Standard Operating Procedure (SOP)

Intentional Largescale Euthanasia of American Bullfrogs (*Lithobates catesbeianus*) Performed by Qualified Environmental Professionals

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FINAL

Table of Contents

Purpose

- To provide current Standard Operating Procedures (SOP) for the humane euthanasia of American bullfrogs (*Lithobates [Rana] catesbeianus)*, where mass depopulation is supervised by Qualified Environmental Professionals (QEP).

Application

- This SOP applies to QEP and those under their supervision who are involved in the mass depopulation of American bullfrogs in British Columbia (BC) to ensure that all animals are euthanized in a humane and safe manner.
- The QEP must directly oversee and ensure compliance with this SOP and that all animals are confirmed to be American bullfrogs prior to euthanasia.
- This protocol applies to both adults and tadpoles.
- If bullfrog egg masses are found they should be removed from the water and buried.

Background

- British Columbia has 11 native species of frogs and toads. These species are protected under the BC *Wildlife Act*. It is illegal to kill, collect, possess or harass them without a permit.
- American bullfrogs are invasive to BC. They have established populations on Vancouver Island, in the Lower Mainland, and on some Gulf Islands. Ongoing removal of these animals is required to reduce and/or eliminate their impacts on native species and ecosystems.
- Field conditions present limitations to methods than can be safe and practical to use, but do not reduce the need for humane euthanasia techniques to be applied.
- Acceptable and unacceptable methods are outlined based on current scientific evidence and field safety. As new information, equipment and pharmaceuticals become available, revisions to this protocol will be required.
- Additional research into euthanasia and mass depopulation methods specific for American bullfrogs is recommended given the scale of the issue and current limitations in methods applicable to mass depopulation under field conditions.

Overview of Methods

- Euthanasia techniques must prioritise methods that minimise or prevent pain and stress and result in rapid loss of consciousness, followed by death.
    - Techniques that do not meet this standard and/or are not currently approved methods in the scientific literature are not considered acceptable.
- Techniques must be reliable and repeatable throughout the depopulation event.

    - Where operator fatigue may cause reduced animal welfare, alternative options or additionally trained personnel must be available.
- Proper handling and restraint of bullfrogs must occur to minimize the amount of pain, and/or distress experienced by bullfrogs.
    - Continuous analysis and refinement of handling methods must occur to optimize the welfare of bullfrogs prior to and during euthanasia.
- All animals euthanized must be confirmed dead.
    - This can be challenging to determine in amphibians due to their ability to cope with hypoxia and hypotension. The heart can beat after brain electrical activity has ceased and therefore, secondary methods of euthanasia should be used prior to disposal to ensure death (AVMA, 2020).
- Bullfrogs must be kept within their preferred optimal temperature and humidity zone (POTHZ) prior to euthanasia.
- Do not transport bullfrogs from one area to another.
- Animals must be handled with wet, powder free gloves, using the same or similar water to what they were found in. Otherwise, bare hands must be free of any sunscreen, lotions, soap, or other chemicals, and moistened in water before handling any bullfrogs.

Equipment

- All equipment must be inspected regularly to ensure good working condition.
- All equipment must be properly cleaned and disinfected between different water bodies and/or populations of animals.
    - Refer to the amphibian and reptile decontamination protocol authored by the Canadian Herpetofauna Health Working Group (CHHWG 2017) for specific details.
        - https://www.canadianherpetology.ca/conservation/doc/HHWG%20Decontamination%20Protocol%202017-05-30.pdf
        - NOTE: If other decontamination protocols are in place for the region where work is being completed, follow the more stringent protocols.
- Containers used to hold frogs should not result in trauma to the animals. If hard plastic containers are used, they should be lined with bubble wrap (Wright, 2001) or some other padding that will prevent trauma.
    - Sealable plastic bags can be used for holding frogs and will prevent trauma when inflated with air prior to sealing.

