

CHAPTER 2 METHODS

2.1 STUDY AREA

Venezuela is located in the northern part of South America between 25° 46' and 0° 43' north latitude and 59° 38' and 73°23' west longitude. It is bordered on the north by the Caribbean sea, on the west by Colombia, on the east by the Atlantic ocean and British Guyana and on the south by Colombia and Brazil (Figure 2-1). This research was done on several cattle ranches in the Venezuelan Llanos: Puerto Miranda (4,000 ha) in the state of Guárico, Santa Luisa (25,000 ha), El Frío (80,000 ha), and El Cedral (54,000 ha) in the state of Apure. The owners of these ranches are keen to protect wildlife and also combine their cattle ranching activities with tourism.

Climate

The data were obtained from the field station in Mantecal (30 Km from the study sites) managed by Ministerio del Ambiente. The average temperature in the lower Llanos is 26.6 C°, the mean diurnal fluctuation is 9.5 C°, and the mean seasonal fluctuation is 3.0C°. The area receives an average of 1,575 mm of rainfall a year with over 90% of the rain falling between April and November. The period between January and April is acknowledged as a dry season when all the water bodies shrink to minimum size and only the surfaces that hold water are large depressions called esteros (see below) and lagoons. From July to October there is a distinct wet season when the savanna floods and there is abundant standing water due to rainfall and overflowing of the rivers. The two months between each season are considered transitional (Figure 2-2). During this study, I encountered marked droughts as well as very wet years (Figure 2-3) in which I gathered data on the population, behavior and reproductive activities.

Landscape and vegetation

The following description of llanos was taken from Berroterán (1985), Lopez-Hernández (1995), Ojasti (1978), Ramia (1967), Rivero-Blanco and Dixon (1979), Sarmiento (1983), Thorbjarnarson (1990), Troth (1979), Vila (1960) and personal observation of the study area.

The llanos is a large geosyncline (252,530 km²) tilted to the East and located in the intersection of the Andes ridge and the Caribbean ridge in the northern part of South America. The most flooded area is located at the eastern part and drains into the Orinoco river; it is transected by its tributaries west to east. Situated over pre-Cambrian basement rocks, the llanos is composed primarily of alluvial deposits from the Tertiary and Quaternary periods. Sediments are quite recent, associated with the upper Pleistocene uplift of the llanos region and deposition due to erosion from the Andes and Caribbean Cordilleras after the last glacial period.

The Llanos includes several topographic areas, but the general profile is flat with a slope of 0.02% to the east. It can be divided by four basic subregions: piedmont region adjacent to the Andes, the high plains, the alluvial overflow region, and the aeolian plains (Sarmiento 1983). The alluvial overflow plains, where this study was carried out, are situated in a central tectonic depression in the middle of the Llanos. The relief is especially flat with high elevations below 80 meters above sea level. The dominant

¹ All figures and tables appear at the end of the corresponding chapter.

vegetation associated with this region is a hyper-seasonal savanna with a few trees or palms. Gallery forest bordering the rivers and patches of dry forest adjacent to them interrupt the otherwise continuous plain. In the wet season, the rivers overflow and flood most of the savanna due to a combination of heavy rainfall, poor surface drainage, and poor permeability. There are three different physiographic positions in the overflow plains: banco, bajío and estero. These differ from each other in relative elevation, drainage soils, and vegetation.

Bancos are the most elevated regions, composed of the riparian areas that run along the river banks and cover 29% of the area. They are elongated in shape, 1 to 2 meters higher than the surrounding areas. The bancos are sandy loams, poor in organic matter, acidic (pH 4.5 to 5.5), and have moderate to good internal and external drainage. The soils of the bancos are classified as Aquultic Haplustalf and Aeric Tropaqualf. The dominant vegetation is a gallery forest with dominant tree species including palms (*Copernicia tectorum*), saman (*Pithecelobium saman*) masaguaros (*Pithecelobium guachapale*), fig (*Ficus* spp.), caruta (*Genipa* spp.), palo de agua (*Cordia collococa*), and Camoruco (*Sterculia apetala*).

Bajíos are lower regions more distant from the rivers where sedimentation of finer particles takes place. It covers 44% of the total surface of the overflow plains. These regions have poorer drainage and most soils contain a high proportion of expandable clay (2:1) and stilt (Vertic Tropaqualf and Udorthentic Pellusterts). The bajíos soil is acidic pH (4.5 to 5.0) and is richer in organic matter than the bancos. In the rainy season, the bajío is partially covered by water, but it dries out completely in November or December. The only trees that occur in the bajío are the palm (*Copernicia tectorum*) and caujaro (*Cordia* sp.). The area is dominated by spiny scrubs called barinas (*Cassia aculeata*), guaica (*Rocherfortia spinosa*), *Mimosa pigra*, *Mimosa dormiens* and *Hydrolea spinosa*, and by grasses including *Trachypogon* spp., *Paspalum* spp., *Paratheria prostata*, *Eleocharis* spp., *Leersia hexandra*, and *Hymenachne amplexicaulis*.

Esteros are the third and lowest region of the low overflow plains and cover 19% of the surface. They are characterized by poorly drained soils with very fine texture (> 60% clays) where the main route of water loss is evaporation. As the dry season progresses, the esteros hold water for longer than any other areas; they dry up only at the end of the dry season (March or April). The soils are heavy, composed 2:1 clays (Udorthentic Pellusterst, Entic Pellusterst and Vertic Tropaqualfs). Deep cracks in the soil are often observed in the peak of the dry season in the dry esteros. Since the esteros are flooded most of the year and have soil with heavy texture, tree growth is inhibited, with the exception of the occasional palms. Instead the esteros are dominated by floating vegetation of which *Eichhornia crassipes* and *E. azurea* occupy a large percentage. Other common elements are *Salvinia* sp., *Pistia stratiodes*, and *Ludwigia* sp.. Some rooted vegetation also occurs: *Thalia geniculata*, *Ipomoea crassicaulis*, *I. fistulosa*, *Eleocharis* spp. and *Cyperus* sp.. Even though there is a continuum among these three physiographic units, it is easy to identify them by the amount of time they remain flooded and the vegetation that grows on them (Berroterán 1985; Ramia 1967).

