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Underwater Anesthesia of Diamondback Terrapins (*Malaclemys terrapin*) for Measurement of Auditory Evoked Potentials

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Investigations into the biology of aquatic and semiaquatic species, including those involving sensory specialization, often require creative solutions to novel questions. We developed a technique for safely anesthetizing a semiaquatic chelonian species, the diamondback terrapin (*Malaclemys terrapin*), for measurement of auditory evoked potentials while animals were completely submerged in water. Custom-modified endotracheal tubes were used to obtain a watertight seal on both sides of the glottis and prevent aspiration of water during testing. No adverse effects were seen after the procedures, and assessment of venous blood-gas partial pressures and lactate concentrations indicated that sufficient gas exchange was maintained under anesthesia through manual ventilation.

Abbreviation: AEP, auditory evoked potential.

Auditory evoked potentials (AEP) are electrical responses that are produced by the synchronous discharge of neurons in the auditory pathway of the CNS after acoustic stimulation detectable by the ear.[2,30] Measurements of AEP in response to acoustic stimuli of varying frequencies and intensities are used to assess hearing sensitivity. This technique has proven to be useful in a variety of domestic and exotic animal species, because it does not require a specific behavioral response and can be performed in subjects that are unlikely to be cooperative.[26] AEP have been used to generate audiograms rapidly and noninvasively for a variety of aquatic species, including marine mammals, fish, and invertebrates.[5,23,24]

Diamondback terrapins (*Malaclemys terrapin*) are a brackish water species native to inshore areas and estuaries of the eastern and southern United States. Because the species spends much of its life in water, we hypothesized that it may have developed sensory specialization to the underwater environment, as have other aquatic turtles.[7] Diamondback terrapins are listed as threatened or endangered in large portions of their natural range[11] and face threats from incidental bycatch in crab traps[3] as well as pressure from increasing human interactions in the form of vehicular trauma: nesting female terrapins are susceptible to automobiles on land,[29] and both sexes are commonly struck by watercraft during foraging, mating, and basking behavior.[6] Previous studies have shown variable behavioral responses in terrapins exposed to low-frequency sounds, indicating that the species may not have a survival-adaptive aversive response to boat noise.[19]

The properties of sound and its measurement references differ between water and air, making measurements of hearing in the 2 media difficult to compare. Because of these differences, it is important to measure hearing sensitivity in both environments for amphibious species to identify the detectable sound frequencies and intensities as well as to reveal differences between related organisms.[26] Accurate results for AEP measurements require the subject to remain still to reduce electrical artifacts caused by muscle movement and to stay in the same position relative to the sound source. During in-water testing, the ears of the subject must remain below the surface and away from the air–water interface, to minimize inaccuracies in hearing measurements resulting from sound reflection and rarefaction. Although aquatic turtle species, including diamondback terrapins, can remain submerged for well over 30 min without obvious adverse effects, unsedated or unanesthetized subjects are unlikely to remain still and in the same position relative to the speaker during auditory testing. Several anesthetic protocols have been used safely in aquatic turtle species.[20] Supplemental ventilation often is required to maintain sufficient blood oxygenation, and there is little available information on the risks of aspiration when anesthetized chelonians are submerged. Diamondback terrapins show reduced reflexive control over the glottis while anesthetized,[21] thus increasing (at least hypothetically) the risk of drowning or other negative sequelae should the animal attempt to take a breath while submerged.

Forced submersion results in lactic acidosis and marked acidemia in aquatic turtle species. Loggerhead sea turtles (*Caretta caretta*) subjected to short-term submersion in trawl nets exhibit an acidemia that was corrected within 30 min of allowing the animals to respire at will at the surface, suggesting that respiratory compensation is critical to maintaining physiologic blood pH in these animals.[10] Apnea secondary to anesthetic medications likely has similar effects on venous blood-gas parameters in the absence of manual ventilation, even though breath-holding diving species may be more tolerant to this complication than are nondiving species.

A previous study investigating the underwater hearing of the red-eared slider (*Trachemys scripta elegans*) used an injectable anesthetic protocol similar to the one presented here, although without intubation and supplemental ventilation.[7] Although no mortalities were reported, the animals' response to submerged anesthesia and subsequent recoveries from anesthesia were not

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described.[7] It is difficult to assess effect of sublethal hypoxic injury in chelonians, but it would be irresponsible to assume the potential does not exist. Hypoxic brain injury can damage the hearing apparatus in other species, including humans,[16] suggesting that the provision of sufficient ventilation and blood oxygenation during anesthesia is essential for accurate assessment of hearing capacity.