Chemical Euthanasia Methods

Benzocaine

Product Information

- Benzocaine is a Health Canada approved drug and an effective topical or immersion bath anesthetic similar to MS-222.
- It is both a CCAC and AVMA approved drug for the first step of euthanizing amphibians (CCAC, 2010; 2021; AVMA, 2020)
- Over the counter formulations of 20% benzocaine (e.g. Orajel® Maximum Strength; Life Brand® Toothache Pain Relief Maximum Strength) used to treat oral pain in people are available at local grocery and pharmacy stores and proven to be effective for amphibian anesthesia and/or euthanasia (Brown et al., 2004; Chen and Combs, 1999; Cecala et al., 2007; Torreilles et. al., 2009).
    - These products do not require a prescription unlike benzocaine hydrochloride powder or MS-222 and therefore, are highlighted in this document due to ease of access to non-veterinary professionals.
- Use only the 20% benzocaine products.
    - Other concentrations are available, but the 20% formulations will result in the quickest anesthesia with the smallest amount of product required, thus improving animal welfare.
- Avoid products that include menthol in the active ingredients.
- Proper storage conditions (room temperature) and adherence to expiry dates are required to ensure the product will remain effective.
    - Products must be protected from direct sunlight, freezing and heat to maintain effectiveness.
- When used correctly, deep anesthesia occurs within minutes and death within 3-5 hours (Torreilles et. al., 2009).

Safety and Disposal

- It is recommended to wear powder free, nitrile gloves when handling this product.
    - Minor side effects to benzocaine in people include localized skin irritation including redness and itching.
    - In some cases, a more severe allergic reaction may occur.
    - In very rare situations, systemic illness (methemoglobinemia) may develop.
    - Avoiding direct contact with the medication will prevent any of these complications.

- Keep the product away from infants and children.
- Refer to the product packaging for more information on product safety and storage conditions.
- Animals euthanized with benzocaine should be handled with gloves and are not fit for human consumption.
- Contact local authorities for specific disposal requirements in your jurisdiction.
    - Generally, disposal of animals can occur at a landfill and disposal of the solution down a sanitary sewer.
    - Do not dispose of benzocaine or benzocaine treated frogs in the field. This will prevent contaminating the water or surrounding environment and eliminate potential harm to scavengers.
    - Unused or expired product purchased at local pharmacies can be returned to the pharmacy for disposal.

Anesthetic Technique

Topical Application

Summary of Equipment Needed:

- A tube of 20% benzocaine gel or paste. Examples include:
    - Orajel® Maximum Strength gel.
    - Life Brand® Toothache Pain Relief Maximum Strength gel.
- Gloves.
- Plastic, sealable bag.
- Tweezers or pliers.
- Towel.

Technique:

- Use a 20% benzocaine product that does not contain more than 1% alcohol.
    - Orajel® Maximum Strength or Life Brand® Toothache Pain Relief Maximum Strength gel or paste are good options.
        - Other products such as Anbesol® gels contain high amounts of alcohol, which should not be used topically due to irritation and aversive behaviour in the frog after application.
        - Avoid liquid products for topical application, which also contain high amounts of alcohol.

- Wear gloves wetted in water before restraining the frog at the waist/hind legs.
- Apply a strip of 20% benzocaine the width of the tube opening along the entire length of the underside of the frog and gently rub it in with a gloved fingertip for 10-20 seconds.
    - The ventral skin quickly absorbs drug comparted to the head or back of the frog and will result in effects 5-10 times quicker (Chen and Combs, 1999).
- Place the animal into a large plastic bag , fill it will air, seal it closed to retain the frog and place a towel overtop.
    - Note that there is no risk of suffocation during this procedure, as frogs have a very low oxygen demand compared to mammals.
- Keep the bag out of direct sunlight and in an area that is quiet and the temperature is similar to where the frog was found.
    - Allow 15 minutes to pass before checking on the frog, at which point it should be deeply anesthetized.
- Confirm anesthesia by testing the following reflexes:
    - The righting reflex will be lost first and indicates light anesthesia has been reached (Gentz, 2007).
        - To perform this test, turn the frog upside down and it will not flip over if anesthetized.
    - To confirm deep anesthesia and lack of pain perception, the withdrawal reflex is tested and will be absent (Gentz, 2007).
        - With gloved hands, forcefully pinch a toe or tail on the hindlimb of the frog/tadpole with tweezers or pliers.
        - Firm pressure is required, otherwise a false result could occur.
        - The animal will not respond to a pinch when deeply anesthetized.
    - If either of these reflexes are present, allow an additional 15 minutes for the drug to take effect, and/or apply another strip of benzocaine.
- Once confirmed to be deeply anesthetized, keep the frog in the bag in a dark and quiet location at ambient temperature for 5 hours.
    - Because the frog is deeply anesthetized it will not be stressed or in any pain during this time.
- After 5 hours the frog should be dead (no heartbeat), but death must not be assumed.
- Confirm death by checking for the following:

