

Standard Operating Procedure (SOP)

Euthanasia of American Bullfrogs (*Lithobates catesbeianus*) Performed by British Columbia Residents

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Purpose

- To provide current Standard Operating Procedures (SOP) for the humane euthanasia of American bullfrogs (*Lithobates catesbeianus*), when performed by British Columbia (BC) residents.

Application

- This SOP applies to BC residents who are involved in the euthanasia of American bullfrogs to ensure that all animals are euthanized in a humane manner.
- BC residents can only apply these methods on their own land or with landowner permission.

Background

- 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 and differing experience levels of BC residents present limitations to methods than can be safe and practical to use, but do not reduce the need for humane 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.

How to Identify a Bullfrog

- BC has eleven native species of frogs and toads, which are protected under the BC *Wildlife Act*. It is illegal to kill, collect, possess or harass them.
- BC residents must ensure that animals are positively identified as American bullfrogs prior to euthanasia.
 - If you are unsure of the species you have found, contact a BC Government invasive species specialist by submitting an online report or an app report:
 - www.gov.bc.ca/invasive-species
 - Or the Invasive Species Council of BC
 - E-mail: info@bcinvasives.ca
 - Telephone: 250-305-1003
 - Toll-free: 1-888-933-3722
 - Numerous online resources (e.g., BC government websites, <https://bcreptilesandamphibians.ca/>, iNaturalist) are also available to help identify an animal.

- Adult bullfrogs are up to twice the size of native BC frogs and toads, growing up to 20cm in length (not including the legs).
- Colour varies from green to brown on the back and sides and a lighter/white belly. Males have a bright yellow throat during mating season (June-August).
- They have a prominent, circular ear membrane (tympanum), which is larger than the eye in males and about equal in size to the eye in females.
- They also have a prominent fold of skin from behind the eye, around the top and back of the tympanum, to just behind the mouth.
 - Bullfrogs lack folds of skin along their back (called dorsolateral folds) that can be seen in Green frogs.



Photo: Adult male bullfrog. Image taken from the BC Reptiles & Amphibians website.

Overview of Methods

- Euthanasia methods prioritise techniques 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.
- Methods that are reliable, repeatable and practical for the typical BC resident are provided.

- Proper handling and restraint of bullfrogs to minimize the amount of pain, and/or distress experienced by bullfrogs is a priority, taking into account resident safety.
- All animals euthanized must be confirmed dead prior to disposal.
- 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 clean water before handling.

Equipment

- All equipment must be properly cleaned and disinfected.
 - A dilute bleach solution (1-2%) can be used.
 - Bleach must be kept away from wildlife and waterbodies and residents should take their own safety precautions when handling bleach.
 - Refer to the amphibian and reptile decontamination protocol authored by the Canadian Herpetofauna Health Working Group (CHHWG 2017) for additional 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 the bullfrog occurs, follow the more stringent protocols.
- Containers used to hold frogs should not result in trauma to the animals.
 - Large, sealable plastic bags (e.g., Ziploc® bags) can be used for holding frogs and will prevent trauma when inflated with air prior to sealing.
 - If hard plastic containers are used, they should be lined with bubble wrap (Wright, 2001) or some other padding that will prevent trauma.
 - Glass and metal containers are not suitable for use due to safety concerns to both frogs and people.

Chemical Euthanasia Methods

Benzocaine

Product Information

- Benzocaine is a Health Canada approved drug and an effective anesthetic for amphibians.
- It is both a Canadian Council on Animal Care (CCAC) and American Veterinary Medical Association (AVMA) approved drug for anesthesia and euthanasia of amphibians (CCAC 2010; CCAC 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 are 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 BC residents.
- Use only the 20% benzocaine products.
 - Other concentrations are available, but the 20% formulation will result in the quickest anesthesia with the smallest amount of product required.
- Avoid products that include menthol in the active/medical 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 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 in the garbage and disposal of the solution down a sanitary sewer.
- Do not dispose of benzocaine or benzocaine treated frogs in the environment. 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 gels or pastes 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 for 10-20 seconds.
 - The ventral skin quickly absorbs the drug compared to the head or back and will result in effects 5-10 times quicker (Chen and Combs 1999).

- Place the animal into a large plastic bag filled with air, seal it closed to 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 should be absent (Gentz 2007).
 - With gloved hands, forcefully pinch a toe on the hindlimb of the frog with tweezers or pliers.
 - Firm pressure is required, otherwise a false result could occur.
 - The animal will not respond to a toe 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 room 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 this must be confirmed.
- 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 breathing indicated by the lack of throat movements.

- No heartbeat.
 - 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.
- Measuring container
- 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.
- When possible, 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 frog was found can be used.
- The depth of water should be no higher than the shoulders of the bullfrog to ensure the head is out of the water.
- 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 plastic bag, add the frog, fill the remainder of the bag with air and seal it closed. .

- 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 on the hindlimb of the frog 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 add an additional 5ml or grams of benzocaine to the bath and stir until dissolved.
- Once confirmed to be deeply anesthetized, keep the frog in the bag in a dark and quiet location at room 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 this must be confirmed.
- 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 breathing indicated by the lack of throat movements.
 - No heartbeat.
 - 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.

Physical Euthanasia Methods

Application

- Acceptable when applied appropriately and result in rapid loss of consciousness.
- When drugs 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 (AVMA, 2020; CCAC, 2023) and other methods are not feasible or timely.

Blunt Force Trauma

- A 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. Otherwise, the benzocaine procedure should be used.
- Must be conducted in a separate area from other animals.

Manually Applied Blunt Force Trauma Technique

Option 1:

- 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.
- 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.

Option 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.

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 deeply anesthetized.

Pithing

- Performed after deep anesthesia via 20% benzocaine or blunt force trauma to the brain.
- Use a small metal probe (e.g., Precision 1.4mm flathead screwdriver; approximately 6cm long, 1-2mm diameter; a similarly sized sewing needle; 20-22 gauge, 1.5 inch needle).
- Hold the frog so it faces away from you, flex the nose down with a finger and locate the soft depression (foramen magnum) at the end/base of the skull.
- Insert the probe into the skull approximately 1-2 cm to enter the brain. 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

- Performed after deep anesthesia via 20% benzocaine or blunt force trauma to the brain.
- 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 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.
 - Some clove oil derivatives are potential carcinogens (AVMA 2020).
 - Clove oil is known to cause stomach 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 is not an acceptable euthanasia method (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 this technique is humane.
3. Pithing and/or Decapitation on Conscious Frogs
 - Animals must be unconscious prior to using these methods.
4. Cervical Dislocation
 - 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. Gunshot

- While gunshot is an acceptable method of euthanizing animals, the risk of ricochet of projectiles at point blank range with amphibians is a safety concern that does not warrant the risks of this method for depopulation of bullfrogs.

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