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Anesthesia with Tricaine Methanesulfonate (MS222) and Propofol and Its Use for Computed Tomography of Red Swamp Crayfish (*Procambarus clarkii*)

Michael Palillo,[1] Jack Palillo,[2] Nonyé Williams,[1] Mary White,[3] Mael Glon,[4] Lauren Pintor,[5] Willie Bidot,[6] Nguyen K Tram,[7] Mitchel R Stacy,[7,8] Genevieve Kendall,[9,10] Dondrae Coble,[11] and Raphael Malbrue[11,*]

Crayfish (Decapoda: Astacoidea and Parastacoidea) are among the few animals that have stem cells in hemolymph, with the capacity to continuously produce differentiated neuronal structures throughout life. As the use of crayfish and other invertebrates increases in biomedical research, we must develop laboratory standards and guidelines for performing clinical procedures. This manuscript presents introductory protocols for anesthesia in crayfish during diagnostic imaging. Five anesthetic protocols were evaluated: immersion in buffered tricaine methanesulfonate (MS222; 50 mg/L); immersion in buffered MS222 (150 mg/L); immersion in propofol (65 mg/L); injection of propofol (50 mg/kg); and injection of propofol (100 mg/kg) into the ventral surface of an abdominal somite. MS222 immersion (50 and 150 mg/L) had no observable effect on crayfish. After an extended period of time, immersion in propofol (65 mg/L) created a sedative effect suitable for short-term handling. Propofol injection (50 mg/kg) into the ventral surface of an abdominal somite created an effective plane of anesthesia without adverse effects during or after recovery. Propofol injection at 100 mg/kg had adverse effects and is not recommended for use in crayfish. CT imaging was performed successfully as proof of concept for handling anesthetized crayfish. These findings provide initial data for the anesthetization of crayfish used in research settings.

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Procambarus clarkii are freshwater crustaceans that have become increasingly popular in epigenetic, microbiome, stem cell, and evolutionary biology research.[5,9,20,21,34,41-44,47,48] This popularity primarily due to the small size, high fecundity, and overall environmental and nutritional adaptability of *Procambarus* spp.[20,33,34,42] In addition, crayfish have clear potential to become a mainstay model for neuroregenerative and immunologic research.[35,37,41] As *P. virginalis* (marbled crayfish) becomes increasingly available as a biologic model, basic information on how anesthetic drugs affect this genus is necessary.[20,28,42]

Recently, researchers have found that *P. clarkii* (red swamp crayfish) has the unique ability to produce neuronal structures continuously throughout adulthood, via selective differentiation of innate immune cells that act as neural precursors.[6] In addition, these systems provide regenerative abilities to sensory organs (for example, eyes) and CNS structures, providing a useful model for neuronal regeneration.[9,10,37] Historically, the crayfish genus *Procambarus* has contributed significantly to the aquaculture industry both globally and in the United States.[13,19,25] Because crayfish are raised intensively for aquaculture, evaluating anesthesia may also be relevant to the industry in terms of humane care. As use increases, so will the need to create *P. clarkii* colonies in biomedical vivaria.[27]

Aquatic animal models are becoming more popular due to the ability to control precise environmental variables, giving insight into xenobiotic chemicals and mechanisms. Moreover, zebrafish (*Danio rerio*) have proven invaluable in advancing myriad research areas, including carcinogenesis, tissue regeneration, genetic disease and disorder pathogenesis, gerontology, and hematopoiesis.[16,18,23,24,30,45,46] Like zebrafish, crayfish have the potential to become an important research model and fill a scientific gap regarding the ability to regenerate neural structures. For that reason, research facilities must develop evidence-based protocols that provide humane methods of husbandry and anesthesia.

Propofol was chosen as a sedation method in the current study because it is known to be safe and have neuroprotective effects.[2,3,38] Although propofol has neuroprotective properties, its use could be counterproductive when evaluating mechanisms of nervous tissue damage.[8] Propofol has been used to effectively sedate other aquatic animals, can be given parenterally, is not a controlled substance, and is widely used in human and veterinary medicine.[40] MS222 was chosen because it is the most widely used anesthetic in aquatic animals and is the only federally approved anesthetic agent for use in aquaculture use of fish, amphibians, and other aquatic, cold-blooded animals.[12]

