

Toe Clipping of Amphibians and Reptiles: Science, Ethics, and the Law¹

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ABSTRACT.—Public concern for the humane treatment of animals in research has led to specific guidelines for appropriate treatment of study organisms. Field research poses special challenges that Institutional Animal Care and Use Committees find difficult to address based on existing guidelines. Toe clipping is a common but contentious example whose use has been called barbaric and whose efficacy has been questioned. We provide a brief review of the ethical bases for such positions, the legal framework they have engendered, and the scientific evidence regarding the impacts of the practice. Leading philosophical views vary but tend to focus on the suffering or distress of individual animals, primarily vertebrates. The law has adopted this individual-centered view. Biologists, in contrast, tend to more wholistic views that focus on populations and ecosystems. Scientific studies of the impacts of toe clipping, most of them relatively recent, have become increasingly sophisticated statistically. Most show little impact of toe clipping on study animals, the exception being the likelihood of recapture of toe-clipped individuals in some frogs. If unaccounted for, effects of methodology can bias scientific findings. The few studies focusing on physiological indicators of distress show no increase resulting from toe-clipping. Thus, toe clipping of reptiles and amphibians meets legal and ethical expectations and should remain acceptable where it meets study needs. Biologists have long been concerned about the possible ethical implications of their methods. Philosophical inquiry has been beneficial in improving our understanding of these methods, but the need of biologists for better philosophical elaboration of ecological ethics has only partially been addressed.

Nations and professional societies have long had ethical standards to guide the behavior of their members. Similarly, concerns about animal welfare are far from new, both in philosophy and in law (Fraser, 2010). The seminal work of Singer (1975) and Regan (1984), and a growing public concern for the welfare and perhaps even rights of nonhuman animals (collectively referred to hereafter as “animal rights” or AR), has led to a change in the laws and the inclusions of specific guidelines regarding what is appropriate by scientific organizations. Philosophy and public concern drive the legal framework that regulates biological research, leading the herpetological community to also set rules for acceptable behavior (HACC, 1987, 2004). There are two primary reasons why such taxon-specific rules are needed in field biology. First, field research, because it is not conducted in the easily controlled confines of a laboratory, poses special challenges that Institutional Animal Care and Use Committees (IACUCs) find difficult to evaluate based on guidelines provided by existing regulations (e.g., FDA, 1989; for a recent review of the relevant laws and regulations, see Boal et al., 2010). Existing regulations are based on—and most appropriate for—laboratory studies, primarily those that focus on a small number of standard models such as white mice (Lindzey et al., 2002; Boal et al., 2010). Second, although there is general agreement that unnecessary pain and suffering should be minimized in scientific studies (e.g., Emlen, 1993), there are disciplinary and organism-specific differences in appropriateness of specific procedures. Thus, “Due to the large range of diversity represented by the over 12,280 species of amphibians and reptiles, no concise or specific compendium of approved or required methods for field and laboratory research is practical or desirable” (HACC, 2004).

As herpetologists seek to answer questions using new and old methodologies, they have to balance the importance of the information sought, the likelihood of success given a particular method, the equipment and manpower costs, and the ethical implications of their actions (e.g., Parris et al., 2010). We use the issue of toe clipping in herpetology as an example of such balancing acts. Data on individual animals, whether documenting survival or movement patterns, are essential in studies

of the reptilian ecology of amphibians and reptiles, and gathering such data naturally requires marking individual animals (e.g., Dunham et al., 1988). Toe-clipping has been used historically to individually mark a variety of organisms (e.g., Donnelly et al., 1994), but the Food and Drug Administration (FDA, 1989) “determined that toe clipping for animal identification is a potentially painful procedure and its use in animal studies should be discouraged.” Although IRAC (1985) did not recommend prohibiting the use of toe clipping for *any* taxonomic group, NRC (2010) only recommended its use in adult rodents when no alternatives are available. The use of the procedure has declined in many taxonomic groups, including many not covered by these documents, because of concerns about its humaneness. In herpetology, however, the method remains very common (Dunham et al., 1988, 1994; Ferner, 1979, 2007). The standard guidelines in the field (HACC, 2004) recommend that the technique “may be used for general marking of free-ranging animals when toe removal is not judged (by observation of captives or of a closely-related species) to impair the normal activities of the marked animal” and specifically suggests that “[t]he IACUC should be receptive to reasonable justification of such procedures.”

