CHAPTER 29

Techniques for Working with Wild Reptiles

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Capturing wild animals for identification purposes, as well as for radio and satellite telemetry applications, is common in amphibian and reptile research and conservation efforts. In the past, these efforts have been predominantly performed by research biologists with minimal veterinary input. Institutional Animal Care and Use Committees (IACUC) are now required by most research institutions and for publication of research in the peer-reviewed literature. Veterinarians are being asked more frequently for their input, advice, and expertise in areas of infectious disease prevention, aseptic techniques, pain management, anesthesia, and surgery, all of which are critical in the topics discussed in this chapter. The techniques described here are not inclusive but are either commonly used by the authors or important techniques used by the scientific community.

AMPHIBIAN AND REPTILE CAPTURE TECHNIQUES

A variety of techniques are available for capturing amphibians and reptiles. Passive capture methods generally do not restrict an animal's movements; thus they are less likely to result in stress or injury to the animal. Active sampling methods require careful monitoring to prevent injury to animals or mortality but can yield valuable data on population size, individual growth rates, health, reproductive state, and survival. A general overview of commonly used passive and active capture methods for amphibians and reptiles is presented, along with selected references that provide more detail on specific methodologies.

Passive Capture Methods

Cover Objects. Terrestrial amphibians and reptiles can often be captured under natural cover objects such as woody debris and logs, which offer a favorable microclimate. Artificial cover objects can be placed in suitable habitats to attract them so that these species may be systematically sampled. For terrestrial salamanders, "cover boards" consisting of untreated lumber should be placed flush with the soil in shaded or moist conditions (Figure 29-1). Cover boards can be of varying sizes, but relatively small, thick boards are most effective for salamanders.[1] Cover boards should be anchored with a metal stake if they are placed adjacent to a stream or river, where flooding could occur.[2] Tin roofing material or black plastic cover sheets can be used to attract snakes and lizards.[3] These materials should be placed in open sunny areas and checked in the early morning during hot times of the year or in early afternoon in winter. Any type of artificial cover should be left undisturbed for 2 to 4 weeks after placement to allow it to weather and attract target animals. Cover objects can be carefully lifted with a snake hook to check for amphibians and reptiles.

Leaf Litter Bags. Leaf litter offers substrate to larval salamanders and their invertebrate prey but can be a limited resource in many streams. Leaf litter bags made from plastic bird netting (1.9-cm mesh size) and packed with aged leaf litter can be used to attract larval salamanders in streams.[4] Bags can be made by cutting the net into various sized squares (50 × 50 cm to 90 × 90 cm), adding a handful of leaf litter and a few small stones to weight the bag, and pulling the corners of the square together with a plastic zip tie to create a bag.[5] The litter bags should be placed in slow moving pools or meanders within streams and anchored to adjacent vegetation. Researchers can check the bags every 2 to 4 weeks by gently removing them from the stream and placing them directly into a shallow pan along with 3 to 4 cm of stream water. The litter can then be carefully searched for larvae.

FIGURE 29-1 Field crew installing cover boards along a streamside to attract terrestrial salamanders.

Polyvinylchloride Pipe Refugia. Polyvinylchloride (PVC) pipe or large diameter sections of bamboo can be used to attract tree frogs.[6-8] Pipes or bamboo can be cut into short lengths and can be placed in the ground or hung vertically on a nail placed on the trunk of a tree (Figure 29-2). The diameter of the pipes may affect the number of captures, but generally pipes of ¾″ to 2″ diameter are used.[9,10] Ground pipes should be approximately 1 m long, and one end of the pipe should be cut at an angle so they can easily be inserted upright into the soil. Tree pipes should be hung between 1 and 2 m above ground on large diameter trees. Tree pipes can be 0.60 m in length, and placement of a cap on the bottom allows rainwater to accumulate, which makes the pipe more attractive to frogs. A small drain hole should be drilled into capped pipes about 10 cm above the cap to prevent the pipe from completely filling with water. Pipes can be checked by removing them from the ground or tree and checking for tree frogs (Figure 29-3). Frogs can be coaxed from the pipes with the use of a small diameter wooden dowel with a small circular piece of sponge on one end that serves as a plunger. Nontarget taxa (e.g., flying squirrels) may enter the tree pipes and be unable to escape. Thus a short section of nylon rope tied through a hole at the top of the pipe and dangled into the pipe is recommended to allow squirrels a means of escape.[11]

Active Capture Methods

Netting. A variety of nets and netting techniques are available to capture aquatic amphibians and reptiles; net mesh size and configuration should be based on the size and habit of the species targeted for capture. For most freshwater taxa, sampling with nets should be concentrated in emergent vegetation and other available microhabitats. If netting is conducted systematically, the method can be used to estimate densities of target organisms.[12] Sturdy dip nets with a mesh size of 3 mm or less are effective for capturing amphibian larvae and work best in water that is less than 1 m in depth (Figure 29-4). Dip nets should be dragged along the substrate to dislodge and capture amphibian larvae. Seine nets require at least two people but can be used to capture larger amphibians and reptiles in waist deep water. Seine and trammel nets are frequently used to capture Diamondback Terrapins *(Malaclemys terrapin)* in estuarine systems during mid-ebb through mid-flood tides[13] (Figure 29-5).

Hoop Traps. Baited hoop traps are a common method for capturing freshwater turtle species.[14] Hoop traps consist of two to three large-diameter hoops enclosed in netting (Memphis Net and Twine Co. Inc., Memphis, Tenn), with a closed end that can be secured above water to a stake or tree and an open end with an inverted funnel that can be anchored to the bottom of a stream or wetland (Figure 29-6). In streams, hoop traps are typically set with the opening pointed downstream, which allows the scent of bait to disperse downstream. In ponds or lakes, hoop traps can be set either perpendicular or parallel to

FIGURE 29-2 Polyvinylchloride (PVC) pipes used to attract tree frogs. Pipes can be mounted on tree trunks *(left)* or placed in the ground *(right).*

FIGURE 29-3 Pine Woods Tree Frog *(Hyla femoralis)* in a polyvinylchloride (PVC) pipe.

FIGURE 29-4 Dip netting for larval amphibians in a forested wetland.

FIGURE 29-5 **A** and **B,** Biologists seining for Diamondback Terrapins *(Malaclemys terrapin).*

the bank (held in place with two stakes), and the top of the trap should be above water to allow turtles to breathe. A fyke net can be extended from the open end of the hoop trap out into a stream or wetland to guide turtles into the trap. Traps can be baited with chicken livers, sardines, fresh or frozen fish, cat food, canned corn, canned green beans, and watermelon rinds, depending on the diet of the target species.[14-16]

Basking Traps. Freshwater turtles can be captured using basking traps, which consist of either a wire basket anchored to an existing basking log or a floating artificial basking platform with a net in the center that is placed in a wetland or stream so that turtles are attracted to bask on it. In the latter case, a shallow basket (of varying size depending on the target species) can be made with chicken wire and attached to the side of a basking log with the use of a staple gun or zip ties. The chance of success with this method is maximized if appropriate basking logs can be identified in advance with the use of binoculars. Once a suitable log is identified, the trap should be placed on the downstream side of the log to capture turtles as they attempt to jump into the water away from the boat. Artificial basking platforms can be made with a square floating frame with treadles that can tip the turtles into a barrel or net in the center or that simply relies on the turtles falling into the trap when disturbed.[17,18] Artificial basking traps are especially effective when placed amidst floating aquatic vegetation in water bodies with few natural basking sites. Traps can be checked when the sun is high and turtles would be expected to be out basking. Traps should be checked frequently to prevent turtles from overheating.

Drift Fences. A drift fence is a barrier that directs amphibians and reptiles toward traps.[19] Fences may be constructed from aluminum flashing, hardware cloth, or siltation fence, a portion of which is buried below ground to prevent animals from digging under the fence. Drift fences can be placed in specific configurations called arrays or may encircle particular habitats, such as a pond, to capture animals migrating to and from a breeding site[19,20] (Figure 29-7). Traps are generally placed every 5 to 10 m along a fence or at the ends of short sections of fence. Pitfall (bucket) or funnel traps (described further on) are most effective for terrestrial amphibians, small snakes, lizards, and turtles. Wooden box traps[21,22] are effective for capturing large snakes; lizards and terrestrial amphibians can also be captured in box traps along drift fences (Figure 29-8). Traps should be shaded with cover boards, and a wetted sponge or shallow water bowl should be placed in the traps to protect animals from desiccation. This method requires considerable effort to install and maintain, particularly if a wetland is completely encircled. Traps must be checked daily or multiple times a day during migration events and extreme weather conditions (hot or cold) to prevent mortality.

Funnel Traps. Various trap designs with inverted funnels at the openings are highly effective at capturing amphibians and reptiles. For example, commercial minnow traps and crayfish traps can be used to capture large aquatic salamanders (*Siren* spp. and *Amphiuma* spp.), juvenile and small aquatic turtles, and aquatic or semiaquatic snakes.[23] These traps can be placed in standing water with a portion of the trap left above water to allow animals to surface and breathe. Traps should be anchored to a tree or stake so they cannot be dislodged by predators and should be clearly marked so they are easy to find. Several iterations of funnel traps can be made by hand with the use of inexpensive, readily available material. For trapping along a drift fence, funnel traps can be made with the use of aluminum window screen (Figure 29-9). Screen funnel traps can be staked down using a wire pin flag or can be floated in shallow water. A recent variation of a funnel trap is made from a 120-L plastic trash can and funnels made from 6-mm mesh galvanized hardware cloth,[24] which was inexpensive to make and highly successful for capturing sirens, amphiumas, and aquatic snakes.

Hook and Line Noose or Cage Traps. Crocodilians can be captured by a hook and line noose or by cage trapping.[25,26] The use of a hook and line noose requires close access to the alligator and considerable strength to catch the animal and pull it to shore for further restraint. A treble hook and fishing line can be used to snag the animal on the osteoderms or bony scutes of the dorsum. Capturing individuals greater than 1 or 1.5 m in length generally requires a large (7 to 11) gauge treble hook and doubled 30-lb line with a 6 ft leader and a hook secured with a bimini twist (Andrews K: Pers. observation, 2007). When snagged, the animal can be gradually pulled close enough that a noose can be eased over the snout and tightened, after which the animal can be pulled to

FIGURE 29-6 **A,** Funnel trap set for Alligator Snapping Turtles *(Macrochelys temminckii)* in shallow water in a river in southwest Georgia. **B,** Pulling the funnel trap into the boat for transport to process. **C,** Pulling an Alligator Snapping Turtle out of a trap.

FIGURE 29-7 Drift fence surrounding an isolated wetland in southwestern Georgia. The fence directs amphibians and reptiles toward pitfall traps (19-L plastic buckets) that are shaded and contain a wetted sponge.

FIGURE 29-8 Researchers are removing an Eastern Diamondback Rattlesnake *(Crotalus adamanteus)* from a box trap associated with drift fences constructed with the use of hardware cloth.

shore. Once on shore, the tail can also be noosed (on larger crocodilians), and the jaws and front and hind legs can be restrained with electrical tape. Hook and line techniques can be time-consuming because multiple throws may be required to properly secure the animal, and it may be necessary to allow the animal to become fatigued. However, this technique is the best approach when capturing animals that are close to shore or in habitats inaccessible by boat (e.g., stormwater lagoons). Elsey and Trosclair[26] describe large steel traps that can be used for crocodilians; smaller wire-cage live traps (Havahart; Woodstream Corp., Lititz, Pa) can be used for small crocodilians.[25]

Hand Capture. A number of amphibians and reptiles can be captured by hand under appropriate conditions. Snakes, salamanders, and frogs are frequently collected on roads at night, particularly during or immediately after rain. Many aquatic species, including snakes, frogs, and alligators, can also be captured in wetlands at night using a bright light or head lamp to detect eyeshine or by homing in on movements or calls. With training, many people can identify frogs by their unique calls. For many species of frogs, it is possible to quietly approach them as they call and catch them by hand or with a dip net. During the day, many frogs can be detected along the margins of wetlands or under natural cover objects such as logs. Scuba, snorkeling, and jump/catch/hand grab techniques have been used for a variety of freshwater turtles, Diamondback Terrapins, and sea turtles.

FIGURE 29-9 Funnel trap made from aluminum window screen and used to capture small amphibians and reptiles in a swamp.

Cage Traps/Bucket Traps for Gopher Tortoises. The two most commonly used methods to trap Gopher Tortoises (*Gopherus polyphemus*) are bucket traps[27] (Figure 29-10) and wire-cage, one-door live traps (Havahart; Woodstream Corp., Lititz, Pa)[28] (Figure 29-11). A bucket trap is made with a 5-gallon light-colored plastic bucket with at least ten 0.25″-diameter holes drilled in the bottom. Smaller buckets, or plastic plant pots, can be used to trap juvenile tortoises. The bucket is placed in a hole dug in front of the burrow entrance. Care should be taken when digging the hole because this is typically where female tortoises lay their eggs (eggs may be in the nest from mid-April through October). Newspaper, wax paper, or brown wrapping paper is used to cover the top of the bucket once it has been sunk into the ground. The paper is cut so that it is approximately 2 inches wider on all sides than the diameter of the bucket and secured tightly around the edge of the bucket with a few slits to ensure that it tears as the tortoise steps onto it. A thin layer of sand is placed on the paper to conceal the trap. Shading each bucket trap will prevent trapped tortoises from being exposed to direct sunlight for prolonged periods. Drowning and overheating are the most common problems occurring with this trapping technique. One advantage of this technique over wire-cage traps is that the tortoise can be captured whether it is inside or outside the burrow.

