# CLINICAL ANESTHESIA AND ANALGESIA IN INVERTEBRATES

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***Abstract***

Invertebrates are an expansive and diverse group of animals that have had little attention regarding anesthesia and analgesia. Economic use, environmental awareness, laboratory research, and increasing demand for invertebrates as pets has lead to a greater desire for knowledge for these animals in the veterinary medical community. With the increasing number of animal welfare regulations, various scientific studies have improved the overall knowledge of invertebrate medicine, but much more research is required to fully understand anesthesia techniques in the different species treated by veterinarians. Analgesia is a controversial and often neglected topic with invertebrates because of the common belief that invertebrates do not feel pain. Recently, the idea that invertebrates do not feel pain has been challenged with the discovery of nociceptive pathways similar to those in vertebrates. This article presents a general overview of anesthetics and analgesics used in selective invertebrate taxa. Copyright 2012 Elsevier Inc. All rights reserved.

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The invertebrates are a collection of animals comprising more than 95% of the earth's species, unified by the lack of a vertebral column, or "backbone." Barnes and Ruppert stated that the invertebrates are a group of unrelated taxa that share no universal "positive" traits.[1]

Depending on the text or specialist(s), there are currently more than 30 recognized phyla of invertebrates (not including the protozoans). Unfortunately, very little is known regarding medicine and, more specifically, anesthesia/analgesia of many of these taxa. A comprehensive review of anesthesia/analgesia for all invertebrate phyla is beyond the scope of this article. Consequently, only the most economically important and familiar metazoan taxonomic groups are included in this review of invertebrate anesthesia and analgesia. We have elected to use the taxonomic terminology currently described in an article by Ruppert and coworkers, with the knowledge that invertebrate zoologists may use a slightly different nomenclature for some groups.[2]

The goal of this article is to provide an overview of anesthetic concerns and techniques in more commonly anesthetized invertebrates, but it should be emphasized that more research is necessary to better understand and improve anesthesia and drugs used in the different, and frequently unrelated, invertebrate species. For example, magnesium chloride is a commonly used anesthetic/muscle relaxant in invertebrate species. Some controversy exists about the ability of magnesium chloride to produce adequate sedation and analgesia via blocking nerve transmission and neurotransmitter release, or acting only as a neuromuscular blocking agent (muscle relaxant).[3] Differences in vertebrate versus invertebrate anatomy/physiology, as well as routes of administra-

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tion, seem to play a role in the pharmacologic effects of therapeutic agents; consequently, the issue remains unresolved and not fully understood.[3] Specific dosing regimens for the compounds mentioned in the text can be found in Table 1 of this article.

## MOLLUSKS

### Anatomy, Physiology, and Natural History

The mollusks are a diverse and large group of animals (approximately 100,000 species) that occupy terrestrial, freshwater, and marine environments. Some mollusk species are extremely critical to the environment because of their ability to filter water and consume debris and detritus. Economically, they are one of the most important taxa on earth, and provide billions of dollars annually as a source of food, animals for pets, display, and research.

Despite their wide variety of function and form, nearly all mollusks have the following traits in common at some point in their life history: gills for respiration, a chitinous radula for feeding, a muscular foot for locomotion, a calcareous shell for protection and a mantle to secrete it, and a ciliated planktonic larval form for dispersal. We have elected to discuss only the most economically important classes.

#### *Gastropods*

*Anatomy, Physiology, and Natural History.* The gastropods are a large and easily recognized group of mollusks that includes the abalone, snails, nudibranchs, and sea hares, among others. Most are aquatic and have a well-developed head with eyes and other sensory organs, an external shell, muscular foot, and gills within a chamber for respiration. However, there are many exceptions to the identifiable criteria listed above, and one need only examine a common garden slug (no shell or gills and terrestrial) to appreciate gastropod diversity.

*Anesthetic Agents Used and Techniques.* Snails can be anesthetized with menthol or 5% ethanol or inhalant agents like isoflurane.[4,5] A 10% Listerine solution (McNeill-PPC, Stillman, NJ USA) in normal saline solution is commonly used to anesthetize snails in research settings.[6] Sodium pentobarbital in water has been reported to have a very slow onset (8 hours) in these species but generates good anesthetic effects and has a low mortality rate.[7]

Anesthesia via isoflurane for terrestrial snails will require an anesthetic chamber with the ability for fresh gas inflow and waste gas scavenging.[5] The mean anesthetic concentration of isoflurane in the pond snail (*Lymnaea stagnalis*) is reported as 1.09.[5] Induction is less than 10 minutes, but an excitatory period is common. The depth of anesthesia may not be adequate for surgical procedures. An anesthesia level reached in snails is defined when body and tentacle withdrawal response to gentle stimulation is absent. Tentacle withdrawal reflex remained under inhalant anesthesia, suggesting an insufficient depth for surgery.[5]

Sea snails (*Aplysia californica*) are commonly anesthetized with intracoelomic administration of magnesium sulfate or magnesium chloride.[3] Induction is fast and smooth, and these compounds provide good muscle relaxation.

*Abalone.* Commercially farmed abalones frequently require physical examination and sizing, pearl seeding, and removal from tanks for maintenance and harvesting.[8] Removal of abalones from the substratum often requires mechanical assistance. This forced removal may result in injury with slow recovery or even death. Therefore, a muscle relaxant or anesthetic agent may be necessary to avoid stress and mechanical injuries related to dislodging the mollusk. Compounds used in abalones for their removal from a tank include 3% ethanol, 2-phenoxyethanol, benzocaine, magnesium sulfate, and sodium pentobarbital.[9,10] Magnesium sulfate is administered in water with dose ranges depending on the size of the abalone (higher doses for larger animals).[8] Induction time is fast and recovery is uneventful. Phenoxyethanol also shows a fast induction period and a good recovery time.[8] Nembutal (sodium pentobarbital) produces good muscle relaxation with an induction time of 15 minutes and complete recovery.[9] Recovery from any anesthetic event includes thorough washing of the abalone and exposure to fresh flowing seawater at their optimal temperature (18°C) until muscle strength returns.