Nearly a decade ago, Parris and McCarthy (2001) and McCarthy and Parris (2004) used statistical models to reevaluate previous data and concluded that removal of multiple toes results in measurable reduction in return rates in mark-recapture studies of frogs, 4–11% per toe removed after the first. Writing in the prestigious journal *Nature*, eminent theoretical ecologist Robert May stated that toe clipping shows “barbarity” (May, 2004). Although some authors agree that the method raises serious ethical or methodological concerns and should perhaps not be used (e.g., Parris et al., 2010), others strongly disagree (e.g., Phillott et al., 2007) because alternative marking methods have their own drawbacks and risks (Reaser, 1995; Parris and McCarthy, 2001; McAllister et al., 2004; Phillott et al., 2007). Although the practice of toe clipping remains common in herpetological studies, it also remains contentious, with some exchanges being rather testy (see Phillott et al., 2007, 2008; Parris and McCarthy, 2008).

To summarize, ethical guidance for field biologists is extremely limited, and toe clipping has been specifically identified as an example of this need (Minteer and Collins, 2005). To give investigators and IACUCs a better understand-

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ing of the bases for the laws they enforce and the strong views they sometimes encounter, we begin by briefly reviewing some of the philosophical literature. Second, because herpetologists are often poorly acquainted with the legal framework they must work within, and may be confused about why they run afoul of the public or IACUC, we provide an overview of the relevant legal documents under which U.S. IACUCs must operate, and which stem from the philosophical views covered in the first section. Third, because few careful evaluations of the critical assumption that removing toes has no effect on the study organism were carried out until relatively recently, we review the scientific literature on the impacts of toe clipping in amphibians and reptiles in order to provide investigators and IACUCs with the most up-to-date analysis possible.

PHILOSOPHICAL OVERVIEW

Of necessity, our brief review glosses over some differences and completely ignores many authors. We begin by surveying the attitudes toward toe clipping within the field and then concentrate on the philosophical literature.

Toe clipping was first described by Bogert (1947). Biologists of the time tended to have relatively little concern for animal welfare. Bogert (1947) nonetheless stated, "Serious discomfort to the toads was not manifest as a result of the wounds; the specimens with clipped toes were apparently as normal in behavior as those not marked," indicating a concern with the welfare of the individual animal, not just the efficacy of the method. Researchers subsequently using the method often referred to methodological details designed to reduce impacts. For example, Tinkle (1967) stated, "Never were adjacent toes clipped, nor more than two on a single foot." Ethical, as opposed to methodological, concerns were typically not mentioned. In compiling methods for marking, Ferner (1979) paid little explicit attention to ethical considerations associated with toe clipping or other methods, although discussing concerns such as toe regeneration in amphibians and citing studies that showed negative effects on clipped animals and discussing the overall lack of such findings in the literature. More recently, when updating his review, the same author added a section specifically devoted to ethics and toe clipping (Ferner, 2007), presumably a response to the greater concern about such issues. Similarly, when the herpetological societies published their first guidelines for use of animals in research they noted only, "The humane treatment of wild vertebrates in field research is both an ethical and a scientific necessity" (HACC, 1987). The newer edition adds, "just as with other vertebrate groups, the use of amphibians and reptiles in research and teaching raises ethical questions that must be carefully considered prior to the initiation of a project" (HACC, 2004). Thus, ethical considerations have always existed within the field, but they have become more explicit in recent decades. What happened?

The views of biologists, leading up to the middle of the 20th century, often arose from a traditional utilitarian world view. Utilitarianism "accepts as the foundation of morals, Utility, or the Greatest Happiness Principle, holds that actions are right in proportion as they tend to promote happiness, wrong as they tend to produce the reverse of happiness.... By happiness is intended pleasure, and the absence of pain" (Mill, 1863). Although some philosophers have had more nuanced views, within the population at large this view traditionally referred only to human well-being or anthropocentrism (e.g., Garlick et al., 2011). Singer's (1975) expansion of the notion of moral consideration to include nonhuman animals, however, changed that. A utilitarian, Singer focused on individuals and argued that we must seek to maximize the aggregate happiness (or reduce the suffering) of all organisms capable of those states. The key to deciding who deserves such consideration is

sentience, with the level of consideration partially being determined by the degree of sentience (which for many is identical to the capacity to feel pain) (Singer, 1987, 1993). How to assess sentience remains a difficult question (Volpato et al., 2007). Entities other than individuals, such as scientific knowledge or ecological integrity, cannot feel pain and are, therefore, never considered.