An alternative method is using a wire-cage trap in front of the burrow. The burrow should be scoped to ensure that the tortoise is in the burrow before setting a trap. A major advantage of this method is that a hole does not have to be dug; thus there is no chance of damaging eggs if a nest is present, and it is less stressful to the tortoise. Small wire-cage traps can be used to capture juvenile tortoises. The trap should be well

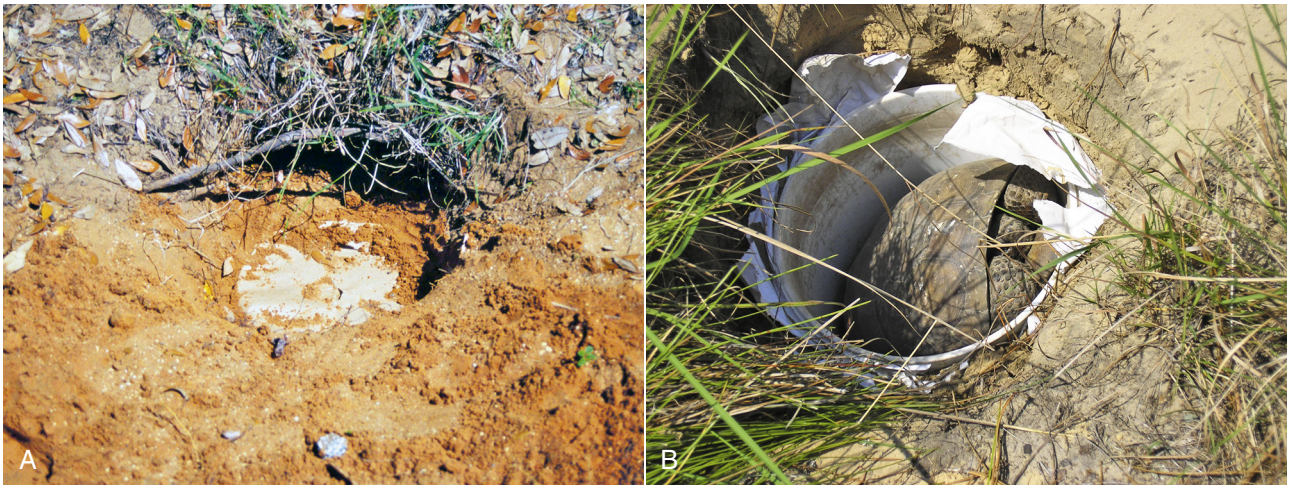

FIGURE 29-10 **A,** Bucket trap set and ready for trapping. **B,** Gopher Tortoise *(Gopherus polyphemus)* caught in a bucket trap. *(Photos courtesy Tracey D. Tuberville, Savannah River Ecology Laboratory, The University of Georgia, Athens, Ga.)*

camouflaged and shaded. It is advisable to check the traps two to three times per day regardless of type. During the colder months of the year (mid-October through mid-April), tortoises may not leave their burrows for extended periods, which makes these techniques less effective. Tortoises generally will not surface to bask until temperatures reach at least 18°C to 21°C (65°F to 70°F).

Shrimp Trawl. Foraging sea turtles can be collected by independent fishery trawlers. Twenty-meter four-seam nets with 20-cm mesh, without turtle excluder devices, are used by one research vessel to capture sea turtles.[29] The trawl duration was limited to 30 minutes (Figure 29-12).

Nesting Beach Encounters. Nesting female sea turtles can be sighted during nightly beach patrols and observed from a distance until they finish nest excavation. Once the turtle starts the process of oviposition, she typically enters a trancelike state and may be safely processed without disrupting normal nesting behavior (Figure 29-13). The turtle should be allowed to complete the nesting process and cover her eggs. Procedures that are more time-consuming, such as satellite tag placement, may require additional restraint. Four slotted boards can be interlocked to create a box around the turtle. Sand can be pushed around the box for further security (Figure 29-14).

Hoop Net and Boat Approach. Foraging Leatherback Sea Turtles *(Dermochelys coriacea)* can be located via aerial surveys and captured from a boat with the use of a 1.5-m diameter breakaway hoop net. Once secured in the net, the turtle is brought aboard the capture boat for processing. Injury to the turtle can be avoided if the boat is equipped with rubberized surfaces and rounded edges, and air-filled plastic or other similar padding should be placed around the turtles to cushion the body. A continuous flow of sea water over the turtle will help keep it cool while on deck (Figure 29-15).

HANDLING

Recommended Precautions to Prevent Disease and Other Health Problems

Infectious diseases can be transmitted within and between amphibian and reptile populations when handled for various procedures by field workers. Some examples of significant

FIGURE 29-11 **A,** Scoping a Gopher Tortoise *(Gopherus polyphemus)* burrow to see if it is inhabited before setting a wire trap. *(Photo courtesy Dr. Terry M. Norton, Georgia Sea Turtle Center, Jekyll Island, Ga.)* **B,** Live wire trap, shaded and set in front of Gopher Tortoise burrow. **C,** Juvenile Gopher Tortoise captured in live wire trap. *(Photos courtesy Tracey D. Tuberville, Savannah River Ecology Laboratory, The University of Georgia, Athens, Ga.)*

FIGURE 29-12 **A,** Leatherback Sea Turtle *(Dermochelys coriacea)* captured with a quick release hoop net. *(Photo courtesy of Connie Merigo, New England Aquarium, Large Pelagics Research Center, NMFS Permit #1557-3.)* **B,** Leatherback Sea Turtle on board for tagging, health assessment, and satellite transmitter placement. *(Photo courtesy of Kara Dodge, Large Pelagics Research Center, NMFS Permit #1557-3.)*

FIGURE 29-13 Female Leatherback Sea Turtle *(Dermochelys coriacea)* being tagged while laying eggs. *(Photo courtesy St. Kitts Sea Turtle Monitoring Network.)*

FIGURE 29-14 Loggerhead Sea Turtle *(Caretta caretta)* surrounded by a wooden restraint box for satellite telemetry placement after finishing the nesting process. *(Photo courtesy of Mark Dodd.)*

infectious and parasitic diseases that may be transmitted when appropriate precautions are not taken include chytrid fungus *(Batrachochytrium dendrobatidis)* in amphibians, iridovirus in amphibians and reptiles, *Cryptosporidium serpentis* in snakes, paramyxovirus in snakes, *Entamoeba invadens* in reptiles, *Mycoplasma agassizii* in tortoises, and herpesvirus (fibropapillomatosis) in sea turtles. The following are some general guidelines for handling and manipulating herpetofauna in the field:

- Avoid using insecticides before handling amphibians and reptiles.
- Wash hands with disinfectant hand wash or wear separate disposable examination gloves after handling each animal.
- Clean any handling equipment with an appropriate disinfectant between each animal.
- Use a separate clean, well-ventilated container or bag for each animal if temporary containment or transport is needed.
- Avoid high or low temperatures during transport of the animal.
- Do not handle or transport amphibians and reptiles from different populations at the same time.
- If wild amphibians or reptiles are held in captivity for various procedures such as surgical placement of radio transmitters, each animal should be isolated in a well-ventilated container and kept at its preferred optimal temperature zone with a thermogradient. The enclosure should be easily disinfected. Wood enclosures should be avoided because they cannot be disinfected.

FIGURE 29-15 **A,** Leatherback sea turtle (*Dermochelys coriacea*) captured with a quick-release hoop net. **B,** Leatherback Sea Turtle on board for tagging, health assessment, and satellite placement. *(Photos courtesy of Kara Dodge, Large Pelagics Research Center, NMFS Permit #1557-3.)*

MARKING

Recommended Precautions to Prevent Disease and Other Health Problems

Field researchers working with amphibians and reptiles should have some basic aseptic technique and disease prevention protocols established for the field site and species under investigation that are practical but effective. Preventing individual animal infections and spreading of disease from one individual to another within a population and, most importantly, preventing population level disease outbreaks should be the ultimate goal. A risk assessment should be conducted for each project that evaluates the appropriate level of precautions that should be taken for that specific situation.

Amphibians. Because of the permeable nature of the amphibian skin, they are very sensitive to many toxins at levels that are well-tolerated without ill effects by reptiles, birds, and mammals. The amphibian skin is very sensitive to human handling. Soaps, lotions, and natural oils can all irritate or damage the skin. Researchers should wear powderless vinyl or plastic gloves when handling amphibians. Many of the common antiseptics and disinfectants are toxic to amphibians (e.g., surgical scrubs with soaps or detergents, iodine products, chlorhexidine, quaternary ammonium compounds, chlorine, alcohol, and ammonia). For these reasons, surgical, injection, and tag sites should be wiped clean of debris and secretions with clean unchlorinated water or saline before the procedure.[30]

Reptiles. Depending on the procedure, the area should be cleaned at least once with a surgical scrub (betadine or chlorhexidine) followed by 70% isopropyl alcohol. For invasive surgical procedures, such as internal placement of radio transmitters, surgical preparation that repeats this aforementioned process four times is recommended. Examination gloves should be worn when minor invasive procedures are performed on reptiles and should be changed between animals. Where feasible, hand washing or an alcohol-based hand disinfectant should be used between animals. Ideally, instruments such as tagging applicators, passive integrated transponder (PIT) tag injectors, and other instruments should be gas sterilized ahead of time or cold sterilized in the field with the use of an appropriate disinfectant such as dilute chlorhexidine solution (2% chlorhexidine gluconate; Phoenix Pharmaceutical, Inc., St. Joseph, Mo) between animals. A review of the advantages and disadvantage of the various antiseptics and disinfectants available can be found in other chapters of this book. For true disinfection of instruments to be achieved, a 30-minute soak time is preferred, but no less than 10 minutes is recommended before use on the next animal. This precaution may not be practical in every situation; thus the researcher will need to establish specific protocols depending on the species, potential for disease transmission, and numbers of animals being processed.

Manual Restraint, Analgesia, and Anesthesia

Many of the procedures discussed in this section can be performed with manual restraint alone. Some procedures, depending on the species, may require local anesthesia, pain management, sedation, or general anesthesia. The research team should consult and collaborate with a veterinarian experienced with amphibians and reptiles when deciding on the implementation of appropriate protocols. Other chapters in this book may be consulted for details in this area.

Temporary or Semipermanent Marking and Identification Methods

Photo Identification. Photographs are frequently taken as a form of identification in amphibians and reptiles and can be particularly useful in the case of a morphologic anomaly or injury. High-resolution digital photographs that clearly show the anomaly, injury, mark, or natural pattern of individuals can be taken each time the animal is captured. Photos can be stored electronically or printed and used in the field. Although noninvasive, this method can be time-consuming for identifying individuals and requires that patterns are stable throughout the life of the animal. Thus photographs are most useful as a

FIGURE 29-16 Spot patterns on the dorsum of an Eastern Tiger Salamander *(Ambystoma tigrinum).* The *upper* photograph was taken in 2007 and the *lower* photograph was taken in 2011. *White arrows* indicate unique spots that can be used to recognize this individual. The *red arrows* show a slight change in one of the spots over the 5-year period.

FIGURE 29-17 Gopher Tortoise (*Gopherus polyphemus*) with very distinct, old, healed pitting lesions that have been used for photo identification.

secondary means of identifying individuals. Some amphibians (e.g., Eastern Tiger Salamander, *Ambystoma tigrinum*) do not develop spots until several months after metamorphosis, and spots may change slightly as the animal ages. However, with careful attention, patterns can be clearly recognized even after several years (Figure 29-16). Photo identification also has utility for monitoring freshwater, marine, and terrestrial turtles, in which shell anomalies and injuries are common (Figure 29-17). Photographs of the pineal spot of Leatherback Sea Turtles can be useful for later identification.[31] This technique can also be useful with patterned snakes, such as rattlesnakes, that have variation in the number or shape of dorsal markings.

Paint Marking. Paint marking has been used as a noninvasive means of identifying reptiles. Different color

FIGURE 29-18 Gopher Tortoise *(Gopherus polyphemus)* hatchlings marked with a paint pen and nail polish.

combinations and paint locations can yield numerous individual marks. Small daubs of model airplane paint applied to the limbs, base of the tail, and dorsum have been recommended for temporarily marking lizards.[32,33] Data suggest that paint marking did not increase mortality of lizards from predation[34]; however, care should be taken to use the minimum amount of paint necessary to identify the lizard. Paint marking has limited utility for most snake species because recaptures are so infrequent. However, paint has been widely used to temporarily mark the rattles on crotalids.[35] This technique is particularly useful for capture–mark–release programs in which thorough visual searches for snakes are conducted over short time frames. In this case, recently captured individuals can be identified visually without having to have them in hand. An individual marking scheme can be developed based on color and the position of the marks on the rattle, or a common mark can be used for a cohort or population. The mark can be lost completely if the rattles break; therefore this technique is most useful for identifying individuals within an activity season.

Paint pens and nontoxic nail polish have been useful in temporarily marking the carapace of a variety of freshwater aquatic and terrestrial chelonian species (Figure 29-18). One example in which this is particularly useful is for identifying individual Gopher Tortoises in their burrow with a burrow camera scope. If the tortoise had already been processed that season, it had a number on its carapace that was readily visible with the scope. This information is essential when setting traps to capture these tortoises (Figure 29-19).

Paint and other temporary marks have also been applied to sea turtles, but the technique is less common than in freshwater and terrestrial turtles. Because of the wear on the carapace and the corrosive nature of salt water, paints do not last as long as in freshwater. Two-part resins have also been used to temporarily mark sea turtles.[36,37] To further increase retention throughout a nesting season, one can use a Dremel tool to create grooves in scutes that can then be filled with paint.[36] Paint, like other temporary marking techniques, is generally used in conjunction with other more permanent marking techniques.[38]

FIGURE 29-19 Adult Gopher Tortoise *(Gopherus polyphemus)* with paint pen number and radio transmitter on carapace going into a burrow.

