

# Anesthetic Considerations for Amphibians

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## Abstract

The popularity of amphibians in research, zoological exhibits, and as pets is on the rise. With this increased popularity comes a need for veterinarians to develop methods for managing these animals for various diagnostic and surgical procedures. For many of these procedures, the provision of anesthesia is a must. Fortunately, there are a number of different anesthetics available to the veterinary clinician for anesthetizing amphibians, including tricaine methanesulfonate, clove oil, propofol, isoflurane, and ketamine. In addition to the variety of anesthetics at our disposal, there is also a wider range of methods for delivering the anesthetics than are generally available for higher vertebrates, including immersion, topical, and intracoelomic routes of delivery. The purpose of this article is to review the different methods that can be used to successfully manage an amphibian patient through an anesthetic event. Copyright 2009 Elsevier Inc. All rights reserved.

**Key words:** amphibian; anesthesia; anesthetic; anuran; monitoring; urodelan

Amphibians are commonly used in research and are popular exhibit animals in zoological collections. Although they are not as popular as reptiles in the pet trade, they are being presented to veterinarians in clinical practice with increased frequency. Because veterinarians are being asked to work with these animals, it is important that we develop standard protocols for managing them in captivity.

Historically, anesthesia has been a specialty in herpetological medicine that has received little attention. Procedures were often completed with an animal being manually restrained or cooled to minimize movement. Because these methods would not be acceptable for birds or mammals, they should also not be considered acceptable for reptiles or amphibians. Fortunately, in the past 2 decades we have seen an increase in the number of scientific studies evaluating anesthetics in amphibians. Although many of these articles have been directed toward a specific species, *Xenopus laevis*, because of its value in research, the information obtained does have application across amphibian species.<sup>1-4</sup> However, it should not be assumed that all 4500+ species of amphibians

will respond to an anesthetic the same way. In cases in which there is no reference for a particular species, special precautions should be taken to minimize the likelihood of complications.

Regardless of the species, success with anesthesia can be achieved by following a consistent, well-planned protocol. For the author, the most pressing points to consider when establishing a protocol are the preanesthetic examination, the selection of an anesthetic that will allow the veterinarian to perform the desired procedure safely, an anesthetic-monitoring protocol that will help to determine the level of anesthesia, and a postanesthetic plan that considers the recovery and management of the animal af-

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ter the completion of the procedure. The purpose of this article is to review the methods that can be used to successfully manage anesthesia in captive amphibians.

## Preanesthetic Examination

A preanesthetic examination should be performed on each patient to determine whether an animal is a suitable candidate for an anesthetic procedure. The examination should include both a physical evaluation of the patient and supportive diagnostic tests, such as bloodwork, to help to characterize the physiologic status of the patient. Before performing an examination, however, it is important to consider the different techniques that can be used to safely restrain the animal for the examination.

Amphibians, like fish, require some special consideration when planning for a procedure that requires restraint. The integument of both of these groups of animals is an important component to their innate immune system, and damage to the skin during an examination could compromise the patient. To minimize the likelihood of causing problems, an amphibian should be handled with care. Latex or vinyl examination gloves are strongly recommended when handling an amphibian. The smooth texture of the gloves will minimize the potential for injury to the amphibian's integument. The gloves should be premoistened with distilled or dechlorinated water. Wearing gloves also has a benefit to the individual restraining the amphibian. Many amphibians can secrete toxins that can be absorbed through the skin of the handler.<sup>5</sup> The integument of an amphibian can also be covered with a variety of opportunistic pathogens (e.g., *Pseudomonas* species, *Aeromonas* species). The examination gloves can serve as a protective barrier against both potential toxins and pathogens.

The physical examination of an amphibian should include both a hands-off and hands-on review of the patient. The hands-off examination will enable the clinician to evaluate the breathing pattern, general demeanor, and locomotion of the patient. Animals that are dyspneic or depressed should be handled carefully. Amphibians should have a natural escape response when approached. Anesthetic procedures should be delayed in animals displaying abnormal behaviors.