Custom-modified endotracheal tubes were developed previously for use in acquiring underwater AEP measurements in juvenile green sea turtles (*Chelonia mydas*).[9] The turtles were anesthetized and intubated with endotracheal tubes designed with 2 independently inflatable balloons, one positioned just inside the trachea immediately below the glottis, and the second inflated within the oral cavity to provide a counterseal on the external aspect of the glottis.[9] The similarly modified endotracheal tubes we used in the current study allowed for continued ventilation and maintenance of blood oxygenation throughout anesthetized submergence of diamondback terrapins for AEP measurements.

Materials and Methods

Animals. A total of 8 adult (6 female, 2 male) terrapins were obtained on loan from the Marine Academy of Technology and Environmental Science (Manahawkin, NJ), which acquired the diamondback terrapins from private owners or aquariums that were no longer able or willing to care for the turtles. All terrapins were estimated to be older than 10 y and ranged in weight from 237 to 1380 g, with straight carapace lengths from 11.1 cm to 19.7 cm. Diamondback terrapins are a sexually dimorphic species: female terrapins were considerably larger (median weight, 1020 g) than were the male terrapins (median weight, 254 g). For the duration of the study, terrapins were housed either individually or in pairs of the same sex in holding tanks of filtered water at brackish salinity (19 ppt). Aquarium water heaters and thermal heating lamps were used to maintain water temperatures between 19 and 21 °C. Water changes were performed as needed. Animals were fed frozen shrimp ad libitum on days not immediately preceding an anesthetic procedure. All procedures were approved by the IACUC of Duke University and North Carolina State University, and scientific holding permits for the terrapins were provided by the New Jersey Division of Environmental Protection's Division of Fish and Wildlife.

Endotracheal tubes. Custom endotracheal tubes were modified from 6-French (outer diameter, 2 mm) Foley urinary catheters by the addition of a solid rubber cuff permanently adhered to the exterior of the tube 2 mm proximal to the existing inflatable balloon (MILA, Erlanger, KY; Figure 1). The tube was inserted into the trachea until the rubber cuff sat snugly on the external opening of the glottis, providing a seal in the oral cavity. The balloon then was inflated with an appropriate amount of sterile saline (approximately 0.04 mL) to provide a watertight seal within the trachea and prevent accidental dislodgement of the tube during manipulations of the subject. Endotracheal tubes were secured in place by using adhesive tape around the skull of the terrapin.

Anesthesia. Animals were sedated with ketamine (KetaVed, Vedco, St Joseph, MO) and dexmedetomidine (Dexdomitor, Pfizer, New York, NY) for both in-air and underwater AEP procedures. Intravenous injections were performed in either the subcarapacial sinus or the circumflex iliac vein dorsal to the hind leg. In several cases, lymph fluid was withdrawn prior to injection in combination with or in place of venous blood. Efforts were made to obtain the 'cleanest' intravascular injection possible, but the presence of lymph fluid did not preclude injection. After AEP testing, anesthesia was reversed by using atipamezole (Antisedan, Pfizer) at 10 times the dose of dexmedetomidine and given by intramuscular injection into a thoracic limb. Induction times were measured from the time of injection to intubation (if the animal was intubated) or to the start of AEP procedure (if not). Anesthesia time was reported as the time from first anesthetic injection to venipuncture after the collection of AEP measurements, just prior to administration of the reversal agent. Recovery times were measured as the time from administration of the reversal drug (atipamezole) to coordinated ambulation and an active escape response to approach and manual handling.

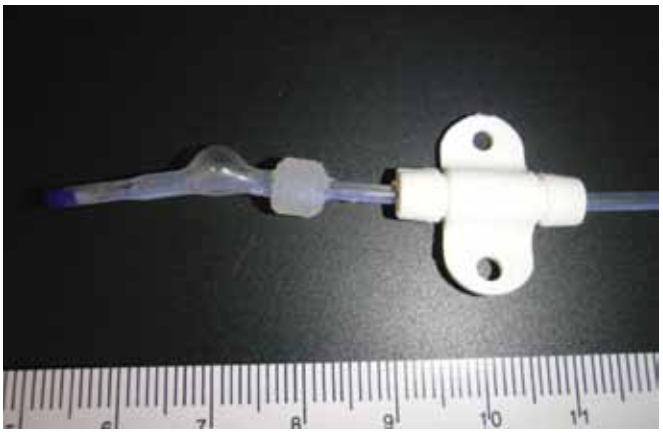

Figure 1. Endotracheal tube modified for use in underwater anesthesia.

