

EASTERN INDIGO SNAKE
(Drymarchon couperi)
**CAPTURING, HANDLING, BLOOD AND TISSUE SAMPLING,
MARKING, PIT TAG IMPLANTATION, AND SURGICAL
PROTOCOL**
U.S. FISH AND WILDLIFE SERVICE
Dated: August 17, 2016

The U.S. Fish and Wildlife Service (Service) authorizes capture, handling, blood and tissue sampling, marking (scale clipping), PIT (passive integrated transponder) tag insertion, and radio telemetry (including surgical implantation of transmitters) of eastern indigo snakes, pursuant to section 10(a)(1)(A) of the Endangered Species Act (Act), through issuance of permits to take eastern indigo snakes in response to requests from individuals for such permits. Permits will only be issued for activities conducted by qualified persons, as described in permit applications, which benefit the recovery of the eastern indigo snake.

The Service has developed this protocol which identifies specific capturing, handling, blood and tissue sampling, marking, PIT tag insertion, and surgical transmitter implantation techniques to be followed when conducting these activities. This protocol will be attached to all future permits authorizing these activities. The Service will update the protocol as new information is obtained from on-going studies.

CAPTURING

Capturing eastern indigo snakes, even in areas where they are known to occur can be difficult. Smith and Dyer (2003) conducted a study to determine whether road running (road cruising), using drift fencing with traps, or using a camera to scope gopher tortoise burrows would be valid techniques for detecting indigo snakes, and thus offer an opportunity either directly or indirectly for their capture. The results were disappointing. In fact, incidental sightings, outside the scope of the research project, resulted in the most encounters with eastern indigo snakes. Researchers familiar with indigo snakes find that this is frequently still the case. The Service is continuing to pursue a more accurate survey method for this species, including the use of detector dogs. With the issuance of a permit, eastern indigo snakes may be captured by hand after being observed during road cruising, or other direct observation during general field surveys, and/or by trapping using stationary cover objects or large box traps (see Rudolph *et al.* 1999 for a description of box traps).

Gopher tortoise burrows

If an eastern indigo snake is present or suspected of being present in a gopher tortoise burrow, a large single-opening funnel trap placed at the mouth of the burrow may be used to capture snakes as they exit the burrow. Shade the funnel traps and check them 3 to 4 times per day during daylight hours.

HANDLING

Captured eastern indigo snakes may be measured, weighed, sexed, photographed, scale clipped and/or implanted with a PIT tag; sampled for genetic studies or diagnostic purposes via blood sampling, skin biopsies, or body (cloacal, oral, and/or skin) swabbing; and/or surgically implanted with a radio telemetry device (see specific protocols for these techniques below). Disease may be transmitted within and among eastern indigo snake populations during handling if appropriate precautions are not taken. Avoid handling multiple snakes at one time if possible. Use extra precautions when handling ill snakes, especially if snake fungal disease (SFD) (*Ophidiomyces ophiodiicola*) is a possibility.

A simple field kit can be made using leak proof containers to hold gauze soaked with a solution of povidone-iodine, such as Betadine[®], to be used for scrubbing, and alcohol for cleaning areas where scales are clipped and/or sites where PIT tags are inserted. Appropriate sized examination gloves can be placed in zip top bags.

When handling eastern indigo snakes, the following protocols must be used when handling snakes for mark-recapture, telemetry, and other purposes:

Disinfection prior to handling

- Examination gloves should be worn whenever possible when performing invasive procedures. If not possible, wash hands with soap and water if a vehicle is available near the processing site, or with disinfectant hand wash. Repeat after handling each snake or wear separate disposable latex gloves for each snake.
- All equipment used for holding snakes should be washed prior to use (e.g. cloth bags or pillowcases) or disinfected between snakes.
- Clean handling equipment used in the field with isopropyl alcohol or other appropriate disinfectant (i.e., dilute bleach, chlorhexidine, or other environmental disinfectant) between each snake. Contact time with the disinfectant is important; a minimum of 10 minutes of contact time should be used.
- Care should be taken to ensure that contaminated items, such as used examination gloves, swabs, gauze, disinfectant wipes, etc., do not contact cleaned or sterile equipment or snakes. Contaminated materials should be isolated and disposed of in sealed bags.