#### *Cephalopods*

*Anatomy, Physiology, and Natural History.* This group of predatory mollusks includes such familiar forms as the chambered nautilus, cuttlefish, octopuses, and squids. Most of these animals are pelagic and have the ability for fast locomotion. They have closed circulatory systems, high metabolic rates, and advanced nervous and sensory systems. All have an internal skeleton (shell) with the exceptions being the octopuses (no shell) and the nautilus (external shell). Other

| TABLE 1. Chemical restraint and anesthetic agents used in invertebrates | | | |
|---|---|---|---|
| **Drug** | **Dosing regimen** | **General comment** | **References** |
| Benzocaine | 100 mg/L | Abalone anesthesia. Not sold as anesthetic in the United States. Available from chemical supply companies. Do not use topical anesthetic products marketed for mammals. Prepare stock solution in ethanol (benzocaine is poorly soluble in water). Store in a dark bottle at room temperature. | 9,44 |
| | 2.5-3 g/L | Euthanasia for cephalopods. | 45 |
| | 400 mg/L | Anesthesia of leeches. This could be applied, with caution, to other aquatic annelids. | 46 |
| Butorphanol | Fish, amphibian, and reptile dosages can be used with care. | For analgesia. Use with caution because dosages are empirical; a biotest is recommended. | |
| Carbon dioxide | Blow gas over terrestrial turbellarians. | | 47,48 |
| | 3% to 5% | Anesthesia of terrestrial arthropods. Isoflurane and sevoflurane may be preferable with regards to recovery; an anesthetic chamber has been developed/described for use in the fruit fly. | 33,49 |
| Chloretone | 0.1% | Anesthesia of turbellarians. | 48,50 |
| Clove oil (Eugenol) | 0.125 mL/L (approx. 125 mg/L) as an immersion | Crustaceans/stock solution: 100 mg/L of eugenol by diluting 1 part clove oil with 9 parts 95% ethanol (eugenol is poorly soluble in water); over-the-counter preparations available at most pharmacies contain approximately 1 g eugenol per mL clove oil but are not usually 100% pure. | 26 |
| Ethanol | 1.5% to 3% solution | Cuttlefish (*Sepia* sp.), may not be effective for cold water cephalopods. | 11 |
| | 3% solution | Abalone anesthesia. | 44 |
| | 5% solution | Anesthesia of aquatic gastropods. | 4,49 |
| | 5% solution | Oligochaetes. Adequate for terrestrial earthworms such as *Lumbricus terrestris*. | 50,51 |
| | 10% solution | Anesthesia of turbellarians. | 48,52-54 |
| | 10% solution | Euthanasia of cephalopods. | 45 |
| Ethanol/menthol (Listerine) | 10% in Lymnaea saline solution | Anesthesia of aquatic gastropods. | 6 |
| Hydroxylamine hydrochloride | 1% solution | Anesthesia of turbellarians. | 48,53 |
| Ice | Used to effect. Pure ice water can also be used. | For turbellarian anesthesia. Cover ice with filter paper to protect the flatworms. | 48,53,55 |
| Isoflurane | | Can be used with an anesthetic chamber. For anesthesia of terrestrial gastropods; arachnids. Fast induction with a possible excitatory period. The anesthetic depth may not be appropriate for invasive surgery of gastropods. The gas is usually applied at a 5% concentration for spiders and other arachnids. | 5,20-22,64 |
| Ketamine | 40-90 $\mu$g/g IM | Anesthesia of crayfish with an induction time of less than 1 min and a duration of 10 min at the low dose and 2 h at the higher dose. | 25 |
| | 0.025-1 mg/kg | Anesthesia of Australian giant crabs. Fast induction (less than 30 sec) with an excitatory phase followed by dose-dependent anesthetic duration of 8 to 40 min. | 26 |