    - No righting reflex.
    - No withdrawal reflex.
    - No corneal reflex.
        - Touch the eyeball and the frog will not blink.
    - No respirations.
    - No heart beat.
        - With the frog on its back, visually check for the heartbeat by looking at the skin along the middle of the chest (sternum) for 30 seconds.
- If confirmed dead, no additional methods are required.
- If a heartbeat is still present or you are unsure, a secondary euthanasia method must be used while the animal is deeply anesthetized.

Immersion Bath

Summary of Equipment Needed:

- 20% benzocaine gel, paste or liquid. Examples include:
    - Orajel® Maximum Strength liquid.
    - Anbesol® Extra Strength liquid.
    - Orajel® Maximum Strength gel.
    - Life Brand® Toothache Pain Relief Maximum Strength gel.
- Clean, de-chlorinated water.
- Gloves.
- Plastic, sealable bag.
- Tweezers or pliers.
- Towel.

Technique

- 20% benzocaine gels or liquids (e.g., Orajel® Maximum Strength liquid or Anbesol® Extra Strength liquid or gel) can be used for immersion baths once the product is mixed in water.
    - Products with high amounts of alcohol can be used in this situation because the alcohol will be sufficiently diluted.
- Advantages of this method are that several animals can be humanely euthanized in the same container, with anesthesia of animals occurring simultaneously.
- Use clean (i.e. free of contaminants) water from where the animal was found.

    - Otherwise, tap water that has been aged for 24 hours to de-chlorinate and closely matches the water temperature of where the frog was found can be used.
    - Avoid using water colder than where the frog came from, which can delay anesthesia.
- Add 5mls (liquid) or grams (gel/paste) of 20% benzocaine per litre of water and mix well until fully dissolved into solution.
- Place the solution into a large plastic bag, add the frog(s) and seal it closed with the bag inflated with air.
- The depth of water should be no higher than the shoulders of the bullfrog to ensure the head is out of the water.
    - With multiple animals in one container, ensure that all frogs remain in the bath (some may attempt to jump onto others).
- Place a towel overtop, keep the bag out of direct sunlight and in a quiet area where the temperature is similar to where the frog was found.
    - Allow 15 minutes to pass before checking on the frog, at which point it should be deeply anesthetized.
- Confirm anesthesia by testing the following reflexes:
    - The righting reflex will be lost first and indicates light anesthesia has been reached (Gentz, 2007).
        - To perform this test, wearing wet gloves, turn the frog onto its back and it will not flip over.
    - To confirm deep anesthesia and lack of pain perception, the withdrawal reflex will be absent (Gentz, 2007).
        - With gloved hands, forcefully pinch a toe or tail on the hindlimb of the frog/tadpole with tweezers or pliers.
        - Firm pressure is required, otherwise a false result could occur.
        - The animal will not respond to a pinch if deeply anesthetized.
    - If either of these reflexes are present, allow an additional 15 minutes for the drug to take effect, and/or apply another 5ml or grams of benzocaine to the water and stir until dissolved and place the frog back into the solution.
- Once confirmed to be deeply anesthetized, a secondary physical euthanasia technique (e.g., pithing) can be used immediately.
- Otherwise, keep the frog(s) immersed in the original solution in a dark and quiet location at ambient temperature for 5 hours.