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[1]Department of Veterinary Preventative Medicine and [2]College of Public Health, The Ohio State University, Columbus, Ohio; [3]College of Veterinary Medicine, Midwestern University, Glendale, Arizona; [4]Department of Evolution, Ecology and Organismal Biology, and [5]School of Environmental and Natural Resources, The Ohio State University, Columbus, Ohio; [6]Office of Animal Resources, Western University of Health Sciences, Pomona, California; [7]Center for Regenerative Medicine, The Research Institute at Nationwide Children's Hospital, Columbus, Ohio; [8]Department of Surgery, The Ohio State University College of Medicine, Columbus, Ohio; [9]Center for Childhood Cancer and Blood Diseases, Nationwide Children's Hospital, Columbus, Ohio; [10]Department of Pediatrics, The Ohio State University, Columbus, Ohio; and [11]Animal Resources Core, The Abigail Wexner Research Institute at Nationwide Children's Hospital, Columbus, Ohio
[*]Corresponding author. Email: raphael.malbrue@nationwidechildrens.org

Other methods of anesthesia in crayfish range from immersion in clove oil to electro-stunning and vary in their time to effect and duration of sedation.[15,17]

The current study investigated anesthetic techniques for laboratory-housed crayfish (*P. clarkii*). We also tested propofol injection into the coelomic cavity through the ventral surface of an abdominal somite. We hypothesized that both propofol and MS222 would be effective anesthetic agents in laboratory-housed *P. clarkia* and that propofol injection directly into ventral surface of an abdominal somite would yield the fastest time to anesthesia and the slowest recovery time.

Materials and Methods

Animals. The study used 79 sexually mature, adult, farm-raised crayfish (*P. clarkii*). Cohorts of 24 crayfish were maintained in an AAALAC-accredited, USDA-registered, OLAW-assured facility (The Abigail Wexner Research Institute at Nationwide Children's Hospital) on a 12:12-h light cycle. All of the 40 female and 39 male *P. clarkii* ($n = 79$; weight: average, 26.3 g; minimum, 11.8 g; maximum, 45.0 g) were individually housed. All animals were obtained from a commercial crayfish producer (Louisiana Crawfish, Natchitoches, LA) and were shipped overnight to ensure minimal transit time. Crayfish were obtained through a series of 5-lb (2.3-kg) shipments as needed by various study arms. Crayfish were given a 5-d acclimation period to ensure that they were healthy on arrival and then were enrolled in the study. Debris and detritus material were removed through 3 freshwater baths with conditioned system water. All animals were acclimated to the housing for 2 wk prior to being used in this study.

Crayfish housing system. Crayfish housing consisted of a primary containment system, substrate, stress-reduction hide, basic biologic filter, and air source. Each container was 33 cm × 19 cm × 11.5 cm in size and held 6.2 L of water (Our Shoebox, Container Store, Coppell, TX). Systems were considered static with individual tank aeration and basic biofiltration. The 79 crayfish used all were housed in identical systems to promote efficacy and reproducibility of this system and decrease confounding factors. All life-support systems were set up 4 wk prior to introduction of the crayfish to allow for appropriate cycling of the systems. Beneficial bacteria (Microbe-Lift Special Blend Water Care, Ecological Laboratories, Cape Coral, FL) were added according to the manufacturer's instructions to hasten the cycling of each individual tank.

The water used in the tanks was purified by a reverse-osmosis system (Indigo RO Reverse Osmosis System, Avidity Science, Long Credon, Aylesbury, UK) to remove chemicals and pathogenic microbes. The system is maintained by vivarium and management staff. The water was treated with chlorine after osmosis to help ensure adequate microbe removal. A commercial aquarium dechlorinator (API, Mars Fishcare, Chalfont, PA) was then added to the water according to the manufacturer's instructions after collection but before being used. Dechlorination was not validated, but water was left to sit for 24 h before being used to allow time for dissolution of chlorine. Because reverse-osmosis purification creates water that is void of the natural anions and cations needed to sustain life for crayfish and other aquatic species, 2.5 mL of Replenish (Seachem Laboratories, Madison, GA) was added to every 5 gallons (approximately 20 L) of water.

Husbandry and water-quality testing. Every 7 d, routine maintenance of the containment systems included removal of 50% of the water, which was accomplished by syphoning the tank using 4.76-mm (3/16-in.) airline tubing (Elite Silicon Airline Tubing, Marina Products, Hagan, Montreal, Quebec, Canada) until 1.6 L of water was removed. This water then was replaced with conditioned water to dilute nitrate and waste products. Water-quality testing was performed daily on days 0 through 15. A smart water tester (Apera PC60-Z Smart Multi-Parameter Pocket Tester, Apera Instruments, Columbus, OH) was used to measure temperature (°F), pH, conductivity (mS/cm), total dissolved solids (ppm), and salinity (ppt). A water-testing kit (API Freshwater Master Test Kit, MARS Fishcare, Chalfont, PA) was used to analyze ammonia (ppm), nitrite (ppm) and nitrate (ppm) levels. A second test kit (API GH and KH Test Kit, MARS Fishcare, Chalfont, PA) was used to measure carbonate hardness (ppm) and general hardness (ppm). All tests were performed according to the manufacturer's instructions, and reagents kept up to date. However, we have been unable to find literature reports of water quality parameters for crayfish.