For all underwater AEP, animals were intubated with the double-cuffed endotracheal tubes described previously and were manually ventilated with room air using a 60-mL catheter-tipped syringe (Figure 2). Ventilations were delivered on an expiration–inspiration–pause pattern, with an inspiratory volume of approximately 15 mL/kg, according to a study that indicated average voluntary respiration volumes of 12.8 to 13.7 mL for diamondback terrapins weighing between 650 and 720 g.[21] Breaths were delivered in groups of 2 to 3 approximately every 2 to 3 min to obtain an average of one breath per minute, with detachment and voiding of the syringe between expiration and inspiration. This protocol was based on a reported normal respiratory pattern for diamondback terrapins, which consists of several cycles of expiration and inspiration followed by a variable period of apnea.[21] This pattern has been reported for several aquatic chelonians, among other vertebrate species, with increasing numbers of ventilatory cycles associated with longer periods of apnea.[8,22,28] Submersion times were limited to 60 min for all terrapins, as a conservative precaution against inadvertent prolonged hypoxia. Terrapin heart rates were measured by using a Doppler ultrasonography unit (Pocket-Dop 3, Carefusion, Middleton, MA) both prior to anesthesia and at the completion of AEP testing (before injection with the reversal agent) for several of the procedures (2 intubated for underwater AEP and 4 in-air AEP without intubation).

For most in-air AEP measurements, the terrapins were not intubated or manually ventilated. Respiratory rate was recorded through visual observation, and if an animal failed to breathe voluntarily after a 10-min period, the AEP procedure was paused and manual tactile stimulation was provided until the animal performed at least one full respiration. Two female terrapins were also intubated and manually ventilated for in-air AEP measurements to ensure appropriate performance of the endotracheal tubes.

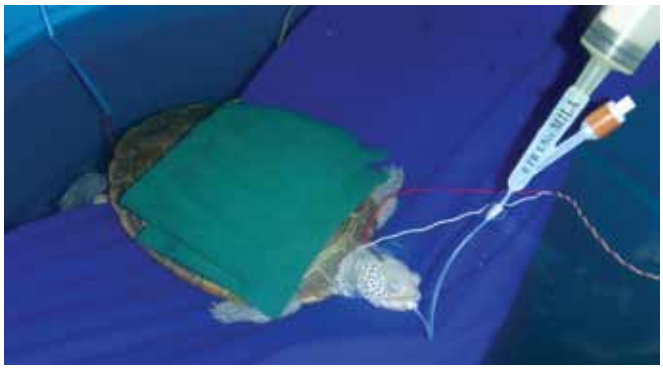

Figure 2. Anesthetized and intubated diamondback terrapin submerged for measurement of AEP.

AEP measurements. AEP measurements were conducted by using methodologies developed for evaluating aerial and underwater sea turtle hearing sensitivity.[25] In-air AEP were recorded with animals resting a standardized distance from the sound source on open cell foam. Underwater AEP were performed with the animal submerged to a depth of 10 cm (from the surface of the water to the tympanic membrane) in an approximately 180-L plastic barrel filled with brackish water (salinity 15 to 16 ppt). The terrapins were suspended within the water column by using a sling of elasticized fabric, and fishing weights were placed over the carapace to maintain the necessary depth (Figure 2). To collect AEP, needle electrodes (27-gauge; length, 6 mm; Rochester Electro-Medical, Lutz, FL) were inserted subdermally on the top of the head (recording electrode); in the deltoid muscle of the neck (reference electrode); and either just beneath the skin of a rear limb for aerial measurements or in the brackish water for underwater measurements (ground electrode). Sound stimuli were delivered by using an amplified underwater speaker (AQ339 Aquasonic Underwater Speaker, Clark Synthesis, Littleton, CO) positioned 80 cm below the turtle (underwater) or an aerial speaker (DI 6.5R Definitive Technology, Baltimore, MD) positioned 40 cm directly in front of the turtle (in-air), and electrode responses were recorded by using an Evoked Potential Workstation (Tucker–Davis Technologies, Alachua, FL) and laptop computer with SigGen RP and BioSig RP software (Tucker–Davis Technologies). AEP results will be reported elsewhere.