    - Because the frog is deeply anesthetized it will not be stressed or in any pain during this time.
- Anesthetic solution should be changed regularly (e.g. between 'batches', daily, or if it no longer effective), or if the pH changes from neutral.
- After 5 hours the frog should be dead (no heartbeat), but death must not be assumed.
- Confirm death by checking for the following:
    - No righting reflex.
    - No withdrawal reflex.
    - No corneal reflex.
        - Touch the eyeball and the frog will not blink.
    - No respirations.
    - No heart beat.
        - With the frog on its back, visually check for the heartbeat by looking at the skin along the middle of the chest (sternum) for 30 seconds.
- If confirmed dead, no additional methods are required.
- If a heartbeat is still present or you are unsure, a secondary euthanasia method must be used while the animal is deeply anesthetized.

MS-222 (Tricaine methanesulfonate)

Product Information

- MS-222 is an isomer of benzocaine and a Health Canada approved drug.
- It is a CCAC and AVMA approved method for the humane euthanasia of amphibians (CCAC 2010; CCAC 2021; AVMA 2020) and is commonly used to effectively anesthetize a variety of amphibians (Cakir and Strauch, 2005; Crook and Whiteman, 2006; Torreilles, et al., 2007).
- Like benzocaine, MS-222 deteriorates in sunlight and breaks down into harmful by-products when in contact with metal containers. Only use this solution in plastic containers and away from sunlight
- When large numbers of frogs require euthanasia, this may be the most practical and cost-effective option.
- The product is commonly available in Canada under the tradename Syncaine® and in 100g and 1kg amounts.
- A veterinary prescription is required.

- Contact a veterinarian with amphibian or fish experience and establish a valid Veterinary Client Patient Relationship (VCPR) to obtain a prescription.

Safety and Disposal

- Wear gloves and eye protection and always work outside with MS-222 whenever possible.
- If working inside with powdered MS-222 a N95 mask should be worn.
- Animals euthanized with MS-222 should be handled with gloves and are not fit for human consumption.
- Contact local authorities for specific disposal requirements in your jurisdiction. Generally, disposal of animals can occur at a landfill and disposal of the MS-222 solution down a sanitary sewer.
    - Do not dispose of animals euthanized with MS-222 or solution in the field.

Anesthetic Technique

Immersion Bath

Summary of Equipment Needed:

- MS-222
- Sodium bicarbonate
- Measuring container
- pH test strips or reader.
- Clean, de-chlorinated water.
- Gloves, eye protection, +/- mask.
- Plastic, sealable bag.
- Tweezers or pliers.
- Towel.

Technique:

- MS-222 is acidic and causes aversive behaviours in animals if not buffered. Buffering also improves the efficacy of the drug, with lower concentrations needed to anesthetize animals (Gentz, 2007).
    - Use sodium bicarbonate (baking soda) to buffer the solution to a final pH of 7.0-7.5 prior to placing the animal in the solution (Gentz, 2007).
    - Generally, this requires 1:1 to 2:1 ratio of sodium bicarbonate to MS-222.

    - Add the sodium bicarbonate to the MS-222/water mixture. Do not pre-mix the powders together.
- Use 10g/L MS-222, which is at the upper range (AVMA, 2020), to ensure rapid loss of consciousness.
    - Measure out 10g of MS-222.
    - Place in 1 litre of water (deionized is best).
    - Mix well until completely dissolved.
    - Add sodium bicarbonate until a pH of 7.0-7.5 has been obtained (use pH test strips or reader).
    - Fresh solution must be made daily.
- Use clean water from where the animal was found.
    - Otherwise, tap water that has been aged for 24 hours to de-chlorinate and closely matches the water temperature of where the amphibian was found can be used.
    - Avoid using cold water, which can delay anesthesia.
- The depth of water should be no higher than the shoulders to prevent drowning.
    - With multiple animals in one container, ensure that all amphibians remain in the bath (some may attempt to jump onto others).
- Place the solution into a large plastic bag, add the amphibian(s) and seal it closed with the bag inflated with air.
- Deep anesthesia should occur within 30 minutes.
- Confirm anesthesia by testing the following reflexes:
    - The righting reflex will be lost first and indicates light anesthesia has been reached (Gentz, 2007).
        - To perform this test, wearing wet gloves, turn the frog onto its back and it will not flip over.
    - To confirm deep anesthesia and lack of pain perception, the withdrawal reflex will be absent (Gentz, 2007).
        - With gloved hands, forcefully pinch a toe or tail on the hindlimb of the frog/tadpole with tweezers or pliers.
        - Firm pressure is required, otherwise a false result could occur.
        - The animal will not respond to a toe pinch if deeply anesthetized.
    - If either of these reflexes are present, allow an additional 15 minutes for the drug to take effect, and/or apply another 5 grams of MS-222 to the water and stir until dissolved.