Fauna

The fauna in the llanos is both abundant and diverse. More than two hundred species of birds form a large group of both residents and migrants that gather in the dry season to feed in the drying waters. Important groups are: herons, (*Ardea cocoi*, *A. herodias*,

Nycticorax nycticorax), ibises (*Bubulcus ibis*, *Eudocimus ruber*, *E. albus*, *Butorides striatus*), egrets (*Egretta tula*, *Casmerodius albus*), storks (*Ciconia maguari*, *Jabiru mycteria*, and *Mycteria americana*), ducks (*Amazonetta brasiliensis*, *Anas discor*, *Cairina moschata*, *Dendrocygna viduata*, *D. Autumnalis*, *Neochen jubata*) shorebirds (*Jacana jacana*, *Actitis macularia*, *Himantopus himantopus*, *Rynchops niger*, *Chloroceryle amazona*, *C. americana*), and many birds of prey (*Heterospizias meridionalis*, *H. nigricollis*, *Buteo magnirosris*, *Parabuteo unicintus*, *Rostrhamus sociabilis*, *Pandion haliaetus*, *Falco femoralis* *F. sparverius*, and *Caracara plancus* among others). There are other also species of more terrestrial birds such as: *Ortalis ruficauda*, *Colinus cristatus*, *Columba ssp*, *Zenaida auriculata*, *Piaya cayana*, *Crotophaga ani*, *C. major*, *C. sulcirostris* and many passerines among the large diversity of species that either live or migrate to this areas (Phelps and De Schauensee 1978).

Among non-volant mammals, the most abundant are Capybaras (*Hydrochaeris hydrochaeris*), followed in abundance by white tailed deer (*Odocoileus virginianus*). Other mammals to be found in the savanna include giant ant eaters (*Myrmecophaga tridactyla*), crab eating foxes (*Cerdocyon thous*), lesser anteaters, (*Tamandua tetradactyla*), armadillos (*Dasypus sabanicola*) raccoons, (*Procyon cancrivorus*), giant river otters (*Pteronura brasiliensis*), opossums (*Didelphis marsupialis*), agouti (*Dasyprocta fuliginosa*), pacas (*Agouti paca*), porcupines (*Coendou prehensilis*), and several small rodents (*Holochilus sciureus*, *Sigmodon asltoni*, and *Zygodontomys brevicauda* among others). Howler monkeys (*Alouatta seniculus*) are common in the tree tops. The least common mammals also present are fresh water otters (*Lontra longicaudis*), pink fresh water dolphins (*Inia geoffrensis*), peccaries (*Tayasu pecari* and *Pecari tajacu*), skunks (*Conepatus semistriatus*), tapir (*Tapirus terrestris*), and felids such as ocelots (*Leopardus pardalis*), pumas (*Puma concolor*), and Jaguars (*Panthera onca*).

Reptiles are very abundant in the llanos. Other than anacondas, we find large numbers of spectacled caimans (*Caiman crocodilus*), side-neck turtles (*Podocnemis vogli*), green iguanas (*Iguana iguana*), and tegu lizards (*Tupinambis teguixin*). These reptiles constitute a great percentage of the biomass of the area. Other reptiles found in much lesser abundance include mata-mata turtles (*Chelus fimbriatus*), hinged turtles (*Kinosternon scorpioides*), river turtles (*Podocnemis unifilis* and *P. expansa*), Orinoco crocodiles (*Crocodylus intermedius*), and dwarf caiman (*Paleosuchus palpebrosus*). We can also find smaller species of lizards such as *Cnemidophorus* spp., *Ameiva* spp., and snakes, including: *Crotalus durissus*, *Liophis lineatus*, *Helicops angulatus*, *Corallus hortulanus*, *Spilotes pullatus*, *Chironius carinatus*, and *Epicrates cenchria*.

Amphibians are very abundant and ubiquitous in the wet season. The most common species include *Bufo marinus*, *Hyla crepitans*, *H. microcephala*, *H minuscula*, *Leptodacylus bolivianus*, *Scinax rostrata*, *Pleurodema brachyops* and *Pseudis paradoxus* among others. Fishes are also very abundant and diverse in the llanos; when the rivers flood the savanna they invade the new wet land foraging and breeding. In the dry season some return to the rivers but a large number of them cannot find their way back and become isolated in temporal ponds where the density of fish increases as the dry season progresses.

2.2 MAIN STUDY SITE: EL CEDRAL

El Cedral is where most of the study was performed and where the project was carried out for longest time (most of 1992 to 1997). It is a 54,000 ha cattle ranch located in Apure

state, Muñoz district (7° 30' N and 69° 18" W). This location was chosen due to its abundant population of anacondas, the active vigilance of the owners to discourage poaching on the ranch, and by its good internal roads for traveling during wet season.

El Cedral is a good representative of the alluvial flood plain described above. A series of human-made dikes have created more permanently flooded habitats (Módulos) where the impact of the dry season is diminished. The gates of the dikes are closed at the end of the wet season to hold the water for pastures and cattle. Each módulo has an approximate extension of 7,000 h. Due to the natural slope, the water gathers at the east of the módulo leaving the higher western surface dry and available for cattle ranching. As the dry season progresses, the cattle move toward the new land that is exposed by the drought, thereby allowing the growth of new buds in these areas.

As a result of this water management, the lower lands suffer a premature drought due to the water sequestered in the upper módulos. During the middle of the dry season, the gates are opened for a short period of time (one or two days) and the lower módulos flood again stimulating the growth of the plants there. The decrease in water on the surface flooded in the upper módulo exposes a large area where the soil is moist and grass growth begins. This management continues throughout the dry season to provide green pasture for cattle despite the lack of rain. When the wet season begins, the gates are opened again to let the water out and prevent overflowing and breaking of the dikes.

The dikes provide good and reliable roads to move around the ranch even in the heights of the wet season. The construction of dikes (and roads) produced another kind of habitat that is used by the anacondas and was advantageous for the study: borrow pits. Borrow pits are large holes left over from where dirt was taken to build the uplifted road. Accordingly, borrow pits are found along the roads and their variable size and depth determines how long into the dry season they hold water. Different arrays of aquatic vegetation grow in them as the season changes

2.3 SAMPLING EFFORT

Data were collected from the beginning in 1992 through 1998, sampling was concentrated in the dry season of each year, but the years 1992 and 1996 were sampled completely. See Table 2-1 for a summary of the sampling effort along the study period.