TABLE 1. Continued

| Drug | Dosing regimen | General comment | References |
|---|---|---|---|
| Lidocaine | 0.4-1 mg/g IM | Anesthesia of crayfish with an induction time of less than 2 min and duration of anesthesia of 5 to 30 min when injected into the tail. | 25 |
| Magnesium chloride | 1:1 isotonic salt and seawater as an immersion | Anesthesia of marine turbellarians. | 48,56 |
| | Intracoelomic | Anesthesia of sea hares (*Aplysia* sp.) with short induction time (2-5 min) and good muscle relaxation. | 3 |
| | 6.8 g/L | Anesthesia of cephalopods. Induction time of 6 to 12 min reported in cuttlefish (*Sepia* sp.). | 13,44 |
| | 30-50 g/L | Anesthesia of scallops. Fast induction time and recovery. | 19 |
| | 1:1 mixture of 7.5% with seawater | Anesthesia of echinoderms. Adjustments in concentration may be required for long procedures. | 34,57 |
| | 7.5% immersion | Anesthesia of polychaetes. | 58,59 |
| | 10% solution | Euthanasia of cephalopods. | |
| Magnesium sulfate | Intracoelomic | Anesthesia of sea hares (*Aplysia* sp.) with short induction time (2-5 min) and good muscle relaxation. | 3 |
| | 4-22 g/100 mL | Abalone anesthesia with fast induction and good recovery. | 8 |
| Potassium chloride | 1 g/kg IV (330 mg/mL solution) | Euthanasia of American lobsters. Inject solution at base of the second walking leg. | 60 |
| 2-phenoxyethanol | 0.5-3 mL/L | Abalone anesthesia with fast induction and a short recovery period. | 8 |
| | 1-2 mL/L | | 44 |
| Procaine | 25 mg/kg IV | Anesthesia of crabs with a very short induction time (less than 30 sec) and prolonged anesthesia (2-3 h). | 27 |
| Propylene phenoxetol | 1-3 mL/L of a 1% solution | Anesthesia of oysters. This concentration should produce anesthesia in less than 15 min with a short recovery time (under 30 min). Higher doses can be used but induce a deeper level of anesthesia. This can also be used for giant clams. | 16,17,44 |
| Sevoflurane | Can be used with an anesthetic chamber at a 5% concentration. | Anesthesia of terrestrial arthropods. See isoflurane for details of administration. | 44 |
| Sodium azide | 100 mmol/L | Anesthesia of nematodes (*Caenorhabditis elegans*). This species can survive for 2 h without apparent harm. Sodium azide is a plant and animal cell mutagen, so proper hygiene and handling are advised. | 61,62 |
| Sodium bicarbonate (Alka-Seltzer; Bayer HealthCare, Wayne, NJ USA) | Two tablets/0.5 to 1.0 L bath | For euthanasia of aquatic invertebrates. This generates $CO_2$ and should only be used when other agents are unavailable. Keep aquatic invertebrate in solution > 10 min after respiration stops. This dosage is based on the piscine literature. | 63 |
| Sodium pentobarbital | 400 mg/L | Aquatic gastropods anesthesia. Very slow onset but apparently safe. This is a controlled drug and should be handled and monitored accordingly. | 7 |
| | 1 mL/L | Anesthesia of abalone. | 9 |

TABLE 1. Continued

| Drug | Dosing regimen | General comment | References |
|---|---|---|---|
| Tricaine methanesulfonate (MS-222; Finquel, Argent Laboratories, Redmond, WA USA) | Dosages and efficacy vary widely depending on species and application. Consult the taxon-specific literature for details. | General anesthesia. Stock solution: 10 g/L, buffer the acidity by adding sodium bicarbonate at 10 g/L or to saturation. Store stock solution in a dark container. The shelf life of stock can be extended by refrigeration or freezing. Stock that develops an oily film should be discarded. Aerate the water during anesthesia to prevent hypoxemia. For euthanasia, keep the animal in solution > 20 min after respiration stops. | 44,49 |
| Xylazine | 16-22 mg/kg IV | Anesthesia of giant crabs. Fast induction (3-5 min) and approximately 30 min of anesthesia (dose dependent). | 26 |

Abbreviations: IM, Intramuscularly; IV, Intravenously.

interesting features include the ability to "ink" when disturbed or threatened and suction-cup discs on the arms. All of the approximately 700 species of cephalopods are marine.

*Anesthetic Agents Used and Techniques.* Cephalopods should ideally be anesthetized in their own seawater to maintain a mineral balance between their bodies and the water. The two most commonly used anesthetic agents are magnesium chloride and ethanol.

Cuttlefish (*Sepia officinalis*) have been successfully anesthetized with 1.5% to 3% ethanol diluted in seawater.[11] At higher concentrations inductions are rapid, but there is a risk of overdosing; at lower doses occasional transient excitement has been noticed.[11] Adequate dilution to a lower concentration immediately after induction will optimize anesthetic outcome. If the procedure cannot be performed in the water, anesthesia is maintained with a recirculating anesthesia system.[12] Anesthetic concentration is adjusted over time to the depth of anesthesia desired (Fig 1).

If magnesium chloride is used as an anesthetic agent in cuttlefish, a 7.5% stock solution is prepared with distilled water.[13,14] This stock solution is mixed with a known amount of seawater to prepare an anesthetic concentration suitable for induction. A final concentration of 6.8 g/L has been reported to have a short induction time without side effects.[13]

Octopuses (*Octopus vulgaris*) are anesthetized in a similar manner with either ethanol or magnesium chloride. Ethanol can stimulate excitement during the short induction time expressed by increased respiratory rate, attempts to climb out of the solution, and/or ink ejection.[15] Induction time has been reported to be 4 minutes and time to full recovery 2.5 minutes.[15] Ethanol is not effective in cold water octopus species (below 15°C) (I. G. Gleadall, personal communication, 2006).

Cephalopods are commonly monitored with visualization of respiratory rate and pattern. Normal conscious values for *Octopus vulgaris* in a weight range from 100 to 800 g is 26 to 30 breaths/minute.[15] The cardiovascular system is assessed by placing a Doppler ultrasound probe (Parks Medical Electronics Inc., Aloha, OR USA) on the dorsal area (above the aorta) or behind the gills (above either branchial heart) to monitor heart rate and blood flow. In transparent spe-

FIGURE 1. A cuttlefish (*Sepia officinalis*) under general anesthesia with dilute ethanol. A recirculating anesthesia apparatus is being used to supply a steady flow of water over the gills.

cies the organs in the dorsal mantle can be observed. Depth of anesthesia is difficult to assess in cephalopods. One guideline is that the level of anesthesia seems adequate when no response to tactile and surgical stimuli is present.[15] Further indicators of anesthetic depth of cephalopod species are the flaccidity of the arms, loss of normal posture, and inability to regain normal bearing after disturbance.[15] Respiration usually remains spontaneous; a depression or cessation is a sign of critically deep anesthesia.