Once daily, 3 shrimp pellets per crayfish were added to each tank. (0.5 to 1 g; Wardley-Hartz, Hartz Mountain Industries, Secaucus, NJ). Guaranteed analysis included 36% crude protein, 8% crude fat, and 36.5% crude fiber. Carbohydrate analysis was unavailable. Any remaining food or debris was left until weekly water changes were performed.

Anesthesia experiments. ***Anesthesia assessment.*** To assess time to anesthesia, *P. clarkii* tactile function was observed after administration of the anesthetic agent (Figure 1). Qualitative anesthesia scores were created. Stage of anesthesia was adapted from previously published work.[11] Assessment of depth of anesthesia was adapted from sedation score tables published for *Limulus polyphemus* anesthesia.[4]

MS222. MS222 (Tricaine-S, Syndel Laboratories, Ferndale, WA) was tested at 50 and 150 mg/L using a time- and dose-dependent immersion design. Working solutions were made on

Stage	Condition	Depth of anesthesia
I	Awake	- Normal purposeful movement - Strong evasion of tactile stimulation - Vigorous righting reflex
II	Sedated	- Slow uncoordinated to coordinated limb movement - Response to tactile stimulation with muscle tone present and weak to absent evasion/withdrawal - Coordinated righting reflex with telson bearing weight - Response to painful stimuli through digital applied pressure to arthrodial membrane of the second limb in dorsal recumbency
III	Surgical plane of anesthesia	- Little to no purposeful limb movement - Minimal to absent muscle tone present with no withdrawal reflex in response to tactile stimulation - No righting response with absent to weak limb movement - No response to painful stimuli through hemostat applied to arthrodial membrane of the second limb while in dorsal recumbency.

Figure 1. Stages of anesthesia in crayfish (adapted from references 3,10).

the day of use by dissolving MS222 in conditioned facility water. To account for the acidic nature of MS222, medical-grade sodium bicarbonate (Sodium Bicarbonate 8.4% Injection, VetOne, MWI, Boise, ID) was added to the working solution until the pH measured 7.0 (PC60-Z Smart Multi-Parameter Pocket Tester, Apera). For each dose of MS222, 4 L of working solution was added to each of 3 totes (30-gallon [113.6 L]; Sterilite, Lowes, Mooresville, NC). Two cohorts of crayfish were used, one for each dose of MS222 tested. Each cohort included 16 crayfish (8 males, 8 females; weight, 11.8 to 21.6 g), independent of all other study arms. While in the working solution, crayfish were separated to prevent interactions by placing them in porous plastic baskets (Y-Weave Cube Storage Basket, 2 in. × 6 in., Target, Minneapolis, MN) placed in the larger 113.6 L totes. The 50-mg/L cohort had a 120-min exposure time; 150-mg/L cohort had a 90-min exposure time. Time to anesthesia and any changes in behavior were recorded. After MS222 exposure, crayfish were placed in preconditioned water for a 24-h recovery period.

Propofol. The effect of propofol (PropoFlo, Zoetis, Parsippany-Troy Hills, NJ) on *P. clarkii* was assessed through a design that involved route of administration, time to effect, and effect duration. Propofol immersion working solution was prepared by diluting propofol in conditioned facility water until the solution had a final concentration of 65 mg/L, which was selected based on our clinical experience. The subjects for this experiment were 16 crayfish (8 males and 8 females; weight, 14.6 to 19.7 g) independent of all other study arms. 4 L of working solution was added to each of 6 totes (30-gallon [113.6-L] Bella, Lowes); crayfish were separated by using porous plastic baskets (Y-Weave Cube Storage Basket, 2 in. × 6 in., Target) in the larger totes (Figure 2 A). Crayfish were immersed until an effect was seen (maximal exposure time, 80 min).

The propofol injection study involved 20 crayfish (10 males and 10 females; weight: average, 25.1 g; minimum, 16.0 g; maximum, 44.0 g), independent of all other study arms. Propofol was injected at dosages of 50 mg/kg and 100 mg/kg into the intersegmental membrane between segments on the ventral abdomen. The operator used a sterile 1-mL syringe with a 25-gauge, 5/8-in. needle and was careful to pull back on the plunger and visualize hemolymph in the needle hub, thus confirming placement into the open circulatory system (Figure 2 B and C). This needle size was chosen specifically to avoid causing trauma to the abdomen and to avoid penetrating the carapace dorsally. The ventral surface of the abdominal somite is located on the ventral side of the abdomen, and injection occurred between the third and sixth segments and 1 to 2 cm off midline. After the 3 independent anesthesia trials, crayfish were placed in preconditioned facility water for recovery. Crayfish were assessed for the ability to recover from anesthesia over a 260-min period. Mortality was defined as lack of any movement for 24 h, with no response to stimuli and lack of muscle tone. Full recovery was considered as an 'awake crayfish' (Figure 1).

