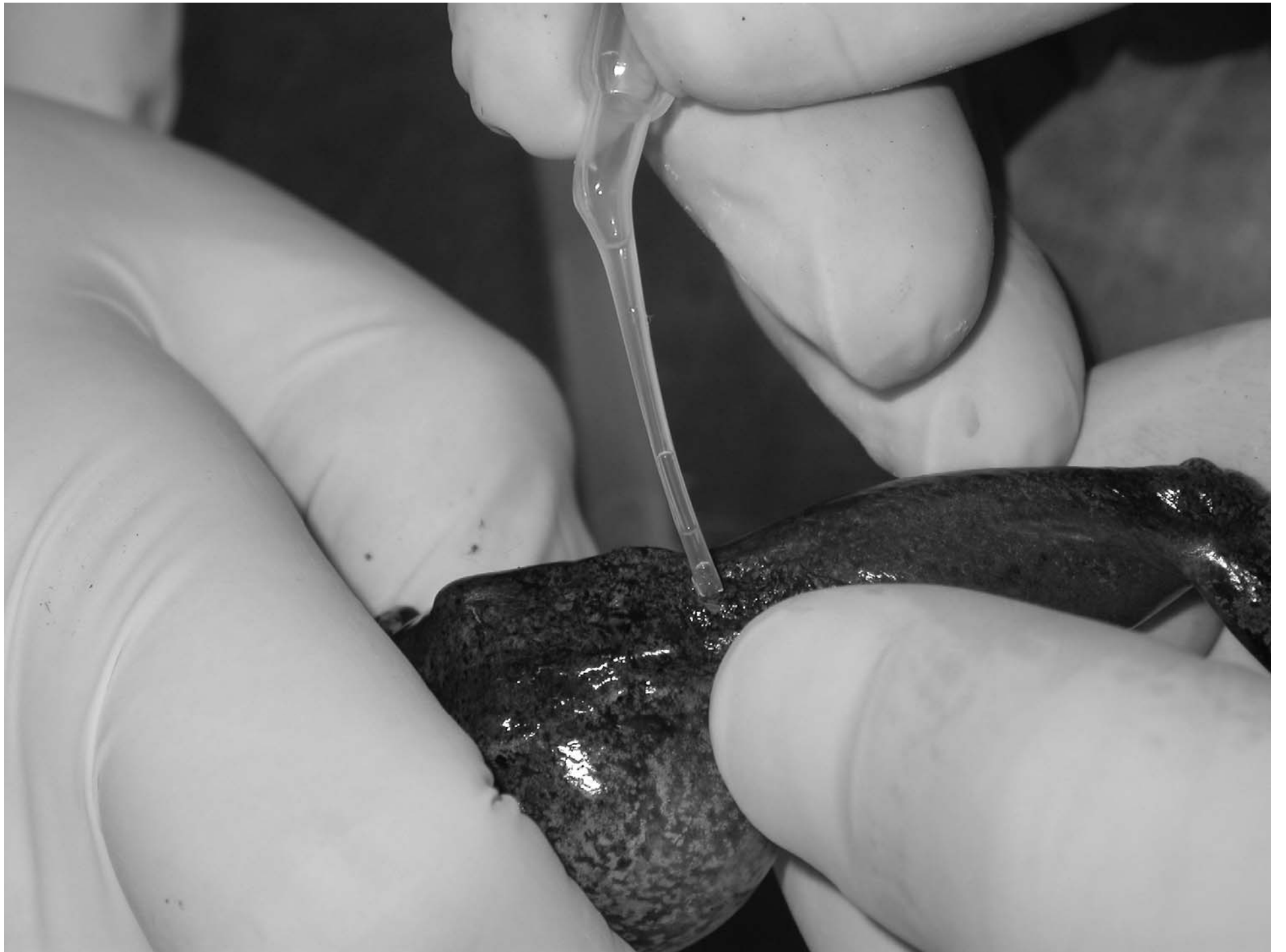














The new generation of “microchips”