For the hands-on examination, the animal may be manually restrained. For small or fractious amphibians, chemical restraint may be necessary to complete a hands-on examination without injuring the patient or handler. Amphibians do not generally

require a high degree of restraint for an examination. Urodelans can often be examined while being held in an open hand. If restraint is required, then the animal can be gently grasped behind the forelimbs with an index finger and thumb, with the examiner's remaining fingers encircling the rear body (legs and tail). Care should be taken when handling the tail, because tail autonomy can occur. Because of their natural escape response, anurans need to be secured quickly. For most anurans, they can be secured by grasping them in front of their rear legs. For larger specimens, such as the horned frogs (*Ceratophrys* species), care should be taken to minimize the likelihood of being bitten. The amount of restraint required for a caecilian depends on the species and general demeanor of the animal. Some caecilians can be examined similar to snakes, whereas others need to be chemically immobilized because they cannot be secured for an examination.

The hands-on examination of an amphibian should be done in a thorough but rapid manner. The oral cavity should be opened atraumatically. Processed radiograph film or a rubber spatula can be used to gently pry open the mouth of an amphibian. The mucous membranes should be moist and pale pink in color. Increased tackiness may be an indicator of dehydration. The integument of the amphibian should be moist and free of defects. Animals that appear dry should be kept moistened with distilled or dechlorinated water. Body condition can be used to determine the general health status of the amphibian. The musculature over the limbs of anurans and urodelans and the tail of urodelans can be evaluated to assess body condition. In many amphibians, transillumination can be done to evaluate the coelomic cavity/viscera. Positioning a pen light or transilluminator on the lateral body wall will enable the examiner to see various organ systems. For most species, respirations can be measured by counting gular movements or rib excursions. A Doppler ultrasound can be used to measure the heart rate. The probe can be placed on the ventral aspect of the pectoral girdle to obtain the heart rate.

Preanesthetic bloodwork can be used to assess the physiologic status of an amphibian patient. Unfortunately, many of the animals we work with in captivity present challenges for collecting samples or obtaining sufficient volumes of samples for screening. Blood samples can be collected from the ventral tail vein of larger urodelans, the ventral abdominal vein of anurans and urodelans, the femoral vein of anurans and urodelans, and the sublingual vessels of anurans. Cardiocentesis can be done, but can result in cardiac arrest in some species. If only a small

volume of blood can be collected, then a packed-cell volume or blood smear can be done at a minimum. This type of data can be used to determine the general health of the patient and provide insight into potential complications. For example, if a patient is anemic, then recovery from the anesthetic event might be expected to be prolonged and the clinician can plan for it. Plasma chemistries can provide insight into the hydration status of the amphibian and general organ function. In the past, there were often limitations with processing small sample volumes; however, there is now equipment that can provide complete biochemistry analysis on samples as small as 100  $\mu\text{L}$  of whole blood (VetScan 2; Abaxis, Union City, CA USA). If electrolytes are found to be imbalanced, they can be corrected with fluids before initiating a procedure. Likewise, if enzymes are found to be elevated or there are alterations in the minerals (e.g., inverse calcium to phosphorus ratio suggestive of renal compromise), then a plan to use an anesthetic that will not affect that system(s) can be made.

## Selecting an Anesthetic for an Amphibian

When selecting an anesthetic for an amphibian procedure, it is important to consider the type and duration of the procedure being performed. Procedures of short duration, such as diagnostic sampling, can be managed using any of the possible anesthetic methods, including immersion, topical, or parenteral administration; however, selection of the anesthetic in these cases should be determined based on a desire to have a rapid recovery. Procedures that demand a longer anesthetic period, such as surgery, require an anesthetic that will maintain a longer surgical plane of anesthesia. The type of procedure can also have an affect on the anesthetic selection. For example, the application of a topical anesthetic may be preferred over an immersion anesthetic for a coeliotomy.