Statistical analysis. The duration of anesthesia and full recovery time after immersion or injection of propofol were tested for normality by using the Shapiro–Wilks method. Due to nonnormality, a Mann–Whitney U (Wilcoxon Rank Sum) test was used to compare mean ranks (JMP Pro 15, SAS Institute, Cary, NC). A 2-sample test of proportions was used to assess differences in mortality between sexes. Statistical significance was set as a P value less than 0.05. Data are presented as means and SD.

In vivo CT imaging experiment. CT scans were completed on 11 crayfish (5 males and 6 females) to test the effectiveness of sedation for brief imaging studies. Males received injections into the ventral surface of the abdominal somite with a dosage of 100 mg/kg of propofol; females were similarly injected with 50 mg/kg of propofol. Separation of the sexes in this way was necessary due to a limited number of crayfish eligible for enrollment in the study. An additional single female crayfish that received a 50-mg/kg dosage of propofol also received a CT contrast agent (Fenestra VC, Medilumine, Montreal, Canada) with a dose of 300 µL/25 g. The contrast agent was injected in the same manner as propofol, into the ventral surface of the abdominal somite, and was used to compare the CT image quality of crayfish that did not receive contrast media. CT imaging was performed by using a µPET–CT system (U-PET^{6}CT^{HR}, MILabs, Utrecht, Netherlands). The crayfish was placed in the prone position on a scanning bed (Figure 3). The scan settings were: full 360° rotation; X-ray tube settings of 0.33 mA and 55 kV; 0.750 degree per step; 1 projection per step; 1 × 1 binning; and exposure time of 40 ms. All CT images were reconstructed by using MILabs reconstruction software (version 12.0) with an 80-µm voxel grid, Hann projection filter, and 160-µm Gaussian volume filter. The reconstructed CT images were analyzed by using commercially available software (PMOD Technologies, Zürich, Switzerland).

Results

Water-quality testing. Quantitative analysis of water-quality data showed typical (expected) variations in water conditions over the study period (Table 1).

MS222 immersion. A concentration of 50 mg/L MS222 produced no observable sedation or anesthesia of crayfish throughout the 120 min of exposure (Table 2). Similarly, no level of sedation or anesthesia of crayfish were observed throughout 90 min of exposure to a concentration of 150 mg/L (Table 2). All crayfish were determined to be fully awake through their respective observation times.

Propofol. All crayfish (n = 16) immersed in propofol at a concentration of 65 mg/L exhibited sedation, with a mean (± 1 SD) time to effect of 64 ± 1 min (Table 2). A total of 20 crayfish received propofol via injection into the ventral surface of the abdominal somite. The time to effect was 54 ± 12 s in the 10 crayfish dosed at 50 mg/kg and 30 ± 6 s in the 10 crayfish given the 100 mg/kg dose (Table 2). Among the crayfish given 100 mg/kg of propofol, 3 (1 female and 2 males) died afterward.

Time to effect differed significantly between the 2 routes of propofol administration. Propofol injection at both doses had a significantly (P < 0.0001) faster median time to effect than did immersion in 65 mg/L propofol. In addition, compared with immersion, the median time to effect was 71 times faster with injection of 50 mg/kg propofol and 127 times faster at 100 mg/kg (Tables 2 and 3). The mean time to effect was 24 s faster (P = 0.0003) in the crayfish that received 100 mg/kg of propofol than in those given the 50-mg/kg dose. Crayfish sex had no effect on the mean time to effect for either the 50-mg/kg or 100-mg/kg cohorts (P = 0.9004 and P = 0.9166, respectively). MS222 data were not analyzed due to the lack of observable effects.

Recovery from anesthesia. Subjects fully recovered from propofol immersion in 7 ± 2 min (mean ± 1 SD). For subjects sedated via propofol injection, the recovery time was 42 ± 15 min at 50 mg/kg and 101 ± 34 min) at 100 mg/kg; these times differed significantly (P < 0.001). Time to recovery also differed (P = 0.0042) between the 2 dosages of injected propofol. Adverse events and mortality were monitored visually for 24 h. In groups that did not undergo CT scanning, no mortality was seen with either propofol immersion or injection at 50 mg/kg,

