Methods to Individually Identify the Amphibian Specimen

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Introduction

The quality of management of animals is greatly enhanced when specimens can be identified as individuals. Without this ability to consistently identify each specimen it becomes impossible to maintain medical histories, pedigree data, and other pertinent information that is directly related to the specimen. Inability to identify individuals will also impact gene diversity maintained in a captive population (see section on Genetic Management).

For the past twenty-five years zoo populations of endangered species have been managed to maintain genetic diversity through selective breedings. Genetic population management is most efficient at the individual level with full pedigrees (Schad, 2007). This requires that each individual can be identified through its life and that parentage can be established for all offspring.

Maintaining the long term identify of an animal requires some type of recordable identifier to connect the individual with its records. In zoos, this is usually an accession number and in some rare cases for particularly noteworthy specimens of the Amphibia, a name. These identifiers can be used as a key for information stored in the records system and thus establish its pedigree.

Individual identification techniques can be divided into two general categories: Those that are invasive and those that are not. It is important to note that all techniques are not entirely full proof. All have failed under some conditions, and in invasive techniques, mortalities have occurred. Some invasive techniques can cause permanent deficits, impair physical activity, and decrease survivability (i.e., tissue amputation). These techniques need to be individually evaluated for the circumstances for their proposed use prior to implementation.

Non-Invasive Techniques

Animal Color and Pattern

One of the simplest and most effective methods for identify amphibians is by their pattern and coloration. In many species, once the animal reaches its adult form, its pattern, marking, glandular structures and coloration usually stabilize for the remainder of their life. Although there might be some ontogenetic changes as the animal ages (i.e., darkening), its earlier markings are usually still visible. These patterns can be document either by drawing (if minimal talent is available) or by photographing the animal. Depending on the species, it may be best to use the dorsum, venter, or lateral areas to delineate specimen differences. In the case of animals with "warty" or granular skin, the position and numbers of these features, as well as the coloration are excellent unique identifiers for animals.

The patterns of juvenile animals may change as the animal matures; however, with repeated photographs being taken every 1-4 month, the staff at the Detroit Zoo successfully tracked the identification of a group of juvenile emperor newts through adulthood and saw minimal changes. With regular updating of the records, it is possible to use photographic pattern IDs on juvenile animals in species where there is ontogenetic changes in pattern and color. However, reliance on this method must include a commitment to regularly updating images during growth.

There are limitations for this technique. Obviously if the animals are visually indistinguishable, this technique is ineffectual. In addition as the number of animals in an enclosure increases, it becomes more tedious to determine one animal from the group, particularly if the differences between individual specimens are minimal. Another important factor is size. If the specimens are very small, it is difficult to identify differences without a magnification device, adding the need for instrumentation and specimen restraint. Depending on the species, this technique becomes somewhat ineffective when there are more than five to ten specimens housed together.

There are anecdotal accounts of using xerographic copy machines of fossorial caecilians to document annul ring patterns. Though this method of identification generally disturbed the office staff and soiled the copier, it was effective and less stressful that trying to hold the animals for photographing or drawing. The animal was placed on the clean glass of the copier and a moist towel was placed over it for restraint.

As technology has improved and digital cameras have become the norm, pattern recognition has moved well beyond researchers with hand drawn renditions of the animals they study. Gamble et al. (2008) have developed a pattern recognition algorithm which uses photographs of marbled salamanders (Ambystoma opacum) taken in the field. Tests of the system proved successful for the identification of an individual in only about a minute with 95% accurate in a database of 1000 images.

Isolation

Another simple technique for maintaining individual identification is by separating animals into different enclosures. If there is only one animal in the cage, you can easily know who it is. This can also be applied to sexual pairs housed together in the same enclosures, as long as you can determine the sex of the individual specimens (which may or may not be the case in some species of amphibians). By attaching a card with the accession number on the cage, the animal or pair of animals is associated with its identifier. This technique has had some failures when the cage marks fade or are rubbed off, or the attached card is removed. This deficiency can be overcome by simple maintenance of the numbers and cards.