## Immersion Anesthetics

Amphibians present veterinarians with a unique method for delivering an anesthetic that is not available in the higher vertebrates: immersion. In many cases, the highly vascular integument of an amphibian is capable of absorbing different anesthetics at a rate that can provide surgical anesthesia. The most common compounds used to anesthetize amphibians via immersion are tricaine methanesulfonate (MS-222; Argent Laboratories, Redmond, WA USA) and clove oil (Spectrum Chemical Corp., Gardena, CA USA), although other compounds, such as

Propofol<sup>†</sup> (PropoFlo; Abbott Animal Health, Abbott Park, IL USA), have also been used.

The water used to make anesthetic water for an amphibian should be of high quality. The author always prefers to use water from the animal's enclosure for the base of the anesthetic solution. To determine the value of the water, it is important to evaluate the ammonia and nitrite levels, hardness, alkalinity, and chlorine/chloramine levels. If the water is deemed to be of good quality (e.g., no ammonia, no nitrite, neutral pH, good buffering capacity and hardness, and no chlorine or chloramines), it should be used as the base for the anesthetic. If it is of poor quality, then dechlorinated tap water can be used. The temperature of the anesthetic solution should be similar to the preferred range of the amphibian. Anesthetic solution that is too cold may result in a delayed anesthetic induction and prolonged recovery. If the anesthetic solution is too warm, the amphibian may process the drug too rapidly and have an inconsistent anesthetic event. Aerating the water with an airstone and aquarium pump will ensure that the water is well oxygenated.

The induction chamber used for an immersion anesthetic should be water tight and have a secure lid to prevent escape. Plastic storage boxes with lids work well as induction chambers (Fig 1). Sealable plastic bags, glass tanks, and Styrofoam coolers have also been recommended.<sup>5</sup> Animals may become agitated during the induction period, and injuries associated with an escape response (e.g., jumping into the side of the induction chamber) are possible. Reducing the light and noise in the room can help to limit complications. Lining the induction chamber with a protective material (e.g., bubble wrap) has



**Figure 1.** A *Ceratophrys* species being anesthetized in an immersion of clove oil. A plastic shoe box was used as the induction chamber.

also been recommended.<sup>5</sup> When inducing an amphibian in an immersion anesthetic, it is important to keep the animal's head and nares above the water line. Failing to do so may lead to accidental drowning. To limit the likelihood of problems, the author recommends keeping the anesthetic solution below the level of the humero-scapular joint.<sup>6,7</sup> It is important to monitor the patient closely during the induction period to reduce the likelihood of complications. Once anesthetized, the amphibian should be removed from the anesthetic solution for the procedure. If additional anesthesia is required, it can be "dripped" onto the animal.

Apnea is a common finding in amphibians that are anesthetized with immersion anesthetics. In higher vertebrates, anesthetic-induced apnea can have fatal consequences. In amphibians, this is also a concern, but it is presumed that most amphibians rely on cutaneous respiration during these types of events.<sup>6,7</sup> Therefore, it is important that the immersion solution have appropriate oxygen levels to limit complications such as hypoxia. Aerating the anesthetic solution with a pump used for aquariums can be done to ensure that oxygen saturation levels are appropriate (>5 ppt).

### Topical

Although immersion anesthetics are certainly applied "topically," there are a select number of anesthetics that can also be applied directly onto the integument of an amphibian. A potential advantage of using an anesthetic topically versus as an immersion is related to the volume and dose of anesthesia required. Because topical anesthetics are applied directly onto the integument, there is no dilutional effect, and a smaller volume and dose can be used. A potential disadvantage associated with this method, however, is a greater potential for toxicity when drugs are rapidly absorbed through the integument. The most common anesthetics used topically are isoflurane and benzocaine.