Transport and Temporary Captivity

- Use a separate clean cloth bag or pillowcase for each snake. Do not reuse until washed with soap, bleach, and hot water.
- Place each individual snake in separate, well-ventilated, plastic containers for transport. Their containers should have some substrate or a towel to make the snake more comfortable and to soak up urine and feces.
- Avoid overheating or cool temperatures during transport. The preferred range of transport temperature is 60 to 72 degrees Fahrenheit (° F) (16 to 22 degrees Centigrade (°C)).
- Containers housing snakes must be secured during transport to prevent sliding, bumping into other surfaces, and/or tipping over.
- Avoid housing snakes in captivity unless necessary for surgery, to treat injuries, or for placement in the captive breeding colony as part of a Service-approved

action. Currently, only the Central Florida Zoo's Orianne Center for Indigo Conservation (OCIC) has approval under this protocol to retain individuals permanently in captivity for the purpose of breeding and reintroductions. Snakes captured for the breeding colony must be transported within 3 days of their capture to OCIC. Each captured snake being temporarily held for the captive colony must have its own well-ventilated enclosure and kept at the indigo snake's preferred temperature zone of 70° to 85° F (21° to 29 ° C). The enclosure should be easily disinfected (dilute bleach, a quaternary ammonia compound such as Roccal[®], or other broad spectrum disinfectant). Wood enclosures should be avoided, because they cannot be disinfected. More specifics on housing are provided below under the **SURGICAL TRANSMITTER INSERTION** section.

TISSUE AND BLOOD SAMPLING

Shed skins

Shed skins may be used for genetic analysis. They should be stored individually (after drying to prevent mold buildup) in paper envelopes, brown paper bags, or zip top bags (The Orianne Society undated). Shed skins should be transferred to a freezer as soon as possible (The Orianne Society undated). Data recorded for each sample should include: a sample identification number, date collected, state and county where sample collected, specific location (latitude/longitude, UTM coordinates), and a physical description of the site where the sample was collected (The Orianne Society undated).

Scale clipping

Examination gloves should be worn when performing invasive procedures such as scale clipping. If examination gloves cannot be worn, then hands should be free of dirt and thoroughly cleaned prior to performing any of these procedures. Ventral scale clipping is one of the most commonly used marking techniques in snake studies. Brown and Parker (1976) described the technique for ventral scale clipping and provided a scale-clipping system which can be used for eastern indigo snakes. Scale clips should be taken from the ventral scales 5 to 25 scales anterior to the vent. The area to be clipped should be cleaned at least once with a povidone-iodine scrub and then with a 70 percent isopropyl alcohol soaked gauze. Examination gloves should be used by the person doing the procedure. Scales are clipped to remove a rectangular section of scute approximately 0.125 to 0.16 inch (in) (3 to 4 millimeters (mm)) deep and 0.16 to 0.31 in (4 to 8 mm) wide. Use sharp-pointed dissecting or micro-surgical scissors that have been heat-sterilized (autoclave) or soaked in isopropyl alcohol or chlorhexidine prior to the procedure. If multiple snakes are to be clipped, sterilize for at least 10 minutes between snakes. Care must be taken to remove all layers of the skin to expose the underlying ventral musculature in a section large enough to cover half of a given ventral scute (Brown and Parker 1976). The incision site should not bleed afterwards, but if bleeding occurs use a sterile compress (cotton ball, gauze) to staunch the bleeding. A liquid bandage may be applied to the site if available. Scale clips may be taken and used for genetic analysis. If used for this purpose, scale clips should be immediately placed in individually labelled 1.5 ml (medical standard) tubes (with screw tops and O-rings) filled with 95 to 100 percent ethanol containing no additives (The Orianne Society undated). Scale clipping should be done by two

individuals whenever possible; one person to hold the snake and the second to do the clipping.

Blood sampling

Small blood samples may be taken from the caudal ventral tail vein for genetic analysis or diagnostic testing. Depending on the diagnostic testing needs, a 25-gauge needle on a heparinized 3-ml (medical standard) syringe can be utilized. Alternatively, if only samples for genetics or infectious disease testing using PCR (polymerase chain reaction analysis) are needed, insulin syringes work well for small samples and are widely available at pharmacies. The caudal ventral tail vein (coccygeal vein) is located on the ventral midline of the tail and can generally be easily accessed. Male snakes should be sampled distal to the hemipenial sacs. The sampling site should be cleaned thoroughly with 70 percent isopropyl alcohol. Only veterinarians, or trained field technicians, with experience taking blood from snakes will be permitted for this procedure.