Invasive Techniques

Freeze/Heat/Chemical Branding

Skin branding is the process of causing a scar to form on the surface of the skin in a manner that makes an identifiable mark. This can be done with heat (direct heat branding or electrocauterization) (Clark Jr, 1971) freeze branding using liquid nitrogen or dry ice (Daugherty, 1976; Paine et al., 1984; Measey et al., 2003); or by using chemicals (using a solution of 0.5% amido Schwartz in 7% acetic acid)(Wolf and Hedrick, 1971). This method is painful; and therefore, requires local or general anesthetic. In addition, due to the nature of amphibian skin these marks are only semi-permanent in some species (CACC, Unknown).

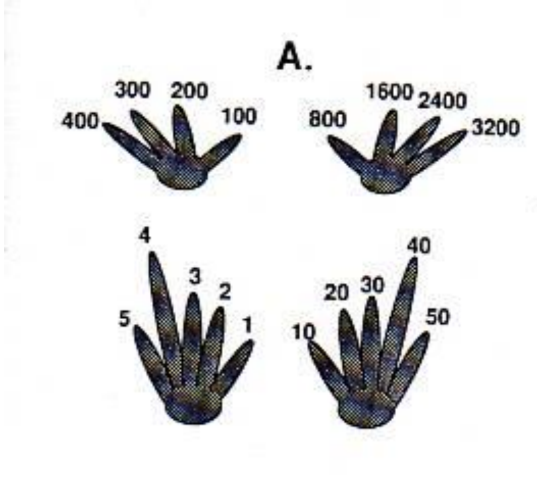
Freeze branding has been employed in field studies for larger species of amphibians such as Cryptobranchids. The animal is “branded” with a mark or number using extreme cold. A metal branding tool is cooled to well below freezing with a refrigerant or dry ice. The branding tool must be of sufficient mass to effectively freeze the skin and pigment cells of the animal. When the very cold brand is placed on the wet skin of an amphibian for a few seconds, the tissues in contact with the brand will freeze and die. The resulting area usually heals without pigment, making a permanent mark on the animal. There are disadvantages to this method. First the brand itself is unaesthetic and visible. Second, the method is crude and the brands are usually large. The actual area frozen by the brand is not easily controlled. The longer the brand is in contact with the skin, the wider the brand. The difference of a small acceptable brand and a large area of scar tissues may be the result of a few additional seconds of contact time between the amphibian and the branding tool.

Though the freeze branding marks have lasted a relatively long time in large species such as Cryptobranchids, there may be limits in smaller species. Both heat and freeze brands have been used in toads. Clark (1971) describes a heat branding method in which wire is formed into the shape of numbers and used to apply unique marks to 311 Gulf Coast toads (*Bufo valliceps*) with mark retention of a year. Paine et al. (1984) tested freeze branding on Puerto Rican crested toads (*Peltophrynes lemur*) at the Buffalo Zoo with success and marks lasting over 2 years.

The photograph to the left shows a freeze brand on a fish; in this case, a single line. It is easily seen here that there are limitations to the number of variations with this crude mark.

Tissue Amputation

Toe clipping has been a common technique in field research projects for reptiles and amphibians for many years. This technique is invasive and creates a permanent deficit in the animal. It has the potential to increase mortality (Clarke, 1972). Still, this is a technique that has been employed in some zoo programs. The technique involves amputating digits from the animal that corresponds to a numbering scheme. Below is an example of a number system for up to 10,000 individuals (Twitty, 1966).



From Donnelly (1994)

A problem with this method is that it may require the removal of multiple digits from a single appendage, leaving the animal little more than a stump. This can compromise the competence of the animal to perform simple essential biological functions such as locomotion, breeding embraces, feeding, etc. In addition, rare infections are noted at the amputation site.

