Dorcas, Michael E. and John D. Willson. 2009. Chapter 1. Innovative Methods for Studies of Snake Ecology and Conservation. pp. 5-37. In *Snakes: Ecology and Conservation*, S.J. Mullin and R. A Seigel (eds.). Cornell University Press, Ithaca, NY.

3107

1

Innovative Methods for Studies of Snake Ecology and Conservation

MICHAEL E. DORCAS AND JOHN D. WILLSON

Snakes are fascinating to many laypeople and scientists alike, and numerous studies of snake ecology and natural history have been conducted. For nearly all snake species, however, a comprehensive understanding of their ecology, and especially population biology, is lacking. Such gaps in our knowledge limit our ability to develop effective conservation and management strategies or, more often, prohibit arguments that conservation is needed at all. We argue that snakes, although often challenging to study, offer many opportunities for ecological study unparalleled by other taxa.

One of the main reasons ecologists often shy away from snakes as study animals is the perception that their secretive natures make them difficult to study. Developing a more complete understanding of snake ecology and its application to conservation has been hampered by this perception (warranted or not). Unfortunately, because of their apparent rarity we often know least about the species that are most in need of conservation. Efforts to study snakes can sometimes be hindered by an enthusiasm for the animals that actually inhibits the development of meaningful questions and study designs. Many researchers who begin snake studies either (1) do not have a question at all, (2) have a question but do not know why that question is important, (3) do not match their question with appropriate methodology, or (4) select a species or group of species that are not particularly amenable to addressing the question(s) of interest (Seigel 1993). For example, many herpetologists have embarked on radiotelemetric studies of a species of snake with no clear question or hypothesis (i.e., the goal becomes the study in itself). Such herpetologists sometimes have a question (e.g., What is the home range of my study species?), but do not know whether or why that question is important. Although we have historically learned much about snake ecology through basic studies of snake natural history, the information required for the effective conservation of snakes nearly always requires answers to specific questions relating to such things as diet, habitat requirements, and population status.

Despite the lack of comprehensive information on many snake species and the perception that they are difficult to study, snakes have been proposed as model organisms (Beaupre and Duvall 1998b; Secor and Diamond 1998; Shine and Bonnet 2000). In fact, snakes are particularly amenable to numerous techniques used in ecology and conservation biology. For example, some snakes are particularly good subjects for mark-recapture studies because they occur at high densities and are easily trapped and marked. Many species are particularly amenable to focal animal studies such as radiotelemetry, allowing a detailed examination of habitat use, movement, and physiological ecology. Although snakes pose significant challenges for effective ecological study in some situations, snakes also offer many ideal opportunities for in-depth investigation of ecological phenomena, especially if the correct questions are matched with appropriate capture techniques, study design, and analyses (see also Seigel and Mullin, Chapter 11).

Our goal here is to discuss innovations in methodology for the design and implementation of ecological and conservation-oriented studies of snakes. We take the approach that the reader can find information on details of the basic techniques elsewhere in this book and in other sources; here we focus instead on the development and use of newer techniques and question-oriented approaches to studying snake ecology.

In this chapter, we discuss techniques related to (1) the capture and marking of snakes in the field, (2) focal studies of individual snakes, and (3) studies of snake populations. In each section, we discuss which types of questions can be addressed and which methodological and analytical techniques are best for addressing those questions. Our hope is that, during the course of a well-designed snake ecology study, researchers will seize the opportunity develop and investigate new and exciting questions (Greene 2005; Blomquist et al. 2008). In this chapter, we also make the reader aware of biases associated with certain techniques and how those biases can affect the interpretations of data. The reader should note that we present information on techniques that we have used or with which we are most familiar. Thus, unlike good snake ecology studies, this review is biased toward techniques used by us and our colleagues.

Capturing and Marking Snakes

In the first volume of the *Snakes* series, an entire chapter is dedicated to describing techniques for capturing and marking snakes (Fitch 1987a).

Although these techniques remain the standards among snake ecologists, numerous refinements have been proposed, along with novel methods employing recent technological advances. In addition, studies have elucidated sampling biases that can hamper the interpretation of capture data. Next, we review advances in methods for capturing and marking snakes, with particular emphasis on how the choice of capture methods can influence the analytical tractability of data and interpretation of results.

Active Capture Methods

Active capture methods involve the observer's searching out free-ranging snakes. These methods take advantage of an a priori understanding of snake behavior and can be among the most effective methods for capturing large numbers of snakes. Because such methods rely on the competence of the observer, they are sensitive to observer bias (Table 1.1). For example, interobserver variability was one of the strongest sources of variation in visual counts of Brown Treesnakes (*Boiga irregularis*) on Guam (Rodda and Fritts 1992b). In addition, visual searches often target snakes only in specific habitats or involved in specific behaviors (e.g., basking, foraging, or hiding beneath cover). Because snake activity is highly dependent on environmental conditions (Peterson et al. 1993), active capture methods may suffer from low repeatability as a result of a variation in capture rates caused by environmental variation (Table 1.1).