Skin biopsies

Snake fungal disease (SFD) (*Ophidiomyces ophiodiicola*) is becoming more common in various species of snakes including eastern indigo snakes (Lorch *et al.* 2015, T. Norton *et al.* 2015 unpublished data). If skin lesions are present, a diagnosis can be made by taking a full thickness skin biopsy for histopathology and PCR. In most situations, some form of local or injectable anesthesia will be needed to perform the procedure. To get accurate results, the biopsy must be full skin thickness. Observing changes in the dermis is important for distinguishing SFD from other infections. Also, collecting adjacent healthy skin with the biopsy is helpful for observing necrosis patterns that are somewhat characteristic of SFD. Finally, detection of *O. ophiodiicola* alone should not be considered a positive diagnosis without supportive histopathology. Samples should be fixed in buffered 10 percent formalin for histopathology and frozen in an ultralow freezer or shipped immediately to the participating laboratory on ice packs for disease identification through use of PCR. Samples must be collected by a veterinarian or well-trained biologist. If skin lesions are on the head, an experienced veterinarian will need to do the biopsies. Sterile technique should always be utilized and post-operative pain management should be considered when appropriate.

Other sampling

Swabbing snakes (oral cavity, cloaca, skin), in the lab or field, may be conducted for diagnostic purposes.

PIT TAG INSERTION

PIT tags are electronic microchips encased in biocompatible glass that vary in size between approximately 0.4 and 0.55 in (10 and 14 mm) long and 0.08 in (2 mm) wide (Gibbons and Andrews 2004). In small snakes weighing less than 7 ounces (oz) (200 grams (g)) and with a total length less than approximately 3.3 feet (100 centimeters), the smallest PIT tags should be used. Once implanted, PIT tags can be used to verify if individuals have been previously captured. PIT tags may be inserted subcutaneously (SQ) by appropriately trained individuals, or implanted into the body cavity by a

qualified veterinarian during radio transmitter placement surgery. Test the tag to be used with a PIT tag reader before it is injected to make sure it is reading properly.

Subcutaneous implantation

Examination gloves should be worn when performing invasive procedures such as PIT tagging. If examination gloves cannot be worn, then hands should be free of dirt and thoroughly cleaned with a disinfectant soap or solution prior to handling each snake. Clean the area selected for tag insertion with a povidone-iodine scrub and 70 percent isopropyl alcohol. Optimally, at least two scrubs of each should be done prior to injecting the PIT tag under the skin with the insertion device and needle provided with it. Only packaged and sterilized needles and PIT tags should be utilized. If reusing needles and placing the PIT tags in the needle manually, they should be gas-sterilized rather than sterilized in liquid disinfectant such as alcohol or chlorhexidine. The PIT tag should be inserted SQ in the area that was previously cleaned, approximately 15 to 30 scale rows anterior to the vent (cloaca), along the side of the body (approximately two-thirds of the snout-vent length from the head). Insertion location is very important in order to help prevent injury to internal organs. The PIT tag should be placed along the upper side of the snake, approximately two thirds of the way up between the edge of the ventral scales and the crest of the vertebrae. To inject the tag, grasp a fold of skin between your fingers; insert the needle into this fold and depress the plunger completely (The Orienne Society undated). Keep the needle parallel to the snake's body; do not force the needle into the muscle tissue or between ribs. Post-injection, sterilize the site using rubbing alcohol and use tissue glue to seal and protect the injection site. Scan the tag to ensure it is reading correctly.

Body cavity implantation

Using sterile technique, a qualified veterinarian may manually place a PIT tag into the body cavity of an anesthetized indigo snake receiving a radio transmitter (see **SURGICAL TRANSMITTER INSERTION**, below). Female snakes will be palpated to determine if eggs are present and, if present, care taken to avoid impacting them during tag implantation.

SURGICAL TRANSMITTER INSERTION

Eastern indigo snakes are particularly amenable to animal studies using implanted radio transmitters due to their large size, long-range movements, and large home range sizes. This technique has been extensively studied and is considered an established method for the study of snake ecology and conservation (Dorcas and Willson 2009). It is described in detail in Hyslop *et al.* (2009).

Radio-telemetry devices may be surgically placed in the body cavity of eastern indigo snakes by a veterinarian** having knowledge of snake anatomy and significant experience with snake anesthesia and surgery, and holding the appropriate permits necessary to treat species listed by State regulations and the Act. All snakes must be in good body condition prior to surgery. Snakes will be prepared for sterile surgery in a standard accepted fashion. Recently there have been advances in snake analgesia (pain

management) and anesthesia. In the past, surgery on snakes involved using gas anesthesia (isoflurane) alone. Isoflurane alone was typically administered while the snake was in a clear plastic tube, and once the snake was relaxed, it was intubated with an un-cuffed endotracheal tube and maintained on isoflurane. However, isoflurane does not have any pain management properties so some form of pain management is needed. A second surgical method provides pain management during surgery and post-operatively and is preferred for eastern indigo snakes unless professional expertise allows for an alternate method of pain management. This method, developed by Dr. Darryl Heard from the University of Florida, has been used successfully on eastern indigo snakes by Dr. Terry Norton from the Georgia Sea Turtle Center and is summarized below by Dr. Norton. Metric units are not converted as these units are standard in medical practice.