One place that this technique has been used is for some conservation release programs. Usually young amphibians are monitored by groups and are all given a single mark coding (i.e., the removal of one digit). Older animals may be marked with an individual code. The use of this technique has to be weighed carefully between the need to track animals and to obtain release survival rates and the potential harm that may be done to the released animals.

In addition, toe regeneration was an issue in some species, especially salamanders (Davis and Ovaska, 2001), so Heatwole (1961) used beryllium nitrate to inhibit the regrowth of the toes in *Plethodon cinereus*, which was very successful for the study. Heatwole acknowledged that beryllium nitrate is known to be toxic and cause edema and death at even low concentrations, but used a dilute concentration carefully applied to the clipped toe (Heatwole, 1961). American Society of Ichthyologists and Herpetologists (2004) recommends limiting the number of toes clipped per animal and avoiding removal of two adjacent toes (Beaupre et al., 2004). May (2004) wrote an article stating that the use of toe clips statistically compromised of studies using these marks.

As molecular ecology and skeletochronolgy have become tools in ecology, toe clipping is often a marking method where the removed tissue has important uses. Lien (2007) recently used toe-clipping to determine the demographics of Taipei Grass Frog (*Rana taipehensis*) and used the toes for skeletochronological investigations. If toe clips are used, all removed toes should be cataloged and stored in an appropriate manner for possible analysis, especially in rare or endangered species.

Tail clipping in salamanders has also been used as a marking technique; however, the regenerative ability of salamanders makes this a temporary method at best. Arntzen et al. (1999) compared marking and tissue sampling methods in the newt *Triturus cristatus* and recommend it even though the clips grew back within about eight months. This is another opportunity for researchers to collect genetic samples. Tail clipping in tadpoles has limited utility as well since the mark is generally lost within 2-3 weeks of amputation (Turner, 1960). Guttman and Creasey (1973) noted that tail clipping also has the risk of causing damage to blood vessels or nerves.

Passive Identification Transponders

Passive identification transponders (commonly referred to as PIT tags) are perhaps the most common method of identification for zoo animals. A microchip is place under the skin in the back or into the coelomic cavity of the animal. This is accomplished by making a small incision in the animal and manually placing the tag, or by using an applicator. The applicator is a large hypodermic device with a 12 gauge needle. The tag can then be detected by a reader that sends radio signals to the transponder, which excites a transmitter in the tag to return a unique alpha/numeric code that is decoded by the reader. There are billions of codes available that assure that the codes are unique. The code appears on a screen in the reader.

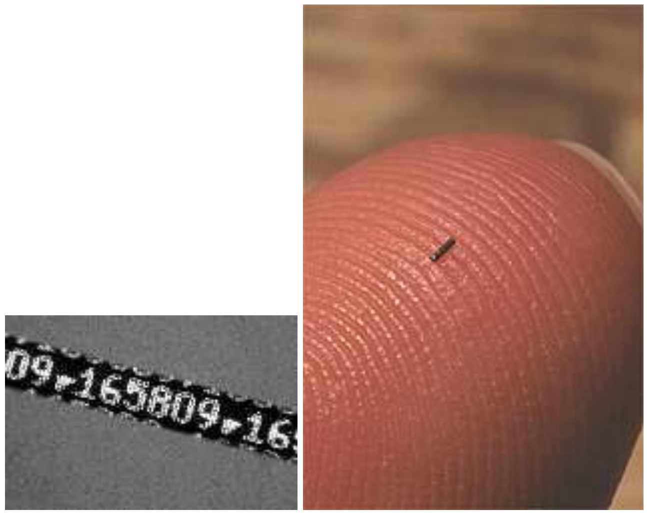
This is an invasive technique that potentially exposes the animal to infection, although this has rarely been report. There are also limitations. The tags are ~1.2cm long and 2.2cm wide. Frogs must be at lease 25-35 mm in SV length to accept the tags. Also, the tags frequently migrate in the body of the animal and it is not uncommon for the PIT tags to be expelled from the amphibian, usually from a different location than the original insertion. This is particularly true when the tag is placed in the body cavity. Tags are known to be passed in the feces. When the tag is expelled, the identification of the animal can be lost.