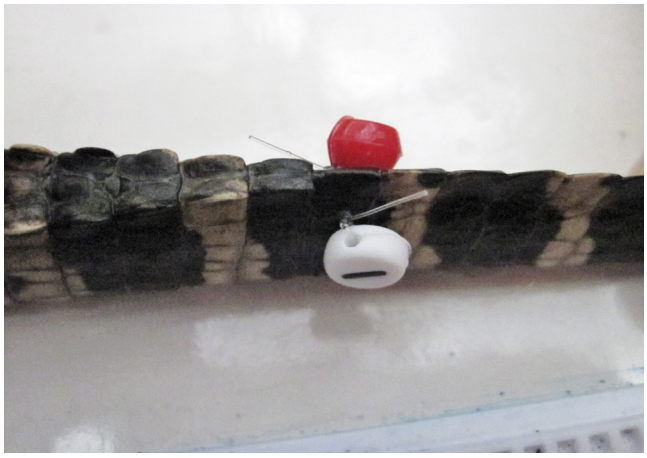

FIGURE 29-20 Photograph of colored and numbered beads used to mark American Alligators *(Alligator mississippiensis). (Photo courtesy Tracey D. Tuberville, Savannah River Ecology Laboratory, The University of Georgia, Athens, Ga.)*

Colored Beads. Small colored and numbered beads have been used to mark American Alligators ([*Alligator mississippiensis*]; Tuberville T: Pers. com., Jan 14, 2012; [Figure 29-20]) and West Indian Rock Iguanas (*Cyclura* spp.; Hudson R: Pers. com.; [Figure 29-21]). In the alligator study, researchers applied two beaded tags on the single row of tail scutes using fishing line. Using two tags that varied in color and number, they were able to identify individuals and to differentiate among experimental groups without having to handle the animals. Marking could involve one to three beads of varying colors and numbers on each individual. Bead retention was sufficiently high in captive animals, but this marking technique would presumably not be viable in the wild where interaction with other animals and habitat debris would decrease retention rates.

Fluorescent Polymers, Pigments, and Tags. Acrylic polymers (also called visible implant elastomers [VIEs]) and fluorescent pigments can be injected into the skin of amphibians in different portions of the body (e.g., the tail of salamanders, sides of the body, and hind legs of frogs). Different injection sites, color combinations, and numbers of marks can allow for large numbers of individuals to be uniquely marked, and marks can be retained for up to 2 years.[39] This technique is one of the few reliable methods for marking larval amphibians; polymers can be injected into the tail fins of larvae.[40]

Small prenumbered fluorescent tags (Figure 29-22); Northwest Marine Technology, Inc., Shaw Island, Wash; Floy Tag, Inc., Seattle, Wash) also can be implanted subcutaneously in frogs and salamanders.[41] These tags come in a variety of colors and sizes and work best in species with lightly pigmented skin. These fluorescent tags can be accurately read using an LED light.[42] Floy tags have been used as tertiary identification in some chelonian research projects. The numbered tags can be secured to the shell with a clear, fast-curing epoxy. As stated earlier, the tags come in a variety of sizes and are small enough to use on juveniles without compromising their growth.

Monel, Inconel, and Cattle Ear Tags. Monel and cattle ear tags are effective and an inexpensive means of marking crocodilians. Monel tags have been attached to the webbing on the rear foot of American Alligators[43]; this technique requires recapturing the animal to read the tag. Monel tags do not corrode as rapidly in freshwater systems as they do in salt water (see further on); thus these tags are effective for most crocodilians. Plastic cattle ear tags have also been used to mark crocodilians. Roto (Sheepman Supply Co., Frederick, Md) and Allflex (Allflex USA, Inc., Dallas, Tex) ear tags, in particular, are inexpensive. These tags experience a higher rate of retention when the scute is pierced on the tail of American Alligators prior to attachment, by using a fine drill bit on the tagging device to facilitate penetration and secure latching of the male and female components of the tag (Andrews K: 2010). However, retention of external tags is generally low in crocodilians because of extensive, aggressive interactions with conspecifics. The use of these tags is beneficial because individuals can be identified from a distance with binoculars when the animal is out of the water.

Both plastic and metal tags are used to mark the flippers of sea turtles. Tags come in varying sizes, and smaller tags should be used on immature turtles. Plastic tags are inexpensive but are susceptible to breakage, wear, and entanglement in nets.[36] Although tags made from metal alloys (Monel, Inconel, and titanium) may last longer than plastic tags, these tags are more expensive than plastic tags and Monel tags can also be susceptible to breakage or corrosion, which can lead to infection at the attachment site.[44] Research entities such as the Archie Carr Center for Sea Turtle Research at the University of Florida and the Wider Caribbean Sea Turtle Network (WIDECAST) work in coordination to facilitate a global database of turtle tags and to reduce potential redundancy in marking. In addition to individual marking codes, contact information can be placed on the back tags to assist with tag recoveries.

In sea turtles, tags are generally attached to the edge of fore flippers, and when they are applied correctly, the risk of infection or injury at the attachment site is generally low. Tags may either be inserted through the keratinized scales on the flippers or in the soft tissue between scales. Although no comparative data are available, tags placed in the middle of the scales have been retained for approximately 20 years in some

FIGURE 29-21 **A** and **B,** Colored beads to identify Caribbean iguanas. *(Photos courtesy of Rick Van Veen.)*

FIGURE 29-22 Alphanumeric tags for marking amphibians. Tags come in a variety of sizes and colors and can be implanted subcutaneously in amphibians via a small incision or with a specially designed syringe.

FIGURE 29-23 Placement of metal Inconel flipper tags on proximal pads on the front flipper of a marine turtle; the curved part of the tag allows room between the tag and flipper to reduce risk of constriction. *(Photo courtesy of S. Dilts.)*

individuals.[36,45] Tags are typically placed either in the most proximal pad on the posterior edge of the fore flipper or on the second pad to prevent the turtle from biting the tag[46] and to reduce drag or contact with other animals (Figure 29-23).[47] If the original tag is lost, replacement tags should be placed in a different pad rather than over the scar from the initial tag. Researchers generally tag both foreflippers, but the proximal pad of rear flippers are frequently tagged on juveniles and Leatherback Sea Turtles.[36]

Many of the factors affecting retention are avoidable through proper application and the incorporation of aseptic techniques to reduce risk to turtle health. A common cause of tag loss is failure of the locking mechanism[44]; tags should be double-checked before release and replaced if necessary. Applicators should be cleaned regularly to reduce rust and debris that can impede proper application of the tag, and they should be replaced when necessary to avoid tag loss. Tags may also be lost if they are snagged by debris (e.g., nets)[44] or substrate, as is the case with Hawksbill Sea Turtles *(Eretmochelys imbricata),* which shelter in coral reefs. Tearing generally results in tag scars that are readily visible and provide some information on whether the animal was marked in the past. Flipper tags can also be lost because of tissue necrosis if they are improperly applied. Tags should be placed such that roughly two thirds of the tag covers the flipper and the remaining one third, specifically the curved part, hangs over the edge of the fore flipper so that it does not constrict

FIGURE 29-24 Syringe and passive integrated transponder (PIT) tag used to mark amphibians and reptiles for individual recognition. Tags are passive (no battery).

circulation or growth. Because of problems with tag retention, biologists regularly tag both fore flippers in addition to using permanent marking methods such as PIT tagging. It is imperative to account for flipper tag loss to avoid overestimation in population abundance and underestimation of survivorship.[48]

Permanent Marking Methods

Passive Integrated Transponder Tags. Passive radio frequency identification (RFID) tags (or PIT tags) are one of the most widely used and reliable tools for marking amphibians and reptiles.[49,50] A PIT tag is an electronic microchip encased in biocompatible glass that ranges in size from 8 to 22 mm long and about 2 mm in diameter; the smallest tags weigh less than 0.10 g (Figure 29-24). Tags are dormant until activated by a handheld reader (Figure 29-25), which generates a close-range, electromagnetic field. Each tag contains a unique alphanumeric code that allows an indefinite number of individuals to be marked. The detection range for PIT tags varies with the size of the tag and type of reader. PIT tags can be expensive when large numbers of individuals are marked but have the benefit of small size and a potentially infinite life span.

There are two mainstream manufacturers of PIT tags: EZid American Veterinary Identification Devices (AVID, Weld, Colo) and Biomark, Inc. (Boise, Idaho), although there are additional manufacturers.[50] Both companies manufacture tags that are reliable and functional for the life of most animals, provided tags are inserted correctly (see further on); however, data on retention in long-lived species are not yet available. Biomark offers waterproof readers that are durable for use in the field. It is best to use one supplier at a field site because some brands are encrypted and cannot be read with the readers of other manufacturers. Further, care should be taken when recording identification codes from the reader because it is easy to accidentally transpose numbers or letters.

In addition to marking individuals, PIT tags can be used to monitor thermoregulatory behavior and movements of animals. BioMedic Data Systems, Inc. (Seaford, Del) produces temperature-sensitive PIT tags that have been used to monitor body temperature in snakes.[51,52] Automated scanning systems placed in areas the animal is expected to cross can be used to track movements of PIT-tagged animals. Scanning systems

FIGURE 29-25 The unique alphanumeric code of a passive integrated transponder (PIT) tag is activated when scanned by a manufacturer-specific PIT tag reader. *(Photo courtesy of D.E. Scott.)*

have been used to monitor migrations of PIT-tagged amphibians to and from breeding ponds or burrows[53-55] and use of passages and culverts under roads by Desert Tortoises *(Gopherus agassizii)*.[56] However, scanning systems are expensive, and the detection range of PIT tags in the field can be very limited.

PIT tags are injected or inserted through a surgical incision, most commonly under the skin, into muscle or the body cavity (Figure 29-26). Application of tissue glue at the insertion site can help with tag retention and can control bleeding (Norton T: Pers. observation, 2011). Tags can be purchased in preloaded sterile syringes or individually and loaded into a sterilized stainless steel syringe for implantation (see Figure 29-24). All PIT tags should be sterilized before insertion if this has not been done by the commercial source. The preferred technique for sterilization is ethylene oxide gas (STERIS Isomedix Services, Mentor, Ohio) or hydrogen peroxide plasma sterilization (Sterrad, Advanced Sterilization Products, Irvine, Calif). Sterility should be maintained during the loading and insertion of the PIT tag.

In frogs and toads, PIT tags can be inserted subcutaneously after the area is cleaned as described earlier, or they can be inserted into the coelom with the use of the procedures described in the Surgically Invasive section of the chapter. For subcutaneous implantation, approximately 4 mm of skin on the ventral surface can be pinched and the tag inserted via syringe.[57] In Eastern Hellbenders *(Cryptobranchus alleganiensis alleganiensis)*, PIT tags were implanted in the dorsal side of the tail approximately 8 cm posterior to the hindleg.[58]

In reptiles, PIT tags are generally implanted subcutaneously, although implantation sites vary by taxa. For example, in crocodilians, PIT tags can be injected subcutaneously at the junction of the ninth and tenth ventral scale rows posterior to the cloaca.[49] In lizards, PIT tags can be inserted

subcutaneously, through a small incision in the skin on the side of the animal[34] or anterior to the left rear leg in large lizards or tuatara.[59] In snakes of all sizes, PIT tags can be implanted subcutaneously between the second and third left dorsal scale rows, approximately two thirds of the way down the body.[50,60] They can also be inserted into the body cavity during transmitter placement. PIT tagging may not be appropriate for all species because tags may be expelled through the walls of the digestive tract of some species (e.g., Brown Water Snakes, *[Nerodia taxispilota]*[61]), and tags placed in the body cavity can migrate.[49] The frequency with which PIT tags are expelled or migrate within the body has not been quantified for any species.

PIT tags in adult freshwater turtles, terrapins, and tortoises can be injected into the loose skin at the shoulder or hind leg on either side of the body. Buhlmann and Tuberville[62] describe a method for implanting PIT tags in small aquatic turtle species such as *Sternotherus* and *Kinosternon* spp. They recommend holding the animal vertically and carefully inserting the syringe above a hind leg, to implant the tag adjacent and parallel to the bridge of the shell. Researchers have surgically implanted sterile PIT tags in hatchling and small turtles and tortoises by making a small incision into the inguinal skin just in front of the back leg, creating a small pocket under the skin with hemostats, and then inserting the PIT tag with the hemostats. Tissue glue is used to close the incision. Local anesthesia with lidocaine or bupivacaine can be used depending on the size of the patient. Care should be taken to avoid toxicity in small turtles. Dexmedetomidine alone or in combination with ketamine and/or an opioid agent is a good reversible sedative that can be used for this procedure. Dosage depends on the species. PIT tags appear to have high retention rates but may migrate from the implantation site; thus scanning the entire body for tags is recommended.

PIT tags are also commonly used to mark sea turtles. The minimum straight carapace length for PIT tag application is 30 cm. Tags are placed in the left front flipper either in the triceps superficialis muscle (shoulder area) or in the flexor carpi ulnaris (posterior aspect of the flipper, anterior and immediately distal to the second large scale). Tags should not be inserted through fibropapillomas or any abnormal skin. Infection rates are low provided that appropriate sterilization and site cleaning techniques are employed.[63] PIT tags are generally used as a secondary marking method in sea turtles because flipper tags are often lost.[64,65] Redundancy in marking methods is critical in sea turtles because of possible migration of PIT tags and the difficulty in effectively scanning such large animals. In addition, differences in PIT tag manufacturers used in different projects around the world may preclude detection.