TABLE 1.1
Strengths and weaknesses of frequently used capture methods for snake population studies

Capture Method	Capture Type	Effort	Capture Rate[a]	Repeatability[b]	Observer Bias
Opportunistic search	Active	– –	++	– –	++
Transect/quadrat survey	Active	++	– –	–	+
Coverboard	Active	–	+	–	–
Road survey	Active	– –	+	–	–
Drift fence/trap	Passive	++	+	+	– –
Funnel trap[c]	Passive	+	+	+	–

Note: Plus and minus signs represent high (+), very high (++), low (–), or very low (– –) values within a category. Thus, pluses are strengths for capture rate and repeatability, but are weaknesses for effort and observer bias.

[a] Efficacy of capture methods varies by snake species (e.g., stand-alone funnel traps are effective for many aquatic and arboreal species but not for many terrestrial species). Thus, capture rate is considered here for species for which the given method is effective.

[b] *Repeatability* refers to comparability of sampling events, independent of differences among observers (observer bias) and, particularly, of sensitivity to environmental stocasticity, changes in snake behavior, or other factors that cause short-term variation among samples. For example, even within a single day, captures under coverboards can vary greatly depending on the environmental conditions at the time of the survey.

[c] *Funnel traps* here refers to stand-alone funnel traps, including aquatic minnow traps and arboreal snake traps.

Visual encounter surveys (VES), the simplest active capture method, are effective for surface-active species or for those that use specific habitat types or bask conspicuously. Although the basics of VES have not changed, increasing standardization by constraining time, effort, or the spatial pattern of sampling (e.g., transects or area-constrained searches) has increased the utility of VES for analytical techniques that rely on standardized sampling (e.g., relative abundance indices). Moreover, several authors have addressed potential sources of bias in VES, improving our ability to interpret results. For example, biotic and abiotic factors that influence census counts have been examined in Shedao Pit Vipers (*Gloydius shedaoensis;* Sun et al. 2001).

Two other active capture methods commonly applied to snakes are the turning of natural or artificial cover objects (coverboards; Fitch 1992; Grant et al. 1992) and road surveys (Fitch 1987a). Although these techniques are essentially variants of VES and suffer from similar repeatability issues, they are less prone to observer bias than VES (Table 1.1). Both methods are highly effective for collecting many snake species, some of which are not sampled effectively using other methods (e.g., traps). However, both coverboards and road surveys have been used relatively infrequently for snake population monitoring (but see Mendelson and Jennings 1992; Sullivan 2000).

Passive Capture Methods

Passive capture methods generally involve trapping animals. Although passive capture methods often yield a lower catch per unit effort than active methods, they are usually preferable for population studies because they are insensitive to observer bias and maximize repeatability by integrating captures over time (Table 1.1; Willson and Dorcas 2003; Willson et al. 2005). Most snake traps are variants of funnel traps (Fitch 1951) that have been used to sample snakes in both aquatic (e.g., minnow traps; Keck 1994a; Willson et al. 2005) and arboreal (Rodda et al. 1999a) habitats. Several new terrestrial funnel trap designs have been developed, most of which are wooden and are used in conjunction with drift fences (e.g., Burgdorf et al. 2005; Todd et al. 2007). Although unbaited funnel traps can be effective, baiting increased capture rates in both aquatic (Keck 1994a; Winne 2005) and arboreal (Rodda and Fritts 1992b; Rodda et al. 1999a) habitats. Escape rates from traps can be high for both arboreal (Rodda et al. 1999a) and aquatic (Willson et al. 2005) traps. Although flaps covering the funnel openings have been shown to reduce rates of entry to the traps, they increase snake retention rates by 170% (Rodda et al. 1999a). As with VES, quantifying biases is crucial to the interpretation of capture data because nearly any trap will not representatively sample all species or demographics within species (see examples in Enge 2001; Willson et al. 2005; Rodda et al. 2007b; Todd et al. 2007; Willson et al. 2008).

Marking Snakes

Individually marking snakes is necessary for mark-recapture studies and allows the researcher to assess movement and changes in body size, condition, or reproductive status. Scale-clipping (Weary 1969; Brown and Parker 1976b; Fitch 1987a) remains one of the most effective and inexpensive methods for marking snakes; even small species can be scale-clipped by using a large-gauge needle to excise a portion of scale (Mao et al. 2006). Clipped scales, however, can regenerate rapidly and, after long periods, marks may be difficult to recognize (Conant 1948; Fitch 1987a).