Another issue with PIT tags is that there are several systems on the market which are incompatible. The most common tags are sold by AVID and Trovan. There has been a patent dispute between these companies and AVID tags may be more easily obtained in the U.S. An AVID tag cannot be read by a Trovan reader. AVID does make a universal reader that will read both tags and another European system, but it is expensive (~$1,200 USD). Some programs have adopted Trovan tags as a standard and some AVID. This may make it necessary to have more than one system availble at your institution.

Portable Avid Transponder Reader

Decimal Coded Wire Tags

A technology developed for the fisheries managers to monitor released fish is to use small sections of magnetized wires with tiny (micro) numbering imprinted on the wire (Donnelly et al., 1994). The presence of the wire is detected with a magnetic detector system. It then must be removed in order to read the number with a magnifying device. The removal of the tag is a surgical procedure and there is risk for mortality, functional deficit, or scarring. This technique has limited applications in captivity, but could be a valuable tool for release programs where large numbers of small animals are released.



From : http://www.nmt.us/products/cwt/cwt.htm.

Visible Implant Fluorescent Elastomer (VIE) tagging system

Another technique using pigmented polymers also has its origins in fish studies. The technique involved injecting a visible fluorescent elastomer subcutaneously into the animal (Donnelly et al., 1994). The elastomers are availble in a variety of colors. Some colors of the elastomer can then be seen by placing the animal under a black light, other do not fluoresce and must be visible without the light for identification. The fluorescing dyes are clearly visible under the skin even with some pigmentation. By placing the elastomer at different sites on the animal and using different colors, an animal can be identified. Below is an example of marking of a frog using the webbing in the hind foot.

From: http://tropicalis.berkeley.edu/home/husbandry/tags/E-ANTs.html

Systems have also been developed for salamanders. This photo shows four sites that can be used to code identification with various colors.

From: http://www.pwrc.usgs.gov/resshow/droege2rs/salmark.htm

Another application for elastomers is to mark larvae. The elastomer becomes a permanent part of the animal. When the animal metamorphoses into the adult form the elastomer tag can remain visible. One problem with technique is that you never really know where the implant will end up on the metamorphosed animal. Tissues move and proliferate during the metamorphosis process. Tagging of larvae often requires that the animals are anesthetized, especially in smaller species, which carries its own risks. The small size makes it impossible to safely and effectively restrain the larvae while tagging. With anesthesia, very small animals can be marked. For example, larval red spotted newts were successfully marked at the Detroit Zoo and retained their tail tags.

Another issue is that some of the elastomer may migrate in body of the animal. In Wyoming toads, it was discovered that elastomers injected in a leg could be detected under UV light in the liver when the animal was later examined during necropsy (Williams, 1995). Due to the skin of amphibians not being directly adhered to the muscle, tags place subcutaneously will often migrate to the lowers part of the body. Multiple color tagging of frogs in the thighs often results in a collection of color spots in the groin area. This can be resolved by injecting the elastomer into the surface of the underlying muscle. Once injected, gently running your finger over the site will indicate if the tag is secure.

The use of elastomer has been compared to other methods of marking, including Davis and Ovaska (2001) who compared elastomer tagging to toe clipping. They found that western red-backed salamander, (*Plethodon vehiculum*) tagged with elastomer showed better weight gain than animals that were toe clipped in the lab and in the field. Heeymeyer et al. (2007) tested elastomer in eastern red-backed salamanders (*Plethodon cinereus*) which have dark skin which could limit the value of this technique. Their results indicate that this marking technique is a viable option though there was some migration of the tags and they there therefore recommend placing multiple marks as far away from each other as possible (Heemeyer et al., 2007). The manufactures of this produce site a number of papers that have tested the applicability of this product including Regester and Woosley (2005) who used VIE to identify and track the egg masses (Northwestern Marin Technology, Application Note APG02, 2007).