2.4 FINDING THE ANIMALS

Intensive searches

Despite their size, anacondas are anything but conspicuous. Their secretive nature in an aquatic habitat with murky water and dense vegetation, and cryptic coloration, combine to make finding these animals a real problem. Early in the study I gathered all the pieces of folk wisdom that I could from local residents, and by testing them in the field I discovered a successful method for finding the animals. The dry season provides the best time for this activity because all the snakes that live in the flooded savanna gather in the few depressions that hold water during that time.

I systematically searched all the water bodies (esteros, borrow pits, lagoons and rivers) of the study area where water depths were less than 50 cm (searches in deeper water proved to be unsuccessful due to problems caused by restricted mobility of the researchers combined with the increased ease of escape by the snake). Searches were conducted by wading, shuffling in the water under aquatic vegetation, and poking poles into the drying

mud. Other places that I found animals were in the caves on the river banks, large cracks on the ground of dry esteros (vertisoils), and spots protected from the sun under bushes neighboring the water bodies. These areas maintain a relatively low temperature even at the height of the dry season.

Early experience indicated that anacondas do not move much in the early hours of the day and their activity levels are also very low during the peaks of the heat of the day. Searching for animals began in the morning (around 0800 hr.) and continued until the afternoon, depending on the success of captures. On some days, by 1000 h I had already caught more animals than I could process during the rest of the day. However, on other days the search went on for several hours without much success. On these days I would stop around 1300 h and resume searches around 1530 h until the end of the day.

Intensive searches were conducted during the height of the dry season in the areas where the water was low enough to successfully catch the snakes. Due to the dikes of the módulos, some areas would dry fairly early, allowing us to make thorough searches in all areas even relatively early in the season. Later in the season the gates flooded the lowest módulos, creating a drop in the water levels in the upper ones and making them available for searching.

Cruising

Another method for finding the animals was conducted by systematically patrolling the area looking for moving animals. This was done either by riding on top of a truck, horseback riding, or by motor boat, depending upon flooding conditions. This method proved useful in the areas that had more water and when the intensive search (wading in the water) was not feasible

2.5 CATCHING AND RESTRAINING THE ANIMALS

The search and capture of snakes was always in teams of 2 to 7 people since I needed a lot of help to catch and subdue the large animals. At first several people needed to be involved in the process of finding the snakes as well as in preventing the large animals from coiling around the person that was holding the head. Volunteers and keepers from the Bronx Zoo or others zoos around the United States came to help at different times.

Submerged snakes were located by detecting them with feet or poles followed by confirmation of their presence by gently touching them with the hand. Wading through a swamp looking for snakes, we often stepped on spectacled caimans that were under the haycynth. Caimans were surprisingly oblivious to being stepped on. They were often confused with logs for their tough texture and immobility. The most reactive animals shook violently, sometimes throwing a person on his or her back, but these animals never made any attempt to bite (in no less than a hundred encounters). In a similar incident with a 4-meter long Orinoco Crocodile in 1998, the crocodile behaved in a similar way. Perhaps the animals under the hyacinth are in some sort of seasonal sopor or estivation that significantly decreases their aggressiveness.

Once the presence of a snake was confirmed, the animal was captured by seizing the head and exhausting it through physical struggle. When the water level was too high to control an animal, we dragged it to shallower water or to the shore where it was slower and more easily subdued. Although strong, anacondas are bulky and heavy, and move slowly on land. When the animals became defensive and started to try to bite the handlers, due to its slow movements, it was always possible to recognize the intentions of the snakes by

carefully watching their behavior. This way we could move out of reach, and avoid being injured. When the snakes were in caves, where the head could not be seized, the animals were gently poked with a stick encouraging them to leave the hole. After a short time the animals would move to the water where they were subdued as described

Once the snake had been controlled, it was put in a large sack or in a 200 l. metallic drum, depending on the size of the animal. At first many people were needed to subdue and process each snake, but as the project advanced I was able to subdue and restrain the snakes with fewer people. A most important procedural advance was the discovery that, once the head had been seized, the snake's defensive mechanism is to form a powerful loop with the first part of the body (the basis of the constriction movements). This loop forces the hand of the holder forward (from neck to nose), making the holder lose his grip. Then the snake proceeds to wrap the hand of the holder with its body, leading to many complicated situations because, at that point, the snake was loose and the "holder" held! I discovered that by keeping the first fifth of the animal's body stretched, I could prevent the animal from developing the loop in first place. This change in procedure made handling of the animals much easier and safer. After making this discovery, I could control all the animals with only the help of my wife (47.7 kilos and 160.7 cm).

Another problem was keeping an animal restrained while it was being processed. Restraining large and potentially dangerous reptiles for field studies without using anesthesia can pose a risk to the researcher as well as the subject. Several techniques developed for safely handling crocodylians and venomous snakes are reviewed by Flower (1978), Almandarz (1986) and Gregory, et al. (1989), but passive restraint of large boids has not been addressed, possibly because few field investigations into their natural history have been undertaken.

Sometimes when handling a large specimen the handler held the snake's mouth closed by applying strong pressure on the snake's head and jaws. On some occasions this resulted in the teeth of one gum cutting into the opposing gum. This was a minor injury for the animal, but it was a circumstance I tried to avoid. In order to reduce stress on the specimen, and to minimize risk during the captures, the number of handlers needed, and the time required to take data from each animal, I developed the following method for safely working with anacondas (Rivas et al. 1995).

While holding the anaconda's jaws closed, a cotton sock of appropriate size was pulled over the snake's head. Once the snake's snout contacted the terminal end of the sock, several loops of plastic electrician's tape were firmly, but not tightly, secured over the sock around the snake's neck (directly behind the quadrate bone) securing the sock on the anaconda's head. Taking care to keep the mouth closed, a second length of tape was secured over the sock and around the snake's snout (midway between eyes and nostrils) to secure the jaws. At this point the anaconda could be released for measuring, scale counting, scale clipping, parasite collection, and blood sampling without risk to the investigators.