Venous blood-gas measurements. Blood samples were collected from the subcarapacial sinus in all terrapins after AEP measurements and immediately prior to reversal of anesthesia. This venipuncture site, consistently accessible in most chelonians, is a vascular complex composed of the caudal cervical branch of both jugular veins as well as bilateral common intercostal vessels arising from the azygous veins.[13] In addition, one animal was opportunistically sampled prior to anesthesia to provide baseline blood gas values for comparison. Syringes were precoated with heparin prior to venipuncture, and the heparinized whole blood was analyzed within 3 min of collection by using an iStat Portable Clinical Analyzer (Heska, Fort Collins, CO) loaded with CG4+ cartridges (Abaxis, Union City, CA). Because the iStat instrument performs analysis at 37 °C, pH-dependent variables must be corrected for temperature when the blood is collected at a lower temperature, as in ectothermic species. Manual corrections were calculated as previously described for other aquatic turtle species:[9]

$$pH_{corr} = pH_{[37\ °C]} + 0.010 \times \left(\Delta T_{[°C]}\right),$$

by using a pH/T value of 0.010 U/°C as determined for the freshwater painted turtle (*Chrysemys picta*).[8] Corrected pCO_2 values were determined by using the formula

$$pCO_{2corr} = pCO_{2[37\ °C]} \times 10^{[-0.019(\Delta T_{[°C]})]},$$

and pO_2 was corrected by using the formula

$$pO_{2corr} = pO_{2[37\ °C]} \times 10^{[-0.0058(\Delta T_{[°C]})]}.\ 1$$

In each case, ΔT was determined by using the air or water temperature in the area of the subject terrapin as measured by a thermocoupler (Barnant Type-T Thermocoupler, Barrington, IL) at the time of AEP completion. Blood-gas measurements of terrapins undergoing in-air AEP testing without intubation or manual ventilation and of terrapins undergoing intubated underwater anesthesia with ventilation were compared qualitatively, because the small sample size precluded reasonable statistical comparison.

Results

Anesthesia. In-air AEP recordings were recorded successfully for all 8 terrapins, and underwater recordings were obtained from 5 female animals. Total anesthesia doses necessary for sufficient sedation ranged from 6 to 16 mg/kg for ketamine and 30 to 80 µg/kg for dexmedetomidine, including supplemental doses that were required for several terrapins (Table 1). For reversal, atipamezole was administered at 300 to 750 µg/kg. Induction times for anesthesia ranged from 8 to 57 min (median, 31 min), and procedure time (length of AEP measurement) was 14 to 81 min (median, 58 min). Recovery times ranged from 0 to 130 min (median, 18 min; Table 1).

On one occasion, terrapin 4 never achieved sufficient anesthetic depth to allow for intubation or AEP measurement, despite receiving high doses of ketamine (10.5 mg/kg) and dexmedetomidine (52 µg/kg). However, this animal was anesthetized successfully for underwater AEP testing the following day (Table 1).

Preanesthesia resting heart rates ranged from 35 to 55 bpm, whereas anesthetized heart rates ranged from 4 to 20 bpm. After reversal of anesthesia with atipamezole, terrapin heart rates rapidly increased to 30 to 40 bpm, thus approximating preanesthesia rates.

Venous blood gases. We compared summary results for terrapins undergoing in-air AEP testing without intubation and manual ventilation with blood-gas measurements from terrapins after underwater anesthesia (Table 2). Qualitative comparison revealed no marked differences in corrected pH, pCO_2, or pO_2 or lactate between the 2 populations.

Discussion

Ketamine combined with dexmedetomidine was a safe and effective intravenous anesthetic protocol for diamondback terrapins and provided sufficient sedation to prevent myogenic artifact in AEP measurements. During the study, we gradually increased the doses of both ketamine and dexmedetomidine, because supplemental doses were needed frequently with the initial dose (6 mg/kg ketamine and 30 µg/kg dexmedetomidine). The most consistently effective dose to allow intubation of terrapins was 10 to 12 mg/kg ketamine plus 50 to 60 µg/kg dexmedetomidine. These doses are slightly higher than many reported doses for other reptiles, especially for intravenous administration, but they are within the range of recommended

Table 1. Drug doses and effective durations for diamondback terrapins anesthetized for AEP measurements.