As with other taxa, PIT tags would seem to have advantages over other marking methods because the tags are small, passive, and may last indefinitely. However, a study found higher levels of corticosteroids in lizards implanted with PIT tags than in those marked by toe clipping.[66] It is possible that such stress responses occur in other taxa, but this effect of marking has not been quantified sufficiently because most of the studies that have been conducted only measure survivorship of the individual and tag retention.

Shell Drilling and Notching. Adult turtles and tortoises can be permanently marked by notching the marginal scales of the carapace; appropriate clean techniques should be used (i.e., the area to be notched or drilled should be cleaned with betadine and alcohol at least once before drilling, and the drill bits should be soaked in an appropriate disinfectant between turtles).[67] Turtles and tortoises have 11 to 12 scales on each side of their carapace; scales at the anterior and posterior portion of the shell generally have a thin margin that can easily be notched with a triangular file (Figure 29-27) or Dremel tool or drilled with the use of a small drill bit (Figure 29-28).[68] Scales on the bridge connecting the carapace and plastron (scales 4 to 7) should not be filed because they are generally not flared and there is a risk of penetrating the body cavity. In addition, drilling holes directly through bone may cause lesions in the bone or bone marrow.

Several different numbering or alphabetical schemes can be derived when scute notches are made on turtles, but in general, scales in each of the four quadrants of the turtle (i.e., front right, front left, rear right, rear left) are assigned a series of numbers (e.g., 1s, 10s, 100s, and 1000s) or letters such that thousands of individuals can be uniquely marked[68] (Figure 29-29). Shell notching has been used successfully to

FIGURE 29-26 Subcutaneous injection of a passive integrated transponder (PIT) tag into an Eastern Kingsnake *(Lampropeltis getula)* with the use of a 12-gauge needle. *(Photo courtesy of D.E. Scott.)*

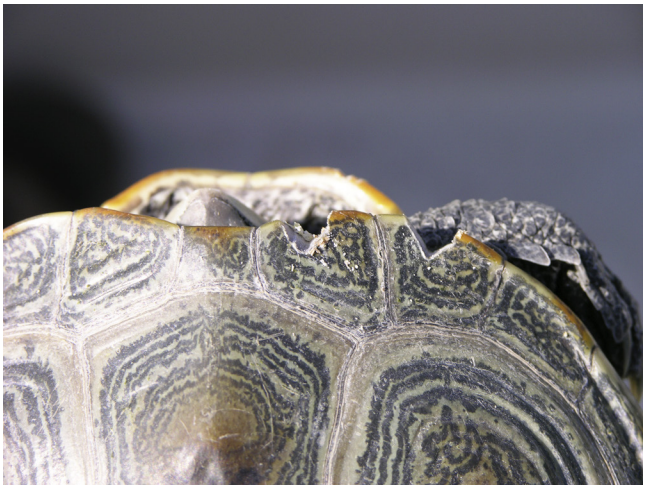

FIGURE 29-27 Shell marginal notched with a triangular file. *(Photo courtesy Dr. Terry M. Norton, Georgia Sea Turtle Center, Jekyll Island, Ga.)*

identify individual turtles over more than 25-year capture intervals (Gibbons W, Greene J: Unpublished data, 2001). Soft shell turtles lack bony marginal scales but can be marked by notching the margin of the shell[69,70] or by clipping claws.[67] Hatchling turtles can be marked with the same numeric and alphabetical system with the use of fingernail clippers to create a notch in the marginal scutes. Most chelonians tolerate notching of the shell without analgesia or sedation, but if a drill is used to create a hole in the shell, adequate sedation and analgesia is indicated. Dexmedetomidine alone or in combination with ketamine and/or morphine derivatives are good choices because they are effective and reversible.

FIGURE 29-28 Use of a handheld drill to mark marginals on larger freshwater turtles. *(Photo courtesy Dr. Terry M. Norton, Georgia Sea Turtle Center, Jekyll Island, Ga.)*

FIGURE 29-29 Example of a turtle shell marking scheme. Marginal scales should be notched such that the turtle can be individually identified. Combinations of scales (e.g., numbers 1 and 2 on the upper right quadrant of the carapace) can be notched to mark the turtle as number 3.

Shell notching does not work as well on sea turtles as on freshwater turtles because of their thick shells, extensive growth, and the frequency of marginal shell injury that can obscure marks. However, a cordless drill can be used to drill holes through the keratinous overlay in the supracaudals (Figure 29-30). As the turtle grows, holes in the keratin migrate toward the edge of the marginals. If a turtle is recaptured, the same drill pattern can simply be reapplied at the highest part of the keratin. On nesting female sea turtles, a flashlight can be shined beneath the shell to determine where the keratin layer extends beyond the bone. Shell notching is not possible on all species, such as Olive Ridley Sea Turtles *(Lepidochelys olivacea)* (Andrews K: Pers. observation, 2011). In other species, such as Loggerhead Sea Turtles *(Caretta caretta),* drilling can be problematic because of high loads of epibiota that can prevent drilling or that can grow over holes and prevent positive identification (Andrews K: Pers. observation, 2011). However, this technique has proven effective and essential in a long-term saturation tagging project of Hawksbill Sea Turtles.[45] Many of the techniques used here are performed by biologists in the field studying large populations of reptiles and amphibians. As this field evolves veterinarians are becoming more involved and can assist biologists in making decisions about practical but appropriate analgesia for painful procedures and where clean and sterile techniques need to be utilized. These techniques are rarely indicated in clinical practice where individual pet reptiles are involved; however, it is helpful for veterinarians dealing with amphibians and reptiles to be familiar with the current identification practices that are performed.

Toe Clipping. Historically, frogs, salamanders, lizards, and juvenile crocodilians were often marked by removal of toes or *toe clipping.*[32,71] There is some controversy about the level of pain produced by this procedure and whether analgesia or sedation should be used when toe clipping is performed.[30] When large numbers of animals are processed, it may be impractical to provide such intensive care. Researchers using this technique should keep this in mind and consider seeking alternative identification methods when available. The advantage of toe clipping is the low cost and large numbers of unique marks available if individual digits and limbs are assigned

FIGURE 29-30 Use of a handheld drill to mark keratinous growth on subcaudals on marine turtles.

a different series of numbers. To facilitate recognition of a toe-clip mark versus a natural injury, researchers can remove multiple toes on different feet. Removal of adjacent toes should be avoided. The thumbs of adult male frogs, which are used in amplexus, should never be removed, nor should more than two digits on any one limb be removed. Scissors should be disinfected with an appropriate product (e.g., chlorhexidine) before use and between animals. A drop of tissue glue can be used to stop the bleeding. Excised toes can be preserved in ethanol for genetic analysis or skeletochronology.[72,73]

In addition to pain from the procedure, there is concern that toe clipping may affect survival of individuals. Studies have shown that the number of toes clipped may negatively affect frog survival,[74] particularly in arboreal species (e.g., tree frogs, *Hyla* spp.). However, a laboratory study on Squirrel Tree Frogs *(Hyla squirella)* found no difference in growth and survival after toe clipping.[75] Many salamanders and some frogs can regenerate toes quickly; thus the reliability of the method is further limited. Both toe clips and injectable fluorescent polymers (mentioned previously) have been used to mark individual amphibians[76]; the advantage of using both methods is increased reliability in identifying individuals over time, although the effects of such invasive marking methods on behavior and long-term survival are not known.[77]

Toe clipping is more effective in lizards and juvenile crocodilians than in amphibians because their toes do not regenerate. However, the technique may have negative effects on behavior of arboreal lizards, which have subdigital toe pads for clinging to vegetation.[78] In contrast, a number of studies have demonstrated that toe clipping does not affect sprint speed, which is a measure of fitness in terrestrial lizards.[79-81] The frequency of natural toe loss in lizards has been used as further evidence that toe clipping in lizards is unlikely to influence survival,[82] yet natural toe loss can preclude recognition of toe clips.[83] Although the technique is commonly used with juvenile alligators, natural toe loss from conspecific interactions is frequent and can reduce accuracy and detectability in recaptures.

Scale Clipping. Scale clipping is an inexpensive technique that is frequently used to mark snakes. Removal of the scale, with small sharp, sterile scissors, leaves a scar that persists after shedding and the marks can be accurately read for many years. The method was originally described for subcaudal scales,[84] but Brown and Parker[85] presented a marking system using ventral scales, which are larger and easier to remove than subcaudals.[86] Depending on the numbering system, the entire scale can be removed, or the left or right half of the ventral scale can be removed.[85] Application of tissue glue can prevent excessive bleeding. This technique can be used on snakes of all sizes, but fewer scales should be removed and smaller scissors may be required for neonates or small snake species. Local anesthesia with lidocaine or bupivacaine provides adequate analgesia for this procedure. A nonsteroidal antiinflammatory drug such as meloxicam provides postprocedure analgesia.

Scute Clipping. Tail scute clipping is one of the more common, affordable, and persistent methods of marking crocodilians. Detectability of clipped tail scutes is reduced with juveniles because of their accelerated growth rates, but overall, this technique is the most efficient in terms of ease of application and the ability to identify large numbers of individuals.[83] Enumeration systems vary and can consist of numeric or alphabetical ordering. Both left and right sides of dorsal scutes can be incorporated to increase the number of potential marks. A sterilized utility knife (X-Acto, Elmer's Products, Inc., Columbus, Ohio) or box cutter can be useful for making a clean cut in the least amount of time. Dull blades will result in multiple cuts to mark the animal, thereby increasing handling time and stress. The tail scute must be removed down into the muscle; the cut should be made just below where the scales merge with the adjoining scales (Figure 29-31). If the scute clipping is performed properly, a small amount of bleeding will occur and indicates that the mark will truly be permanent. Appropriate aseptic technique, as described earlier, should be used. Local anesthesia with lidocaine or bupivacaine will provide appropriate analgesia.

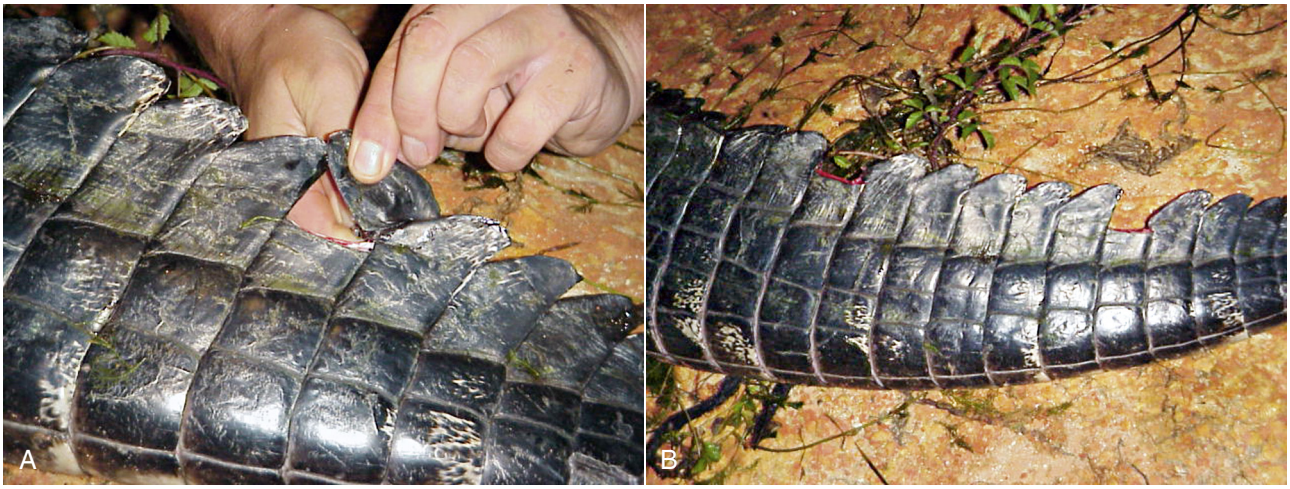

FIGURE 29-31 **A,** Removal of a scute on the tail of an American Alligator *(Alligator mississippiensis).* **B,** Completed pattern of a double clip on an American Alligator tail. *(Photo courtesy of C. Hagen.)*

Heat Branding. Heat branding is a fast, affordable, and straightforward method for marking snakes. Although the method was originally described as a method to mark amphibians and reptiles,[87] it is most appropriate for use with snakes.[88] It could be appropriate for some lizards with thick skin, but it was not effective in a pilot test with *Calotes* spp. (Whitaker N, Andrews K: Unpublished data, 2008). Pilot tests have been conducted on heat-branding in juvenile American Alligators, but the mark was insufficient for long-term identification (Tuberville T, Andrews K: Unpublished data, 2012). Disposable medical cautery units can be used to brand individual scales in patterns similar to those recommended for scale clipping[89]; however, the brands should also be applied to four to five lateral scales that correspond to the marked ventral scales to differentiate between marks and scars from natural injuries (Figure 29-32). High and low temperature settings are available on some cautery units; the low temperature units setting should be used with neonates or small snake species. This technique has been used successfully on a broad array of snake species and both neonates and adults.[89] If applied correctly, brands can persist for many years; however, long-term durability of the mark for longer lived species (e.g., *Crotalus* spp.) is not known. Cautery tips will last longer if overhanging scale material is trimmed off on keeled-scale snakes before branding.

Freeze Branding. Freeze branding, which alters the pigmentation of the scales, can also be used to mark lizards, snakes, and crocodilians.[90] Coolants include dry ice and ethyl alcohol or liquid halocarbon (Freon). Coolants can be applied to the skin with copper wire with a rounded tip or a synthetic sponge-tipped dowel for 20 to 30 seconds. Freeze branding has several shortcomings over heat branding. The marks are variable depending on the original color pattern of the animal and can be difficult to read.