Although often lumped together with Singer by nonphilosophers, Regan comes from a deontological tradition. In his seminal work, Regan (1984) argues that animals have rights if they are "subjects of a life," although other AR deontologists have espoused different grounds for assigning moral standing and rights to animals. Singer, Regan, and many others would agree that an animal with a complex brain should be considered, and perhaps even be thought of as a "person" (Varner, 2008). Whether Singer or Regan would consider a frog or a lizard worthy of consideration, and if so, to what extent, is debatable. Outside of the philosophical realm, however, such a debate would be moot for two reasons. First, other proponents of the AR view have extended the notion of sentience. Although recent work yields no consensus about whether fish are capable of consciousness (Chandaroo et al., 2004), others have broadened the notion to include even insects (Lockwood, 1987; Draney, 1997). About a decade ago, one of us witnessed a distraught woman who demanded to see the "gas chamber" where *Drosophila* larvae were euthanized. Second, the legal framework governing the activities of IACUCs, although somewhat variable from document to document, generally treats all vertebrates as sentient and, therefore, deserving consideration (see below). Thus, we should assume that amphibians and reptiles are included among the groups that merit consideration.

Our experience is that most field biologists, herpetologists included, hold views that are broadly similar to those ascribed to Leopold, for whom the complexity, diversity, and integrity of ecosystems have intrinsic value in addition to the utilitarian benefits they provide for humans (Leopold, 1949). We expect most also share with Brady (2006) an appreciation of the intrinsic value inherent in knowledge, whether applied or basic. These are commensurate with the sentiments of Bogert (1947) when he first described the procedure of toe clipping, and may be acceptable to Singer, for whom useful scientific research that improves lives is of value. However, it is incompatible with Regan's approach described above, as well as with how many in the AR community view things, because entities other than individuals, such as ecological integrity and scientific knowledge, are neither sentient nor subjects of a life. We suspect few field biologists would be comfortable with the claim that "when we see animals as only the products of a competitive struggle for survival, we risk overlooking the positive qualities of their lives. Pleasure has moral import ... for it amplifies the moral burden of depriving animals the opportunity to lead fulfilling, enjoyable lives" (Balcombe, 2009). Although Singer and Regan only value individuals (although they are not opposed to protection of other values or acquisition of knowledge), Leopold (1949) and Brady (2006) place paramount value on knowledge, species, and ecosystems (although they are not opposed to protection of individuals).

LEGAL OVERVIEW

Responding to increased public pressure, politicians in many countries passed legislation that affects animal researchers, either directly or via rules made by various agencies. It is beyond the scope of this review to cover this entire body of regulation, which changes frequently and varies from country to country. Even within a country, rules vary based on the organism and context. For example, in the United States, insects are not legally considered animals for the purposes of

animal welfare regulations, something that biologists find perplexing. In this paper, we shall focus on the United States and limit ourselves to laws and rules likely to affect herpetological research.

In the United States, organizations receiving federal funding for animal research, and all their employees, are subject to the Animal Welfare Act (AWA; 7 U.S.C. 2131, Public Law 89-544), which is enforced by the U.S. Department of Agriculture (USDA). The AWA excluded several categories of animals from its purview, including ectotherms, but the Public Health Services Policy (PHS, 2002) later defined "animal" to include all live vertebrates, thus bringing herpetologists into the fold. Which of these definitions of animal applies to a particular study is a function of funding sources and institutional decisions. Meeting applicable standards of the AWA is a legal requirement, but electing to follow additional policies, allows institutions access to certain funding sources. Many institutions choose to meet the stricter interpretations of policies set and monitored by the American Association for Accreditation of Laboratory Animal Care, a nonprofit organization. As with the USDA, this group conducts site visits to ensure compliance. All scientists working at such institutions are bound by the rules, not just those whose work is supported by a particular funding agency.

Universities discharge their oversight responsibilities through IACUCs, and many other types of institutions have similar committees. Guidelines for IACUCs are provided in NRC (2010), FASS (2010), and policies of USDA and PHS implementing the Federal regulations. Given that a violation by a single employee, even one who is not funded by that particular granting source, can endanger funding for the entire institution, IACUCs and those who oversee them tend to take their job seriously. To ensure compliance, IACUCs will conduct regular oversight of all facilities where animals are held for 12 h or longer. In the case of off-site locations, documentation such as video may be required instead. The requirements that individual researchers face depend upon the interpretation that their IACUC gives to ever-changing legislation and regulatory documents produced by the government and nongovernmental organization, such as the guidelines on euthanasia produced by the American Veterinary Medical Association (AVMA, 2007), and professional societies (e.g., HACC, 2004). Those wishing to conduct research in a U.S. institution should expect to fill out a detailed form, explaining what they want to do, how they want to do it, and justifying both using support from the literature. The IACUC will need a justification for the numbers of animals to be used and for any suffering that might be caused by the procedures to be carried out. Most IACUCs and their published regulations, unfortunately, are focused on laboratory animals and may have little experience or understanding of field research (Lindzey et al., 2002; Laber and Young, 2007; Boal et al., 2010). Similarly, AVMA rules being applied may not be appropriate for field studies (Julien et al., 2010). In field research, predetermining the exact number of animals needed or likely to be encountered is difficult. A colleague who worked on explosively breeding amphibians told us about exceeding what they thought was the total number to be used during the entire year on the first breeding night. In a situation like this, the protocol can be amended.