This technique for rendering anacondas safe to work with proved quite reliable. No health problems were attributed to the use of this technique. Anacondas treated in this manner frequently struck with great accuracy, but they were unable to inflict injury and usually settled down after a short period. Sometimes the snake managed to bite the sock at the moment of muzzling so some teeth stuck out of the sock enabling it to snag the handlers during processing, but no important wounds occurred. Some small animals that

had relatively loose fitting tape managed to remove the sock during the processing, but this did not create much of a problem either. On some occasions, the sock on the muzzle became wet and produced breathing problems for the animal. Luckily we detected it early on and prevented any damage to the animal as a result of this restricted breathing, wet socks should be avoided when using this technique. Releasing the snakes into cloth bags or steel barrels for transportation may be accomplished by removing the tape and the sock while the animal is restrained. This passive restraint method could be used effectively on many species of large, non-venomous snakes in both field and captive situations.

2.6 PROCESSING THE ANIMALS

Data collected

For each animal captured, I recorded the following data: total length, tail length (to the nearest 0.5 cm in large animals and to the nearest 0.1 cm in snakes smaller than 1 m long), mass (to the nearest 100 g in large animals, to the nearest 5 g in small ones), and sex. Since snake's length is critical data in the analyses I used here and throughout herpetology, a separated section (see below) is devoted to the methods I used. In this report, unless it is clearly explicit, all lengths are given in centimeters and all masses are given in grams or kilograms.

To identify the sex of a squamate reptile a metallic probe may be introduced into the cloacal pouch of the animal. Males have deeper pouches than females; if the animal is a male the probe would go in a longer distance than if it is a female (Rivas and Ávila 1996). At the beginning of this study, all animals were probed to identify sex until I learned to identify the sex by external characters (see below). For sub-sample of 56 individuals (18 females and 38 males), I also measured the left spurs to compare sizes between sexes. From a sub-sample of 56 males and 38 females, I recorded the injuries and scars present on the animals. I classified the scars and wounds based on the estimated length. Scars smaller than 2 cm were recorded as small, scars larger than 2 cm and smaller than 5 were recorded as medium, and scars larger than 5 cm were recorded as large.

Marking and identification

Snakes were marked by scale clipping at both sides of the spurs. Each animal was identified by a unique combination of clipped scales that allowed for future recognition. Even though scales do regenerate, regenerated scales have a darker color than the original ones, so it is possible even after several years to reliably identify the animal. A backup method for identification of individuals was to copy the pattern of spots the snakes have in the first 15 subcaudal scales. Every animal I recaptured was identified by the clipped scales and confirmed by the pattern of the spots.

Each animal captured was released within 24 hours at the same place of capture unless it showed evidence of having fed recently, in which case the animal was kept in a drum with water until it defecated (usually within 7 days). Feces samples from these animals were analyzed immediately. Items found in these samples that could not be identified to the species level were labeled and preserved in formaldehyde for future identification. Some animals were radio-tagged by force-feeding the transmitters to them. Since this technique revealed important traits of the snakes ecology they are described in a different section (see below). All animals were assigned with a number for identification. Some

individuals that were radio implanted or that were captured in several occasions were also assigned with a name for easy of reference.

2.7 WHAT IS THE LENGTH OF A SNAKE?

Introduction

The way that herpetologists have traditionally measured snakes is by stretching them on a ruler and recording the total length (TL). However, due to the thin constitution and slim muscular mass of most snakes it is easier to stretch a snake more than it is to stretch any other vertebrate. The result of this is that the length we record of a snake is influenced by how much we stretch the animal. Stretching the animal as much as we can is perhaps a precise way to measure the length of a specimen but it might not correspond to the actual length of a live animal. Furthermore, it may seriously injure a live snake. Other methods consist of placing the snakes in a clear plexiglass box and pressing the snake with a soft material (e. g. rubber foam) against a clear surface. Measuring the length of it may be done by following the snake's body with a string (Frye 1991). This method, though, is restricted to small animals that can be placed in a box. In this contribution I propose an alternative way of measuring snakes that is more accurate than stretching the animals on a ruler. I further analyze the precision of this method by using a sample with a large range of sizes.

Methods

To record a more naturalistic measure of the length of the animal we followed a middle line of the body from head to tail with a string and then measured the length of the string by laying it loosely on a ruler (Figure 2-4). This allowed us to record the actual length of the animal regardless of its position and without having to stretch it. I used newborn anacondas born from 14 females that had been kept in captivity during gestation for a study of female's reproductive output (Chapter 5). A total of 82 newborn live babies and 42 stillborn were measured for this study. Repeated measures of the same animal with the string were slightly different due to the errors caused by the snake struggling and moving from under the string. Thus each measure was taken three times and the average of the three measures was calculated.

I also recorded the TL of each snake in the sample using the conventional method of stretching them on a ruler. I used a sign test to compare both measures of each animal. I divided the measurements obtained by the stretching method by the measurements obtained by the string method in order to calculate a relationship between the two measures. In order to analyze the changes of this relationship in respect to size, I used the mass as an independent measure of the size of the animal. I performed a Spearman correlation test between the variables. The use of stillborn in this study was to remove the effect of the error introduced by the struggle of the animal. By removing this I attempted to determine the actual TL of the animal.

Another sample of 81 animals from a wild population (ranging from 84.7 cm to 494.7 cm TL) was measured by three different people without allowing any of them to know the value recorded by the other people. All of the animals were measured by one of two researchers who had three years of experience performing the procedure, and by two people well instructed in the technique but without much previous experience.

In order to assess the accuracy of the measurements collected from 60 animals by different researchers, I used a sign test to compare the values collected by an experienced researcher with the values obtained by one of the inexperienced researchers. A t-test was used to compare the means of both sets of measures. I also compared the measurements taken by the two researchers that had experience, using a sign test on 13 animals that were measured by both.

I calculated the coefficient of variation (CV) on the three measures collected on animals from the wild to study the changes on the precision of the measurement of snakes of different sizes. The CV was calculated by dividing the mean by the standard deviation (see formula in Sokal and Braumann 1980) and provides a measurement of the variance that is not dependent on the absolute value of the variable measured. This is especially important when dealing with variables that vary in a wide range of values. All statistical analysis were made with SSPS 8.0.

Results and discussion

The string technique described here is comparable to using it with the squeeze box except that it can be used on larger animals that cannot fit in a box or that cannot be pinned and restrained. Thus it has a larger applicability. Measurements taken with the string were consistently shorter than when measured with the ruler ($Z = 6.82$; $p < 0.000$; Table 2-2). The quotient among the measurements is smaller in larger animals ($r = -0.362$; $p < 0.001$; Figure 2-5). This suggests that smaller animals are being significantly stretched when measured on a ruler.