Magnetized Wire Tags. Magnetized wire tags have been used to mark large numbers of sea turtles. They are less expensive than PIT tags, have high retention rates (95% to 100%),[91] and they appear to be effective for the lifetime of the animal.[92] Magnetized tags cannot be used to differentiate individuals unless used in conjunction with another marking

FIGURE 29-32 **A,** Ventral scale pattern after heat branding on a Black Racer *(Coluber constrictor).* This image displays code 146 (anal plate is skipped). **B,** Lateral scale pattern after heat branding for the same individual code as in **A**. **C,** Image of healed lateral marks from heat branding on a Banded Water Snake *(Nerodia fasciata). (Photos courtesy of J.D. Willson.)*

FIGURE 29-33 Carapacial and plastral placement of living tags. *(Photo courtesy Dr. Jynette Wyneken, Florida Atlantic University, Boca Raton, Fla.)*

technique.[93] Wire tags are typically placed in foreflippers[94] and require a magnetometer for field identification. Tags can also be detected on x-ray films. The disadvantage of this technique is that magnetometers can be expensive and may detect false-positive animals if there are other magnetic components in the area of the animal.[94]

Living Tags. Living tags have been used to identify cohorts or year-classes of hatchling and juvenile sea turtles.[36,95,96] Living tags are created by autografting a piece of light-colored plastron tissue into a darker scute on the carapace (Figure 29-33).[94] Tags grow with the turtle and appear as spots or streaks in older individuals. It does take a trained eye to identify a mark, particularly on adults that received the tags as hatchlings. However, living tags provided confirmation of the return of head-start Kemp's Ridley Sea Turtles to Padre Island National Seashore in Texas 11 years after release.[96]

Grafts can be obtained with the use of a surgical biopsy punch and can be secured with surgical glue.[95] Initial experimentation of the technique tested the use of melanin-destroying substances to emphasize the contrast of the plastral material from the carapace.[97] Generally, living tags are applied to different scutes to represent different year classes.[93,98]

RADIO TELEMETRY, SATELLITE TELEMETRY, BIOTELEMETRY, AND ARCHIVAL DATA RECORDERS

The purpose of this section is to introduce some basic principles of telemetry and describe techniques that have worked well for the authors and other researchers and likely have a broad application; an exhaustive review of the literature will not be provided. Amphibians and reptiles are difficult animals to study because they are often cryptic and wary. They also live in habitats that are often inaccessible and can spend considerable periods of time submerged in water or below ground. The ongoing advancement and miniaturization of electronic tagging technology has provided the means to effectively study these animals in the wild with minimal disturbance.[99] In addition, data on physiologic function (e.g., heart rate, body temperature, and feeding habits) may be assessed. Telemetry refers to an active transmission of data coming from an animal, with the objective of remotely collecting data on an animal's location, behavior and physiology, and characteristics of the environment.[88] Acoustic, electric, magnetic, and electromagnetic (e.g., visible light, radio wave, and microwave) forms of energy can be used to transmit data.[88]

The most commonly used spatial technology in herpetofauna is radio telemetry, which utilizes high-frequency radio waves. The equipment to conduct radio telemetry studies includes a transmitter, a power source (e.g., battery), and a transmitting antenna, all of which must be attached to the animal. Transmitter packages can be externally attached through a variety of methods or surgically implanted internally. Radio signals are detected by receiving antennae, which can be handheld, positioned on land at specific locations, or carried on land via vehicles, ships, planes, or satellites.[88] Satellite telemetry, including global positioning systems (GPS), enables information to be relayed from the transmitter to a receiver via satellites. Biotelemetry refers to the transmission of biological information from sensors on the animal, without direct contact between the transmitter and receiver. Internal physiologic data such as heart rate, body temperature, or electrocardiographic results, behavioral data such as diving time and depth, and environmental information such as ambient temperature, light, or salinity can all be relayed. Archival data recorders are self-contained biotelemetric units that collect and archive data for later recovery. This technology allows for researchers to gather information on wide-ranging (e.g., sea turtles) or secretive species that would otherwise be difficult or impossible to study. A wide range of designs, sizes, and methods of application are now available and allow for study of a variety of species.

Disadvantages and limitations of this technology include (1) high cost and complexity of use, (2) battery capacity and size limitations on transmission longevity and strength, and (3) the physical presence of the device may affect the animal's behavior. Internal implantation of transmitter packages requires anesthesia and surgery. Attachment and retention of the transmitter package often presents many challenges. In most instances, transmitting devices should eventually detach from the animal or be removed, which may require recapture. Recovery of archival recorders is required to retrieve data, which may also necessitate recapture and even another surgery. The researcher should select a transmitter package and attachment method appropriate to the animal. The general rule of thumb is that a transmitter package (i.e., battery, transmitter, and antenna) should not exceed 5% of the animal's body mass[86] and the attachment and placement should not impede the animal's movements, physiology, reproductive success, and survival. Tradeoffs between transmitter attachment methods and transmitter weight, transmitter longevity, and transmission range should be determined to optimize performance so that the particular study's objectives can be met.

Radio Telemetry: Nonsurgical Placement

Amphibians. Telemetry placement in amphibians is complicated by their generally small body size and sensitive skin. Most manufacturers can configure a transmitter based on the body size and life history of the focal species. However, projected battery life of the transmitter is limited by battery size, so small transmitters may need to be refurbished with a fresh

FIGURE 29-34 Southern Toad *(Anaxyrus terrestris)* with belt attachment of a radio transmitter package. *(Photo courtesy of S. Sterrett.)*

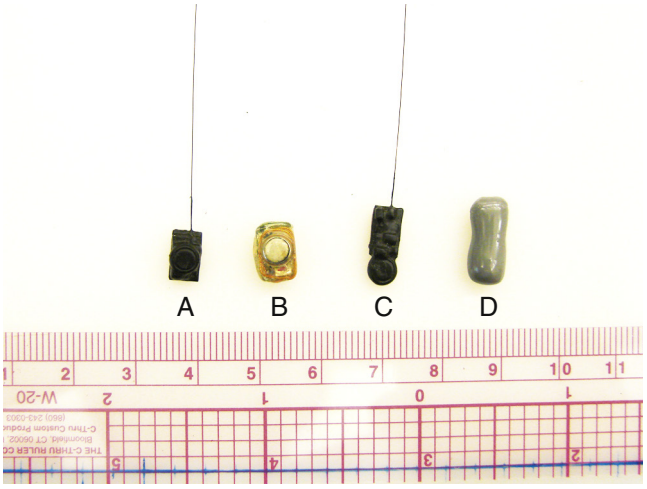

FIGURE 29-35 Different configurations for small radio transmitters used in amphibians. **A,** The transmitter has the battery mounted on top with a free hanging antenna. **B,** The transmitter has the antenna coiled around the battery, and the entire package is encapsulated in acrylic for implantation into the body cavity. **C,** The transmitter has the battery mounted at the end of the transmitter, creating a longer but thinner package, also with a free hanging antenna. **D,** A fully encapsulated transmitter with the same configuration as in *C.*

battery or replaced frequently (every 1 to 2 weeks to up to 3 months, depending on the size of the transmitter package). Transmitters may be attached externally to frogs with the use of belts or harnesses lined with small beads to prevent abrasion of the skin (Figure 29-34). The belt must have enough slack to prevent wear on the skin but must be tight enough to prevent the animal from jumping out of the harness or becoming entangled. For external transmitter placement, the antenna is loose and will trail behind the animal (Figure 29-35). Because of the body shape and fossorial habit of many salamanders, external attachment of transmitters is generally not used (see section on surgical placement of radio transmitters further on).

Reptiles

CROCODILIANS. In a recent publication, methods for acquisition and monitoring of behavioral and physiologic variables from free-ranging crocodilians through the use of data loggers and via satellite, radio, and acoustic telemetry were described.[100-104] Radio telemetry has been most widely used to track the movement patterns of crocodilians in addition to body temperatures and heart rates. The nuchal shield has been most commonly used as the site for transmitter attachment; however, measuring body temperature via radio telemetry requires that the transmitter be located internally either via ingestion[105] or by surgical implantation into the coelomic cavity.[106,107]

Archival tags (data loggers) have been used to record diving behaviors, fine-scale movement patterns, and heart rates of crocodilians.[108] Large volumes of data can be stored within these tags and downloaded at a later time. The greatest challenge with this technology is retrieving the tags. To maximize chances of retrieving them, researchers use other technologies simultaneously (e.g., radio telemetry or satellite telemetry) to locate the tag or animal. The tags are designed to release and float.[103] describe a technique using a tag and transmitter encased in a neoprene sleeve that incorporated a polystyrene float. The nuchal shield was used as the external attachment site so that the tag could surface each time the animal was on the surface. Manual restraint and a local anesthetic were used to place the tag. Holes were drilled through the center of four of the scutes to align with wires from the data package. The wires were threaded through the holes in a manner that minimized any loops of wire being exposed so that the telemetry package would not become snagged. Plastic-coated wire was secured with crimp sleeves.

A time-release mechanism was designed to deploy an archival tag in an effort to study Saltwater Crocodile *(Crocodylus porosus)* diving behaviors and fine-scaled movement patterns.[103] These animals inhabit waters with varying salinities from freshwater to saltwater, and it was very unlikely that they could be recaptured. The data-logging units were positioned on the nuchal shield with a similar attachment protocol as already described. Radio and acoustic telemetry were used to retrieve the archival tags. A novel self-release mechanism was designed and used a fluid-filled, spring-loaded syringe and hypodermic needle that could be set to release the tag for deployment periods of days to weeks. The spring-loaded syringe slowly advanced the plunger, and once the plunger moved past an islet on the flotation casing, the unit detached from the aluminum base plate, which eventually corroded off as the crimps dissolved. The time of release was controlled by the amount of fluid in the syringe, the viscosity of fluid, the gauge of needle, and the strength of the spring (Figure 29-36).

LIZARDS. Radio telemetry has been used to study aspects of behavior and spatial ecology in a variety of lizard species including Beaded Lizards *(Heloderma horridum),* Gila Monsters ([*Heloderma suspectum*]; Goode M: Unpublished data, 2011), Yarrow's Spiny Lizard *(Sceloporus jarrovi),* Lace Monitor *(Varanus varius),* Southern Alligator Lizard *(Eligaria multicarinata),* Land Mullet Skink *(Egernia major),* Eastern Bearded Dragon *(Pogona barbata),*[109] and Water Dragons *(Physignathus lesueurii).*[110] Goodman[109] reviewed a variety of telemetry techniques that have been used in West Indian iguanas (*Cyclura* spp.), including ingested transmitters, transmitters externally

FIGURE 29-36 Data logger (archival tag) with self-release device attached to the nuchal scutes of an Estuarine crocodile (*Crocodylus porosus*). The data logger recorded water depth, acceleration in three dimensions, compass bearing, speed, and body temperature. *(Photo courtesy of C.E. Franklin.)*

FIGURE 29-37 Caribbean iguana with radio transmitter sutured to the dorsal crest. *(Photo courtesy of Tandora Grant.)*

applied with duct tape, suture of transmitters to the dorsal crest (Figure 29-37), transmitter collars, belts, and harnesses (Figure 29-38), and use of various adhesives to attach transmitters. The signal range did not differ dramatically between most methods but did differ across habitats and among transmitter models, especially in relation to free antenna length. An additional consideration is the tradeoff between signal strength and battery life. Different researchers using the same model of transmitter may select reduced signal strength to increase battery life if the focal species is not wide-ranging; thus there was substantial variation in signal range and battery life among research projects.[109] Knapp and Owens[111] conducted radio telemetry on hatchling Andros Iguanas *(Cyclura cychlura cychlura)* by placing transmitters on the dorsal side of the pelvic girdle with test monofilament line wrapped around the lizard's body to secure it. This method may also be useful for hatchlings and small lizards of other species.

A harness to attach radio transmitters to Water Dragons was made by cutting a sheet of nylon mesh or fly screen in

FIGURE 29-38 Caribbean iguana fitted with a harness transmitter. *(Photo courtesy of Rick Hudson.)*

the shape of a square with two extending straps.[110] Cutting the square and straps to fit the individual lizard is critical: if they are too large, it may get caught on vegetation, and if too small, it may cause discomfort or abrasions to the skin. The radio transmitter was glued to the harness with adhesive (Super Glue; Super Glue Corp., Rancho Cucamonga, Calif) before attaching the harness to the animal. The square part of the harness was placed firmly on the lizard's back, above the forearms, while the straps were then pulled over the shoulders, crossed on the chest, and pulled up under the lizard's forearms. After pulling the straps snug, they were adhered to the square with Super Glue and to one another where they crossed on the chest. The mesh was held together at glue points with forceps. The harness was easily removed by cutting the straps with scissors. Water Dragons were monitored for up to 2 months without any harness losses or any noticeable problems for the lizards.

SNAKES. Radio transmitters can be attached externally to snakes to collect short-term movement data.[112] The method is ideal for use with juvenile snakes or small-bodied species.[113] Transmitters should be attached dorsolaterally, approximately two thirds of the snout-to-vent length behind the head with adhesive (Super Glue) gel (Figure 29-39). Transmitters are typically shed during ecdysis and can be retrieved for use on another animal.