When evaluating a project, IACUCs seek to avoid or minimize the discomfort, distress, and pain inflicted (NRC, 2010). The USDA requires IACUCs to place each project into one of four categories. Even though herpetofauna are not included in the USDA mandates, these categories are normally used for all vertebrates. The lowest-impact category, B, is rarely relevant for field researchers, because it is reserved for breeding colonies and animals that are not used for any research procedure. Research that at most includes only momentary pain, which does not require analgesia, is

categorized as C. Many herpetologists would consider toe clipping to belong in this category. Where enough pain is caused to warrant the use of anesthetics or analgesia, category D is called for. If such drugs cannot be used, normally because they would interfere with the research, a study falls into category E, which requires additional justification and reporting. Note that the IACUC is required to focus on individual animals and on pain and suffering and excludes from its considerations the ecological or physiological parameters about which field biologists tend to think. Unfortunately, "unless the contrary is known or established it should be considered that procedures that cause pain in humans may also cause pain in other animals" (IRAC 1985). This places a potentially unrealistic, human-centered standard, requiring the IACUC to assume that procedures that are harmful to humans are also painful to other animals, rather than relying on the best available animal science.

REVIEW OF THE SCIENCE

In late December 2010, we searched for relevant studies using Google Scholar, Medline, Web of Science, and Wilson-SelectPlus. In many cases, we also reviewed the literature cited by papers found, as well as those that cited them. Some of these sources are hard to find, even in the age of electronic access. Published studies looking at the effects of toe clipping on a variety of taxa have proliferated in the past decades, adding to a previously limited and less carefully designed set of studies. In addition, anecdotal reports on related topics have also appeared in the non-peer-reviewed literature (e.g., Halliday, 1995), indicating the considerable interest in this issue within the herpetological community. Finally, we located subjective statements made by authors who studied the topic. For example, Phillott et al. (2007) noted that they "have rarely observed a clinical pain response after toe-pad removal" and went on to say "toe-tipping/clipping amphibians cannot be considered as causing the equivalent distress and pain as digit amputation in a human or other mammal." However, because such observations rely on subjective assessment, they are not included in our summary. Similarly, the study of Carothers (1986), who showed that removing the toe fringes of *Uma scoparia*, affected running performance on some substrates, was not included because the toes themselves were not removed.

Ideally, a meta-analysis of past studies can enhance the value of such reviews and increase the power of the analysis to identify patterns that may be missed in analyses of smaller data sets (e.g., Stewart, 2010). However, the nature of the data, collected over several decades using a variety of collection and analyses methods, focusing on a variety of response variables, and not addressing the need for a broad and balanced phylogenetic representation, preclude such an analysis from being conducted with validity.

We identified 69 instances in which the effects of toe clipping were evaluated on at least one parameter in at least one species (Table 1). In most of these, the baseline for comparison (the "control") is animals marked only once, rather than the methodologically preferable but methodologically difficult true control of no marking (for an exception see Davis and Ovaska, 2001). Of those, 11 addressed salamanders, 45 focused on frogs, 12 looked at lizards, and one involved crocodylians. Although some authors tested for multiple possible impacts, others only looked at one response variable. Most parameters evaluated were ecological (most common: survival, $N = 18$ studies; probability of recapture, $N = 17$; growth, $N = 10$) or physiological (locomotor performance, $N = 13$; stress, $N = 3$). No effect was seen in 40 studies (58%). In 22 cases (32%), a statistically significant reduction in the response parameter was measured, almost always a biologically negative effect. We found five cases (7%) where the effect was an increase in the

TABLE 1. Studies assessing impacts of toe-clipping on amphibians and reptiles that were published through the end of 2010. A reduction statistically significant in the response variable is indicated by a “-”; this almost always indicates a biologically negative effect, such as a reduction in return rates. Lack of reported statistical effect is denoted by “O,” a statistically significant increase in the measured trait by “+,” and ambiguous results by “?”.