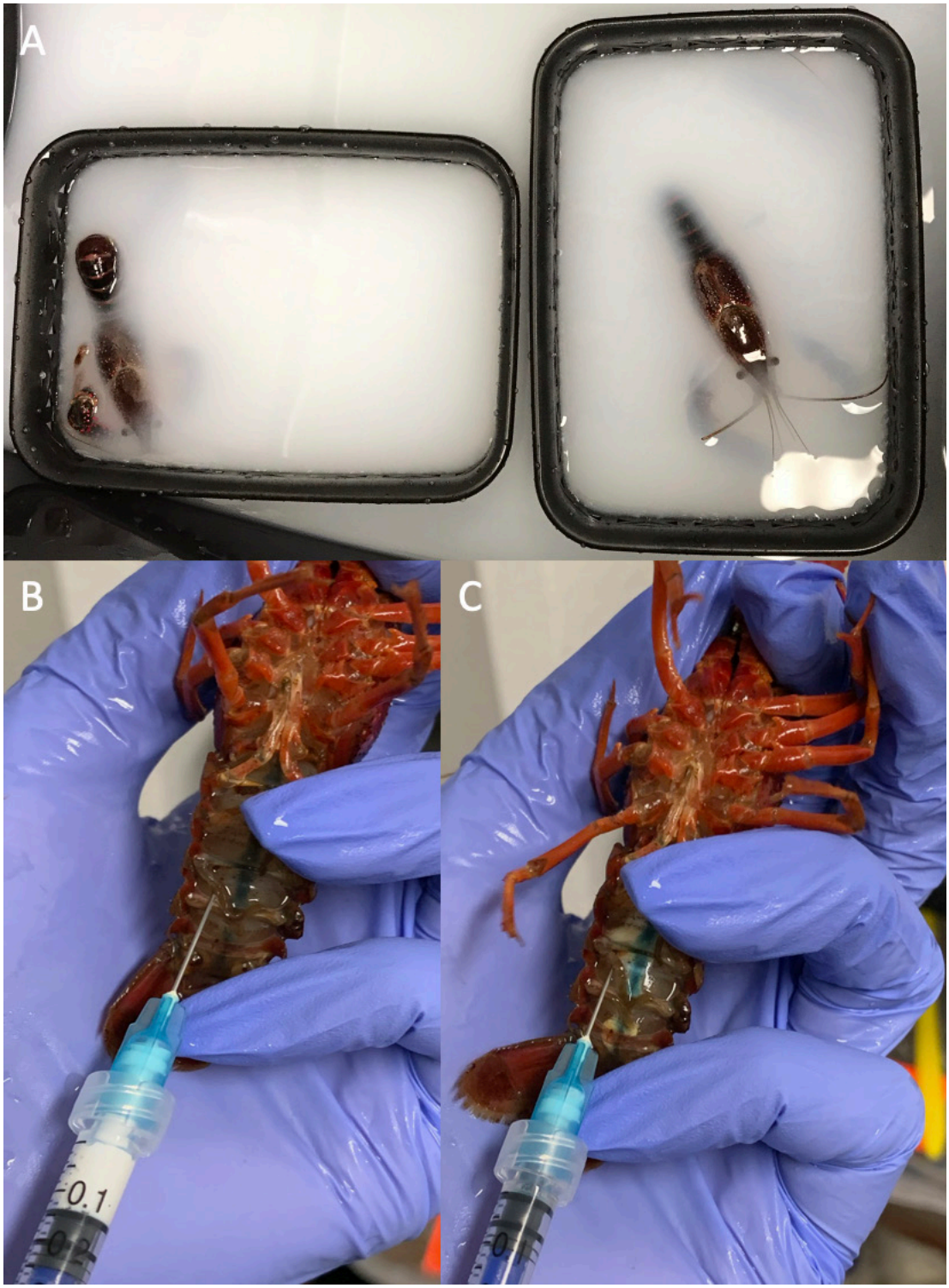

Figure 2. Routes of administration for propofol. (A) Crayfish immersed in conditioned water containing 65 mg/L of propofol. (B) Correct administration of propofol via the intracoelomic route. (C) The ventral abdominal surface after propofol administration; syringe is offset from midline.

and 30% mortality occurred with propofol injection at 100 mg/kg (Tables 2 and 3). A total of 6 deaths occurred among all propofol cohorts: 5 male and one female crayfish died during recovery, all of which had received propofol injection at 100 mg/kg. Of those animals, 3 were male crayfish that underwent CT scans, 2 were male crayfish that were observed only, and one was a female crayfish that was observed only. A sex-specific difference ($P = 0.0143$) in mortality emerged for the 50-mg/kg observation-only cohort.