Freshwater Turtles, Terrapins, and Tortoises. The predominant way transmitters have been attached to turtles is by mounting the tag onto the carapace. Different techniques have varying levels of success depending on the life history traits, age, and size of the study animals (Figure 29-40). Boarman and collegues[114] reviewed 113 scientific papers, reports, and semitechnical articles on radio tracking projects with turtles and concluded that little information was available in the literature to evaluate the effects of transmitters on the study of animals. In this review, transmitters were attached to the carapace by a variety of methods including epoxy, silicone sealant, dental acrylic, and other types of adhesives. Additionally, there were descriptions of the transmitters being strapped on with harnesses or attached via bolts, wire, cable, or nylon ties or with monofilament line, which was passed through the holes drilled into the posterior carapace or marginal scutes. In these cases, the transmitter was either attached to

FIGURE 29-39 **A** and **B,** External attachment of a radio transmitter to a neonate Timber Rattlesnake *(Crotalus horridus). (Photos courtesy of E.M. Schlimm.)*

FIGURE 29-40 **A.** Juvenile Gopher Tortoise (*Gopherus polyphemus*) with radio transmitter on carapace. *(Photo courtesy Tracey D. Tuberville, Savannah River Ecology Laboratory, The University of Georgia, Athens, Ga).* **B.** Adult Gopher Tortoise with radio transmitter on carapace. *(Photo courtesy Dr. Terry M. Norton, Georgia Sea Turtle Center, Jekyll Island, Ga).*

the carapace or allowed to trail behind the turtles. There were many possible behavioral (e.g., mating disruptions) and physical problems that were potentially caused by transmitters. Shell deformities occurred on hatchling Gopher Tortoises because the epoxy holding transmitters encroached on growth areas.[115] This should be avoided when transmitters are placed on the carapace, especially in young, growing animals. Transmitters may become entangled in vegetation, usually between gaps between the shell and the transmitter or the antenna. Transmitters may attract the attention of predators and humans and therefore should be camouflaged in some way (Figure 29-41). Boarman and collegues[114] recommended a process for transmitter placement in Desert Tortoises that is applicable to other nonmarine chelonids:

1. Test the transmitter to confirm that it is functional.
2. Clean dirt off the carapace.
3. Preposition the transmitter to first left or right coastal scute and fit as flush to the carapace as possible.
4. To position the antenna, cut short sections of flexible 3-mm plastic tubing and attach each section to the first

FIGURE 29-41 Radio transmitter and wrapped antenna that has been painted to camouflage the equipment on an Eastern Box Turtle *(Terrapene carolina).(Photo courtesy of J.E. Colbert.)*

four vertebral scutes using epoxy. Each section should be cut slightly shorter than its associated scute. Super Glue can be used to hold each section of tubing in place while quick drying, pliable putty epoxy is spread over each section of tubing in a continuous layer from the scute on the side of the tube to the scute surface on the opposite side of the tube. Avoid getting any epoxy on the scute sutures or neighboring scutes to allow for shell growth.

5. Place the antenna through the tube sections leaving approximately 50 to 120 mm hanging loose beyond the posterior of the animal.
6. Attach the transmitter with putty epoxy, using care not to bridge the scute margins and leaving no spaces between the transmitter and the carapace to get caught in vegetation.
7. Paint the transmitter and putty to reduce reflectivity and contrast.

In addition to these points, the transmitter placement site should be disinfected, as described earlier in the chapter. The transmitter should not be applied to an abnormal part of the shell (e.g., deformation or site of infection). The carapace can be roughened slightly with sandpaper or a Dremel tool before the epoxy is applied. The authors have had success with using a marine epoxy putty (WaterWeld; J-B Weld Company, Sulphur Springs, Tex) for the initial attachment of the transmitter and then coating it with a liquid epoxy (Power-Fast+, Fast Set Formula; Powers Fasteners, Inc. Brewster, NY). The epoxy putty alone may be successful for securing transmitters to terrestrial species, but it is recommended that liquid epoxy be used in aquatic species. A digital, distant-laser thermal monitoring device (Raynger ST; Raytek Corporation, Santa Cruz, Calif) is used to make sure the epoxy does not rise above 37.8°C (100°F) during the curing process. If necessary, the area is sprayed with cool water. In immature growing turtles, it is important not to cross scute suture lines to avoid interfering with the growth of the shell. Hatchling and juvenile tortoises can be radio tracked using small, lightweight radio transmitters attached to the costal scutes with silicon aquarium sealant (Dap; see Figure 29-40, *A*). This sealant is readily removed without damaging the shell of the tortoise.

Bolts and other fasteners can be used to attach radio transmitters to the carapace of aquatic turtles.[110] A surgical preparation and sterile technique should be used in this procedure because it requires drilling through the bone of the shell. Transmitter packages with aluminum plates with holes at either end for attachment and whip antennae are available through several manufacturers (e.g., Holohil Systems, LTD; Carp, Ontario, Canada; and Sirtrack, North Liberty, Iowa). The preferred site of attachment is on top of the posterior marginal scutes and underlying the peripheral bones of the carapace, typically in a region above one of the hindlimbs.[111] Attachment at this site is less likely to create drag or impede movement through substrate or vegetation. The most posterior scute should not be used because it will likely interfere with mating behavior. It is important to pre-place the transmitter on the shell to mark the spots to be drilled and to assess how the transmitter fits in relation to the curvature of the shell. If necessary, a small amount of epoxy can be used to create a flat surface for attachment. Holes drilled too close to the outside edge may break down from wear, allowing the fastener to slip, and holes drilled too close to the scute seams often result in excessive bleeding. For Pig-nosed Turtles *(Carettochelys insculpta),* the epoxy was replaced with neoprene strips cut to the size of the transmitter, which softened the pressure of the transmitter against the shell. Additionally, Pig-nosed Turtles have no scutes, so it was critical to ensure that the holes were drilled on or near the seams between the marginal bones, which are clearly visible beneath the skin in this species. Tags were attached with stainless steel bolts and locking nuts threaded with nylon to prevent loosening and corrosion-resistant metal backing plates. The nut should be on top (transmitter side) to allow full leg movement and prevent abrasion of the skin on the legs. Gaps between the tag and the shell were filled with putty epoxy to prevent debris and vegetation from becoming entangled. This technique has been successful in a large number of species. Turtles have been tracked up to 3 years in some cases.

Radio Telemetry: Surgical Placement

Only healthy animals should be selected for surgical radio telemetry placement. The signal strength and frequency of the transmitter provided by the manufacturer should be verified with a receiver before placement in the animal to avoid implanting a nonfunctioning transmitter. Surgical procedures should be conducted during the appropriate time of year so that surgical sites can heal adequately. Anesthesia and surgeries should be conducted by a veterinarian with extensive experience with amphibians and reptiles using sterile technique. Although not ideal, individuals without a veterinary degree will likely continue to conduct surgeries but still must have IACUC approval. At a minimum, these individuals should receive specialized training in anesthesia, surgery, and sterile technique and have close consultation with an experienced veterinarian.

All telemetry devices and data loggers should be sterilized with either ethylene oxide gas or hydrogen peroxide plasma sterilization before implantation, as previously described for PIT tags. Arrangements can be made with local human hospitals, veterinary schools, or other specialty facilities if this is not available to the researcher. The benefits far outweigh the costs. Cold sterilization is not recommended.

General Guidelines for Amphibian Surgery. In general, because of their size, many amphibians are not well-suited for internal placement of telemetry packages. Internal placement of radio telemetry had been described for large frogs[116,117] and salamanders (e.g., Tiger Salamanders and Spotted Salamanders *[Ambystoma maculatum],*[118] and the Eastern Hellbender[58]). Presurgical preparation of the patient includes ensuring good hydration and electrolyte balance by soaking the animal in a shallow water bath for 60 minutes before surgery. The amphibian can be weighed preoperatively and postoperatively to monitor hydration. The amphibian should be fasted before surgery for a minimum of 4 hours.[119] Appropriate analgesia and anesthesia should be used for internal transmitter placement and other invasive and potentially painful procedures. Although other anesthetic options are available, buffered tricaine methanesulfonate (MS-222; Argent Chemical Laboratories, Inc., Redmond, Wash) has been used widely by veterinarians and researchers for anesthesia in amphibians and appears to have a wide margin of safety.[119] After being anesthetized, the amphibian is removed from the anesthetic bath to avoid overdosing or prolonged anesthesia. Well-oxygenated clean water should be provided during anesthesia, and additional anesthetic can be added, if

needed. Although amphibian skin secretions are known to have antimicrobial properties, sterile techniques should be followed. Because of the potential for toxicity from the various available antiseptics, it is recommended that the patient be rinsed with sterile water before placement of sterile drapes around the patient. Surgical instruments should be heat or gas sterilized. Anesthetized amphibians should not be released until they regain their ability to right themselves. A small incision is made into the skin, thin muscle layer, and coelomic lining to enter the coelomic cavity. The authors have used transmitters in which the antenna is tightly coiled around the battery and the entire package is encapsulated in acrylic (see Figure 29-35, *D*). The transmitter is placed into the coelom, and the incision is closed. The least reactive suture material should be used: polydioxanone (PDS) is recommended for internal sutures and nylon is recommended for skin sutures.[120] Internal transmitters will need to be removed or replaced, which is a consideration that researchers should be aware of when working with rare species, those that are difficult to capture, or species that are particularly susceptible to stress from invasive procedures.

PIT tags can also be implanted into the body cavity of frogs and salamanders, either by syringe or through a small (2-mm to 3-mm) incision in the skin with the use of surgical methods previously described (Figure 29-42). In salamanders, the tags can be inserted along the side of the body, approximately 5 mm anterior to the hindleg[121]; tags should be inserted anteriorly, parallel to the long axis of the body. Incisions can be closed with a small amount of tissue glue.

General Guidelines for Reptile Surgery. A presurgical health screening is recommended. Appropriate analgesia and anesthesia should be used and needs to be tailored to the particular reptile species undergoing surgery. In most cases, injectable sedatives and anesthetics will be followed by intubation and gas anesthesia. Appropriate cardiac and respiratory monitoring equipment should be available. Recommended suture material includes polydioxanone (PDS II; Ethicon, Inc., Somerville, NJ), an absorbable suture that is used internally and is made of a nonabsorbable nylon (Ethicon; Ethicon, Inc., Somerville, NJ) for the skin. A study conducted by Govett and collegues[122] found that PDS was the preferred suture material for sea turtles.

FIGURE 29-42 Surgical implantation of a passive integrated transponder (PIT) tag in an Eastern Tiger Salamander *(Ambystoma tigrinum).*

Radio transmitters used in all of the procedures described further on were manufactured by Holohil Systems, Ltd (Ontario, Canada), unless indicated otherwise. These transmitters are available in a variety of sizes, and the larger transmitters have longer battery life.

CROCODILIANS. Intracoelomic radio transmitter implantation in crocodilians has been conducted under injectable anesthesia with subsequent intubation and manual ventilation with a bag valve mask (Ambu bag).[100-104] A small skin incision was made between scales in the flank of the abdominal region, just posterior to the right hindlimb. Muscle layers beneath the skin were carefully separated to reveal the coelomic lining. Hemostats were used to secure the coelomic lining, and an incision was made to expose the coelom, where the transmitter was inserted. To avoid disturbance when the animals were tracked, the researchers used an automatic and continuous recording station positioned at least 100 m from the waterhole to simultaneously monitor up to 10 individuals. Radio signals from the temperature-sensitive transmitters were received by a four-piece Yagi aerial antenna, mounted 25 m directly above the waterhole that the crocodiles inhabited.[103]

Alternatively, using a similar surgical approach, researchers implanted a multichannel radio telemetry system to measure blood flow and pressure, electrocardiographic data, and body temperature in several captive American Alligators.[123] The technique has been reported to be promising for free-ranging crocodilians.[103]

The authors (TMN, KMA) have developed a technique for surgical implantation of radio transmitters in the muscle of the proximal tail of American Alligators. The technique has been used on six alligators to date. Anesthesia used included dexmedetomidine (Dexdomitor, 0.5 mg/mL; Phizer Animal Health, New York, N.Y.) at 0.010 to 0.15 mg/kg, ketamine (Ketaject, 100 mg/mL; Phoenix Inc, St. Joseph, Mo) at 5 mg/kg, and butorphanol (Butorphic, 10 mg/mL; Lloyd Laboratories, Inc, Shenandoah, Iowa) at 0.2 mg/kg given in combination intramuscularly. After 15 minutes, if deeper anesthesia was needed, propofol (PropoFlo, 20 mg/mL, Abbott Laboratories, North Chicago, Ill) was administered as a slow bolus intravenously to effect at 3 mg/kg. Lidocaine (2%; Butler Animal Health Supply, Dublin, Ohio) was infiltrated into the incision area. Intubation and ventilation with a bag valve mask were performed in all cases. Gas anesthesia can be used to further control anesthesia but has not been necessary with the injectable drug combination used. Atipamezole (Antisedan, 5 mg/mL; Pfizer Animal Health, New York, N.Y.) was used for reversal at the same volume as the dexmedetomidine, given half intramuscularly and half intravenously.

The skin was surgically prepared. A 3-cm to 5-cm skin incision was made between scales about a third of the way down from the dorsal aspect of the tail. Blunt dissection was used to create a pocket between muscle planes, where the transmitter was inserted (Figure 29-43). Holohil Systems Ltd. (Ontario, Canada) transmitters (Model SI-2, 50 mm × 11 mm, 36-month battery projected life) developed for snakes have been used. The antenna was threaded subcutaneously caudally with the

use of rigid copper tubing as described in the Snakes section further on. Similarly, a small skin incision at the distal end of the tubing was made with the same technique. It is a bit more difficult to thread the tubing because of the stiffness of the scales; thus more than one incision was usually needed. The surgical site was closed using 2.0 PDS in a simple interrupted suture pattern for the muscle and skin.