Terrapin (sex)	Weight (g)	Intubated?	Ketamine (mg/kg)	Dexmedetomidine (μg/kg)	Induction time (min)	Procedure time (min)	Recovery time (min)
1 (M)	237.4	No	8.5[b, c]	42.5[b, c]	14	81	not noted
		No	16[b]	80[b]	35	55	4
2 (M)	269.9	No	6	30	32	79	0
3 (F)	840	Yes[a]	8.4[b]	42[b]	14	48	30
		Yes	10	50	31	56	28
4 (F)	1340	No	6	30	31	78	130
		Yes	14	70	36	54	0
5 (F)	1200	Yes[a]	8[b]	40[b]	30	67	10
		Yes	15[b, c]	75[b, c]	8	58	not noted
6 (F)	1380	No	11.6[b, c]	58[b, c]	25	61	23
		Yes	12	60	35	55	19
7 (F)	760	No	10[c]	50[c]	25	62	10
		Yes	12	60	35	59	18
8 (F)	440	No	8[c]	40[c]	30	57	5
		No	12	60	57	14	30

[a]Terrapins were intubated and manually ventilated but were not submerged during in-air AEP measurements.
[b]Includes a supplemental anesthetic injection to achieve sufficient depth for AEP measurements.
[c]Initial anesthetic injection was delivered mostly or entirely into lymph fluid; total doses given are reported.

Table 2. Temperature-corrected median (n = 5 each group) blood-gas values (range) for terrapins after in-air or underwater AEP measurements.

	Anesthesia time (min)	Corrected pH	Corrected pCO_2 (mm Hg)	Corrected pO_2 (mm Hg)	Lactate (mmol/L)
In-air	114 (110–119)	7.431 (7.391–7.549)	26.6 (26.0–30.9)	44 (34–56)	3.04 (0.30-6.73)
Underwater	105 (100–119)	7.461 (7.252–7.639)	26.9 (16.5–40.0)	49 (24–88)	1.96 (1.28-4.31)

Grossly lymph-contaminated samples were not included in this summary. The underwater group consists of terrapins that were intubated and manually ventilated, and the in-air group consists of those that were not; the 2 terrapins intubated and ventilated for in-air AEP measurements were excluded from this comparison.

doses for freshwater turtles.[4] The use of a combined protocol including a reversible α2 agonist (dexmedetomidine) achieves reliably rapid recovery and accommodates much lower doses of ketamine than might otherwise be required. At the end of AEP testing, the reversal agent atipamezole was given intramuscularly at 10 times the total dose of dexmedetomidine. In most cases, reversal was rapid (less than 30 min) and allowed the animals to be returned to water shortly thereafter. No anesthesia-related complications were noted in any of the terrapins.

There are a number of potential venous access sites in the diamondback terrapin, but all present challenges. The site accessed most often in the current study was the subcarapacial sinus, which is located midsagitally cranial to the eighth cervical vertebra.[13] This site is easily accessible and consists of vessels large enough to support rapid injection of anesthetic medications in small chelonians; however, there is frequently contamination of blood samples with lymphatic fluid from the surrounding tissues. A second venipuncture site that we used several times is the circumflex iliac vein, which is accessed dorsal to the hindleg. This vein originates from the dorsal coccygeal vein and splits into right and left branches, running dorsally over the gluteal muscles.[14] Samples collected from this site also were contaminated frequently with lymph fluid. Other potential access sites in chelonians include the jugular and femoral veins, which may have a decreased incidence of lymph contamination but are often difficult to locate and access in small chelonians.[20] Although the ketamine–dexmedetomidine anesthetic combination can be delivered via intramuscular injection, effectiveness is reported to be best with intravenous injection.[27] Furthermore, in our experience, intramuscular injection results in highly variable and prolonged induction and recovery times, making the slight extra effort required to obtain venous access worthwhile in many clinical situations.

Voluntary respiratory patterns were highly variable for nonintubated terrapins in air, although we noted a general respiratory pattern consisting of short periods of apnea broken by episodes of several rapid breaths in many of the animals. This pattern is not unexpected in an aquatic animal that must forage for food underwater, and attempts were made to approximate this pattern during manual ventilation of submerged animals. In the case of underwater AEP measurements, the duration of submergence was limited to a maximum of 60 min to prevent the development of unrecognized hypoxemia. This time frame was established purely as a safety measure, but several of the terrapins showed signs of spontaneous recovery from anesthesia (voluntary respiration, purposeful limb movements) by this

time. Because these movements interfered with the collection of adequate AEP measurements, supplementary doses of anesthesia would be needed for longer procedures.