For recovery, the cephalopod is placed into a container with aerated, anesthesia-free seawater. If spontaneous respiration is not present, gentle and slow mantle massage can be used until respiration is restored. The tentacles remain extended and flaccid in the first phase of the recovery and will retract in response to light pinching as the animal regains consciousness.[11] Resuscitation of cephalopods includes squeezing and relaxation of the whole mantle/body for water circulation over the gills and hemolymph through the body.[11] Anesthesia-free water can be directed over the gills for a washout effect to enhance the recovery phase.

### Bivalves

*Anatomy, Physiology, and Natural History.* This large and economically important group of highly evolved mollusks includes the clams, mussels, oysters, and scallops. Bivalves lack a well-developed head, feed by filtration using the gills for food transport, and use a muscular foot for locomotion. All of the approximately 8000 described species are aquatic with nearly 80% being marine.

*Anesthetic Agents Used and Techniques.* Oysters. Propylene phenoxetol is used as a 1% solution. High doses induce a rapid and relative deep level of anesthesia and may require dilution during a procedure to decrease the recovery time.[16,17] The oyster should be placed hinge down in the solution and leaned against the wall of an aerated container to facilitate monitoring. Induction time is reported to be between 6 and 15 minutes, and adequate anesthesia is reached when the oyster gapes wide enough to part the gill curtain and shows no responsiveness to handling or contraction of the tissue to a stimulus.[16,17] Decreasing handling stress, with a rest period before placing oysters into the anesthesia container, will improve the anesthetic effects. Recovery tanks should be aerated, with recovery time dependent on length of procedure, concentration of anesthetic, and temperature.[17]

Magnesium chloride has variable effects on oysters, with some investigators describing little effect of magnesium chloride in pearl oysters, mainly because of long induction times (1-2 hours).[16,17] Culloty and Mulcahy reported good anesthetic effects, but also long induction and recovery times (90 minutes) at 3.5%.[18] The effect of magnesium chloride seems dependent on species and concentration of the anesthetic agent used.

*Scallops.* Anesthesia in scallops is required mainly for muscle relaxation. In general, the depth of anesthesia/relaxation is adequate when handling and stimulating of the mantle tissue fails to stimulate shell closure. Recovery is often defined as the animal's regained ability to close its shell.[19]

Magnesium chloride is the anesthetic drug of choice in scallops because of its rapid and consistent induction and recovery. The agent is predissolved in seawater and then added to the aerated induction container to reach a concentration of 30 to 50 g/L. Induction times at these concentrations are quick and in the range of 2 to 6 minutes. Recovery time in scallops anesthetized with magnesium chloride is consistently short regardless of concentration used or water temperature.[19] Seawater should be used in aerated recovery tanks and for continuously flushing the animal to facilitate recovery.

*Giant Clams.* Giant clams (*Tridacna* sp.) have been anesthetized with propylene phenoxetol.[16]

## ARACHNIDA

The arachnids are a large group of animals comprised of approximately 70,000 described species of terrestrial, carnivorous chelicerates.[2] All Chelicerata, which includes the horseshoe crabs, scorpions, and sea spiders (Pycnogonida), belong to the phylum Arthropoda. Mites, spiders, and ticks make up the bulk of the arachnid species. Tarantulas (Mygalomorphae) represent an important group of arachnids that occasionally require veterinary medical care.

### Spiders (Aranae)

***Anatomy, Physiology, and Natural History.*** There are approximately 40,000 described species of spiders (Araneae) belonging to 3000 genera, with thousands more species that have not been described.[2]

Spiders range in size from a body of less than a millimeter to over 9 cm.[2] The basic spider body plan includes two large segments, the cephalothorax and the abdomen, connected to each other by the pedicel. All spiders have four pairs of walking legs, paired chelicerae, and paired pedipalps. The venomous fangs, used to immobilize prey, are located at the tip of the chelicerae. The spinneret, located at the distal end of the abdomen, spins silk, an ability that virtually all spiders possess.

Spiders breathe through book lungs, trachea, or both. The heart is large and located in the dorsal abdominal segment. Spiders possess an open circulatory system with the hemolymph functioning in oxygen transport, immune defense, waste removal, and limb mobility.

Spiders have two main cephalothorax nerve centers, the subesophageal and supraesophageal ganglia, and sense their environment with vision, tactile reception, chemoreception, and vibration detection. Their sensing ability is accomplished with four pairs of eyes, tactile hairs, chemosensory hairs, and slit sense organs.[2]

***Anesthetic Agents Used and Techniques.*** Spiders are commonly anesthetized with inhalant anesthetic agents, with many compounds having been used over the years. Of the potent inhalant anesthetics, halothane is least desirable for invertebrate anesthesia, because of the high likelihood of potential toxicity for the veterinarian and support personnel during gas delivery.

Several different induction chambers have been described and used successfully for delivering inhalant anesthesia to spiders.[20-22] These chambers are either commercially available (invertebrate-specific or regular small mammal induction chambers) with appropriate fresh gas inflow and scavenging outflow, or simple self-made clear plastic containers (Fig 2).

**FIGURE 2.** An anesthetic induction chamber constructed from a plastic container attached to inflow and outflow hoses. The animal (a tarantula in this image) is placed within the chamber and anesthetic gas is applied accordingly. Photograph courtesy of Michele Mehalick.