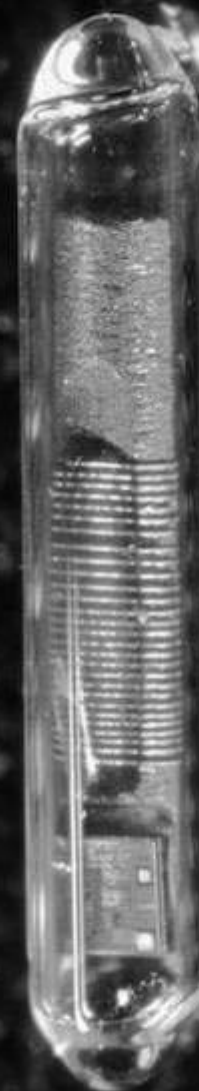
NONATEC TRANSPONDER

www.nonatec.net

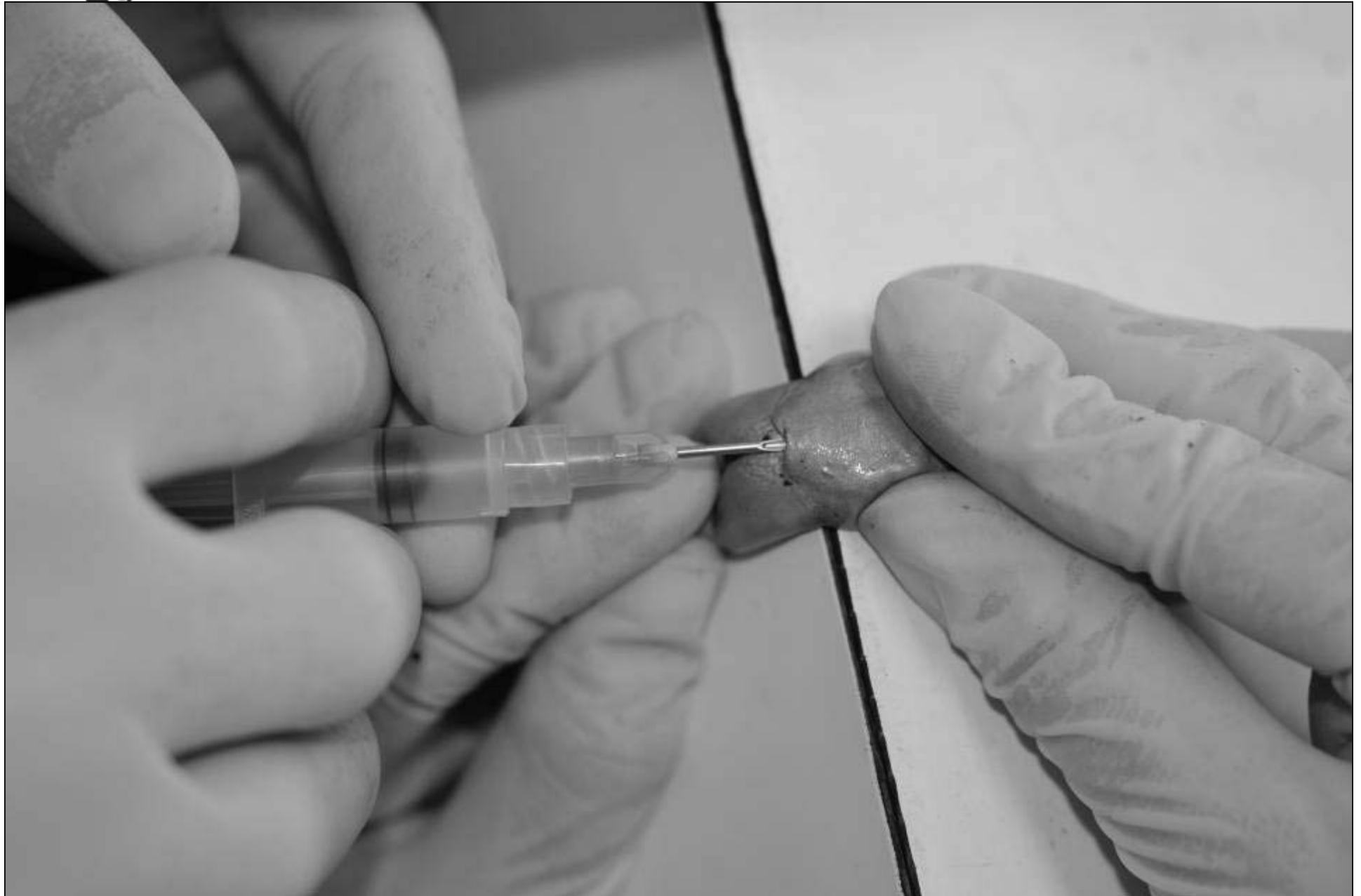
- Size: 1mm dia * 6 mm long
- Weight: 7,15 mg
- Operating frequency: 13,56 MHz
- Needle diameter: 18 G (1,2 mm)

