Viewpoint

Assessment of the cooling-then-freezing method for euthanasia of amphibians and reptiles

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There is general agreement that methods used to euthanize animals should be as humane as possible. There is less agreement, however, about how to evaluate the humaneness of specific methods or how to determine whether those methods are sufficiently humane to recommend their use, especially when dealing with nonmammalian species.

The physiologic effects of a wide variety of drugs have been extensively studied in mammalian species, making it possible to develop recommendations for acceptable and unacceptable drug-based methods for euthanasia of mammals.[1] In contrast, developing similar recommendations for ectothermic vertebrates (ie, fish, amphibians, and nonavian reptiles) poses great challenges. Most importantly, the physiologic systems of ectotherms differ so greatly from those of mammals that it is difficult to extrapolate from mammals to ectotherms.[2,3] Instead, data specific to ectotherms are needed to develop evidence-based recommendations for euthanasia of these species.

The debate about the suitability of hypothermia (specifically, cooling then freezing) for euthanasia of ectotherms illustrates the difficulties involved. We have previously suggested that cooling then freezing represents an acceptable method for euthanasia (and perhaps anesthesia) of many ectothermic species.[4,5] We based our conclusions on results of an experimental study[4] in which we measured peripheral and core body temperatures and brain activity (determined by means of electroencephalography [EEG]) in cane toads (*Rhinella marina*) while they were cooled then frozen and on results of a review[5] of published literature on ectotherm physiology and ecology related to cold and pain. Recently, however, Warwick et al[6] disagreed with our recommendation, arguing that too little is known about the issues involved to reach firm conclusions and invoking the precautionary principle to suggest that the lack of evidence that cooling then freezing is humane meant that the method should not be recommended.

Authors of early reviews agreed with the idea that more extensive evidence was needed before the cooling-then-freezing method could be recommended for euthanasia of ectotherms. In 2011, for example, Sharp et al[7] wrote that "[c]ooling followed by freezing is also NOT appropriate for field euthanasia of cane toads since the efficacy of cooling as a means of euthanasia is unclear." While this statement was true at the time, additional research has subsequently been published. In fact, it was this call for additional research that stimulated our own study[4] on the use of cooling then freezing for euthanasia of amphibians and reptiles.

Much is known about the thermal biology of amphibians and reptiles, including the physiologic effects of exposure to low temperatures, and this topic is one of the most intensively researched aspects of ectotherm biology. We know, for instance, that thousands of species of amphibians and reptiles have high body temperatures by day (often, > 30°C [86°F]) and low body temperatures at night (often, < 10°C [50°F]).[8] Desert-dwelling species, in particular, experience large day-night thermal differentials.[9] Pain sensation is generally recognized to be a means of alerting an organism to a situation that could result in negative consequences.[10,11] We argue, therefore, that it would be unlikely that amphibians and reptiles adapted to habitats with a strong diel variation in temperature would experience pain when exposed to cold temperatures. In fact, many of these species actively select lower rather than higher temperatures, thereby reducing rates of metabolic expenditure or optimizing specific physiologic processes.[12,13]

Of course, even if we accept that amphibians and reptiles do not feel pain when exposed to the types of temperatures they routinely experience in the wild, we are still left with the question of whether they experience it when exposed to the lower temperature associated with the cooling-then-freezing method of euthanasia. However, the evidence to date suggests that cooling of amphibians and reptiles can virtually eliminate brain activity, eliminating the ability to perceive nociceptive stimuli. In addition, there are no signs of increased activity, as would be expected if the animals were in pain.[14]

Electroencephalographic examinations of fish confirm that pain induced by inserting a pin into the fish's side causes an increase in brain activity even in fish that are concurrently exposed to low tempera-

tures, yet freezing alone does not.[15] Likewise, cane toads are capable of coordinated activity at temperatures as low as 10°C[16] and, therefore, could be expected to exhibit overt behavioral signs of cold-induced stress, but do not. Nor do turtles that move to follow environmental gradients in very cold, near-freezing, water.[17] We believe that this lack of increased EEG activity and overt movement in amphibians and reptiles exposed to cold temperatures is compelling evidence that they do not experience pain during this process. Moreover, some ectotherms lack receptors that respond to cold.[5]