*Of the potent inhalant anesthetics, halothane is least desirable for invertebrate anesthesia, because of the high likelihood of potential toxicity for the veterinarian and support personnel during gas delivery.*

Plastic containers without an air flow system may be used by placing a cotton wool swab soaked with a small amount of inhalant agent into the chamber. The spider should be placed in a separate smaller container with ventilation pores. The porous container holding the spider is placed into the larger box allowing the inhalant to reach the spider while ensuring that the animal cannot come into direct contact with the cotton swab. This method is not ideal because of the higher anesthetic exposure risk to personnel, less control of the anesthetic given, and a higher risk of overdose. An induction chamber using a precision vaporizer is much preferred. The advantages of the chamber technique are convenience, low cost, and safety to the patient. The disadvantage is that the animal can only be temporarily sedated or anesthetized. For any physical examination or surgery the animal is removed from the chamber, limiting the time for any procedure before recovery, and may require repeated inductions; this also increases the exposure of humans to the anesthetic gases. A surgery chamber has been developed that allows the clinician to perform the surgery or other manipulations on the patient without removal from the chamber.[21]

Another interesting technique is to induce the spider with an anesthetic chamber and then place its abdomen (with the associated respiratory organs) into a smaller chamber "sealed" with a latex glove. This technique appears to have some merit when a procedure must be performed on the cephalothorax or limbs (D. Dombrowski, personal communication, 2006).

The most commonly used anesthetic agents for spiders are carbon dioxide (Fig 3), isoflurane, and sevoflurane.[20-22] To increase filling time in larger chambers the oxygen flow rate is high at the beginning (1-3 L/min), but can be decreased to a minimum if there are no major leaks in the system (300-1000 mL/min). Oxygen flow below 200 mL/min decreases vaporizer accuracy and may reduce the amount of anesthetic agent in the chamber because of the uptake by the animal; the amount of carbon dioxide in the chamber may also be elevated with low gas flows. An


FIGURE 3. Although inhalant agents like isoflurane and sevoflurane are preferred, carbon dioxide can be used for anesthesia of air-breathing invertebrates, like this tarantula. Photograph courtesy of Larry S. Christian.

appropriate scavenging system is necessary to decrease pollution and human exposure to anesthetic gases. Other agents like carbon dioxide and nitrogen as well as hypothermia have been used to immobilize spiders.[22,23]

Observing the spider for righting reflexes assists monitoring depth of anesthesia. During induction it may take 10 to 15 minutes until full immobilization has occurred. Throughout the medical procedure monitoring the patient for leg movements in response to stimuli is an obvious sign of insufficient anesthetic depth. An increase in heart and respiratory rate is another sign of inadequate depth of anesthesia. A deep level of anesthesia is more difficult to evaluate, and slow respiratory rate and low heart rates are often the only way to assess a patient for excessive anesthesia. An analgesic administered for painful stimulations may make it easier to maintain a consistent "surgical plane" of anesthesia.

Respiratory rate is observed at the cranial lateral side of the animal with the heart lying under the dorsal surface of the body. A heart rate can be obtained by placing a Doppler transducer over the dorsal surface of the spider's body (Fig 4). A pinpoint or regular Doppler transducer can be used with a drop of coupling gel. Normal heart rates are considered to be 30 to 70 beats per minute in large spiders and up to 200 in smaller species. Monitoring of heart rate is usually reserved to larger spiders.

After turning off the inhalant anesthetic gas and maintaining the animal on fresh oxygen or room air, recovery from the procedure is gradual and can take between 3 and 20 minutes. When fully awake the animal should be returned to its enclosure and maintained at its preferred ambient temperature. Feeding should be withheld for 48 hours after anesthesia.[22]

## Scorpions

***Anatomy, Physiology, and Natural History.*** There are about 1200 species of scorpions; most are nocturnal and found in the tropics and subtropics.[2] They belong to the order Scorpiones and share many traits with spiders.

The basic anatomy of scorpions consists of a cephalothorax, segmented abdomen, and telson, or stinger.[2] Although scorpions are infamous for their stinger and venom, a scorpion envenomation is rarely fatal to humans, usually inducing only pain and discomfort.

***Anesthetic Agents Used and Techniques.*** Scorpions are anesthetized similarly to spiders (see previous section). An induction chamber using anesthetic

FIGURE 4. A Doppler ultrasound device (Parks Medical Electronics Inc., Aloha, OR USA) being used to find the heart rate of an anesthetized tarantula. A properly functioning unit can detect cardiac pulses in various invertebrates.

gas in oxygen administered via a precision vaporizer is recommended.

## Crustaceans

***Anatomy, Physiology, and Natural History.*** The crustaceans are a large and diverse group of arthropods that are all aquatic at some stage in their life. Some biologists consider this group, with over 40,000 species, a subphylum of the phylum Mandibulata.[2] Other scientists still consider crustaceans a class of the mandibulates with an aquatic nauplius larva, two pairs of antennae, compound eyes, biramous appendages, segmented excretory organs, and a well-developed protective carapace.[24] The majority of the most conspicuous crustaceans belong to the order Decapoda, which includes the crabs, crayfish, hermit crabs, lobsters, and shrimp.

***Anesthetic Agents Used and Techniques.*** Crustaceans can be anesthetized with various agents. Depending on the animal's size and procedure, MS-222 (Fig 5), isobutyl alcohol, and intramuscular injections of lidocaine, ketamine, or xylazine have been reported (Table 1).[25-28]

***Anesthetic Monitoring.*** Crustacean heart rates can be assessed by applying electrocardiograph pads with ample conduction gel on the shell above the heart (Fig 6). The normal heart rate for lobsters is between five and 20 beats per minute with a circadian influence (higher at night) and 30 to 70 beats per minute for the shore crab, depending on pH and temperature of water.[29,30] The depth of anesthesia is evaluated in crustaceans by the relaxation of the body, ability to withdraw extremities, and very slow antennae withdrawal.