All the measurements estimate a unique parameter: “the size of the neonate”. However, measurements from the two methods of measuring using stillborn snakes had a higher discrepancy between them than measurements on live individuals (Table 2-2). Measurements of stillborn snakes with the ruler were the largest of all and the measurements of stillborn with the string were the shortest of all (Table 2-2). An ANOVA shows significant difference between the measurements of all the groups ($F = 70.47$; $df = 3$; $p < 0.0001$) I used in the analysis only stillborn animals that were completely formed and whose cause of death was most likely due to dystocia of the female or other problems at the end of the gestation (Ross and Marzec 1990). I believe that the size of the stillborns was not significantly different than the size of live neonates, supported by the fact that there was no significant difference in mass ($t = 1.252$; $df = 120$; $p = 0.21$; Table 2-2). Thus the difference in the measurements of the live babies and the stillborns are most likely due the struggling of live animals.

If we assume that the “real” length of the animal is the length when it is relaxed, and not struggling or being over-stretched (as is usually the case when most other vertebrates are measured), then the length of the stillborn measured with the string should be the closest estimate. This is the most accurate way to measure a live animal, but the data suggest that this method is not error-free.

Repeated measurements collected with the string on the same animals showed a relatively high variance. The average variance in animals around 80 cm was 0.514 cm and the maximum was up to 2.35 cm. It was clear while processing calmer animals that the repeated measures on them were more similar than measures of more active animals. In animals that the struggled a lot, the first measurement tended to be the most different. After the process was done once on the individual, it tended to calm down more.

The struggle of the animal during the measurement can potentially influence the repeatability of the measure. The data collected by experienced researchers and by the newer ones were significantly different ($Z = -3.13$; $p < 0.002$), where inexperienced researchers consistently obtained shorter measurements than experienced ones. The data collected by the two experienced researchers were consistent between each other and were not significantly different in a Wilcoxon sign test ($z = -0.27$; $p < 0.79$).

The variance of the measurements changed with the size of the animal being measured (Figure 2-5). Notice that between the size of 2 to 3 meters the variance is particularly high, mostly due to a few animals that had a very high CV. This might be a consequence of the higher level of struggling found in some smaller animals. The smallest animals can be easily subdued during the process and the measures are more consistent with each other (but see below). Beyond a certain size the snakes are stronger and some of them are able to put up more of a struggle, which decreases the precision of the measurements. Larger animals are calmer and although they could make the measuring much harder they tend to be easier to measure consistently (Figure 2-7). Notice, however that the CV is high in all the early sizes and goes down after three meters. Thus the lower variance found in figure 2 for smaller sizes is probably an artifact of smaller values. I noticed that the first measurement of each animal tended to be more different than the following two; this was especially true in medium-sized animals. Larger animals are only females and the medium-sized ones are mostly males so some differences in the behavior of each sex could be involved in this trend. However, these two effects can not be disentangled easily because adult males are always smaller and females are typically larger (Chapter 6).

Stretching a snake apparently has a considerable effect on the measurements collected on the length of the snake. Smaller animals seem to provide less resistance to being stretched than larger ones, thus studies involving measuring animals among several size classes must consider this issue. This method is not different in theory from the method of the squeeze box (Frye 1991) but this has a much broader application to larger sizes. The size of newborn anacondas is within what is considered a small snake. Herpetologists have traditionally considered that this size range can be reliably measured by stretching it on a ruler, yet I have shown that this is not the case. This trend must be particularly critical on larger animals (e.g. exceeding 130 cm) as they would present more resistance to be stretched. Stretching the animal on a ruler is less time consuming and in some situations it might seem appropriate. However, the degree that the animal is stretched can be influenced by the size and behavior of the animal, or even the mood of the researcher! Measuring the animals with a string is a more reliable method especially if it is done by people properly trained in the technique. Research involving mark and recapture, or growth studies must consider these issues.

2.8 FORCE FEEDING TRANSMITTERS: A TECHNIQUE TO STUDY SNAKE'S REPRODUCTIVE BIOLOGY IN THE FIELD

Introduction

The secretive nature of the snakes imposes a serious challenge for field studies. Snake's mating systems, for instance, have been hard to study by direct observations unless in exceptionally large aggregations (Gardner 1955, 1957). These exceptional events may bias the observations to particular situations that do not necessarily reflect the typical mating system of the species.

Radiotelemetry has been used in studies of snake biology typically oriented for research of home range and habitat use (Reinert 1992), and thermoregulation (Peterson et al. 1993). Telemetry also has been used to study mating systems and reproduction of squamate reptiles (Bock et al. 1985, 1989; Duvall et al. 1992; Duvall and Schuett 1997). However, to surgically implant radiotransmitters requires a higher degree of invasive manipulation than is desirable if we do not want to perturb the natural behavior of the animals. Force-feeding transmitters to the snakes to be studied can be done much faster and with less perturbation. It has been used in the past to study ecology of snakes (Madsen and Shine 1994); however, we do not know details of the duration of the transmitters and how effective the technique was to study the biology of the animals. In this chapter I document the efficiency of a force-feeding radio transmitters to study the mating system in anacondas (*Eunectes murinus*).

Methods

The transmitters used were model 15A2 built by Advanced Telemetry System Inc. containing the antenna coiled inside the unit, and covered with a waterproof resin. The dimensions of the units were 15 cm long by 2 cm radius and 91 g; with two batteries in series, of 3.6 volts each. The frequency of these units was in the range of 164-165 MHz range. These units were set to last for 8 months. I lubricated the transmitter with vegetable cooking oil and, holding the snake vertically below the head, forced it down the digestive track of the animal by palpating it down to the stomach, or as far down as possible (Figure 2-9). In larger females, the muscles of individuals tended to prevent the maneuver too far down, but I always could push it far enough to prevent the animal from regurgitating it. Due to their small size, in males (Chapter 6), I could push it all the way to the stomach or even palp it out later and recover the transmitter if I needed to implant it in another animal.