It is true, as Warwick et al[6] indicated, that the number of amphibian and reptile species that survive seasonal freezing and thawing is relatively small. However, a large number of species demonstrate marked shifts in body temperatures in response to diel temperature fluctuations, which are closer to the timeframe associated with the initial stages of the cooling-then-freezing method.[4] Similarly, Warwick et al[6] suggested that "if cooling and freezing were genuinely humane because of progressive suppression of nerve and brain function, then size of the animals would not matter," but this ignores the fact that the thermal differential between an animal's peripheral tissues and its brain depends on the animal's body size.[4,5] Because of thermal inertia, cooling a large reptile will reduce the temperature at its body surface more rapidly than in its body core. It remains possible (albeit, we believe, unlikely) that the thermal differential could be great enough for the animal to perceive pain from its extremities before brain activity ceases. More trivially, larger body size reduces the challenge and stress of drug injection. Thus, for both of those reasons, body size is relevant to the choice of euthanasia method.

Although diel variations in body temperature are less extreme in fish than in terrestrial vertebrates, available evidence suggests that cooling then freezing should be considered an acceptable euthanasia method for aquatic species as well. For example, Warwick et al[6] cited 3 studies that investigated methods for harvesting and slaughtering fish, and all 3 concluded that iced-water chilling is more humane than other methods of euthanasia (eg, electric stunning and asphyxia) on the basis of lactic acid concentrations,[18] rigor indices,[19] and EEG activity profiles.[15] Another study[20] similarly reported that cooling then freezing induced less stress than did euthanasia with methanesulfonate (MS222), a widely used fish anesthetic.

We agree with Warwick et al[6] that the precautionary principle is a useful guideline; however, we argue that it must be interpreted in light of the large numbers of amphibians and reptiles that are killed every year and the fact that killing of these animals will not cease while we work to definitively determine the best way to do so. This takes on practical importance when killing animals en masse, as occurs with invasive species, for example. Notably, members of the general public kill millions of cane toads every year in Australia. Obviously, these individuals do not have access to the legally restricted drugs typically used for euthanasia. Therefore, up until now, the most commonly used method to kill cane toads has been blunt trauma, which carries substantial risks both for the individuals using this method[21] and for the many animals that are maimed but not killed.[a] In contrast, most members of the general public have access to domestic refrigerators that can provide suitable temperatures for cooling and freezing. On the basis of our research,[4] we suggest that cooling then freezing offers a practical and more humane alternative to blunt trauma.

We also agree with Warwick et al[6] that it is difficult to extrapolate from mammals to ectotherms. It is not surprising that regional hypothermia can induce pain in human beings (as opposed to general hypothermia, which has anesthetic effects[22]), because human beings maintain a relatively constant, high body temperature. In contrast, ectotherms routinely experience a wide range of body temperatures on a daily basis, and their body and brain temperatures passively adjust to match the ambient temperature. Thus, we do not believe that one can assume hypothermia causes pain in ectotherms just because it does so in people.

Finally, many institutional ethics committees do not allow cooling then freezing for euthanasia of amphibians and reptiles on the basis of arguments similar to the one put forward by Warwick et al[6] that we currently do not have sufficient information to definitively state that this method is humane. We believe that this response may, at least partly, be attributable to the taxon-specific expertise of ethics committee members, most of whom work with laboratory rodents rather than ectothermic vertebrates. Therefore, we strongly urge ethics committees to include scientists who study ectotherms.

When it comes to euthanasia of amphibians and reptiles, we believe that each situation should be considered on its own merits. Euthanasia of large reptiles would likely best be performed with drugs, but small amphibians or reptiles could perhaps be more humanely euthanized with methods involving hypothermia. That said, it remains possible that species inhabiting areas where ambient temperatures fall to very low levels at night may maintain neuronal activity under thermal conditions that would abolish such activity in species inhabiting warmer regions. We need additional research on a wider variety of ectothermic species to establish guidelines for best practice. At the present time, however, we believe that the available evidence indicates that cooling then freezing offers a humane method for killing most kinds of ectothermic animals.

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Footnotes

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