**FIGURE 5.** This spiny lobster (*Panularis* sp.) is under anesthesia with 0.1% (1 mL/L of 100 mg/L stock solution) eugenol. The depth of anesthesia and gill irrigation is being managed with a large syringe and red rubber catheter. Photograph courtesy of Larry S. Christian.

**FIGURE 6.** The heart rate of this blue crab (*Callinectes sapidus*) is being monitored with both a Doppler ultrasound and electrocardiograph leads.

## Insects

***Anatomy, Physiology, and Natural History.*** The insects, or Hexapoda, are an incredibly diverse group with nearly a million described species. Most are terrestrial, some are aquatic, and the only habitat they have not exploited is the ocean. Insects are arthropods with three major body segments (head, thorax, abdomen) and three pairs of legs. Most have keen eyesight, sensory antennae, well-developed mouthparts, and wings. They have an open circulatory system that contains hemolymph; gases are exchanged through spiracles that open into a system of tracheae.

***Anesthetic Agents Used and Techniques.*** Carbon dioxide remains a popular agent to immobilize insects for entomological research, although negative effects, including convulsion and excitation at induction, are well recognized and the risk of mortality is high.[31,33] The use of carbon dioxide as an anesthetic agent for insects remains controversial and a more progressive approach would be a volatile anesthetic agent (e.g., isoflurane, sevoflurane). This requires a chamber apparatus to allow for appropriate delivery and scavenging of the inhalant agent.[33]

## ECHINODERMS

### Anatomy, Physiology, and Natural History

The echinoderms are a phylum of about 6000 marine species in six classes that all share, at least at some point in their life history, pentamerous radial symmetry. Familiar members include the brittle stars, feather stars, sea lilies, sea stars, sand dollars, sea biscuits, sea urchins, and sea cucumbers. Echinoderms have a water vascular system used for feeding, locomotion, and transport of coelomocytes (immune response cells). Nearly all echinoderms have a skeleton composed of calcareous ossicles, and in some cases, these ossicles are fused to form an external skeleton or test (e.g., sea urchins). The nervous system is composed of a nerve ring with associated radial nerves without ganglia.

### Anesthetic Agents Used and Techniques

Echinoderms can be anesthetized/immobilized with magnesium chloride or MS-222.[34] Ideally they are anesthetized in their own seawater, to keep temperature and water content consistent. Other reported anesthetic agents are menthol and propylene phenoxetol (2 mL/L in seawater).[35-37]

*Whether invertebrates "feel pain" is still an unanswered topic of debate. The crux of the debate may lie in the differentiation between nociception and pain.*

## PAIN MANAGEMENT

Whether invertebrates "feel pain" is still an unanswered topic of discussion. The crux of the debate may lie in the differentiation between nociception and pain. Nociception describes the neurophysiologic components leading to the sensation of pain but excludes the central perception of nociceptive input that leads to pain sensation. Strictly defined, pain is the "subjective sensation or emotional experience" resulting from nociception and is an experience that is created in the cerebral cortex. Although invertebrates do not possess a central nervous system with a well-described cortex or similar structure, it has been shown that a nociceptive response is present in invertebrates, that nociceptor cells are present, and that opioid systems have a functional role in invertebrate nociception.[38-42] No definitive answer exists for the debate of whether invertebrates perceive pain and would suffer emotional stress from it. The animals' response to mechanical, chemical, and electrical stimulus is seen by withdraw-and-escape behaviors. This response can be decreased or slowed when an analgesic is used.[43] It is not clear if this decreased response is due to analgesia, muscle relaxation, or sedation. Until the question of pain in invertebrates is answered, an analgesic should be administered to any animal that is subjected to a painful procedure, considering that some drugs used for invertebrates may have muscle-relaxing properties and lack some anesthetic potency.

Hypothermia and $CO_2$ do not possess analgesic properties and an inhalant agent is preferred. Although inhalant agents are not true analgesic properties, they do render the mammalian patient insensible to painful stimuli when administered at sufficient anesthetizing doses. Unfortunately, the insensitivity to painful stimuli only lasts as long as the animal is anesthetized; therefore, if the procedure is expected to be associated with significant postoperative pain, the administration of an analgesic would be advisable. Diluted lidocaine may be used as a topical analgesic agent on the body surface above a surgery site.

## CONCLUSION

We are still far from understanding all of the details of the physiology, pathophysiology, and anatomy of invertebrates. Much more research is needed to improve the use and increase veterinary medical knowledge of anesthetics and analgesics for invertebrate patients. A guideline of drugs and doses is offered in this article, but at this time some creativity is also needed to successfully anesthetize invertebrate animals.[32]