Species	Parameter	Effect	Source
Salamanders			
<i>Ambystoma jeffersonianum</i>	Probability of recapture	-	McCarthy et al., 2009
<i>Ambystoma jeffersonianum</i>	Survival	O	McCarthy et al., 2009
<i>Ambystoma opacum</i>	Growth	O	Ott and Scott, 1999
<i>Ambystoma opacum</i>	Survival	O	Ott and Scott, 1999
<i>Desmognathus fuscus</i>	Stress	O	Kinkead et al., 2006
<i>Desmognathus monticola</i>	Stress	O	Kinkead et al., 2006
<i>Plethodon vehiculum</i>	Growth	O	Davis and Ovaska, 2001
<i>Plethodon vehiculum</i>	Growth	-	Davis and Ovaska, 2001
<i>Plethodon vehiculum</i>	Locomotion	O	Davis and Ovaska, 2001
<i>Plethodon vehiculum</i>	Probability of recapture	-	Davis and Ovaska, 2001
<i>Plethodon vehiculum</i>	Survival (lab)	O	Davis and Ovaska, 2001
Frogs			
<i>Bombina variegata</i>	Body condition	O	Hartel and Nemes, 2006
<i>Bufo bufo</i>	Survival	O	Heusser, 1958 in Honegger, 1979
<i>Bufo bufo</i>	Survival	O	Van Gelder and Strijbosch, 1996
<i>Bufo bufo</i>	Days males spent at breeding pond	-	Castellano and Giacoma, 1993
<i>Bufo calalmita</i>	Inflammation	+	Golay and Durrer, 1994
<i>Bufo calalmita</i>	Survival	O	Golay and Durrer, 1994
<i>Bufo fowleri</i>	Probability of recapture	-	Clarke, 1972
<i>Bufo fowleri</i>	Probability of recapture	-	Parris and McCarthy, 2001
<i>Bufo fowleri</i>	Probability of recapture	-	McCarthy and Parris, 2004
<i>Crinia signifera</i>	Probability of recapture	O	Lemckert, 1996
<i>Crinia signifera</i>	Inflammation	+	Lemckert, 1996
<i>Crinia signifera</i>	Reproduction	-	Lemckert, 1996
<i>Crinia signifera</i>	Probability of recapture	O	Bull and Williamson, 1996
<i>Crinia signifera</i>	Probability of recapture	-	Parris and McCarthy, 2001
<i>Crinia signifera</i>	Probability of recapture	-	McCarthy and Parris, 2004
<i>Hemius marmoratus</i>	Survival	O	Grafe et al., 2011
<i>Eleutherodactylus coqui</i>	Survival	O	Grafe et al., 2011
<i>Hyla cinerea</i>	Survival	-	Waddle et al., 2008
<i>Hyla labialis</i>	Homing ability	O	Lüddecke and Amézquita, 1999
<i>Hyla labialis</i>	Locomotor performance	O	Lüddecke and Amézquita, 1999
<i>Hyla labialis</i>	Probability of recapture	O	Lüddecke and Amézquita, 1999
<i>Hyla labialis</i>	Reproductive behaviors	O	Lüddecke and Amézquita, 1999
<i>Hyla labialis</i>	Site fidelity	O	Lüddecke and Amézquita, 1999
<i>Hyla labialis</i>	Probability of recapture	-	Parris and McCarthy, 2001
<i>Hyla labialis</i>	Probability of recapture	-	McCarthy and Parris, 2004
<i>Hyla squirella</i>	Growth	O	Liner et al., 2007
<i>Hyla squirella</i>	Survival	O	Liner et al., 2007
<i>Hyla squirella</i>	Probability of recapture	?	Waddle et al., 2008
<i>Hyla squirella</i>	Survival	O	Waddle et al., 2008
<i>Hyperolius nitidulus</i>	Survival	+	Grafe et al., 2011
<i>Leiopelma pakeka</i>	Probability of recapture—long term	O	Bell and Pledger, 2005
<i>Leiopelma pakeka</i>	Probability of recapture—short term	-	Bell and Pledger, 2005
<i>Leiopelma pakeka</i>	Survival	O	Bell and Pledger, 2005
<i>Litoria nasuta</i>	Jump distance—immediate	-	Schmidt and Schwarzkopf, 2010
<i>Litoria nasuta</i>	Jump distance—two weeks	O	Schmidt and Schwarzkopf, 2010
<i>Phrynobatrachus guineensis</i>	Survival	-	Grafe et al., 2011
<i>Rana luteiventris</i>	Growth	O	Hatch et al., 2006
<i>Rana luteiventris</i>	Survival	O	Hatch et al., 2006
<i>Rana pipiens</i>	Growth	-	Daugherty, 1976
<i>Rana pipiens</i>	Growth	+	Hatch et al., 2006
<i>Rana pipiens</i>	Survival	O	Hatch et al., 2006
<i>Rana pretiosa</i>	Inflammation	O	Reaser and Dexter, 1996
<i>Rana pretiosa</i>	Survival	O	Reaser and Dexter, 1996
<i>Rana virgatipes</i>	Growth	O	Standert, 1967
11 spp.	Probability of recapture	O / -	Humphries, 1979 in Parris and McCarthy, 2001
Lizards			
<i>Anolis carolinensis</i>	Clinging performance	-	Bloch and Irschick, 2004
<i>Carlia pectoralis</i>	Endurance—immediate	-	Schmidt and Schwarzkopf, 2010
<i>Carlia pectoralis</i>	Endurance—two weeks	O	Schmidt and Schwarzkopf, 2010
<i>Carlia pectoralis</i>	Sprint speed—immediate	-	Schmidt and Schwarzkopf, 2010
<i>Carlia pectoralis</i>	Sprint speed—two weeks	+	Schmidt and Schwarzkopf, 2010
<i>Cnemidophorus sexlineatus</i>	Sprint speed	O	Dodd, 1993
<i>Cnemidophorus sexlineatus</i>	Survival	O	Dodd, 1993
<i>Eulamprus heatwolei</i>	Stress	-	Langkilde and Shine, 2006

