Is "cooling then freezing" a humane way to kill amphibians and reptiles?

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Abstract
What is the most humane way to kill amphibians and small reptiles that are used in research? Historically, such animals were often killed by cooling followed by freezing, but this method was outlawed by ethics committees because of concerns that ice-crystals may form in peripheral tissues while the animal is still conscious, putatively causing intense pain. This argument relies on assumptions about the capacity of such animals to feel pain, the thermal thresholds for tissue freezing, the temperature-dependence of nerve-impulse transmission and brain activity, and the magnitude of thermal differentials within the bodies of rapidly-cooling animals. A review of published studies casts doubt on those assumptions, and our laboratory experiments on cane toads (Rhinella marina) show that brain activity declines smoothly during freezing, with no indication of pain perception. Thus, cooling followed by freezing can offer a humane method of killing cane toads, and may be widely applicable to other ectotherms (especially, small species that are rarely active at low body temperatures). More generally, many animal-ethics regulations have little empirical basis, and research on this topic is urgently required in order to reduce animal suffering.

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ABSTRACT
What is the most humane way to kill amphibians and small reptiles that are used in research? Historically, such animals were often killed by cooling followed by freezing, but this method was outlawed by ethics committees because of concerns that ice-crystals may form in peripheral tissues while the animal is still conscious, putatively causing intense pain. This argument relies on assumptions about the capacity of such animals to feel pain, the thermal thresholds for tissue freezing, the temperature-dependence of nerve-impulse transmission and brain activity, and the magnitude of thermal differentials within the bodies of rapidly-cooling animals. A review of published studies casts doubt on those assumptions, and our laboratory experiments on cane toads (Rhinella marina) show that brain activity declines smoothly during freezing, with no indication of pain perception. Thus, cooling followed by freezing can offer a humane method of killing cane toads, and may be widely applicable to other ectotherms (especially, small species that are rarely active at low body temperatures). More generally, many animal-ethics regulations have little empirical basis, and research on this topic is urgently required in order to reduce animal suffering.

KEY WORDS: Animal welfare, Bufo marinus, Ectothermy, Evidence-based practice

INTRODUCTION
Concern about the ethical treatment of animals has prompted extensive discussion of how to minimise suffering. Unfortunately, human intuition may fail to predict the stress and suffering of species that are only distantly related to us (Rose, 2002, 2007; Stevens, 2004; Langkilde and Shine, 2006). For example, mammals and birds typically react to falling ambient temperatures by attempting to maintain body temperature (by increasing metabolic heat production). Thus, exposure to low temperatures may cause intense discomfort. In contrast, many amphibians and reptiles exhibit highly variable body temperatures in the course of their day-to-day lives, and react to falling temperatures by becoming torpid (Pough, 1980; Frankenhaeuser and Langkilde and Shine, 2006). For example, mammals and birds are only distantly related to us (Rose, 2002, 2007; Stevens, 2004; Langkilde and Shine, 2006). More generally, many animal-ethics regulations have little empirical basis, and research on this topic is urgently required in order to reduce animal suffering.

RESULTS
Review of published literature
There is significant scientific debate about whether or not ectothermic vertebrates experience “pain” in the way that humans experience pain. This debate is complicated by the fact that most animals do not have a concept of “pain” in the way that humans do. Instead, animals may experience pain in a more basic form, such as discomfort or distress. This discomfort can be caused by a variety of factors, including temperature changes, physical injury, and chemical irritants.

The most humane way to kill amphibians and reptiles is a matter of scientific debate. Some researchers argue that cooling followed by freezing is a humane method, while others argue that it is not. The most important factor in determining the humane method of killing is the species being killed. For example, the cane toad (Bufo marinus) is commonly used in research, and it is important to use a method that is humane for this species. Cooling followed by freezing is a humane method for the cane toad, as it is a cold-blooded animal that is not capable of warming itself up. However, other species may require a different method.

In conclusion, the most humane method of killing amphibians and reptiles is a matter of scientific debate, and it is important to consider the species being killed when determining the method.

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understand the term; several authorities suggest that we should talk of “nociception” instead (Rose, 2002, 2007; Stevens, 2004). Even if a noxious stimulus induces activity in the brain, the process may not involve anything comparable to “pain” as perceived by humans (Key, 2015). Nonetheless, although the subjective experience of a cane toad may be very different to that of a human, most animal ethics committees (and the wider community) continue to believe that amphibians can feel pain.

Even if we accept the contested point that an amphibian is capable of feeling pain, our review of published literature does not support the idea that placing a pre-cooled ectotherm into a freezer is inhumane, for at least four reasons:

1. When a small ectotherm is pre-cooled and then placed into a freezer, thermal differentials within its body are minor (Hillman et al., 2009; Wilson et al., 2009; Bicego et al., 2007) and thus, the animal’s brain cools almost as rapidly as do its limbs. Its neural network is likely to be close to freezing by the time that ice crystals form in peripheral tissues.