Over a four year period, I gathered males before and during the mating season that were actively searching for females, and or females that were involved in breeding aggregations (Chapter 6). I equipped 16 males and 15 females with transmitters and monitored the snakes' behavior during the mating season and throughout pregnancy. On several instances, when a male found a female, I removed the transmitter from the male by palpating it out. I also palpated out the transmitter of all the males at the end of the breeding season of each year to recover them for future use. Retrieving the transmitter from females was not possible due to their more muscular body, which prevented me from feeling or pushing the transmitter by palpating.

Results and discussion

The method of force feeding transmitters proved to be an effective means of studying the mating system of anacondas. No animal died or showed any ill effect as a consequence of either the force-feeding or the extraction of the transmitter. In fact, after the procedure all the females continued with the mating and all the males continued with their trailing activities. In no instances was the transmitter regurgitated after the implantation and all animals were followed for at least a week. The transmitters were 0.3% of the average females size and 1.3% the average size of the males (Chapter 6). Perhaps due to its small size, the transmitter was not perceived as a meal or an obstacle to the animal's movements.

I removed transmitters from 13 males. In two cases, after 21 and 23 days respectively, the transmitter had to be palpated out through the cloaca. In eleven cases the transmitter was

still in the stomach, even after more than 30 days, and was extracted through the mouth. In three males I allowed the transmitter to pass naturally, which took 21, 43, and 45 days, respectively. Notice, however, the large variance in the time that the transmitter remained in the animals (Figure 2-10). Even though I extracted most radios before they came out naturally, it must be noted that the transmitters did stay in the male's tract for long enough to follow them during the courtship and mating. Most females (9 of 13) kept the units until delivery, as they do not feed during pregnancy. Only four females defecated the transmitter before parturition (in 12, 14, 24, and 36 days). The extreme difference between these females and the others suggest that they might have had food in their digestive tracts at the time of the procedure so the transmitter might have passed along with the stomach contents. Two females were not captured after mating and I could not record the exact time that they kept the transmitters. These animals had not defecated the transmitter after 61 and 68 days respectively when the rainy season started, and presumably they kept it until the parturition since they do not fit the pattern of the animals that defecated soon. The retention times found in females that defecate early are not conspicuously different from those of males (Figure 2-9). There does not seem to be a correlation between the passage time and the size of the animals. The variation in retention time in females seems to be strongly influenced by the effect of pregnancy on feeding. Thus, the time that the transmitter is retained is highly variable, and perhaps it is most related to whether the animals were digesting or not. Breeding females do not eat during pregnancy or breeding (Chapter 5), and courting males seem not to eat during the mating season either, judging for the long time that most transmitters lasted in many animals.

I implanted transmitters in 16 males of which 8 found breeding females (the time and distance traveled will be published elsewhere). This is not necessarily an accurate reflection of males success in finding females, because in three cases I removed the transmitter before the end of the season. Thus 50% might be a minimum estimate of the actual success rate.

Due to their particular feeding morphology, it is easy to force feed a transmitter to a snake to study its biology. This technique proved to be reliable for short-term follow-ups, since none of individuals implanted regurgitated the transmitter. The procedure did not seem to interfere with the animal's natural behavior, as suggested by the large number of males that found females and all the females whose mating was studied. This technique can be used quite successfully for studies of mating systems, or even reproductive biology, if care is taken in not implanting the transmitters in animals that have recently fed. Even though force feeding transmitters to snakes to study their behavior has been done in the past the duration of the transmitters in anacondas makes this technique specially useful in anacondas. The presence of the transmitters in the anaconda's tract does not prevent it to feed due to its relatively small size. The long time that the transmitter last on the animals is a consequence of the low feeding rate of the individuals especially in mating season. I believe that this method can be used successfully with other species, however, it might be less effective in smaller species with shorter passage times and higher feeding frequency.

Table 2-1. Field time spent in the study along with the name of the individual performing the work. MM= María Muñoz, CC= Carlos Chávez, RA = Rafael Ascanio, CM= Cesar Molina, JR = Jesús Rivas.

	1992	1993	1994	1995	1996	1997	1998
January	JR	JR			JR	JR	
February	JR	JR			JR	JR	
March	JR	MM	MM	JR	JR	JR	JR
April	JR	MM	MM	JR	JR	JR	
May	JR	MM	JR	JR	JR	JR	
June	JR				CM		
July	JR	CC	RA		CM		
August	JR	CC	RA		JR		
September	JR	CC	RA		JR		
October	JR		RA		JR		
November	JR		RA		JR		
December	JR		RA		JR		

Table 2-2 Total length of neonate green anacondas measured by stretching them on a ruler and by following their midbody line with a string. Lengths are the mean of the three measures of each snake.

	Length Ruler (cm)	Length String (cm)	Mass (g)	N
Live	79.72	77.57	228.11	82
Stillborn	85.12	76.0	225.54	42

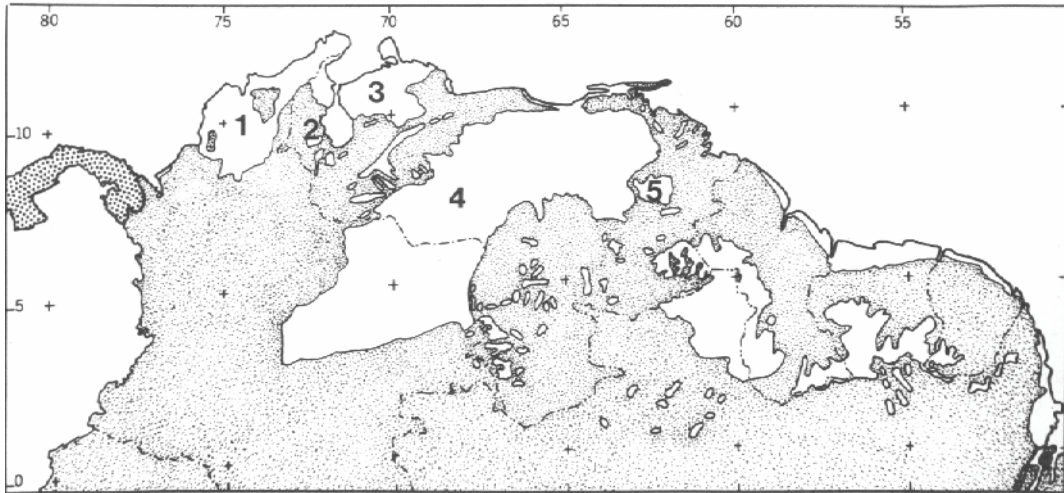


Figure 2-1 Map of Northern South America. The area of the llanos is marked with the number 4 (from Rivero-Blanco and Dixon 1979).