TABLE 1. Continued.

Species	Parameter	Effect	Source
<i>Eulamprus quoyii</i>	Sprint speed	O	Borges-Landáez and Shine, 2003
<i>Hemidactylus turcicus</i>	Clinging performance	O	Paulissen and Meyer, 2000
<i>Oligosoma maccanni</i>	Probability of recapture	O	Jones and Bell, 2007
<i>Sceloporus merriami</i>	Sprint speed	O	Huey et al., 1990
Crocodilians			
<i>Alligator mississippiensis</i>	Growth	O	Jennings et al., 1991

parameter measure, usually a biologically positive effect, and two (3%) where the effect was mixed or unclear.

By far, the greatest likelihood of a reduction in the response parameter occurred in studies of frog recapture probability. Only two studies assessed stress responses, one (Kinkead et al., 2006) in two species of salamander and the other (Langkilde and Shine, 2006) in a single lizard species. Neither showed any increase in stress as a result of toe clipping, as measured by levels of stress hormones. Interestingly, long-term captive geckos did not show elevated levels of stress hormones either (Barry et al., 2010). An additional study assessed the stress effects of capture alone (Cree et al., 2000). The authors found few and variable responses in *Amphibolurus barbatus* and suggested that species that do well in captivity, such as *A. barbatus*, may partially do so because handling causes them less stress than it does to some other species.

DISCUSSION

Inflicting unnecessarily high levels of stress on research organisms is ethically generally considered indefensible (Association for the Study of Animal Behaviour, 2003). Public concern over animal rights issues has increased over the past half century and with it the regulatory burden that researchers must deal with. This trend is not likely to change, and “regulatory creep” (increasingly restrictive regulation not resulting from an actual change in the legal and regulatory landscape) remains a concern (Bennett, 2005). The herpetological community has responded by better defining what it considers acceptable. Initially, the HACC (1987) stated that “[t]he humane treatment of wild vertebrates in field research is both an ethical and a scientific necessity.” The second edition of the guidelines (HACC, 2004) modifies this to read “The humane treatment of both captive and wild vertebrates is an ethical, legal, and scientific necessity” (emphases added). In clear response to changes that have happened since 1987, the second edition also states, “Ultimate responsibility for the ethical and scientific validity of an investigation, and the methods employed therein, must rest with the investigator.” This makes intuitive sense to researchers, but even though IACUCs tend to accept taxon-specific standards, such as those of the herpetological societies (HACC, 1987, 2004) as authoritative guidance, the final word is in fact determined by legal requirements, which, in turn, stem from philosophical developments. Thus, it is important for us to understand both the philosophical and legal framework in which we must function.

Our review of the scientific literature shows another way in which herpetologists have often been ignorant of that framework. With only two exceptions (Kinkead et al., 2006; Langkilde and Shine, 2006), the studies we found focused on response parameters such as survival or locomotor performance that are of obvious interest to biologists. In contrast, the decisions of IACUCs must, under the law, focus on individual pain and distress. Studies of ecological impacts indicate the ongoing concern of the scientific community regarding the methodologies used but do not directly address the concerns that laws and regulations identify. We return to the biological and ethical implications of our findings below and here focus

on the two exceptions and on a handful of other studies that look at stress in other organisms.