To date, reliable signal transmission has been maintained for 12 to 24 months. Although full battery life has not been sustained for these tracked individuals, researchers (Andrews K: Unpublished data, 2010) also received faulty transmitters, so it is unclear whether the reduced battery life can be attributed to malfunctioning equipment or internal crimping of the antenna, which would thereby reduce signal transmission. Further, signal transmission is reduced in brackish water habitats, which could have reduced relocation of the animals. Regardless, the technique was proven effective, so data collection will continue with future individuals to further articulate the technique. Internal implantation of devices in American Alligators is the technique of choice in the field, where it is preferable that tracked individuals need not be visually identified.

A technique recently described subcutaneous implantation of temperature- or pressure-sensitive acoustic telemetry devices in Saltwater Crocodiles.[103] The animals ranged from 2 to 5 m in length. The crocodiles were manually restrained, and a local anesthetic was infiltrated into the surgical site. A 2-cm skin incision was made, and a small pocket was formed under the skin, along a skinfold, with the use of blunt-ended scissors

FIGURE 29-43 **A** and **B,** Surgical placement of a radio transmitter in an American Alligator *(Alligator mississippiensis)* tail. *(Photos courtesy Kimberly M. Andrews, Jekyll Island Authority, Georgia Sea Turtle Center and The University of Georgia, Savannah River Ecology Laboratory.)*

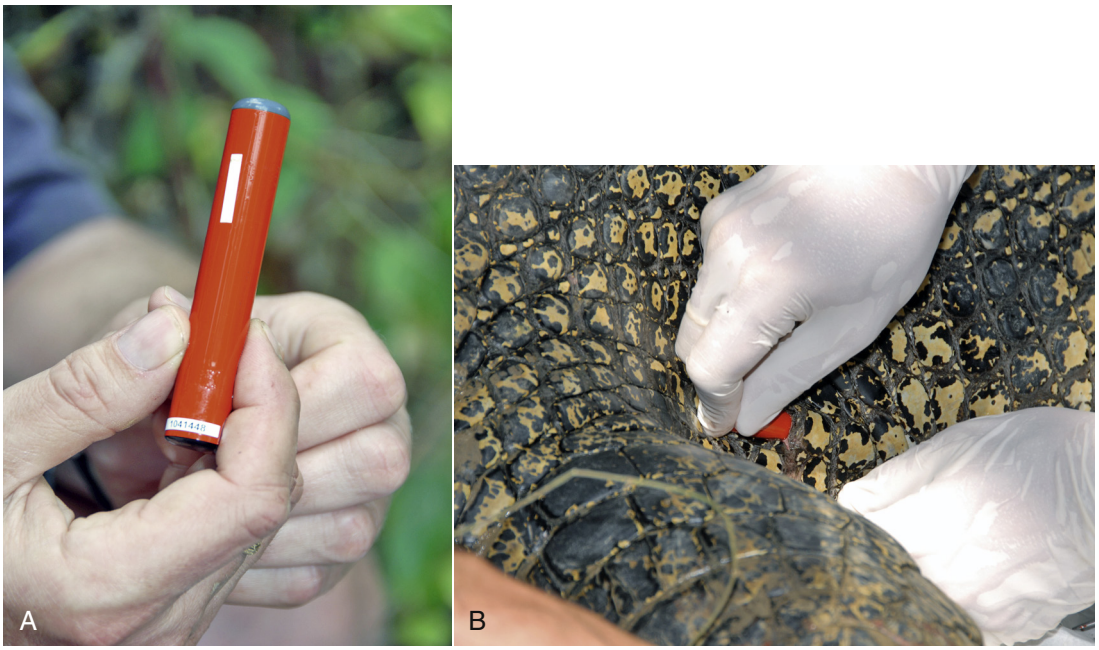

FIGURE 29-44 **A,** Vemco acoustic tag. **B,** Surgically implanting a temperature-sensitive or pressure-sensitive acoustic transmitter subcutaneously in a Saltwater Crocodile *(Crocodylus porosus). (Photos courtesy of C.E. Franklin.)*

(Figure 29-44). The transmitter was then inserted (V16, Vemco-cylindrical, 72 mm long and 15 mm in diameter with rounded ends). The transmitter was positioned so that when the animal surfaced it remained well below the water. The researchers used fixed receiver stations. The technology has potential to be extremely useful in crocodiles because the signal transmission occurs through water and transmission operates irrespective of salinity. Furthermore, the transmitters can be programmed to have a life span of more than 10 years. A variety of sensors are available to measure temperature and pressure (water depth). The signals can be picked up by automatic stationary receivers or actively tracked. The authors reported excellent transmission; animals were detected more than 500 m away with the use of a directional hydrophone and active tracking receiver. At the time of the publication, the transmitters had been functioning well for more than 9 months. Further study is needed to evaluate transmitter retention time.

LIZARDS. An effective technique for surgically implanting transmitters in the coelom of Anegada Ground Iguanas (*Cyclura pinguis*) has been reported and has potential for use in other lizard species.[124] With this technique, two-point fixation of the transmitter to the internal body wall was achieved. Antenna placement in the coelom was a critical part of the procedure. Of 23 iguanas, 20 transmitted good signals for at least 10 months after surgery (Figure 29-45).

Another method was described by Doody and collegues[110] for surgical implantation of radiotransmitters in a semiaquatic monitor lizard *(Varanus mertensi)*. Gas anesthesia was used. The site was aseptically prepared for implantation. A 2-cm to 3-cm skin incision was made longitudinally on the lateral side of the abdomen, approximately 2 to 3 cm anterior from the hindlimbs. Muscle was incised to gain access to the coelomic lining, which was then incised. Transmitters (Holohil Systems, Ontario, Canada, Model 2I-T, 3 g, 2.5 cm × 0.6 cm) with a 10-cm external whip antenna were inserted into the coelom. The transmitter was positioned longitudinally within the coelom. A thin-walled brass tube was threaded subcutaneously from the anterior point of incision along the lateral fold of the animal to just behind the head. The external antenna was positioned inside the tube, and the tube was removed through a small incision made just behind the head, leaving the antenna in place under the skin. Only minimal long-term effects were noted, according to the authors, over a 2-year study period. Fifty individuals were implanted, and 37 transmitted more than 16 days; 4 were tracked successfully for 2 years.

SNAKES. Radio telemetry devices have been surgically placed in the coelomic cavity of a variety of snake species by one of the authors (TMN). Complete healing takes approximately 4 to 8 weeks in reptiles maintained in ideal thermoregulatory conditions. The methodology that follows is a modification of that described previously.[125]

Snout-to-vent and snout-to-tail measurements are taken. The snout-to-vent measurement is divided by three; this distance is measured from the cloaca (vent) anteriorly and used for the incision site for transmitter placement. A permanent marker can be used to mark the skin incision site. The snake is prepared for sterile surgery in a routine fashion. A 3- to 5-cm lateral skin incision is made between the second and third scale rows and then reflected ventrally past the ribs so that an incision can be made in the coelomic lining, which allows access to the coelomic cavity. Care should be taken not to incise the air sac during this process so that the transmitter is not inserted into the respiratory system.

An appropriately sized transmitter should be selected. Temperature-sensitive transmitters are available from select manufacturers, or microtemperature data loggers (iButtons; Maxim Integrated Devices, Sunnyvale, Calif) that have been coated in multipurpose rubber coating (PlastiDip; Plasti Dip International, Blaine, Minn) can also be implanted intracoelomically caudal to the transmitter to record body temperatures. Sterilized copper tubing, a rigid dog urinary catheter, or a similar rigid sterile instrument is threaded subcutaneously anteriorly. The antenna is threaded into the lumen of the tubing. A small skin incision is made in the area of the

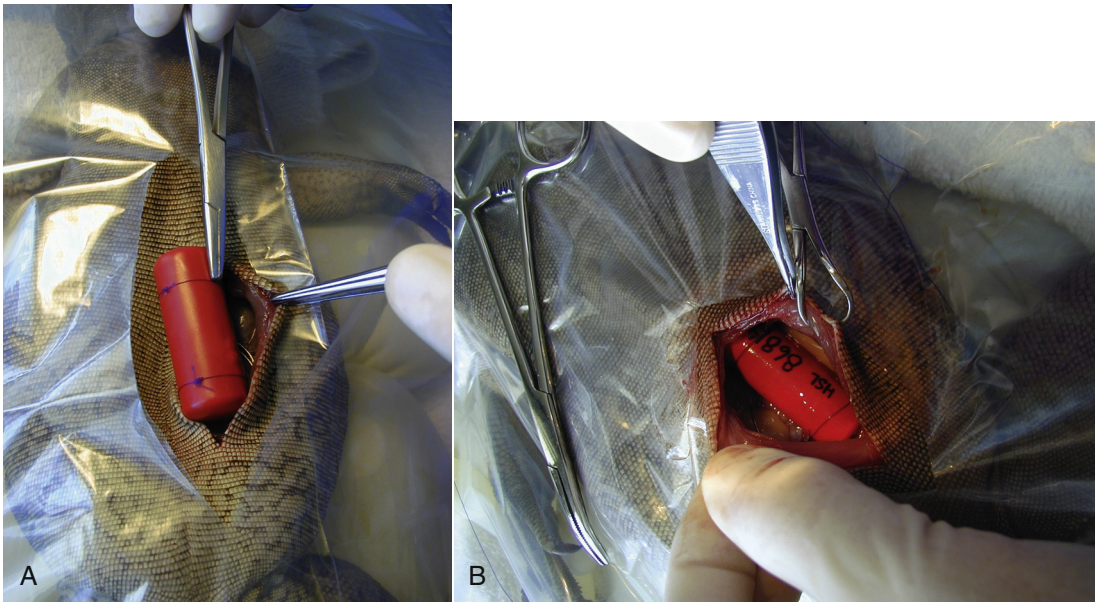

FIGURE 29-45 **A** and **B,** Surgical placement of radio transmitters in Caribbean iguanas. *(Photos courtesy of Fort Worth Zoo Animal Health.)*

anterior end of the tubing. The tubing is removed, leaving the antennae in place (see further on). The antenna can be anchored to musculature by one suture (placed approximately 0.5 cm anterior to the transmitter), which reduces the chances of the antennae protruding from the incision site. Care is taken to ensure that the antenna is not crimped where it enters the transmitter (Figure 29-46). An appropriate suture material and suture pattern should be used to close the surgical site as previously described in this chapter for other reptiles.

Some species of snakes, such as the Eastern Indigo Snake, are prone to developing bacterial infections at the transmitter site. Recent studies on the Eastern Massasauga Rattlesnake *(Sisturus catenatus catenatus)* indicate bacterial infections still occur with some frequency, despite proper incorporation of sterile techniques.[126]

Satellite Telemetry

Satellite telemetry has been used most extensively to study long-term large spatial movements and activities of sea turtles and estuarine crocodiles. This technique is especially important in the marine environment, where very high frequency (VHF) signals are greatly attenuated and distances to be covered are usually large.[127] The primary disadvantage of this technology is the cost of the transmitters and satellite time.

Most sea turtle tracking research that has been published has focused on postnesting females because of their accessibility. For example, a study was conducted on the Georgia coast to evaluate internesting intervals and postnesting migratory behavior in female Loggerhead Sea Turtles.[128] In this study, a variety of methodologies including acoustic, radio, and satellite telemetry were used to evaluate short- and long-term movement patterns, nest site fidelity, and interactions with shrimp trawl fishery (Figure 29-47). Comparisons between the distributions were used to produce potential management scenarios (closures and fleet reductions) designed to reduce the likelihood of interactions. Through application of predictive modeling, spatial closures were identified to have only limited merits as management options, whereas fleet reductions were most likely to produce actual decreases in interactions between turtles and shrimp trawlers during nesting seasons. Until recently, very limited information was available on the spatial ecology of male Loggerhead Sea Turtles during the breeding season. Arendt and colleagues evaluated the movement and diving patterns