Other Injectable Color Markings

Over the years, a variety of methods involving the injection of a highly visible product into the animal have appeared. All these methods were initially applied to the fisheries industry where they were refined and sometimes even automated. The techniques used most often appear to be Panjet™ (Wright Health Group, Ltd. Dundee) and other tattooing methods, Injectable acrylic polymers, and Visible Implant Elastomer (which we have discussed) (Northwestern Marine Technology, Salisbury UK).

Tattooing in general implants some kind of dying into the skin at a depth to avoid the coloration washing off. The Canadian Council on Animal Care (CACC, Unknown) manual for amphibian and reptile care recommends selection of a tattooing method based on 1) use of a dye that contrasts with the animals skin and 2) use of a tattoo that maintains legibility over time, with diffusion into the skin, and in ultraviolet degradation (CCAC Species-Specific Recommendations on: Amphibians and Reptiles, date unknown). However, even with these considerations Murray and Fuller (2000) recommend using tissue removal, branding, freeze branding, and electrocauterization over tattooing due to the potential for problems with visibility and legibility. Herpetological Animal Care and Use Committee (HACC) of the American Society of Ichthyologists and Herpetologists (Beaupre et al., 2004) approves of the use of tattooing as a marking method, but cautions that the dye being used has the potential for absorption and, if the toxicity is unknown in amphibians, possible deaths. In addition, the permeable nature of amphibian skin makes a tattoo marking prone to diffusion.

Panjet™ tattooing is an automated method of injecting dyes intracutaneously through pressurization instead of using a needle. This process is commonly used in fisheries as the small aperture and high pressure of the device essentially forces the dye into the skin of the animal. Measey (2003) used this technique in caecilians and found the marks to be reliable and, based on observation only, did not to impact the survival or behavior of the animals.

Though the name brand Panjet™ was not mentioned, Nishikawa and Service (1988) used a high pressure, needless method like it in a comparison of this technique and toe clips for recapturability in the salamanders *Plethodon jordani* and *P. glutinosus*. They altered the previously used method by decreasing the size of the aperture of the gun using a small tube and placing marks in various locations on the body and limbs. The results of this study showed that this marking method was successful, with results better than those of the toe clipped animals for recapture (Nishikawa and Service, 1988). Taylor and Deegan (1982) studied the effectiveness of this method in green frog (*Rana clamitans*) tadpoles and found it to be successful in marking large numbers of larva, however, they do warn that the pressure may be an issue if trying to mark small, delicate animals. They also did not investigate the possible impacts of marking on larval growth (Taylor and Deegan, 1982).

Another injectable marking protocol used in a variety of amphibian studies is injectable acrylic polymer. Wooley (1973) used this method in salamanders with success, though individual markings were not possible. This process gave marks that were visible from 4-5 feet away; however, there was some slight fading along the perimeter of the mark and slight instability of the marks over time in a few animals. The main advantage noted by Wooley was the ability to observe the animals without capture and handling after the initial marking. However, increased visibility to the researchers would imply increased visibility to predators which may impact survival. Cecil and Just (1978) used the same procedure to mark larval bullfrogs (*Rana catesbeiana*). Though there was some difficulty discriminating similar colors (such as white and yellow) the method was successful and cost effective overall (Cecil and Just, 1978).

Marking larval amphibians presents a unique challenge due to their size and structure. Seale and Boraas (1974) used mixture of dye, petroleum jelly, and mineral oil and injected it into the tail and back of the tadpoles. They found the markings to be permanent (until metamorphosis) and have no impact on the animals. If not place correctly, however, there were problems with the swimming motion of the tad forcing the mark out and would therefore require remarking. Others had much less success with this method since the ratio of mineral oil to petroleum jelly must be exact, and there is a lack of consistency in the available products to allow for regular success.