Most studies examining toe clipping have empirically reached the conclusion that, when properly conducted in herpetological research, this method poses few risks in most herpetofauna (Ferner, 2007; Phillott et al., 2007). With the notable exception of return rates in frogs, our review supports this conclusion as do some studies on stress in other organisms. Two additional studies examined the effects of capture and confinement on stress hormone levels in snakes, and both of them found significantly elevated levels of stress hormones resulting from these noninvasive procedures (Mathies et al., 2001; Bailey et al., 2009). Another two examined the effect of toe clipping on rodents (Castelhamo-Carlos et al., 2010; Schaefer et al., 2010). Similar to the herpetological studies, they found that toe clipping resulted in similar or lower levels of stress hormones than alternative methods often considered more humane. Finally, a recent study of skin and blubber biopsy in whales found rapid healing, reduced risk associated with alternative capture methods, and valuable scientific data production (Giménez et al., 2011). Thus, all available research (Table 1) suggests that toe clipping is, in fact, one of the less stressful marking methods available. Although the kind of “vote counting” we conducted has its inferential limitations (e.g., Stewart, 2010), current data do not allow a more powerful meta-analysis to be conducted with validity. There would be great benefit in conducting such an analysis, once sufficient appropriate data are available. In addition, studies that use a true control (animals from which no toes have been removed), perhaps by using alternative marking methods such as elastomers (Bailey, 2004), would be extremely advantageous.

Alternative methods to toe clipping may not always be feasible (Funk et al., 2005), and studies that examine standard alternatives to toe clipping also show drawbacks (e.g., Boone and LaRue, 1999; Phillott et al., 2007). Despite the rodent data showing toe clipping to be relatively benign, the Office of Animal Care and Use of the National Institutes of Health recently concluded that for rodents, “a principal investigator must continue to justify to the Animal Care and Use Committee (ACUC) that no other methods of identifying the animal are feasible as required by the *Guide for the Care and Use of Laboratory Animals*” (NIH, 2010). This should perhaps not be surprising because of the IRAC (1985) injunction to imagine the effects of unstudied procedures as if they were carried out on humans. PIT tags seem small and harmless to a human used to injections but are relatively huge and probably uncomfortable to a small lizard. A colleague pointed out that the equivalent for a human would be the insertion of a soft drink bottle into our body—something most of us would probably rather avoid. In contrast, toe loss is a common phenomenon in nature (Middelburg and Strijbosch, 1988; Hudson, 1996; Vervust et al., 2009). The *Guide* (NRC, 2010) specifically refers to “small rodents,” leaving application of this method to amphibians and reptiles completely open to the discretion of individual IACUCs. Given the data presented above, we see no legal reason for such permission to be denied for most species. Nonetheless, there is room for additional studies of stress response to this and other common techniques to be conducted.

Legality does not necessarily make a technique methodologically desirable. Much of the debate over toe clipping in herpetology has revolved around whether this method introduces unacceptable biases (see e.g., Standert, 1967). Despite the long-established potential for toe regeneration in some amphibian species, toe clipping remains a standard method for marking salamanders and frogs (Ferner, 2007). With one exception, our review (Table 1) shows few areas where research consistently suggests a problem exists. Studies of survival, growth, and locomotor performance almost invariably show no effect (for an exception, see Davis and Ovaska, 2001), but a robust number of studies suggest that the probability of recapture can be reduced in frogs. Even here it is sometimes difficult to be certain, as repeated analyses of the same species, and sometimes the same data sets, can give different answers. Typically, however, it is the more recent and sophisticated analyses that show a significant effect, at least partially because such tests often offer greater statistical power to identify an effect (Parris and McCarthy, 2001; McCarthy and Parris, 2004; but see Waddle et al., 2008). As first suggested by Clarke (1972), researchers whose studies could be affected negatively by reduced rates of return should carefully consider alternative marking methods or employ statistical analyses that allow this parameter to be isolated and accounted for. Similarly, researchers should carefully consider the contradictory data on effects in climbing species (Paulissen and Meyer, 2000; Bloch and Irschick, 2005).