## REFERENCES


1. Barnes RD, Ruppert EE: Invertebrate Zoology (ed 6). Philadelphia, PA, Saunders College Publishing, 1994
2. Ruppert EE, Fox RS, Barnes RD: Invertebrate Zoology: A Functional Evolutionary Approach (ed 7). Belmont, CA, Thompson-Brooks/Cole, 2004
3. Clark TR, Nossov PC, Apland JP, et al: Anesthetic agents for use in the invertebrate sea snail, *Aplysia californica*. Contemp Top Lab Anim Sci 35:75-79, 1996
4. Flores DV, Salas PJI, Vedra JPS: Electroretinography and ultrastructural study of the regenerated eye of the snail *Cryptomphallus aspera*. J Neurobiol 14:167-176, 1983
5. Girdlestone D, Cruickshank SGH, Winlow W: The actions of three volatile anaesthetics on withdrawal responses of the pond-snail, *Lymnaea stagnalis* (L.). Comp Biochem Physiol C 92:39-43, 1989
6. Woodall AJ, Naruo H, Prince DJ, et al: Anesthetic treatment

blocks synaptogenesis but not neuronal regeneration of cultured *Lymnaea* neurons. J Neurophysiol 90:2232-2239, 2003
7. Martins-Sousa RL, Negrao-Correa D, Bezerra FSM, et al: Anesthesia of *Biomphalaria* spp. (Mollusca, Gastropoda): sodium pentobarbital is the drug of choice. Mem Inst Oswaldo Cruz 96:391-392, 2001
8. White HI, Hecht T, Potgieter B: The effect of four anaesthetics on *Haliotis midae* and their suitability for application in commercial abalone culture. Aquaculture 140:145-151, 1996
9. Aquilina B, Roberts RA: Method for inducing muscle relaxation in the abalone, *Haliotis iris*. Aquaculture 190:403-408, 2001
10. Edwards S, Burke C, Hindrum S, et al: Recovery and growth effects of anaesthetic and mechanical removal on greenlip (*Haliotis laevigata*) and blacklip (*Haliotis rubra*) abalone. J Shellfish Res 19:510, 2000
11. Harms CA, Lewbart GA, McAlarney R, et al: Surgical excision of mycotic (*Cladosporium* sp.) granulomas from the mantle of a cuttlefish (*Sepia officinalis*). J Zoo Wildl Med 37:524-530, 2006
12. Lewbart GA, Harms CA: Building a fish anesthesia delivery system. Exot DVM 1:25-28, 1999
13. Gore SR, Harms CA, Kukanich B, et al: Enrofloxacin pharmacokinetics in the European cuttlefish, *Sepia officinalis*, after a single i.v. injection and bath administration. J Vet Pharm Therap 28:433-439, 2005
14. Scimeca JM, Forsythe JW: The use of anesthetic agents in cephalopods. Proc Int Assoc Aquat Anim Med 127:88, 1999
15. Andrews PLR, Tansey EM: The effects of some anesthetic agents in *Octopus vulgaris*. Comp Biochem Physiol C 70: 241-247, 1981
16. Mills D, Tlili A, Norton J: Large-scale anesthesia of the silver-lip pearl oyster, *Pinctada maxima* Jemeson. J Shellfish Res 16:573-574, 1997
17. Norton JH, Dashorst M, Lansky TM, et al: An evaluation of some relaxants for use with pearl oysters. Aquaculture 144:39-52, 1996
18. Culloty SC, Mulcahy MF: An evaluation of anesthetics for *Ostrea edulis* (L.). Aquaculture 107:249-252, 1992
19. Heasman MP, O'Connor WA, Frazer AWJ: Induction of anaesthesia in the commercial scallop, *Pecten fumatus* Reeve. Aquaculture 131:231-238, 1995
20. Cooper JE: Invertebrate anesthesia. Vet Clin North Am Exot Anim Pract 4:57-67, 2001
21. Melidone R, Mayer J: How to build an invertebrate surgery chamber. Exot DVM 7 5:8-10, 2005
22. Pizzi R: Spiders, in Lewbart GA (ed): Invertebrate Medicine (ed 2). Ames, IA, Wiley-Blackwell Publishing (in press)
23. Madsen B, Vollrath F: Mechanism and morphology of silk dawn from anesthetized spiders. Naturwissenschaften 87: 149-153, 2000
24. Noga EJ, Hancock AL, Bullis RA: Crustaceans, in Lewbart GA (ed): Invertebrate Medicine (ed 2). Ames, IA, Wiley-Blackwell Publishing (in press)
25. Brown PB, White MR, Chaille J, et al: Evaluation of three anesthetic agents for crayfish (*Orconectes virilis*). J Shellfish Res 15:433-435, 1996
26. Gardner C: Options for humanely immobilizing and killing crabs. J Shellfish Res 16:219-224, 1997
27. Oswald RL: Immobilization of decapod crustaceans for experimental purposes. J Mar Biol Assoc 57:715-721, 1997
28. Ferraro EA, Pressacco L: Anesthetic procedures for crustaceans. An assessment of isobutanol and xylazine as general anaesthetics for *Squilla mantis* (Stomapoda). Mem Biol Mar Oceanogr 12:471-475, 1996
29. Aguzzi J, Abello P, Depledge MH: Endogenous cardiac activity rhythms of continental slope *Nephrops norvegicus* (Decapoda: Nephropidea). Mar Fresh Behav Physiol 37: 55-64, 2004
30. Styrishave B, Andersen O, Depledge MH: In situ monitoring of heart rates in shore crabs *Carcinus maenas* in two tidal estuaries: effects of physico-chemical parameters on tidal and diel rhythms. Mar Fresh Behav Physiol 36:161-175, 2003
31. Nicolas G, Sillans D: Immediate and latent effects of carbon dioxide on insects. Ann Rev Entomol 34:97-116, 1989
32. Valles SM, Koehler PG: Influence of carbon dioxide anesthesia on chlopyrifos toxicity in the German cockroach (*Dictyptera: Blattellidae*). J Econ Entomol 87:709-713, 1994
33. Walcourt A, Ide D: A system for the delivery of general anesthetics and other volatile agents to the fruit-fly *Drosophila melanogaster*. J Neurosci Meth 84:115-119, 1998
34. Harms CA: Echinoderms, in Lewbart GA (ed): Invertebrate Medicine (ed 2). Ames, IA, Wiley-Blackwell Publishing (in press)
35. Costello DP, Henley C: Methods of Obtaining and Handling Marine Eggs and Embryos (ed 2). Woods Hole, MA, Marine Biological Laboratory, 1971
36. Van den Spiegel D, Jangoux M: Cuverian tubules of the holothuroid *Holothuria forskali* (Echinodermata): a morphofunctional study. Mar Biol 96:263-275, 1987
37. Hendler G, Miller JE, Pawson, DL, et al: Sea Stars, Sea Urchins and Allies: Echinoderms of Florida and the Caribbean. Washington DC, Smithsonian Institution Press, 1995
38. Nicholls JG, Baylor DA: Specific modalities and receptive fields of sensory neurons in the leech. J Neurophysiol 31:740-756, 1968
39. Kavaliers M: Evolutionary and comparative aspects in nociception. Brain Res Bull 21:923-931, 1988
40. Kavaliers M, Hirst M, Teskey GC: A functional role for an opiate system in snail thermal behavior. Science 220:99-101, 1983
41. Fiorito G: Is there "pain" in invertebrates? Behav Proc 12:383-388, 1986
42. Smith JA: Question of pain in invertebrates. ILAR J 33:25-31, 1991
43. Kavaliers M, Hirst M: Tolerance to morphine-induced thermal response in terrestrial snail, *Cepaea nemoralis*. Neuropharmaology 22:1321-1326, 1983
44. Gunkel C, Lewbart GA: Invertebrates, in West G, Heard D, Caulkett N (eds): Zoo Animal & Wildlife Immobilization and Anesthesia. Ames, IA, Blackwell Publishing, pp 147-158, 2007
45. Scimeca J: Cephalopods, in Lewbart GA (ed): Invertebrate Medicine (ed 2). Ames, IA, Wiley-Blackwell Publishing (in press)
46. Cooper JE, Mahaffey P, Applebee K: Anaesthesia of the medicinal leech (*Hirudo medicinalis*). Vet Rec 118:589-590, 1986
47. Makino N, Shirasawa Y: Biology of long slender land planarians (Turbellaria) in Tokyo and environs. Hydrobiologia 132:229-232, 1986
48. Bodri M: Turbellarians, in Lewbart GA (ed): Invertebrate Medicine (ed 2). Ames, IA, Wiley-Blackwell Publishing (in press)
49. Gunkel C, Lewbart GA: Anesthesia and analgesia of invertebrates, in Fish R, Danneman P, Brown M, et al (eds):