### Parenteral

Parenteral anesthetics have been evaluated and advocated in amphibians, but are less commonly used than immersion or topical delivery routes. The parenteral routes for administering anesthetics in mammals, including intramuscular, intraosseus, and intravenous methods, can also be used in amphibians. In addition to these common routes of drug delivery, injections into the dorsal lymph sacs and coelomic cavity (Fig 2) can also be used in amphibians.<sup>5,7,8</sup> Amphibians do have a renal portal system, so most clinicians avoid intramuscular injections into the



**Figure 2.** Certain anesthetics, such as propofol, can be injected intracoelomically in amphibians.

hindlimbs to limit the likelihood of complications. Intravenous routes of anesthetic delivery are generally reserved for larger amphibians. The author has had some success delivering anesthetics into the sublingual vessels, ventral abdominal vein, and femoral veins of different anurans. Intraosseus routes of delivery are generally limited to the tibia or femur in anurans or large urodelans. Whether the renal portal system has an effect on anesthetics being delivered via this route is unknown. Intracoelomic injections appear to be well absorbed by amphibians, although there is often a delayed clinical response with this method compared with that of other delivery routes.

### Inhalant

Inhalant anesthetics are routinely used to anesthetize amphibians via immersion or topical routes; however, they can also be delivered with the standard route via oxygen flow through the respiratory tract. One of the limitations with the standard method of delivery is related to the small size of many of our captive amphibian patients. The author will commonly intubate and maintain larger amphibians with the standard route of delivery. In many cases, intravenous catheters, feeding tubes, or nasogastric/endotracheal tubes (1.0-1.5 mm tubes; Specialty Vet Products, Daphne, AL USA) can be used to intubate amphibians. Because many amphibians have a proportionally small cervical region, it is important to premeasure endotracheal tubes to prevent intubating a single lung. Amphibians are prone to desiccation when induced with an inhalant in an induction chamber; therefore, it is important to line the chamber with moistened paper towels to minimize the likelihood of complications.

## Anesthetic Options for Amphibians

### Tricaine Methanesulfonate

MS-222 is a water-soluble compound that is a derivative of benzocaine. This anesthetic is primarily used as an immersion anesthetic in amphibians, although it has been used parenterally too.<sup>9,10</sup> Although most of the original references to MS-222 anesthesia in amphibians were related to anurans, more recent work suggests that this anesthetic is also useful in urodelans.<sup>11,12</sup> MS-222 is strongly acidic (pH: 3.0) in solution, so it is important that the anesthetic water is buffered to a neutral pH (7.0-7.5) before adding the amphibian. Not buffering the anesthetic water can have 2 negative impacts: 1) the acidity is toxic to the amphibian and can lead to cutaneous lesions, and 2) the anesthetic becomes ionized and cannot be absorbed. Buffering can be accomplished by adding sodium bicarbonate at a 1:1 ratio (dry weight) in most cases; however, it is important to measure the pH before starting an anesthetic event.

The recommended range of doses for MS-222 immersion anesthesia in amphibians is wide, with doses of 0.2 g/L to 5.0 g/L depending on the age (e.g., larval form vs adult), size, and life history (e.g., larger terrestrial bufonids may require a higher dose than an aquatic species) of the animal.<sup>5,9</sup> Downes<sup>9</sup> has suggested that the doses used for amphibians could be reduced if the water is buffered appropriately. Likewise, the author has found that lower dosing regimens can be used if the anesthetic solution is buffered before use. In addition, induction itself appears to be smoother when the anesthetic solution is buffered, because some animals appear to be irritated by a more acidic solution. Most amphibians can be induced within 10 to 30 minutes with these dosing regimens.

It has also been suggested that MS-222 can be delivered by the parenteral route, with recommendations for injecting the anesthetic into the dorsal lymph sacs and intracoelomically.<sup>9,10</sup> Although these routes can be used, it is important to consider the acidic nature of the drug. If the drug is to be used via these routes, it should be buffered. Downes<sup>9</sup> has described a method for preparing MS-222 for parenteral delivery, although the additional compounds required may not be readily available at most practices. Unless there is a specific need for delivering MS-222 via a parenteral route, such as an experiment or limited access to high-quality water, the author recommends delivering this drug via an immersion method.