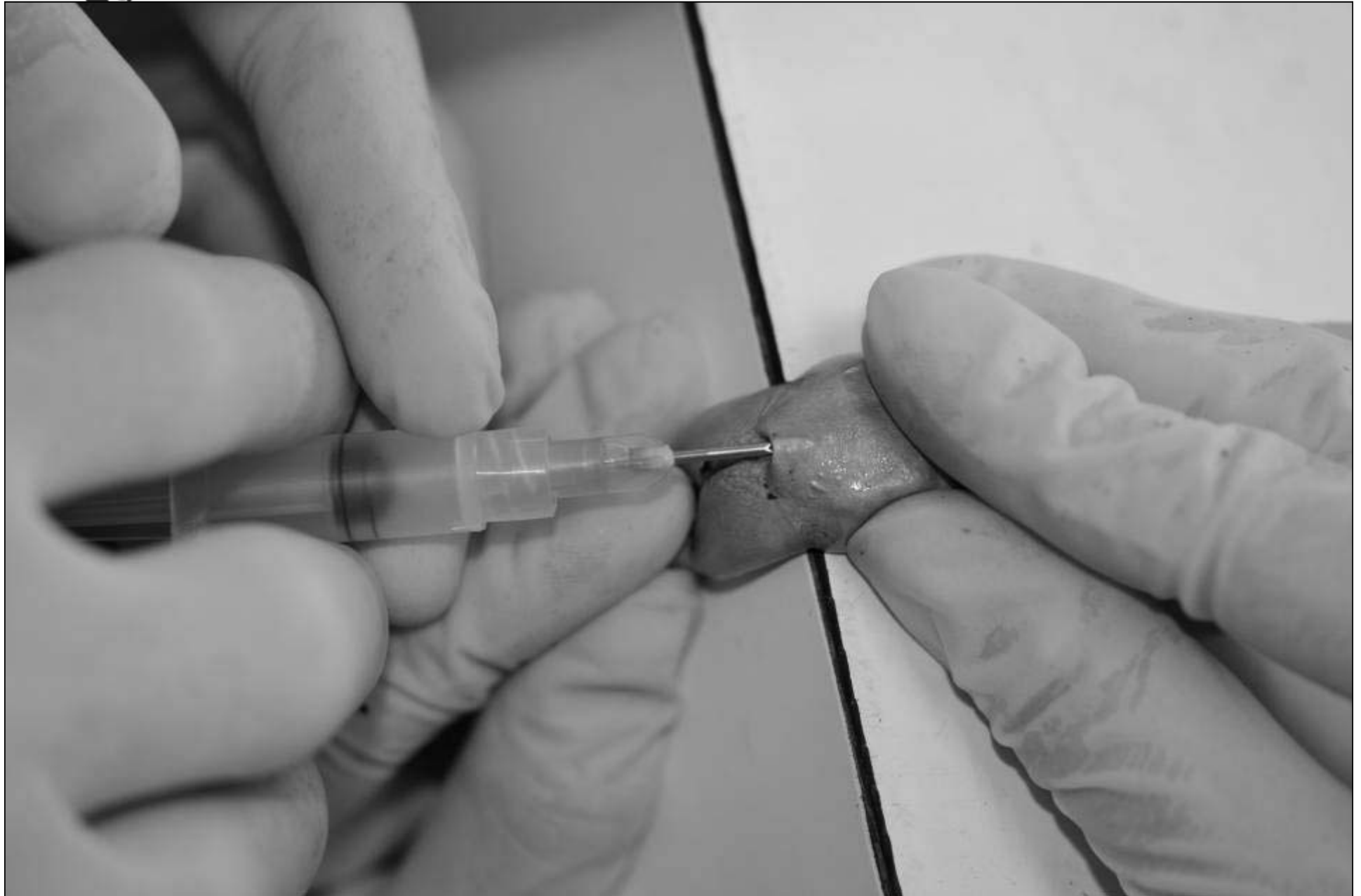


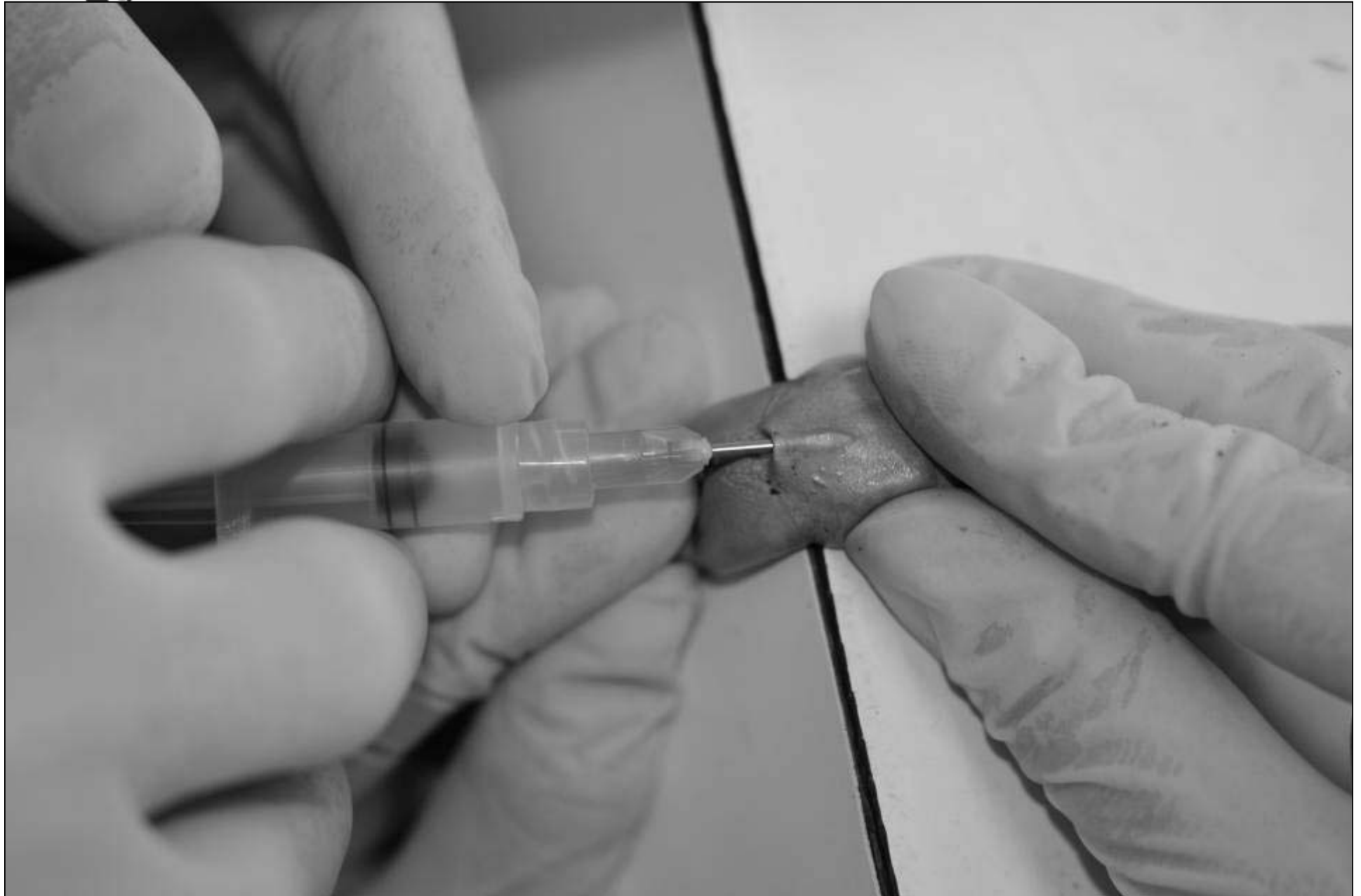
























































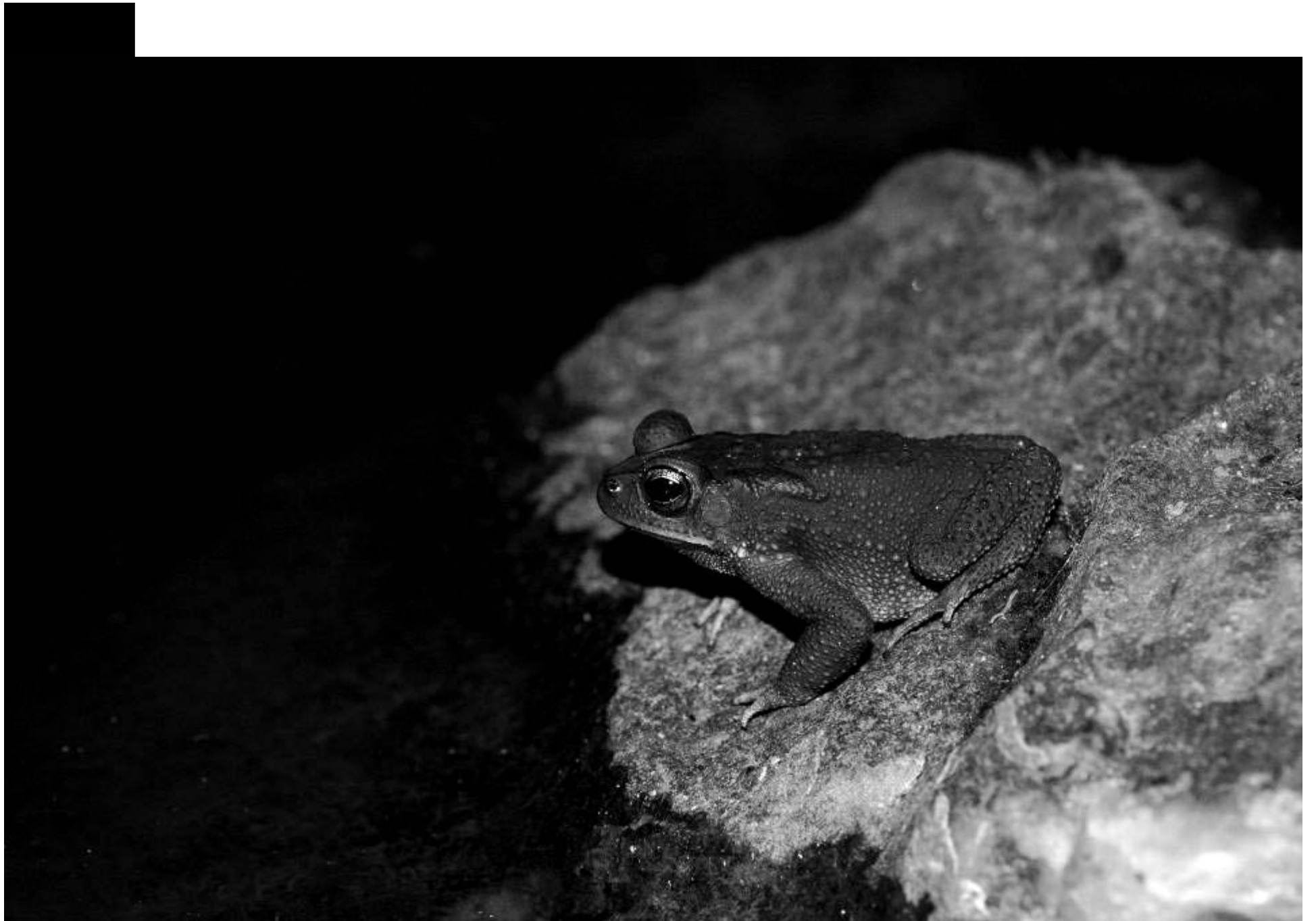
NONATEC™

Power up

Bluetooth®

ID: READ OK
1394613490745984





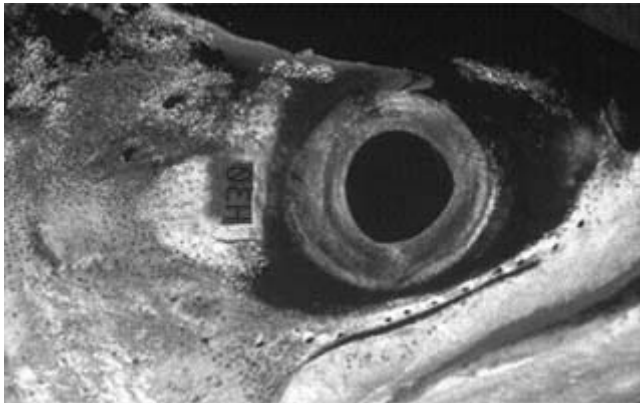


Other marking techniques

- 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



Post-metamorphic anurans: Invasive Techniques



VI Alpha Tags



Decimal Coded Wire Tags™ (CWT)



Panjet Innoculator I





Panjet Innoculator II

- Glass reservoir for a dye solution of Alician-Blue (Wisniewski 1980, British Journal of Herpetology 6: 71-74)
- 5 mm or less from the under surface at 45 ° angle for marking
 - Positive: Few injuries; mark easy to see; retention over 2 years (Jersey > 2 years)
 - Negative: Marks are small; low individual combination



Marking Caecilians

- Panjet tattoos
- Soft visible implant alphanumeric tags
- VIE
- Freeze-branding



Other Marking Techniques

- Injecting powdered dye with a Painjet Inoculators / Tattooing:
Alicia-Blue (microscopy stain)
Few injuries, mark easy to see, retention over 2 years, marks are small but low individual combination.
- Freeze or Chemical Branding
- Knee Tags
 - plastic, numerically-coded fingerling tags are tied to the knee
- Radio-active Tags
 - marking only