Anesthesia and Analgesia in Laboratory Animals (ed 2). St. Louis, MO, Elsevier/Saunders, pp 535-546, 2008
50. Cooper EL: Transplantation immunity in annelids. Transplantation 6:322-337, 1968
51. Marks DH, Cooper EL: *Aeromonas hydrophila* in the coelomic cavity of the earthworms *Lumbricus terrestris* and *Eisenia foetida*. J Invert Pathol 29:382-383, 1977
52. Best JB, Morita M: Planarians as a model system for in vitro teratogenesis studies. Teratogen Carcinog Mut 2:277-291, 1982
53. Pennak RW: Turbellaria (flatworms), in Pennak RW (ed): Freshwater Invertebrates of the United States (ed 2). New York, NY, John Wiley and Sons, pp 114-141, 1978
54. Winsor L, Johns PM, Barker GM: Terrestrial planarians (Platyhelminthes: Tricladida: Terricola) predaceous on terrestrial gastropods, in Barker GM (ed): Natural Enemies of Terrestrial Molluscs. Cambridge, CABI Publishing, pp 227-278, 2004
55. de Campos-Velho NMR, Lopes KAR, Hauser J: Morphometry of the eyes in regenerant of genus *Dugesia* (Platyhelminthes, Turbellaria, Dugesiidae). Braz J Biol 64:1-9, 2004
56. Blair KL, Anderson PAV: Properties of voltage-activated ionic currents in cells from the brains of the triclad flatworm *Bdelloura candida*. J Exp Biol 185:267-286, 1993
57. McCurley RS, Kier WM: The functional morphology of starfish tube feet: the role of a crossed-fiber helical array in movement. Biol Bull 188:197-209, 1995
58. Lewbart G, Riser N: Nuchal organs of the polychaete *Parapionosyllis manca* (Syllidae). Invert Biol 115:286-298, 1996
59. Müller MCM, Berenzen A, Westheide W: Experiments on anterior regeneration in *Eurythoe complanata* ("Polychaeta," Amphinomidae): reconfiguration of the nervous system and its function for regeneration. Zoomorphology 122:95-103, 2003
60. Battison A, MacMillans R, MacKenzie A, et al: Use of injectable potassium chloride for euthanasia of American lobsters (*Homarus americanus*). Comp Med 50:545-550, 2000
61. Avery L, Horvitz HR: Effects of starvation and neuroactive drugs on feeding in *Caenorhabditis elegans*. J Exp Zool 253:263-270, 1990
62. Bodri M: Nematodes, in Lewbart GA (ed): Invertebrate Medicine (ed 2). Ames, IA, Wiley-Blackwell Publishing (in press)
63. Gratzek JB (ed). Aquariology: The Science of Fish Health Management. Master volume (Aquariology Series). Morris Plains, NJ, Tetra Press, 1994
64. Dombrowski D, De Voe R: Emergency care of invertebrates. Vet Clin North Am Exot Anim Pract 10:621-645, 2007