- Once confirmed to be deeply anesthetized, a secondary physical euthanasia technique (e.g., pithing) can be used immediately.
- Otherwise, keep the amphibian(s) immersed in the original solution in a dark and quiet location at ambient temperature for 5 hours.
    - Because the amphibian is deeply anesthetized it will not be stressed or in any pain during this time.
- Anesthetic solution should be changed regularly (e.g. between 'batches', daily, or if it no longer effective), or if the pH changes from neutral.
- After 5 hours the amphibian should be dead (no heartbeat), but death must not be assumed.
    - There are known species differences to MS-222 and different studies have found variations in effectiveness and time to death using high concentrations (Balko, et. al., 2019; Navarro, et al., 2022).
- Confirm death by checking for the following:
    - No righting reflex.
    - No withdrawal reflex.
    - No corneal reflex.
        - Touch the eyeball and the frog will not blink.
    - No respirations.
    - No heart beat.
        - With the frog on its back, visually check for the heartbeat by looking at the skin along the middle of the chest (sternum) for 30 seconds.
- If confirmed dead, no additional methods are required.
- If a heartbeat is still present or you are unsure, a secondary physical euthanasia method must be used while the animal is deeply anesthetized.

Physical Euthanasia Methods

Application

- These methods are acceptable when applied by properly trained and skilled operators and result in rapid loss of consciousness.
- Provides a practical method of euthanasia if pharmaceutical agents are not available or there are concerns about contamination of the carcass with chemicals (CCAC, 2023).
- When emergency euthanasia is required to alleviate immediate suffering of an animal due to a severe injury (e.g., broken leg) in the field (CCAC, 2023; AVMA, 2020) and other methods are not feasible or timely.

Overview

- A field technique that is conditionally acceptable when it results in rapid loss of consciousness and is immediately followed by a secondary physical method to ensure death (AVMA, 2020; CCAC, 2010; CCAC, 2023).
- Must only be performed by experienced and skilled operators.
- Must be conducted out of the sensory range of other animals.

Blunt Force Trauma Technique

- The first step of a physical euthanasia technique.
- The bullfrog is held by the back legs/waist with the head against a hard, flat surface that will not move or compress when the technique is applied (e.g., against a flat rock or a piece of hardwood. Soft ground is not acceptable as it will compress on impact.

Option 1:

- Use a metal hammer, large rock or similar hard object with a large, flat, impact surface larger than the head of the frog.
- Strike the skull just behind the eyes to cause immediate unconsciousness (Wright, 2001).
    - Draw an imaginary "X" between the eyes and back of the jaw.
    - Where the lines intersect at midline is the target area.
    - Apply this technique perpendicular to the top of the skull to ensure maximum impact.

Options 2:

- Alternatively, while holding the frog by the waist/ back legs, in one smooth motion rotate the frog so the top of the head is facing the impact surface and forcefully move your hand downwards so the top of the skull makes contact with the edge of a hard object (Cooper et al., 1989).

- For both of these methods, death must be confirmed following unconsciousness by immediately applying a secondary method to ensure a painless death.
- Pithing alone or decapitating followed by pithing are used in this situation (see below for a description of these methods).
- Freezing is not acceptable.
- These techniques should be limited to small numbers of animals (<10 animals) to prevent operator fatigue unless mitigations are taken.
    - Monitoring of the operator(s) for effective application of this technique is required to ensure effectiveness.