Larval Dying:

Dying whole tadpoles has been used in a variety of studies over the years. In all these methods, the main variable is the dye used. Regardless of the dye, a solution is made and the tadpoles are placed in the dye solution for a set amount of time. The dye absorbs into the semi-permeable skin of the larvae and colors the whole animal. Guttman and Creasey (1973) found that methyl blue killed tadpoles and stained internal organs, neutral red only lasted two days, Bismarck brown Y caused sluggish behavior. If the purpose of the study is to understanding survival and behavior in larval amphibians, the temporary effects of neutral red appears to be preferable. Travis (1981) used neutral red to stain *Hyla gratiosa* tadpoles and assess the impact of dying on tadpole growth and survival. Their data indicates that dying tadpoles decreased the growth of the tadpoles. This should be a consideration in the decision process for considering its use.

Visible Alpha-Numeric Tags

Visible alpha-numeric tags that can be applied to amphibian identification are commercially available (Donnelly et al., 1994). These tiny tags are inserted subcutaneously by making a small incision and placing the tag under the skin. Closure of the skin is performed using surgical glue (cyanacrylic glue, e.g., Crazy Glue). One recommended site for implantation is the inside of the thigh on frogs (see http://tropicalis.berkeley.edu/home/husbandry/tags/E-ANTs.html). These tags are read by using a special blue LED flashlight and amber viewing glasses available from the manufacturer. The tags come with alpha-numeric numbers that provide about 46,000 unique variants. These tags are printed with unique numbers and come in two sizes, (standard at 1.0 mm x 2.5 mm and large at 1.5 mm x 3.5 mm) and in a variety of colors. Workers (Measey et al., 2001; Measey et al., 2003) used this method successfully in the caecilian (*Gegeneophis ramaswamii*) which have previously only be individually identifiable in small captive groups where the annulations pattern was distinguishable. This method did require anesthesia due to the overall difficulty in handling legless amphibians, but would not require general anesthesia in species that were easily restrained.

From: http://tropicalis.berkeley.edu/home/husbandry/tags/E-ANTs.html

Bands and Tags

Some older field studies used cords around the waists of frogs as marks. Some of the cords were color coded while other held small tags. The Canadian Council on Animal Care sites Bull (2000) for comparing waist and arm bands and finding both to cause abrasions in frogs (CACC, Unknown). Raney (1940) sites a study by Breder, (Breder Jr et al., 1927) using a cord with a small tag and his concerns were the lack of permanence and the potential for injury when the cord was tied too tight Raney (1940). However, Lien (Lien et al., 2007) successfully used waist bands for individual identification in Taipei Grass Frogs (*Rana taipehensis*), so there are current uses for this method.

Generally, waist bands, with or without tags, appear to have a limited life span and have the potential to snag of items and potentially inhibit movement. In addition, the colors of the bands may attract predators and I have found no studies on the possible implications to survivorship. However, as a temporary marking technique in captive animals, it may be useful and viable.

Bands around the forelimb were apparently used by Dely (1954) in *Rana esculenta* and *Bombina bombina* according to Honegger (Honegger, 2007). Honegger was primarily interested in marking techniques for zoos and; therefore, dismissed this marking technique due to its aesthetically displeasing characteristics rather than practicality and utility. The other concern with this marking was that it was only seasonal. Depending on the material used to construct the bands, there is also risk of abrasions or injury, interference with amplexus, and attraction of predators. The forelimbs of many male anurans are robust and would not lend themselves to having a band stay in place easily without injury.

Honegger (2007) also refers to a study where bands were placed around the digits of amphibians. This was a temporary making method performed on an unidentified frog species by Dely (1954). I was not able to find the original paper, but it would seem that the anatomical structure of a frog's foot would make tags easy to lose, and if place tight enough to hold in place, would cause strictures which could result in the loss of toes. In addition, any of the bands could interfere with shedding in amphibians as well, depending on how they are applied.