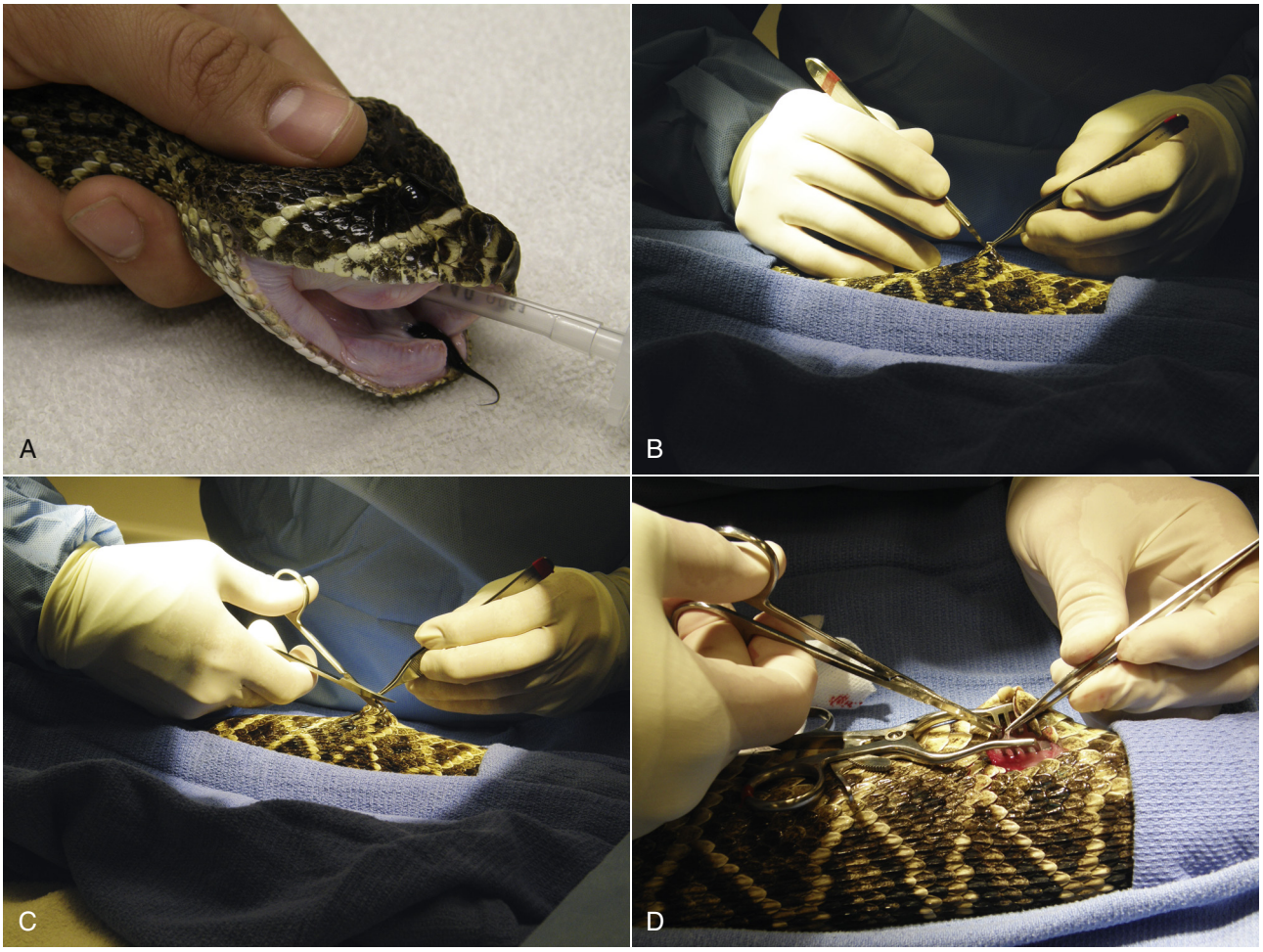

FIGURE 29-46 Surgical placement of a radio transmitter into the coelom of an Eastern Diamondback Rattlesnake *(Crotalus adamanteus)*. **A,** Isoflurane anesthesia is administered through an endotracheal tube. **B,** Initial stab incision into the skin with a scalpel blade. **C,** Skin incision is completed with scissors. **D,** Incision of coelomic lining with scissors. Retractors improve exposure.

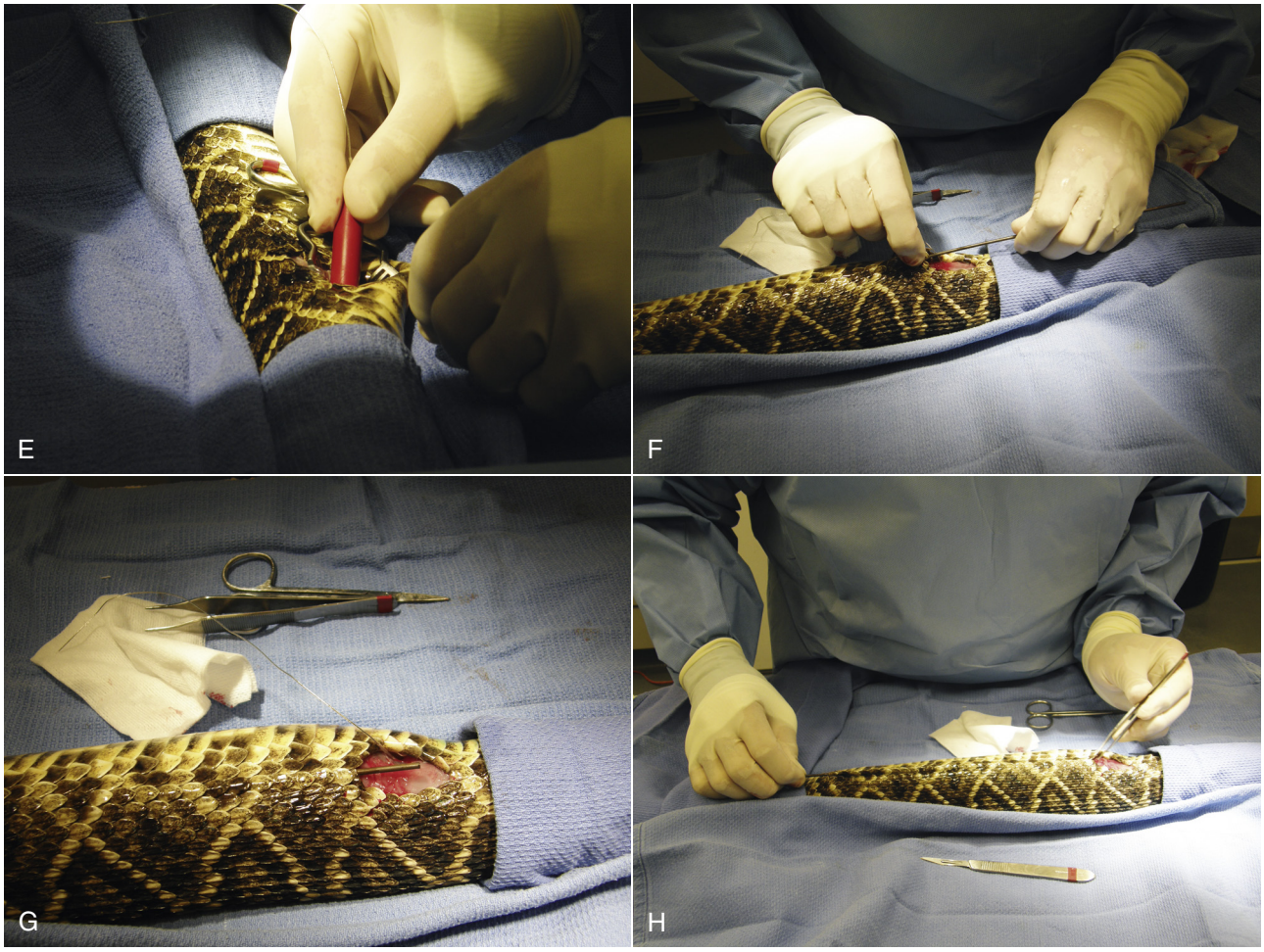

FIGURE 29-46, cont'd **E,** Radio transmitter is being placed into the coelom. **F,** Threading copper tubing subcutaneously. **G,** Copper tubing in place for threading antenna. **H,** Small incision is made to pull out the copper tubing while leaving the antenna in place.

FIGURE 29-47 Loggerhead Sea Turtle *(Caretta caretta)* with satellite, sonic, and radio transmitters on carapace. *(Photo courtesy of Mark Dodd.)*

of resident and migrant adult male Loggerhead Sea Turtles during the breeding season in Cape Canaveral, Florida[129] and after dispersal postbreeding.[130] Satellite telemetry in this study provided critical information for assisting in future conservation management of this species that did not previously exist.

A variety of satellite attachment techniques have been used in sea turtles, including tethering of buoyant transmitter housing to the posterior end of the shell, attachment of "backpack" style transmitters to the anterior vertebral scute with fiberglass cloth and polyester resin, fiberglass filler, two-part epoxies, or putties, and combinations of these techniques.[131] Retention time of satellite tags has been and continues to be problematic. Integration of antifouling paints[132] and an alternative adhesive, Sonic-Weld steel-reinforced epoxy putty,[133] has increased retention time.

The Georgia Sea Turtle Center (GSTC) has utilized this technology to track rehabilitated Loggerhead Sea Turtles after release (Figure 29-48). Satellite placement techniques have been similar to those previously described,[134] except that the transmitter is partially covered with epoxy with only the saltwater switches exposed, as depicted in (Figure 29-49). A laser temperature gun is used to monitor the temperature of the epoxy; when it reaches above 37.8°C (100°F), water is used to reduce the temperature. Although these transmitters could

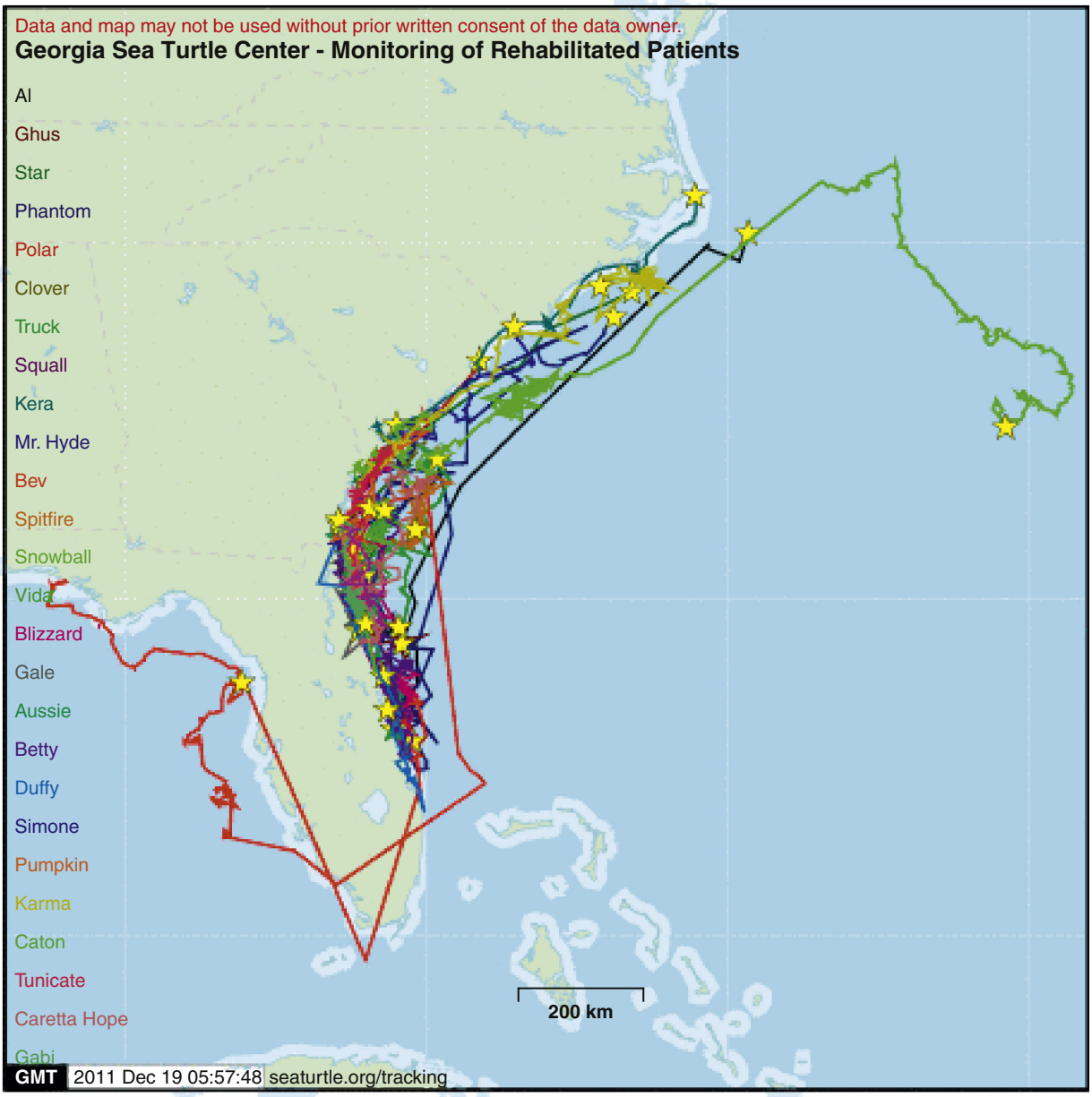

FIGURE 29-48 Satellite tracks of rehabilitated sea turtles released by the Georgia Sea Turtle Center.

FIGURE 29-49 **A** and **B,** Satellite transmitter placed on a Loggerhead Sea Turtle *(Caretta caretta)* carapace.

theoretically last for 3 years, data collection for more than 1 year has been rare, most likely because of physical damage to the antennae or transmitters and/or biological fouling of the saltwater switch sensor. On October 13, 2011, a rehabilitated juvenile female Loggerhead Sea Turtle that had been released with a satellite transmitter from Jekyll Island, Georgia, United States, on July 13, 2009 was recovered by a research vessel near Cumberland Island, Georgia. The turtle was healthy and in good body condition, and the transmitter was still in place. The transmitter itself was covered with epibiota and was missing the antenna (Figure 29-50).

FIGURE 29-50 Rehabilitated Loggerhead Sea Turtle *(Caretta caretta)* released with a satellite transmitter was recaptured more than 2 years later.

Satellite telemetry has also been used successfully to study long range movements of estuarine crocodiles.[102] Before attachment of the device, the area around the nuchal shield was surgically prepared after a local anesthetic was administered. Two holes were drilled into each of four scutes in the nuchal shield, and wires were fed through the holes. A Kiwi-iSat 101 transmitter was attached to the scutes with the use of plastic coated stainless steel wire threaded through four steel loops on the sides of the transmitter and fastened with lead crimps (Figure 29-51). Fifty percent of the animals sent signals for more than 10 months, and four sent signals for more than 15 months.[102]

CONCLUSIONS

Capture techniques, marking of individuals for identification purposes, and telemetry placement techniques are used extensively in amphibian and reptile research and conservation activities. It is critical that veterinarians and biologists continue to work together to develop sound protocols that provide the vital information that this research sets out to obtain while causing minimal negative effects to the individual animals and populations that are being studied and preserved.

FIGURE 29-51 **A,** Satellite/GPS tag being attached to the nuchal scutes of an Estuarine crocodile *(Crocodylus porosus)*. **B,** Satellite/GPS tag attached to the nuchal scutes of a 2.8 m female Saltwater Crocodile *(Crocodylus porosus). (Photos courtesy of C.E. Franklin.)*

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