### Benzocaine

Benzocaine can be used as an anesthetic for both diagnostic and surgical procedures in amphibians. Induction and maintenance of anesthesia with this immersion anesthetic are similar to those methods described previously. Dosing regimens for adult amphibians are generally between 0.01% and 0.03%, with lower doses recommended for larval and paedomorphic amphibians (0.005%-0.01%).<sup>12-15</sup> Benzocaine, like the other immersion anesthetics, can induce apnea in amphibians. A recent study in leopard frogs found that immersion in 0.02% benzocaine resulted in apnea and a need to provide respiratory assistance.<sup>15</sup> It is important that the clinician is prepared to manage these complications to ensure success with their cases. Although MS-222 is generally preferred over benzocaine for most amphibians, a recent study comparing benzocaine with MS-222 in metamorphic and paedomorphic tiger salamanders (*Ambystoma tigrinum*) suggested that benzocaine was more effective than MS-222. However, it is important to note that the authors used a lower dose of MS-222 (0.02%) than is generally recommended for amphibians and that they did not buffer the MS-222.<sup>12</sup> Because of the availability and consistency of MS-222 and clove oil, the author does not routinely use benzocaine for amphibians. However, individuals interested in anesthetizing large numbers of animals may want to consider benzocaine over these other compounds because it is less expensive.<sup>12</sup>

### Clove Oil

Clove oil is a naturally occurring compound that has been used as an anesthetic in humans, mammals, fish, and amphibians.<sup>3,6,7,16-19</sup> The active ingredient of clove oil is a phenolic compound, eugenol. Clove oil has been found to produce surgical anesthesia in both frogs and salamanders.<sup>3,6,7</sup>

Dosing regimens for clove oil may vary depending on the species and the life history of the animal. For example, dosing requirements for leopard frogs (*Rana pipiens*) and African clawed frogs, both aquatic species, were lower (318 -350 mg/L) than those required for tiger salamanders (450 mg/L), a terrestrial species.<sup>3,6,7</sup> The immersion methods used to anesthetize amphibians with clove oil are similar to those described previously.<sup>6,7</sup> Attempts to deliver clove oil topically or parenterally through the dorsal lymph sacs in African clawed frogs were unrewarding.<sup>3</sup> Induction times for anesthesia with immersion in both frogs and salamanders were less than 15 minutes. Surgical anesthesia was achieved in all (12/12) of the leopard frogs and the majority (67%; 8/12) of the salamanders exposed to clove

**Figure 3.** Gastric prolapse was a common side effect noted in leopard frogs anesthetized with clove oil.

oil. The salamanders were not exposed to the anesthetic solution for as long (10 minutes) as the frogs (15 minutes), and this may have accounted for the decreased success with inducing surgical anesthesia. All of the animals in both studies had uneventful recoveries from the clove oil anesthesia, with recovery rates in the frogs and salamanders averaging 89 minutes and 75 minutes, respectively.

Clove oil was found to cause respiratory depression in the leopard frogs but not in the tiger salamanders. Bradycardia was noted in both species, although it was transient. Another side effect noted with clove oil in leopard frogs was that 50% (6/12) of the frogs prolapsed their stomach after being removed from the clove oil solution (Fig 3). The gastric prolapse resolved spontaneously in all 6 animals and was attributed to the bad taste of the compound. The topical application of clove oil has been associated with cutaneous necrosis in African clawed frogs, so care should be used when considering the application of clove oil directly onto the skin of an amphibian.<sup>2</sup>