Knee tags were used by McAllister et al. (2004) in a comparison in marking techniques with radio telemetry. He cites the use of these tags by Elmberg (1989) using elastic cord. The tags were plastic, numerically coded fingerling tags tied at the knee. They found the tags to cause skin irritation and lacerations of the skin and muscle.

As part of a small (2 frog) demonstrational telemetry project, the Detroit Zoo placed transmitters on adult male Bullfogs using a small chair waist band covered in plastic. Though the tags were eventually lost, no injury was noted on the recaptured animal prior to the loss of his tag. Banding may be more applicable to captive management as the animals are monitored daily and a single band loss could be resolved easily.

External Tags:

We distinguish external tags from bands because tags generally involve passing some part of the marker through the skin and/or muscle of the animal. Tags are more invasive and have a potential for greater discomfort for the animals, injury, and infection; however, they do have a higher degree of permanence and reliability. There have been a wide variety of external tags used in amphibian studies over the years. Some of these include jaw tag (Raney, 1940; Raney and Lachner, 1947), tail clips(Raney and Ingram, 1941), bead tags (Nace and Manders, 1982), knee tags (McAllister et al., 2004), and toe tags (CACC, Unknown). Researchers studying amphibians 50-70 years ago appeared to show less concern for the comfort of the animal during the placement of the tags and over the life of the animal with the tag, though its comfort was not completely ignored. The attitude of researchers regarding humane placement of marks is evident with a review of the literature. In addition, recent researchers are required to view test animals in the same light as mammals and birds for permitting. Amphibians were, until recently, classified with the fish by universities and labs, which are not as regulated for testing and handling.

One of the earliest accounts of using a tag was Raney (1940) who used jaw tags on bullfrogs (*Rana catesbeiana*) and green frogs (*Rana clamitans*). During the study he marked 606 frogs using numbered metal tags placed through the lower jaw. This is a method that was used on fish, and Raney's dissatisfaction with toe clipping (due to bookkeeping) and waist bands (due to permanence) led him to use the jaw clips in frogs. He started with 50 frogs as a test group since he was concerned about the implications of the tags on the frogs' survival and behavior. This procedure was done with no anesthesia and Raney reports some of the frogs pulling on the tags with their feet after insertion but "no serious tearing of the flesh was noted". He claimed there was not apparent impact on the animal's ability to eat since the stomachs of recaptures contained food upon palpation and that the call was not noticeably changed. He concluded that the jaw clip method was satisfactory and superior to the other methods available at the time (Raney, 1940). Between 1940 and 1946, Raney and Lacher investigated the impact of the jaw clips on the growth of the toad (*Bufo terrestris americanus*) (Raney and Lachner, 1947). They found that the tagged animals had a slower growth rate than the untagged animals. Woodbury (Woodbury et al., 1956) sites a study by Stebbins, et al. (1954) who found jaw clips to be an unacceptable method of marking salamanders. They found the tags caused inflammation and were slough off through the jaw bone. Recent use of jaw clips is nonexistent from the literature for apparently good reasons.

Tail clips on salamanders were attempted by Raney (1941) where he attached metal bands through the tail and back, however these marking methods failed. In some cases the clips that he used were so heavy that aquatic newts had difficulty resurfacing in water. He also saw a large number of lost tags, tail injuries, at least on completely dropped tail, and what he called "putrid" skin, which I assume to mean an infection of some type. Raney does, however, mention that another researcher used tail clips in *Necturus* with success during the period of the study (Raney, 1941). Overall, using tags on salamanders have been less than successful and are not recommended.