Radio tracking



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



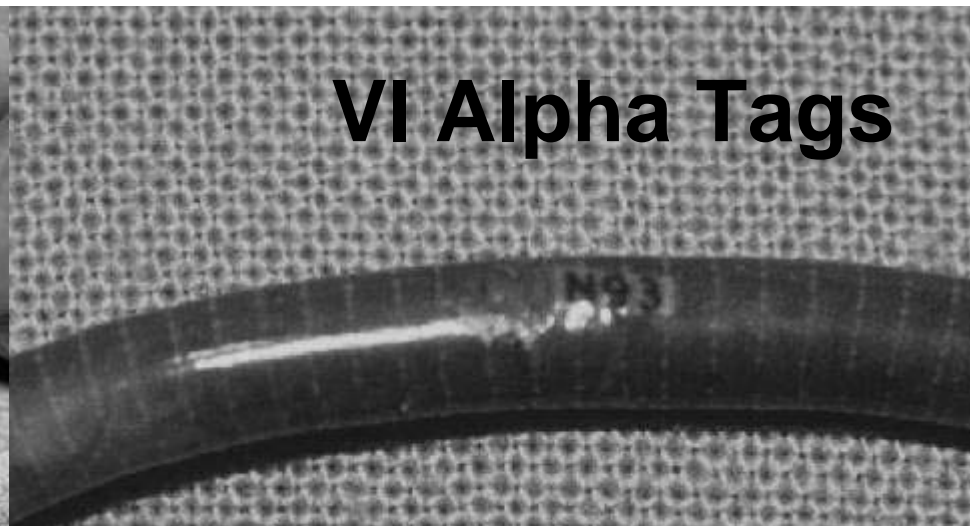


Marking techniques

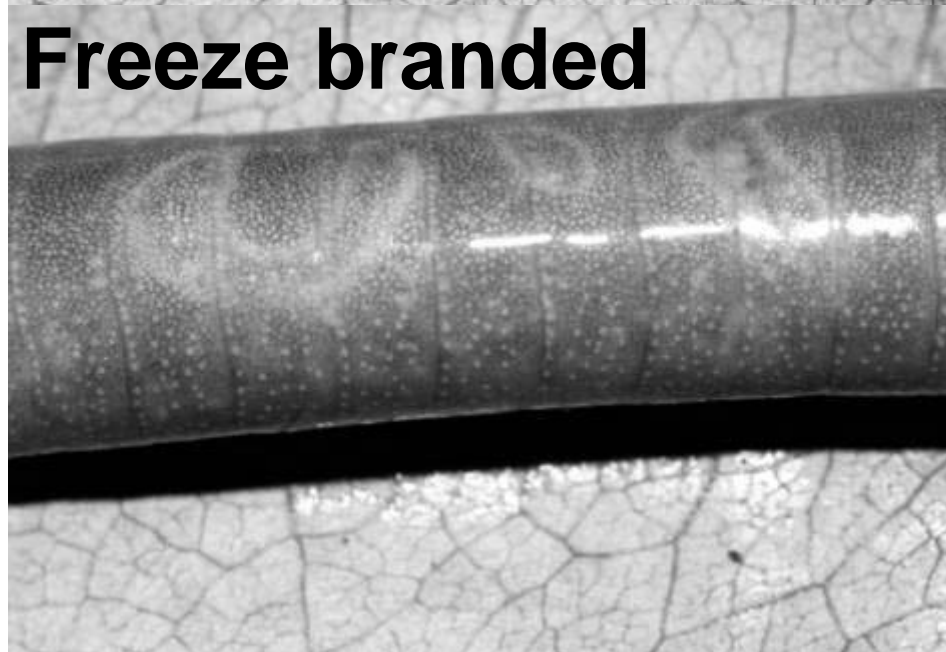
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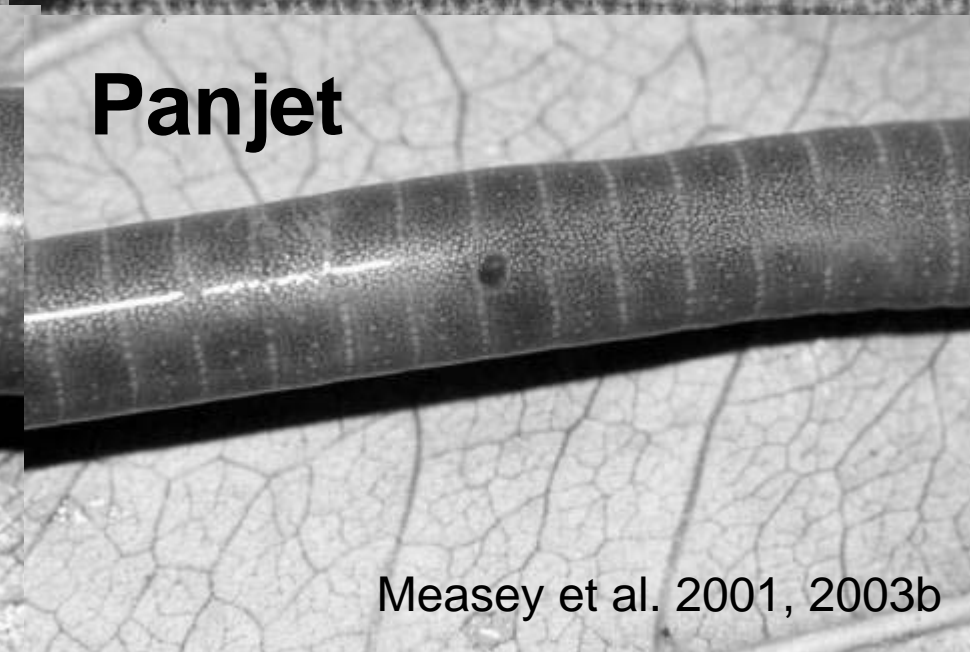
VI Alpha Tags