### Isoflurane

Isoflurane is a popular anesthetic that can be delivered to amphibians as an immersion, topical, or inhalant. Attempts at administering isoflurane parenterally (e.g., intracoelomically, intramuscularly, and subcutaneously) have been made in African clawed frogs, but the results were not as consistent or safe as those reported for immersion or topical exposures.<sup>1</sup> Stetter and coworkers<sup>20</sup> used isoflurane as an immersion anesthetic in both African clawed frogs and *Bufo* species. Direct immersion in a 0.28% solution of isoflurane was found to provide surgical anesthesia. The duration and depth of anesthesia

with a direct bath can be prolonged, so caution should be taken when using isoflurane with this method. The best results can be achieved by removing the animal from the solution once it shows signs of sedation (e.g., loss of righting reflex, slowed response to superficial and deep pain). Aerating the isoflurane (5%) into the immersion water was also found to be effective, but induction and recovery rates were faster and longer, respectively, than those described for topical application or a direct bath of the inhalant. Aerating the anesthetic into the immersion solution is not recommended because of the health risk to the humans performing the procedure. Topical application can be achieved by dripping the isoflurane directly onto the animal, saturating a pad with isoflurane and placing the pad on the animal, or mixing the isoflurane with a water-soluble carrier and applying it on the animal.<sup>1,20</sup> Different doses are generally required for amphibians based on their life history. For example, African clawed frogs require a lower dose (e.g., direct application: 0.007 mL/g body weight; mixed with water-soluble gel: 0.025 mL/g body weight) than toads (e.g., direct application: 0.015 mL/g body weight; mixed with water-soluble gel: 0.035 mL/g body weight).<sup>20</sup> For larger specimens, isoflurane can be used as it was intended, as an inhalant. Induction rates for amphibians are generally best achieved at 5%, whereas maintenance levels generally range between 1% and 3%, depending on the type and duration of the procedure.

### Propofol

Propofol is a nonbarbiturate anesthetic that is routinely used to anesthetize reptiles, birds, and mammals. The primary benefits associated with propofol are that it is noncumulative, not a perivascular irritant, and is not associated with prolonged recoveries. The primary disadvantages associated with propofol include cardiopulmonary depression and route of delivery (e.g., in higher vertebrates, it must be given intravenously or intraosseously). Although propofol can be used intravenously or intraosseously in amphibians, it has also been evaluated via intracoelomic injection and immersion.

Intravenous injections of propofol can be given in the femoral vein, sublingual vessels, heart, ventral abdominal vein, or ventral tail vein of amphibians. Caution should be used when injecting compounds into the heart of an amphibian, because this can prove fatal. Intraosseous injections can be given in the tibia or femur. Intracoelomic delivery of propofol has been attempted in White's tree frogs (*Pelodryas caerulea*) and tiger salamanders.<sup>7,8</sup> In the White's tree

frogs, the propofol was administered at 3 different doses: 9.5 mg/kg, 30 mg/kg, and 53 mg/kg. These doses produced moderate anesthesia, surgical anesthesia, and death, respectively. In tiger salamanders, propofol was dosed at 25 or 35 mg/kg. Surgical anesthesia was achieved in the majority (83%; 5/6) of the animals given the higher dose (35 mg/kg), whereas less than half (40%; 2/5) of the animals given 25 mg/kg achieved surgical anesthesia. Cardiopulmonary depression was observed in both groups, with extended periods of apnea. Immersion of African clawed frogs in a propofol solution was found to have few benefits.<sup>4</sup> Propofol doses of 88 and 175 mg/L were found to cause sedation and death, respectively. However, at the lower dose (88 mg/L) the animals were still responsive to noxious stimuli, suggesting that this route of delivery would have minimal benefit.