Surgery using a pain management methodology:

Administer ketamine 5 mg/kg (body weight) + dexmedetomidine 50 micrograms per kg + buprenorphine 0.03 mg/kg intramuscularly (IM). Place the snake back in a secure container for 15 to 20 minutes. After this time period, the snake will be sedated but not fully anesthetized. Then, place the snake in the plastic tube, start isoflurane at 1 percent and work up to higher level until sedation/anesthesia level allows intubation with an appropriate sized, un-cuffed endotracheal tube. Intubate and maintain on isoflurane. Manually ventilate 1 to 2 breaths per minute and less frequently during recovery. Turn isoflurane off prior to the end of the procedure and keep as low as possible to maintain the snake under appropriate levels of anesthesia throughout. Once the isoflurane has been discontinued, administer oxygen for a few minutes, and then switch to a manual resuscitator such as an Ambu[®] bag to allow CO₂ levels to rise which will stimulate the snake to breathe. Reverse the dexmedetomidine with atipamezole at the same volume as the dexmedetomidine given IM. Use ultrasound for monitoring heart rate; Total CO₂ monitoring may also be used. Use meloxicam at 0.2 mg/kg SQ at the start of the procedure for additional pain management.

During surgery, maintain snakes at a temperature around 80° F (27° C). Avoid sudden temperature changes. Using standard sterile techniques, make a 1.2 in to 2 in (3 to 5 cm) lateral skin incision and reflect skin ventrally. Follow with a ventral midline incision through the coelomic (main body) lining to allow access to the coelomic cavity. Place the telemetry device intracoelomically (within the body cavity). Use transmitters of appropriate weight and shape. For reptiles, it is generally recommended that transmitters should not exceed 5 percent of body mass (Plummer and Ferner 2012). However, since the eastern indigo is a protected species under State and Federal laws, the Service is requiring that a more conservative approach be taken and that transmitters be no more than 3 percent of body mass (British Columbia Ministry of Environment 1998). Since 0.2 oz (5 g) transmitters are now available, juvenile snakes of 7 oz (200 g) or greater may be implanted as long as the required body mass ratio is met. Researchers should be mindful that a transmitter, meeting the less than 3 percent of body mass standard, may be too wide, especially in young, slim, snakes. Researchers must strive to minimize the ratio of transmitter width to body width to the extent possible. Therefore, this might mean that in some cases, elongate, flattened transmitter packages will be preferred over short,

cylindrical ones depending on their weight and width. Thread sterilized copper tubing, or similar product, subcutaneously anteriorly, and then thread the transmitter antenna through it. Make a small skin incision in the area of the anterior end of the tubing and remove the tubing, leaving the antenna in place. This skin incision is typically very small so suturing here is usually not needed. Suture material (for example, size 3.0 to 4.0 polydioxanone (PDS[®]) or nylon) for the main incision in the body cavity is used in a horizontal mattress or simple interrupted suture pattern to close the skin incision. The coelomic lining is too thin to suture. To support full recovery, snakes must be held post-surgery at least 2 to 3 days to monitor them for medical complications and to document their resumption of normal behaviors; all else being equal, the less time held in captivity, the better.

All indigo snakes in captivity for surgery or other health reasons should be kept in strict isolation. They must be kept in a separate room from all other reptiles, including indigo snakes from different sites or permanent captives. When holding snakes prior to and after surgeries, large, well-ventilated terraria under temperature-controlled conditions with supplemental heating, hide boxes, water bowls, and paper bedding must be supplied for each individual snake. Snakes must be monitored at least daily and supplied with clean water and paper bedding as needed. Protective clothing such as gloves and coveralls should be worn when handling these captive snakes. Antibiotics are not typically needed if sterile technique was used during surgery. However, ceftazidime at 20 mg/kg SQ or IM, given every 72 hours, is a good broad spectrum antibiotic to use if deemed necessary.

Eastern indigo snakes should not be captured for transmitter implantation unless daytime temperatures rise to at least 70° F (21° C). Prior to release, ensure that a minimum of 10 days of thermoregulatory temperatures (70 to 80° F (21 to 27° C)) are predicted in order to ensure proper healing in the wild, post-surgery. If these conditions are not likely to be met post-surgery, do not implant radio transmitters.

At the end of studies, all radio transmitters must be surgically removed from eastern indigo snakes by a qualified veterinarian.

****WARNING: Caution to veterinarians that Ivermectin is NOT to be used as a prophylactic. It has caused mortality in eastern indigo snakes.**

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