Nace and Mander (1982) created a unique marking system for captive *Xenopus* which utilizes a surgical wire with colored beads stung onto it. The wire is placed through the fore or hind limb around the bone to assure its stability. In this study, the female frogs were marks on the forelimb and the males on the hind limb to avoid complications and snagging during breeding. Tags placed only through the skin were occasionally lost

(Nace and Manders, 1982). Using bead wire tags through the thigh or tail of amphibians is also possible, though the risk of snagging does exist (CACC, Unknown). In larger frogs, toe tags are an option for marking animals. These tags are placed through the webbing of the hind foot and include a disc-type tag which contains a unique code (CACC, Unknown). Honegger (2007) refers to the use of toe tags by Heusser (1958) who encountered swelling at the site and retention of about one season, with a maximum of two seasons.

Conclusions

There are many options for maintaining the individual identity of an amphibian through its life. Many have been employed in field work and later adopted for captive animals. In captivity the animals are monitored continuously; unlike many animals in field studies that are marked and then released into the wild. If there is an impact on survivorship or morbidity caused by a marking technique it can be directly observed for the entire life of the animal. For long lived taxa this can be decades. Modern veterinary science and pathology can now identify issues from marking techniques that have not been evident in the past (Williams, 1995). This has provided additional information on the suitability of some techniques.

Today current animal ethics limit the options available for zoo animals. Considerations for animal welfare, specimen aesthetics, and long-term health of the specimen are essential. Non-invasive techniques are preferred if they are reliable, functional, and workable. When the techniques are invasive, it is vital to balance the benefits of the technique (i.e., animal pedigree) and the potential costs in pain, morbidity, fecundity, and mortality to the specimen.

Mutilation through tissue amputation is considered an unacceptable technique at some institutions. This is particularly true when there are multiple amputations involved in the marking. These techniques can limit function, create the opportunity for infection, reduce functionality, and increase mortality. Tissue regeneration is also clearly evident if it occurs to captive animals. The pros and cons with some comments for captive situations of many of the techniques described in this chapter are provided in tables 1 and 2 below.

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Table of Pros and Cons of Marking Techniques

Table 1 - Non-Invasive Techniques

Technique	Application	Pros	Cons
Photographic ID	Animals with unique patterns or structures (i.e., warts)	Noninvasive	1. Requires some photographic expertise. 2. Patterns can change with age. 3. Postmortem changes may make it difficult to identify the animal
Pattern drawings	Animals with easily identifiable different patters	Noninvasive	1. Requires some photographic expertise. 2. Patterns can change with age. 3. Postmortem changes may make it difficult to identify the animal
Isolation	Animals are held individually or in pairs (sexually Dimorphic)	1. Noninvasive 2. Does not require pattern	Cage labels or cards may be lost

Table 2 – Invasive Techniques

Technique	Application	Pros	Cons
Freeze Branding	Large Amphibians	Permanent marking	1. Markings are crude 2. Can cause infection 3. Animals must be fairly large
Toe Clipping and tail clipping	Frogs and Salamanders	An easy technique	1. May require multiple digit amputations on one limb. 2. Salamanders may regenerate digit and tail 3. Can cause infections
Other amputation	Not recommended	An easy technique	1. Leaves mutilation 2. May compromise function
Decimal Code Wire	Groups of animals	Can be used to mark many animals	1. Requires reader 2. Animal may have to be euthanized to read wire
Bands	Frogs and salamanders	An easy technique	1. Cannot be used on small animals 2. Tag may fall 3. May compromise function
External Tags	All amphibians	Easy Id	1. Cannot be used on small animals 2. Tag may fall 3. May compromise function
Passive Integrated Transponders	Animals above 25g mass	Unique ID and common use in zoos	1. Cannot be used on small animals 2. Tag may be shed 3. Tag may fail
Visual implants - Elastomer	Marking of any amphibian	Can be used on larvae and adults	1. Elastomer may migrate to internal organs 2. Limited coding systems 3. Requires black light
Other Injectable Implants	Marking of any amphibian	Can be used on larvae and adults	1. Elastomer may migrate to internal organs 2. Limited coding systems 3. Requires black light

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