### Dissociative Agents

Ketamine hydrochloride (HCl; Ketaset<sup>®</sup>, Fort Dodge Animal Health, Fort Dodge, IA USA) and tiletamine HCl (Telazol<sup>®</sup>, Fort Dodge Animal Health) have both been evaluated in amphibians. Although ketamine HCl has been found to provide effective and consistent anesthesia in amphibians, tiletamine HCl has been found to have limited value.<sup>5,21</sup> The primary advantages associated with using ketamine HCl are that it is inexpensive, can be administered intramuscularly, and provides good somatic analgesia. Disadvantages associated with this compound include cardiopulmonary depression and prolonged recoveries. Dosing recommendations for ketamine hydrochloride range from 20 to 210 mg/kg.<sup>5</sup> It is important to use caution with this compound at very high doses, because it cannot be reversed. The author generally recommends ketamine as a preanesthetic, administering doses to animals at 20 to 40 mg/kg intramuscularly. Because dissociatives are not considered to provide visceral analgesia or muscle relaxation in higher vertebrates, the author does not recommend using these compounds as a sole anesthetic for a surgical procedure.

### Medetomidine

The alpha-2 agonists are popular anesthetics in veterinary medicine. The primary advantages associated with these compounds are that they provide sedation, muscle relaxation, visceral analgesia, and are reversible. They are commonly combined with dissociative agents to provide a more complete anesthesia. The primary disadvantage associated with these compounds is cardiopulmonary depression. A study evaluating medetomidine (0.15 mg/kg) in leopard frogs

found that the animals achieved no detectable level of sedation, but cardiopulmonary depression did occur.<sup>6</sup> Additional studies evaluating different species of amphibians and different dosing regimens should be pursued to determine if these compounds have any value as anesthetics for amphibians.

### Anesthetic Monitoring

Anesthetic monitoring of higher vertebrates has improved significantly over the past decade. We now have the ability to use point-of-care anesthetic equipment to measure oxygen saturation, heart rate, respiratory rate, electrocardiograms, end-tidal carbon dioxide, and indirect and direct blood pressures. Unfortunately, for many of our amphibian patients, this monitoring equipment is not sensitive enough to detect their respiratory or circulatory outputs. Although our ability to monitor amphibians may be limited compared with higher vertebrates, there are certainly some methods available that can be used to measure the physiologic and anesthetic status of our amphibian patients undergoing an anesthetic procedure.

### Cardiac

One of the physiologic parameters that can be consistently measured in our amphibian patients is heart rate, and there are a number of different methods available for measuring an amphibian's heart rate. The author's preferred method is to use an ultrasonic Doppler. The heart rate can be measured by placing the Doppler on the ventral pectoral girdle or axillary region of urodelans or anurans (Fig 4).<sup>6,7</sup> An appropriate contact gel should be used to help facil-



**Figure 4.** An ultrasonic Doppler can be placed on the ventral pectoral girdle of an anuran to measure heart rate.

itate the transmission of the heart beat. Because of the sensitivity of amphibian skin, it is important to remove the gel after completing the procedure. A physiologic saline solution (0.9%) can be used to irrigate the area. An electrocardiogram can also be used to measure the heart rate of an amphibian. A 3-lead system can be used as in higher vertebrates. Because alligator clips can damage the fragile skin of an amphibian, the author prefers to place hypodermic needles (25 or 26 gauge) through the skin and to connect the alligator clips to the end of the needles. A pulse oximeter with an appropriate probe can also be used to measure heart rate in larger amphibians. Placing a C-clip probe over a peripheral vessel (e.g., femoral) or inserting a linear probe in the esophagus or cloaca with the probe directed dorsally toward the aorta can be done to measure heart rate. Caution should be used when interpreting oxygen saturation levels on a pulse oximeter in an amphibian. Pulse oximeters were developed for evaluating mammalian values, but can certainly be used to determine oxygen saturation trends in an amphibian.

It is important to obtain a baseline heart rate before starting an anesthetic procedure. Amphibian heart rates are generally much lower (25-80 beats per minute) than those found in higher vertebrates. Knowledge of the baseline heart rate is especially important when attempting to diagnose abnormalities such as bradycardia. The heart rate of an amphibian is dependent on its environmental temperature. Therefore, the animal should be kept at its optimal temperature during the preanesthetic, anesthetic, and postanesthetic periods. This will ensure that the animal can be induced, managed, and recovered from the anesthetic in a consistent matter.