Researchers should always carefully consider the methods they use and make sure that they are both scientifically and ethically sound (Boal et al., 2010). At the same time, we recognize the frequent need for individually marking study animals in field studies and that any research method also carries potential risk of some level of stress to animals. Each marking method has advantages and disadvantages that are often species or context specific, and the challenge is minimizing undesirable effects. For example, because toe clipping can lead to infection and necrosis (Golay and Durrer, 1994), many researchers use local antiseptics. Similarly, a common recommendation is to avoid toe clipping adults of species with fleshy toes, such as Gila monsters (*Heloderma* spp.) and iguanas (*Iguana* and *Cyclura* spp.), in which the procedure is more likely to result in infection. The work of Cree et al. (2000) suggests that, when possible, studies should perhaps be conducted on species that are relatively insensitive to human presence and even handling, such as those common in the pet trade. Nonnative species, which presumably thrive because of their ability to cohabitate with humans and many of which originate in the pet trade (Kraus, 2009), may also make appealing study organisms in that studying them also reduces environmental ethics concerns. Other recent work involving measurements of stress hormone levels suggests that captivity is a source of considerable stress in some reptiles (Sykes and Klukowski, 2009) but that performing "mildly invasive protocols" has little hormonal or behavioral effect (Kahn et al., 2007). If such findings turn out to be the norm, then a study in which animals are captured quickly, toe clipped, and released may well be ethically preferable to one in which animals are held in captivity without such marking.

Toe clipping amphibians and reptiles appears to have relatively little effect in terms of stress and most biological response variables measured but, nonetheless, may be ethically unappealing or even unacceptable. Recalling his first encounter with toe clipping, May (2004) says, "I remember being impressed by the elegance of the experiments concerned—but even more impressed by the casual barbarity of the toe clipping." May's position is based on disgust, rather than a more cerebral ethical principle. However, revulsion is a poor substitute for ethical judgment and a weak basis for law (Nussbaum, 2004:5). May (2004) concludes by stating, "I for one

welcome the attention increasingly given to the rights of non-human animals by philosophers such as Peter Singer." Although we disagree with the opinion May (2004) expressed regarding the technique, we fully agree with his views on the benefits of philosophical scrutiny. Although the AR community is often perceived as inimical to good research and management, it is, in fact, quite diverse (Thompson and Lapointe, 1995), and the perception of hostility is not necessarily correct (Perry and Perry, 2008a,b; Fraser, 2010). In some cases, those concerned about animal welfare seek to address challenges also concerning conservation biologists, although often for different reasons and using different tactics. AR proponents have questioned the validity and humaneness of our long-held methodologies and helped spur the recent flurry of research into the effects of toe clipping and other methods in field research. By doing this, they forced us to bring a more detailed, science-based understanding to traditional methods, which may not have been as carefully scrutinized in the past as they should have been. Further, by better elaborating the ethical considerations involved, philosophers have provided researchers the tools to make individual choices that are consistent with both their personal moral principles and with good science. One method to explicitly take into account a variety of methodological and ethical considerations in choosing study techniques was recently elaborated by Parris et al. (2010). The authors discussed marking techniques and concluded that toe clipping was, overall, inferior to all other options considered. Although we do not necessarily agree with the conclusion of Parris et al. (2010) regarding the efficacy or desirability of toe clipping, we believe that the technique for ethical decision making that they propose has wide application and may be of interest in this, as well as other contexts where researchers face ethical dilemmas. The method allows individuals with differing value systems to reach divergent conclusions as they differentially weigh particular elements. For example, if one's definition of fish welfare is "the internal state of a fish when it remains under conditions that were freely chosen" (Volpato et al., 2007), one would doubtlessly not conclude much about research on fish, which presumably would not freely choose to participate in most studies.

Ironically, although the herpetological community has responded to philosophical and legal changes, the philosophic and legal communities have yet to provide moral and legal theories that meet the identified needs of ecological field-oriented science. Minter and Collins (2005) identified the inability of existing philosophical schools to deal in a plausible way with larger environmental issues. In our context, the schools represented by both Singer (1975) and Regan (1984) fail to consider as valid most considerations not at the level of the individual (Thompson and Lapointe, 1995). Consequently, in the view of many AR proponents, harming an individual is unacceptable even if great benefits accrue to science as a whole or to that individual's species or population. The law continues to ignore the moral standing of entities other than individual animals or people—the very entities that are the focus of wildlife research, such as populations and ecosystems. The philosophical school represented by Leopold (1949), meanwhile, fails to acknowledge the value of the individual nonhuman and the undesirability of harm to individuals. Minter and Collins (2005) called for erection of a new field, ecological ethics, which would be able to simultaneously address all these kinds of issues. However, they elaborated neither the tensions between existing philosophical schools nor the approach this new field would take to resolve the conflicting standpoints. Ecologists have begun venturing into this void (Reiners and Lockwood, 2010), although perhaps without the benefit of a full philosophical education. Further development of ecological ethics would be of great value in confronting the ethical complexities of toe clipping and other field research, in herpetology and outside it.

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