CT study. A total of 11 crayfish underwent CT scanning (Table 3). The average recovery time for the 5 female crayfish injected with the 50-mg/kg dose of propofol was 33 min; none of these crayfish died, and the average CT scan time was approximately 4 min. The single female crayfish given 50 mg/kg propofol and CT contrast agent had a recovery time of 45 min. Among the 5 male crayfish given the 100-mg/kg dose of propofol, only 2 had recovery times that were less than 24 h, and 3 died (mortality rate, 60%). In particular, 2 of the male crayfish that

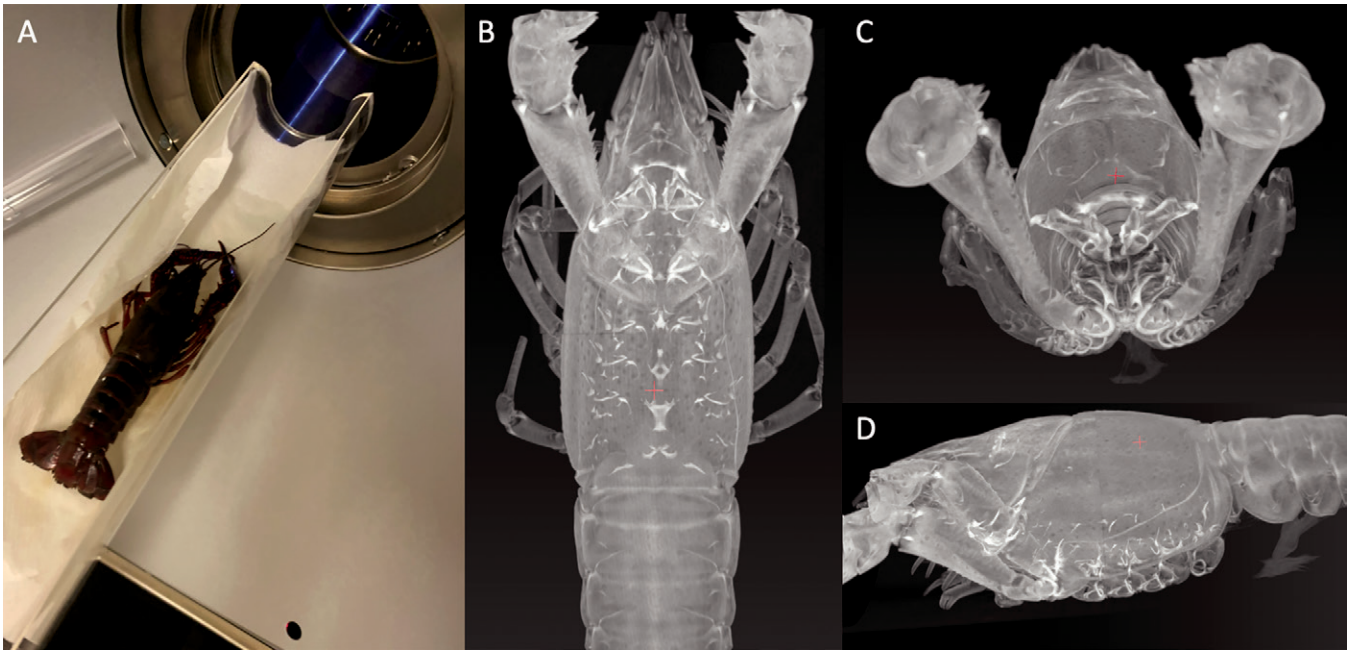

Figure 3. (A) Crayfish in prone position for µCT scanning. Volume rendering of the (B) frontal, (C) transverse, and (D) sagittal views of a crayfish CT image.

Table 1. Daily water-quality testing results during days 0 through 15

	Mean ± 1 SD
Temperature (°F [°C])	72 ± 1 (22 ± 0)
pH	8.0 ± 0.1
Conductivity (mS/cm)	336 ± 22
Total dissolved solids (ppm)	239 ± 16
Salinity (ppt)	0.2 ± 0.0
Ammonia (ppm)	0.8 ± 0.3
Nitrite (ppm)	2.3 ± 0.2
Nitrate (ppm)	6.4 ± 2.3
General hardness (ppm)	159 ± 8
Carbonate hardness (ppm)	63 ± 6

died had recovery times that exceeded 24 h, with very limited improvement, and ultimately were euthanized. The remaining male crayfish that died did so before recovery. CT scans were performed successfully in all 11 crayfish. The CT images noninvasively delineated various anatomic structures, including gills, stomach, and heart, especially when CT contrast was administered (Figures 3 B through D and 4).

Discussion

This study compared propofol and MS222 as potential anesthetic agents for *P. clarkii* in biomedical research. We were able to provide proof of concept by handling anesthetized crayfish and performing CT scans that lasted an average of 4 min. The CT study showed that the 50-mg/kg dosage of propofol was sufficient for performing at least one scan. This dose is suitable for research that requires sedation of crayfish for experimental purposes.