- Additional skilled operators should be used in rotation to prevent operator fatigue when used on multiple animals in close succession.

Secondary Euthanasia Methods

Application

- These methods are unacceptable as the primary means of euthanizing bullfrogs and must only be used when the animal is already unconscious and/or has no deep pain sensation.
- They must only be performed by experienced and skilled team members.

Pithing

- Pithing with a metal probe (e.g., 20-22 gauge, 1.5 inch needle), is performed by holding the frog facing away from you, flexing the nose down and locating the soft depression (foramen magnum) at the end/base of the skull.
- Insert the probe into the skull 1-2 cm and move it from side to side to destroy the brain.
- Without completely removing the probe, redirect it towards the body to sever the spinal cord.

Decapitation Then Pithing

- Decapitation will require a sharp metal knife, large pruning shears or guillotine.
- Remove the head just behind the back of the jaw where the neck meets the skull.
- Because the central nervous system (CNS) of amphibians can tolerate low oxygen and blood pressure, decapitation must be followed by pithing to ensure immediate death (AVMA, 2020).
- Decapitation is used in situations when locating the site for pithing is challenging.
    - Once decapitated, the brain and spinal cord are exposed, making the pithing location obvious.

Unacceptable Methods

1. Clove Oil (Eugenol) is not a Health Canada approved drug, nor a CCAC approved method for euthanizing amphibians (CCAC, 2021).
    - Over the counter products have unknown concentrations and ingredients that do not meet current standards for licensed drugs.
    - The AVMA notes that some clove oil derivatives are potential carcinogens (AVMA, 2020).

- Clove oil is known to cause gastric prolapse and skin necrosis in amphibians under certain conditions (Lafortune, 2001; Ross et al., 2006).
- Since suitable alternatives with known concentrations and ingredients that are approved for use in amphibians are readily available in Canada (e.g., 20% benzocaine products), approved products must be used instead of clove oil for anesthesia and euthanasia of amphibians.

2. Cooling then Freezing or Freezing Alone:
    - Cooling then freezing or freezing alone of either conscious or unconscious frogs is not an acceptable euthanasia method in Canada for amphibians greater than 4 grams (CCAC, 2010; CCAC, 2021; CCAC, 2023).
    - While there is debate about this technique in the scientific community (Lillywhite, et al., 2017; AVMA, 2020; CCAC, 2021), current research is lacking that conclusively demonstrates cooling then freezing is a humane euthanasia method for bullfrogs.
    - Freezing after deep anesthesia/unconsciousness is unacceptable, as animals may regain consciousness prior to death due to the ineffectiveness of the anesthetics at cold temperatures or ineffectiveness of the blunt force trauma technique.

3. Pithing and Decapitation on Conscious Frogs
    - Animals must be unconscious prior to using these methods.

4. Cervical Dislocation:
    - Due to CNS of amphibians being able to tolerate hypoxia and hypotension, cervical dislocation will not result in rapid loss of consciousness or death and therefore, is unacceptable.

5. Alcohol Immersion
    - This method does not result in rapid loss of consciousness prior to death.
    - Animals become distressed when placed in concentrated alcohol solutions and therefore, this method is unacceptable.

6. Captive Bolt:
    - In theory, either penetrating or non-penetrating captive bolts used for poultry could be used as a 2-step euthanasia process for bullfrogs. However, there is a lack of information on the proper equipment and

force required. Further research conducted in a controlled settings is needed before this method can be recommended.

7. Gunshot:
    - While gunshot is an acceptable method of euthanizing animals, the risk of ricochet of projectiles at point blank range while the animal is restrained is a safety concern that does not warrant the risks of this method for mass depopulation of bullfrogs.

8. Carbon Dioxide:
    - Carbon dioxide is not an acceptable method of euthanasia for bullfrogs, as there is a lack of information on the humanness of this technique including pain response, onset of action and time to death.