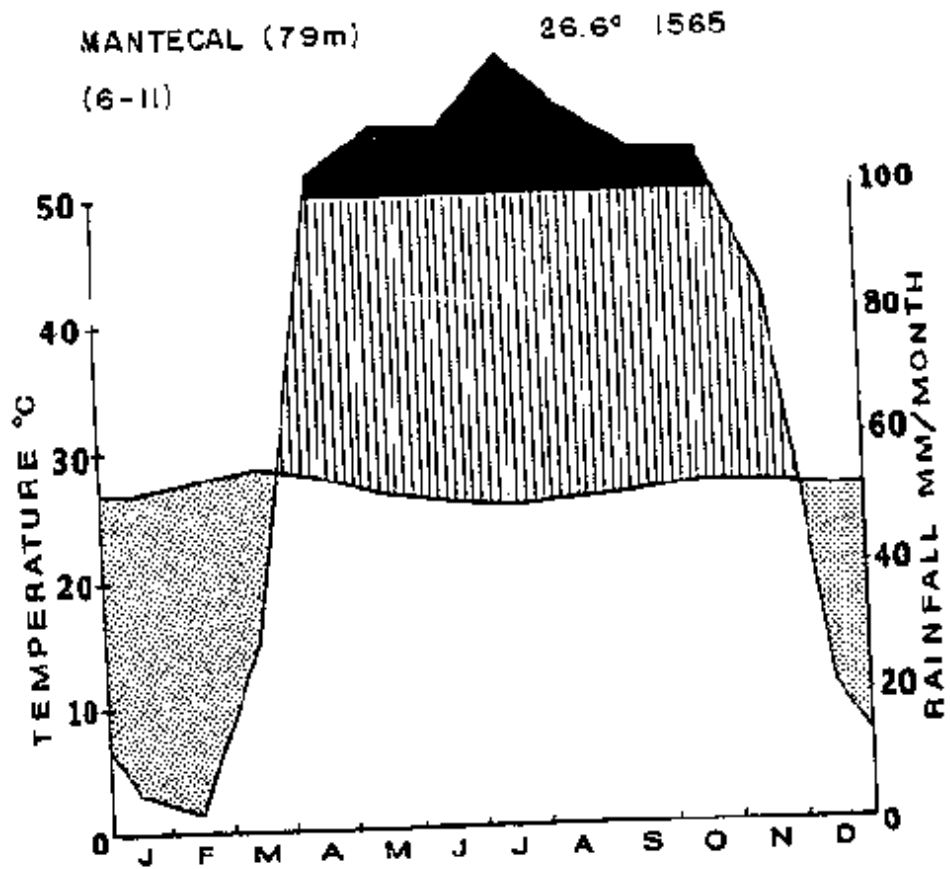


Figure 2-2. Holdridge's representation of climate of Mantecal (from Ojasti 1978). Located approximately 30 Km from the study site.

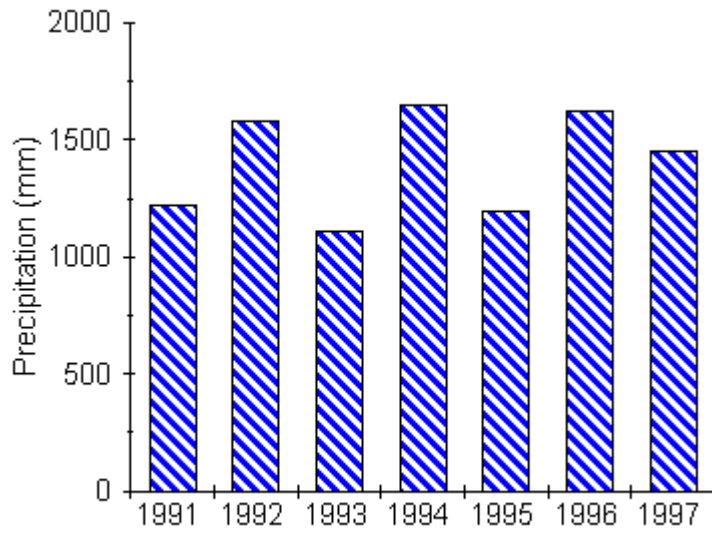


Figure 2-3. Annual precipitation during the years of the study from the Estación meteorológica de Mantecal (Ministerio del Ambiente y de los Recursos Naturales Renovables) located approximately 30 km from the study area.



Figure 2-4 Measuring technique stretching the string over the back of the anaconda to assess its length.

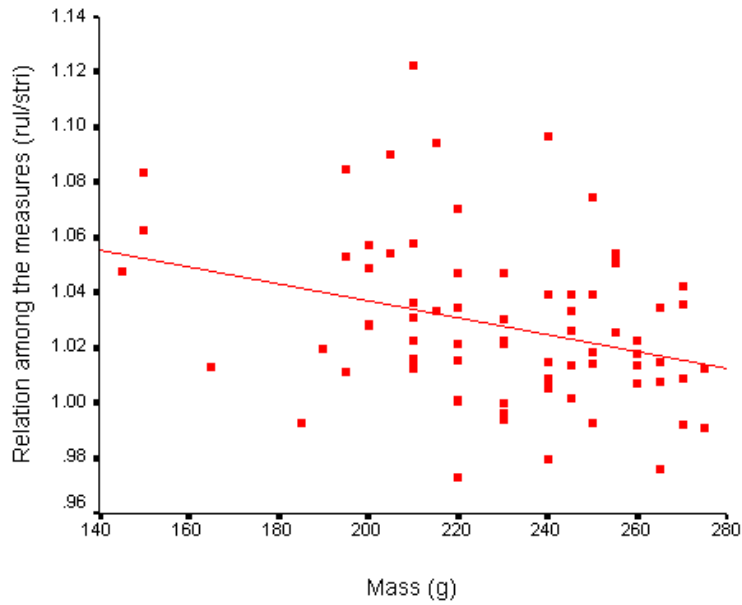


Figure 2-5. Scatter plot of ontogenetic change of the quotient between the measures of neonate anacondas obtained stretching them on a ruler and following the midline of the body with a string. Notice how the relationship between the two measures changes with the size ($r = -0.362$ $p < 0.001$; $n = 124$).