Freeze branded



Panjet



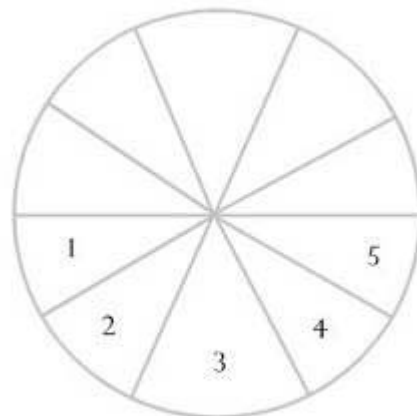
Measey et al. 2001, 2003b



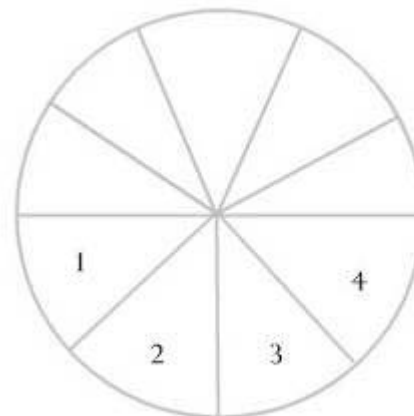




DISCS



TYPHLONECTES COMPRESSICAUDA



TYPHLONECTES NATANS











| | Panjet tattoo | Freeze brand | VIE | VIAIpha |
|------------------------------------|---|---|--|--|
| Date of mark | 26/06/00 | 28/06/00 | 28/06/00 | 18/08/00 |
| Size range of animals marked (mm) | 60 – 252 | 140 – 288 | 84 – 196 | 58 – 255 |
| Anaesthetic | Optional | Optional | Required | Required |
| Average time taken to apply mark | 5 seconds | 10 seconds (per digit) | 1 minute | Up to 5 mins |
| Portability | Good | Poor | Good | Good |
| Immediate effect on individual | Localised swelling where marked on annular groove | Animals disturbed by placement of brand | Animals disturbed by insertion of needle | Animals disturbed by insertion of needle |
| Immediate visibility of mark | Clear | Faint | Generally clear – better in sunlight | Generally clear – better in sunlight |
| Skin puncture | Yes | No | Yes | Yes |
| Visibility of mark on 12/7/00 | Clear | Clear | Generally clear – better in sunlight | n/a |
| Visibility of mark on 29/8/00 | Clear | Clear | Generally clear – better in sunlight | Generally clear – better in sunlight |
| Scars from marking seen on 29/8/00 | No scar visible | Scar tissue formed in branded areas | No scar visible | Scar visible at needle insertion point |
| Visibility of mark on 11/10/00 | Clear | Clear | Generally clear – better in sunlight | Generally clear – better in sunlight |

A summary of the different marking techniques tested on *Gegeneophis ramaswamii*.



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