9. Other:
    - No other methods are acceptable unless pre-approved by the BC Government.

References

American Veterinary Medical Association – AVMA. 2020. AVMA Guidelines for the Euthanasia of Animals: 2020 Edition. Schaumburg IL: AVMA.

Balko JA, Posner LP, Chinnadurai SK. Immersion in tricaine methanesulfonate (MS-222) is not sufficient for euthanasia of smokey jungle frogs (*Leptodactylus pentadactylus*). 2019. J. Zoo Wildl. Med. 50(1):89–95.

Brown, H.H.J., Tyler H.K., and Mousseau T.A. 2004. Orajel® as an amphibian anesthetic: refining the technique. Herpetol. Rev. 35:252.

Cakir, Y., and Strauch, S.M. 2005. Tricaine (MS-222) is a safe anesthetic compound compared to benzocaine and pentobarbital to induce anesthesia in leopard frogs (Rana pipiens). Pharmacol. Rep. 57:467–474

Canadian Council on Animal Care – CCAC. 2010. CCAC guidelines on: euthanasia of animals used in science. Ottawa ON: CCAC

Canadian Council on Animal Care – CCAC. 2021. CCAC guidelines: Amphibians. Ottawa ON: CCAC

Canadian Council on Animal Care – CCAC. 2023. CCAC guidelines on: Wildlife. Ottawa ON: CCAC

Canadian Herpetofauna Health Working Group – CHHWG. 2017. Decontamination protocol for field work with amphibians and reptiles in Canada. The Canadian Herpetologist 7(1):24.

Cecala, K.K., S.J. Price, and M.E. Dorcas. 2007. A comparison of the effectiveness of recommended doses of MS-222 (tricaine methanesulfonate) and Orajel® (benzocaine) for amphibian anesthesia. Herpetological Review 38:63–66.

Chen MH, Combs CA. 1999. An alternative anesthesia for amphibians: ventral application of benzocaine. Herpetol Rev. 30:34.

Crook A.C. and Whiteman H.H. 2006. An evaluation of MS-222 and benzocaine as anesthetics for metamorphic and paedomorphic tiger salamanders (Ambystoma tigrinum nebulosum). The American Midland Naturalist 155(2):417-421.

Cooper JE., R Ewbank, C Platt, C Warwick (eds). 1989. Euthanasia of Amphibians and Reptiles. University Federation for Animal Welfare.

Gentz E.J. 2007. Medicine and surgery of amphibians. Institute for Laboratory Animal Research Journal 48(3):255-259.

Lafortune M., Mitchell MA and Smith JA. 2001. Evaluation of medetomidine, clove oil and propofol for anesthesia of leopard frogs, Rana pipiens. J Herpet Med Surg. 11: 13-18

Lillywhite H.B., Shine R., Jacobson E., Denardo D.F., Gordon M.S., Navas C.A., Wang T., Seymour R.S., Storey K.B., Heatwole H., Heard D., Brattstrom B. and Burghardt G.M. 2017. Anesthesia and euthanasia of amphibians and reptiles used in scientific research: should hypothermia and freezing be prohibited? BioScience67(1):53-61.

Navarro K, Jampachaisri K, Chu D, Pacharinsak C. Bupivacaine as a euthanasia agent for African Clawed Frogs (*Xenopus laevis*). 2022. PLoS ONE 17(12): e0279331.

Ross, A., Guénette, S.A., Hélie, P. and Vachon, P., 2006. Case of cutaneous necrosis in African clawed frogs Xenopus laevis after the topical application of eugenol. *The Canadian Veterinary Journal* 47(11), pp.1115-1117

Torreilles SL, McClure DE, Green SL. 2009. Evaluation and refinement of euthanasia methods for Xenopus laevis. J Am Assoc. Lab Anim Sci. 48:512–516.

Wright KM. 2001. Restraint techniques and euthanasia. In K.M. Wright, B.R. Whitaker (Eds.), Amphibian Medicine and Captive Husbandry, Krieger Publishing, Malabar, FL pp. 114-120.