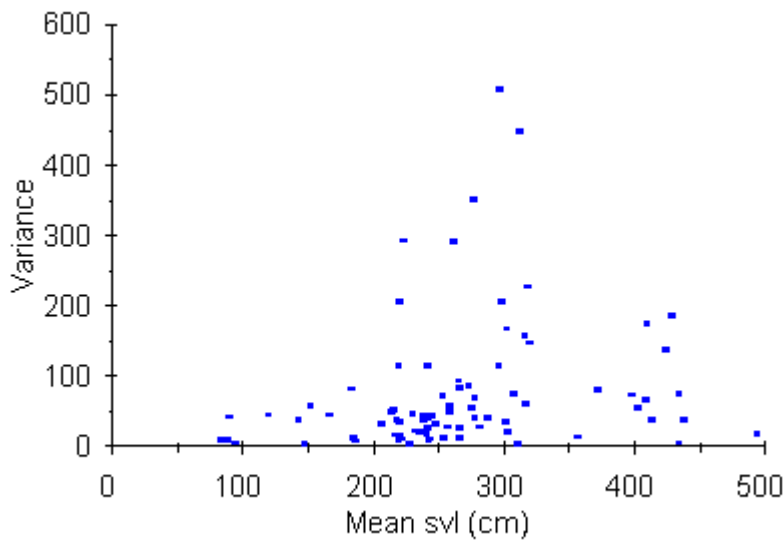


Figure 2-6. Size related change of variance of three measurements of SVL obtained from each anaconda using a string to follow the middle line of the body.

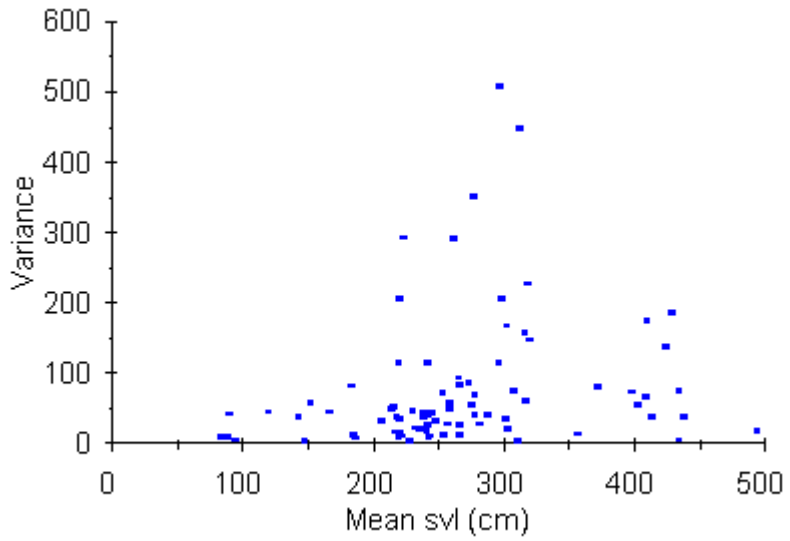


Figure 2-7. Size related change of variance of three measurements of SVL obtained from each anaconda using a string to follow the middle line of the body.

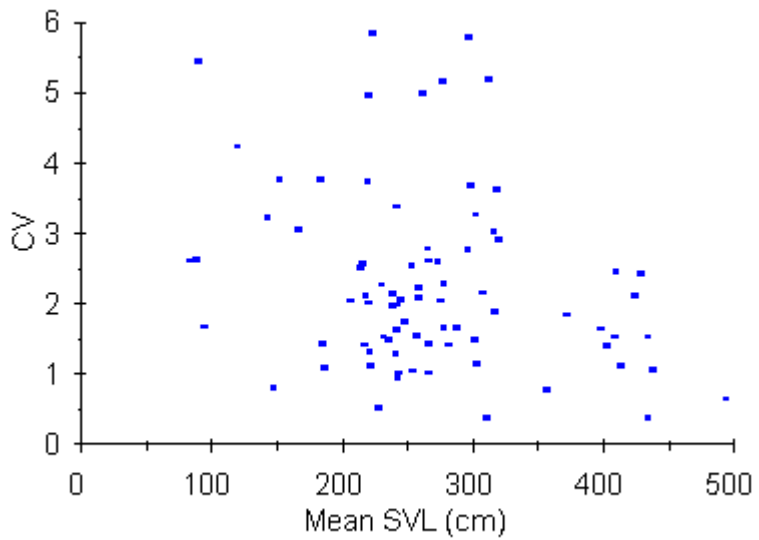


Figure 2-8 Relationship on the coefficient of variation from three measurements on the same individual anaconda measured with the string. Notice the decrease in larger sizes.



Figure 2-9. Force feeding the transmitter to an adult male anaconda. The unit is oiled and pushed gently down the snake's throat. Photo Phillips Bourseiller

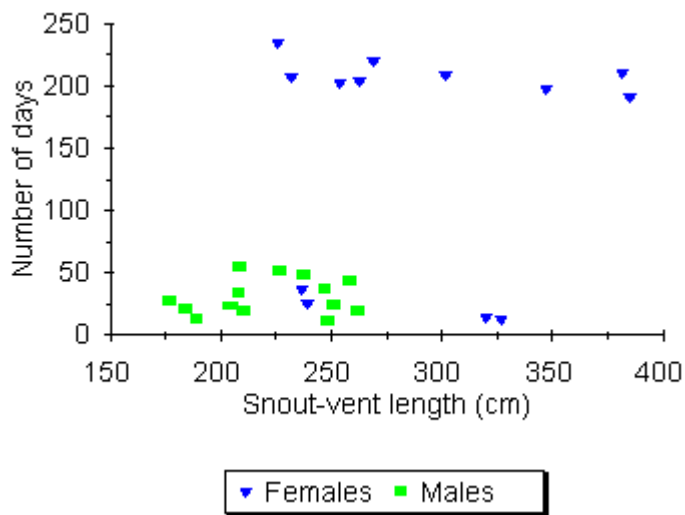


Figure 2-10. Time that the transmitter stayed in the tract of anacondas that had received a forced unit. All the females passed the transmitter naturally. Most males had the transmitter removed artificially at the end of the breeding season or when they found a female. Thus, the duration time for males is a minimum estimate.