Although measuring the heart rate of an amphibian can be invaluable during an anesthetic procedure, it is important to recognize that the heart of an amphibian can beat after it is clinically dead. Many of us learned this unfortunate lesson during high school biology. Because of this, it is important to recognize that heart rate is but one method we can use to monitor anesthesia in an amphibian, and that measuring heart rate should not be the only monitoring method used.

## Respiratory

In higher vertebrates, respirations can be visually characterized by counting thoracic excursions. In some amphibians this is possible, whereas in others it is not. One of the methods that the author has used to measure respirations is gular movements. In some species, such as chelonians, gular pumping is

thought to be done to assist with olfaction. In anurans, it was found to be consistent with respirations.<sup>6</sup> In some species, such as tiger salamanders, gular pumping did not occur for extended periods of anesthesia with clove oil and propofol.<sup>7</sup> Fortunately, amphibians have developed a highly vascular integument that can assist with respirations. Although there is comfort in knowing that these animals can respire through their skin, it does tend to lead to difficulties when trying to determine whether an animal is apneic or not.

## Reflexes

There are a number of reflexes that can be used to assess the anesthetic depth of an amphibian patient. The methods most commonly used by the author include the escape response, righting reflex, superficial and deep pain responses, and the palpebral reflex. It is important to characterize these reflexes in the patient before initiating an anesthetic procedure, because animals may show different responses to each reflex. A rank scale can be used to assess the different levels of a reflex. The author prefers to record the reflexes as 1 = present, 2 = delayed, or 3 = absent. Surgical anesthesia is generally determined to be when the reflexes are all recorded as being absent.<sup>6,7</sup>

The escape reflex can be assessed by placing the animal in an open palm and determining whether it tries to walk or jump away. Most amphibians have a natural desire to “escape” a perceived predator. Animals that are naturally stoic or long-term captives may not display an active escape response. The righting reflex can be measured by placing the animal on its dorsum and counting the time required to correct



**Figure 5.** The righting reflex can be assessed by placing the animal in dorsal recumbency and measuring the time it takes for the animal to recover. This anesthetized leopard frog had no righting reflex.



**Figure 6.** Superficial and deep pain can be assessed by pinching the skin or digit of an amphibian.

its position (Fig 5). Animals that are sedate generally have a delayed response, whereas those that are at a surgical plane of anesthesia will have no righting reflex. The palpebral reflex can be assessed by touching the upper or lower palpebrae with a cotton-tipped applicator. Animals that are alert will blink on response to this stimulation, whereas animals at a surgical plane of anesthesia will not respond. Superficial pain can be assessed by pinching the skin of an amphibian. The author prefers to use the dorsal or ventral surface of the rear feet (Fig 6). Deep pain can be assessed by grasping a digit. The superficial pain reflex is lost before the deep pain reflex; however, the deep pain reflex returns first.

## Postanesthetic Plan

Amphibians should be recovered from an anesthetic event with the same standard of care used for other vertebrates. The recovery area should be quiet and the environmental temperature and humidity appropriate for that species. Lining the recovery enclosure with paper towels or towels that are premoistened with dechlorinated water will help prevent desiccation. Amphibians can be recovered on room air. Return of the reflexes is important for determining when an animal is fully recovered. Replacing an amphibian into an aquatic environment before being fully recovered could result in an accidental drowning.

## Conclusions

As amphibians are presented to veterinarians for various diagnostic and surgical procedures, it will be important to develop complete and consistent anes-

thetic protocols for these animals. Although amphibian anesthesia has improved over the past decade, there is certainly much more that can be done. In addition to finding new and consistent methods for anesthetizing amphibians, it is also important to develop better methods for monitoring amphibians to minimize the likelihood of complications.

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