With the use of an extended endotracheal tube, the potential for excessive ventilatory deadspace must be considered. We calculated the interior volume of the 6-French modified catheter (including attachment cup) to be 1.04 mL, as measured by the weight of water that completely fills the tube. This figure likely overestimates the actual deadspace, because the tip of the syringe used to ventilate the animals takes up a large portion of the space within the attachment cup. To our knowledge, a normal physiologic deadspace has not been determined for the diamondback terrapin; however, mean tidal volume for the anatomically similar freshwater turtle *Pseudemys scripta* has been recorded as 18.5 ± 7 mL/kg.[15] Assuming a similar value for the intubated terrapins in our study, tidal volumes can be estimated as 14 to 25 mL. The volume of the endotracheal tube thus contributes only 4% to 7% of the total volume of a single respiration and is unlikely to have a significant effect on the concentrations of inhaled gases.

To our knowledge, venous blood-gas values of healthy diamondback terrapins have not been reported previously. Whether the measurement of venous blood gases is truly representative of the acid–base status of these animals is unclear. Extensive research with mammalian species (including humans) has shown that, with the exception of pO_2, values from venous samples are generally comparable to those obtained from arterial blood samples, at least for pH, bicarbonate, and (with less precision) pCO_2.[17] Although chelonian cardiovascular anatomy and the potential for shunting of blood between the arterial and venous systems may complicate interpretation, venous blood pH and lactate values have been reported for other species of aquatic turtle, both marine and freshwater.[10,18] The evidence suggests that disturbances of respiratory function in these species result in alterations of venous blood gases similar to that of other vertebrates. Both loggerhead sea turtles and painted turtles showed a marked decrease in venous blood pH and an increase in lactate values (frequently greater than 10 mmol/L) when subjected to forced submersion and apnea in fishing nets.[10,18]

The terrapins in our study all had lactate values well under 10 mmol/L after anesthesia (air median, 3.04 mmol/L; water median, 1.96 mmol/L), suggesting that our anesthesia and respiration protocol maintained sufficient oxygenation to prevent anaerobic metabolism related to hypoxemia. The range of venous blood pH values seen in the terrapins in our study lies between that of loggerhead sea turtles and painted turtles, albeit closer to that of loggerheads, and may reflect differences in the physiology and anoxia tolerance threshold of this euryhaline species.

When we compared venous blood gases after underwater AEP measurement with those of animals undergoing in-air measurements, there were no clear differences in pH, pCO_2, or pO_2. This finding indicates that the intubation and ventilation protocol we used provides gas exchange comparable to that of terrapins breathing spontaneously. For one terrapin, preanesthesia blood pH had a corrected value of 7.421, very close to its postanesthesia value of 7.469. This terrapin was not submerged or intubated and breathed regularly throughout the procedure. Both pre- and postanesthesia pH values of this terrapin were comparable to those seen in the other terrapins, suggesting that submerged anesthesia and manual ventilation for diagnostic procedures does not have detrimental effects on the acid–base balance of diamondback terrapins. The range of lactate concentrations seen in manually ventilated terrapins overlaps completely with that of the nonintubated animals, indicating no relevant increase in anaerobic metabolism to suggest that they exceeded the aerobic dive limit.

The custom-modified endotracheal tubes we used in the current study appear to have provided a sufficient seal within the trachea for safe and effective ventilation during underwater anesthesia. No respiratory or buoyancy abnormalities that might indicate accidental aspiration of water during the submerged procedure were noted in any of the terrapins. The small size of the glottal opening in the smaller male terrapins prevented the use of these endotracheal tubes and the collection of underwater AEP measurements from these animals. It may be possible to adapt smaller-diameter catheters into endotracheal tubes, but there is likely a minimal size limitation on this procedure due to excessive air resistance and the potential for occlusion of the tube by secretions or foreign objects. Resistance to air flow is a grave concern in regard to the intubation of very small animals, because this factor varies inversely with the 4th power of the radius of the tube, thereby creating large increases in resistance related to very small changes in tube diameter.[12] We addressed this concern in our current study through the provision of positive pressure during inhalation and negative pressure during exhalation (by using a catheter-tip syringe), but there likely is a point at which it is not practically possible to overcome the excessive resistance during ventilation.

Sensory biology is one field that may benefit from this technique of providing safe underwater anesthesia, which might be used to evaluate hearing or vision in a variety of air-breathing species. Many reptile and amphibian species perform nonpulmonary (bimodal) respiration, usually through the skin, when submerged. Submerged intubation of these animals could allow for more extensive studies on the extent and efficiency of these processes. Similar techniques and endotracheal tube modifications might also be valuable in studying the respiratory and diving physiology of aquatic and semiaquatic mammals or birds.

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