MS222 is currently the only FDA-approved drug for use in food fish, indicating the need for assessment in crayfish.[39] We saw no observable anesthetic effects of MS222 when administered to crayfish via immersion, in contrast to other studies that reported sedative and analgesic-like effects in similar species.[31,36] These previous studies used higher dosages of MS222, perhaps explaining the lack of effects in our current study. However, a study on *Faxonius* (formerly *Orconectes*) *virilis* (virile crayfish) observed no anesthetic effects at an MS222 concentration of 1000 mg/L.[7] We chose the 2 MS222 doses (50 and 150 mg/L) for evaluation in light of our clinical experience with related species and due to safety and toxicity concerns.

We had success in using propofol to produce anesthetic effects in crayfish when it was administered via immersion or injected into the ventral surface of the abdominal somite. Immersion of crayfish in propofol had a prolonged time to effect, whereas injection was much more effective and efficient. Our results are similar to other studies done on both aquatic animals and crustaceans.[32,38] In contrast to these previous studies, we used higher doses of propofol, which provided both quicker time to effect and longer periods of sedation, thus allowing for safe handling of crayfish. Compared with other anesthetic agents, such as clove oil, propofol injection has a much faster time to effect on crayfish.[17] One limitation of propofol immersion is that this drug is only slightly soluble in water; future studies should take this characteristic into consideration when making working solutions.[14] Furthermore, we acknowledge that methods involving electrical stunning may also be a feasible option but were not considered for the current study.[15]

We were able to perform 4-min CT scans on crayfish given either a 50- or 100-mg/kg injection of propofol. We chose to assess CT scans because they are used in regenerative medicine.[29] Given the average recovery time of 41 min, each crayfish could have undergone numerous scans. However, the 100-mg/kg dose of propofol can have adverse effects in crayfish, and we therefore suggest using the 50-mg/kg dose. Our data shows that the lethality of the 100-mg/kg of propofol may be sex-associated, but further confirmatory studies are needed.

We estimate that the crayfish were removed from the conditioned water for no more than 10 min but acknowledge that this exposure period might have contributed to the higher rate of mortality in the 100-mg/kg propofol cohorts of crayfish that were scanned and handled (3 of 5 died) as compared with those that were observed only (3 of 10). Although crayfish were not submersed in water during scans, they were both sprayed and placed on paper towels that had been moistened with

Table 2. Times (min) to effect and recovery after administration of MS222 and propofol to crayfish via immersion (N = 48)

Anesthetic	Dose (mg/L)	Time to effect (min)			Time to recovery (min)			Mortality (%)
		Median	Mean	1 SD	Median	Mean	1 SD	
MS222	50	not applicable	not applicable	not applicable	not applicable	not applicable	not applicable	0%
MS222	150	not applicable	not applicable	not applicable	not applicable	not applicable	not applicable	0%
Propofol	65	64.2	63.9	1.4	7.9	7.4	2.4	0%

Time to effect was determined to as surgical anesthesia or sedation.
Time to full recovery considered as an awake crayfish.

Table 3. Times to effect (s) and recovery (min) of propofol injected into crayfish via ventral surface of the abdominal somite (N = 31)

Treatment	Time to effect (min)			Time to recovery (min)			No. of crayfish	Mortality
	Median	Mean	1 SD	Median	Mean	1 SD		
50 mg/kg Propofol	51.0	54.0	12.1	36.0	41.7	14.7	10	0%
100 mg/kg propofol	30.5	30.2	5.7	118.0	101.1	33.8	10	30%
50 mg/kg propofol + CT imaging	N/A	N/A	N/A	37.0	32.8	8.8	5	0%
50 mg/kg propofol + contrast media + CT imaging	N/A	N/A	N/A	45.0	45.0	0	1	0%
100 mg/kg propofol + CT imaging	N/A	N/A	N/A	42.0	42.0	19.8	5	60%

Data shown are from the 11 crayfish that also underwent CT scanning.
Time to effect was determined as surgical anesthesia.
Time to full recovery considered as an awake crayfish.
Time to recovery includes only the 2 crayfish with recovery times of less than 24 h.