2. Small animals cool to well below 0°C before freezing begins, allowing time for deep-body temperatures to fall to lower values. In anurans, ice crystals form at −1 to −4.3°C (Hillman et al., 2009). Thus, the critical issue is whether peripheral nerves can transmit nociceptive signals when the superficial tissue of the animal falls below −1°C. In ectotherms, transmission velocities for nerve impulses fall rapidly at low temperatures (Frankenhaeuser and Moore, 1963), and cease at temperatures close to 0°C; for example, 1.3–4°C in tortoises (Rosenberg, 1978), 0–2°C in frogs (Roberts and Blackburn, 1975). In frogs, the nociceptive peripheral neurons cease functioning at higher temperatures than do those transmitting signals such as touch or proprioception (Roberts and Blackburn, 1975).

3. Cold is an anaesthetic (Wilson et al., 2009). Even a modest reduction in skin temperature reduces painful sensations in mammals (Bicego et al., 2007). In amphibians, minor cooling of one limb (to 6°C) reduces the animal’s reaction to noxious stimuli (Suckow et al., 1999). In cane toads, low temperatures have the same structural and electrophysiological effects on myelinated nerves as do local anaesthetics such as lidocaine (Luzzati et al., 1999). Thus, nerve endings close to the peripheral tissues being frozen are unlikely to transmit nociceptive (“pain”) signals.

4. Normal brain functioning is dependent upon temperature in ectotherms. The brains of cane toads fail to respond to electrical stimulation below 3.2°C (LaManna et al., 1980), broadly similar to many other ectotherms (Hunsaker and Lansing, 1962).

**Experimental study**

A toad’s limb and deep body temperature closely followed ambient temperature (Fig. 1A,B,C); differentials between the animal’s skin and its core averaged <1°C (Fig. 1D). Accordingly, we recorded a continuous smooth decline in brain activity from fridge to freezer (Fig. 2). We saw no evidence of increased EEG activity, across any frequency bandwidths, as has been reported in animals exposed to painful stimuli (Lambooij et al., 2002; Zulkifli et al., 2014). Instead, the brain became electrically ‘quiet’ with decreasing temperature.

**DISCUSSION**

Collectively, general features of the thermal dependency of nerve and brain function in ectotherms suggest that for cane toads (and potentially, for many species of amphibians and reptiles), cooling-then-freezing can offer a humane death. By the time that ice crystals form in peripheral tissues, the brain is almost as cold as those
We implanted electroencephalogram (EEG) electrodes for recording brain activity in four wild-caught adult female cane toads (115–133 mm snout-urostyle length, 201–235 g). After the animals were anaesthetised with MS 222 (tricaine methanesulphonate; 3 g/l), we drilled four holes (0.5 mm diameter, two per cerebral hemisphere) through the exposed cranium to the level of the dura overlying the dorsal cortex. A fifth hole was drilled over the olfactory bulbs for the ground. All electrodes were gold-plated, round-tipped pins (0.5 mm diameter) glued in place using cyanoacrylate adhesive. Electrode wires (AS633, Cooner Wire, Chatsworth, California, USA) terminated at a connector fixed on the head with two stainless steel screws (25/095/0000, Hilco, London) and light-curing dental acrylic (Dentsply, Mt Waverley, Victoria). Electrode position was verified by dissection at the end of the study. Toads were then transferred to a damp cage, monitored until they regained normal motor function, and allowed a 10-day period of post-operative recovery at 30°C with food and water available ad libitum. EEG activity was recorded at 100 Hz using a head-mounted, miniature (25×25×9 mm) and lightweight (8 g, including battery) Neurologger 2A datalogger (Vyssotski et al., 2009; Lesku et al., 2012).

To record body temperatures during cooling and freezing, we inserted a thermocouple wire subdermally into each toad’s left hind limb (to measure temperature immediately below the skin) and into the cloaca (to measure deep body temperature, respectively). These thermocouple leads, as well as one measuring ambient temperature, were connected to a TC-2000 thermocouple meter (Sable Systems, Las Vegas, NV USA) and logged each minute using ExpeData software via a UI2 analogue/digital converter thermocouple logger (Sable Systems, Las Vegas, NV USA). Prior to experiments, toads were transferred into a Faraday cage (22×17×12 cm) then placed into a standard household refrigerator (Kelvinator, Charlotte, NC USA). Once the toad’s core reached fridge temperature (∼5°C), it was transferred to a household freezer (Fisher and Paykel, Auckland, New Zealand) for 30 min. Toads removed from the freezer after this time were dead (did not regain consciousness).

Fast Fourier Transforms were performed on 4-s, artefact-free epochs to calculate power in 0.39 Hz bins between 1.17 and 49.61 Hz using RemLogic 3.2 software (Embila Systems, Broomfield, CO USA). Cumulative EEG power was calculated as a measure of brain activity in 10-min bins, starting at the time placed in the new thermal regime. We also quantified power in the bandwidths typically used in analysis of the mammalian EEG (delta, theta, alpha, beta and gamma) and recently applied to amphibians (Fang et al., 2012).

All procedures were approved by the University of Wollongong Animal Ethics Committee (protocol no. AE10/05).

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Competing interests
The authors declare no competing or financial interests.

Author contributions
R.S. conceived the study; J.A., A.J.M., J.A.L. and M.S. gathered the data; A.L.V. designed and provided neurologgers; J.A.L. analysed the EEG data. All authors contributed to manuscript preparation and gave final approval for publication.

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References