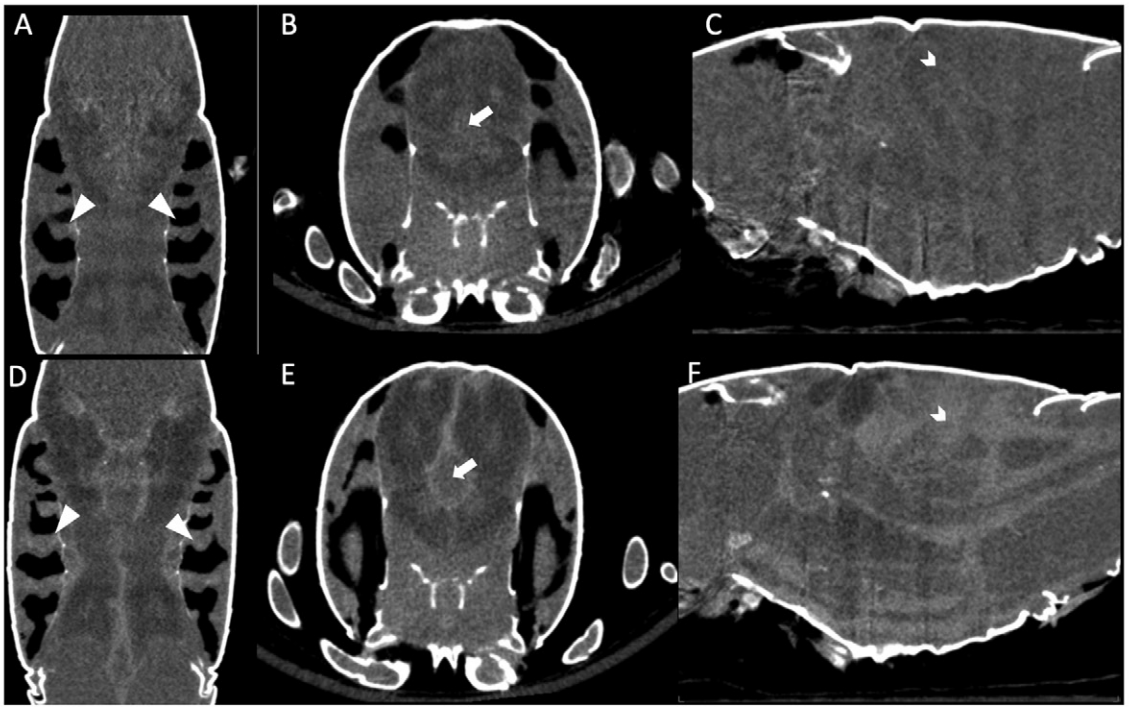

Figure 4. CT images of the (A, D) front view showing gills (triangles), (B, E) transverse view of stomach (white arrows), and (C, F) sagittal view of the heart (white arrowheads). Crayfish CT images in panels A through C were obtained without contrast media; the images in panels D through F were acquired with the use of contrast media.

conditioned system water. Crayfish have been shown to survive out of water for more than 20 h,[5] so the far shorter time period in our current study likely did not contribute to mortality. Further research is needed prior to making any recommendations on safe time out of water for *Procambarus* spp. Necropsies were not performed on the animals that died.

A propofol dose of 75 mg/kg merits evaluation, as it may provide a longer sedation time appropriate for performing surgery or alternative experiments. In addition, injections through the abdomen of crayfish must remain within the ventral surface of the abdominal somite and avoid the midline. The ventral midline houses the nerve cord, which is synonymous to the vertebral column of mammalian species.[2] If the needle is inserted too far cranially, it can potentially enter the cephalothorax and damage internal organs.

Finally, we were able to adequately house crayfish in a biomedical research setting. We describe a viable approach to the housing, husbandry, and routine clinical techniques in *P. clarkii*. The most important water-quality parameter associated with crayfish production is the dissolved oxygen concentration.

Although we were limited by our inability to monitor this concentration with our testing device, we believe that this limitation had minimal influence, because the dissolved oxygen concentration typically becomes problematic if the water temperature rises above 80 °F (26.6 °C).[25,26] Although little guidance is available regarding specific water quality parameters for crayfish, we hope that the data we provide (Table 1) may help inform other studies regarding satisfactory ranges for housing crayfish. During the early phases of the current study, we consulted our institution's IACUC office, which indicated that protocol approval was not necessary in light of current regulations in the United States and the goal of reducing regulatory burden.[22] We also consulted our institutional laboratory animal veterinarians and the Institutional Biosafety Committee to obtain guidance and oversight. Furthermore, although invertebrates are not covered under the USDA's Animal Welfare Act or addressed in the *Guide for the Care and Use of Laboratory Animals*,[1,22] continued development and publication of husbandry and use protocols for this genus will increase experimental validity and decrease confounding factors.

In summary, our results suggest that MS222, at both the 50 and 150 mg/L doses, may not be as effective of an anesthetic agent for crayfish as propofol. Both doses of MS222 provided no anesthetic effects on crayfish in this study. Conversely, propofol had much higher levels of sedation with both routes of administration (immersion and injection) showing promising results. For immersion, the average time to effect for propofol at a 65 mg/L dosage was 63.9 min and the average time to recover was 7.4 min. The average time to effect of propofol at the 50 mg/kg dose was 54.0 s while the 100 mg/kg dose was 30.2 s. The average time to recovery for propofol at the 50 mg/kg dose was 41.7 seconds while the 100 mg/kg dose was 101.1 s. Further research should focus on varying dosages of both MS222 and propofol.

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