An alternate method for marking snakes involves the implantation of passive integrated transponders (PIT tags; Camper and Dixon 1988; Gibbons and Andrews 2004). PIT tags are typically injected into the body cavity using a large-bore needle and provide a presumably permanent and unambiguous unique identification number when a reader passes within a short distance (usually <7 cm). Disadvantages of PIT tags include cost (US$6–8 per tag) and size—most snake ecologists agree that they should not be used in very small snakes. Some companies (e.g., BioMark) are now making smaller PIT tags that may be amenable to smaller snakes. Studies have documented no detrimental effects of PIT tags on the growth and movement of Pigmy Rattlesnakes (*Sistrurus miliarius;* Jemison et al. 1995) or on the growth and crawling speed of neonatal Checkered Gartersnakes (*Thamnophis marcianus;* Keck 1994b). PIT tag loss can occur either through the skin (Germano and Williams 1993) or via expulsion through the gut (Roark and Dorcas 2000).

An effective and inexpensive method has been described for branding snakes using field-portable cautery units designed for ophthalmic surgery (Winne et al. 2006a). Cautery units can be used to brand the ventral scutes and adjacent dorsal scales (Fig. 1.1) and have been shown to be effective over several years and useful even on small individuals or species (Winne et al. 2006a).

Focal Animal Studies

Focal animal studies are ecological studies that rely on the in-depth examination of individual animals. Although the focus of many conservation-oriented studies is assessing population status (size or trends), measuring only population status often does not provide information about the mechanisms underlying population dynamics, which are critical for effective management (Beaupre 2002). The secretiveness of some species makes evaluation of population status impractical and thus, focal animal studies provide the most feasible way to obtain the information necessary to make reasoned conservation or management decisions (Seigel et al. 1998).

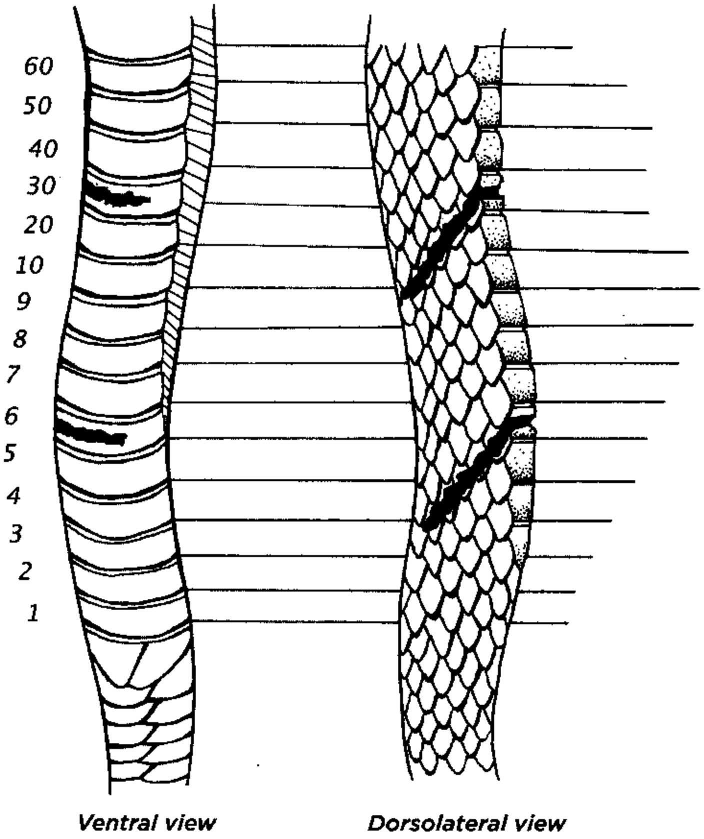

Fig. 1.1. Illustration of a snake heat-branded with ID #36 using a medical cautery unit. For each mark, the researchers branded the anterior portion of the ventral scale and extended the mark diagonally onto the adjoining dorsal scales. (Illustration drawn by R. Taylor; used with permission of Society for the Study of Amphibians and Reptiles from Winne et al. 2006a)

Focal animal studies can be used to address questions about spatial ecology, habitat use, diet, energy acquisition and allocation, reproductive ecology, behavioral ecology, and predator-prey relationships. These studies can also provide information useful for the control of invasive snake species such as *B. irregularis* on Guam (Rodda et al. 1999d) or Burmese Pythons (*Python molurus bivittatus*) in Everglades National Park (Snow et al. 2007). Although the basic techniques used in focal animal studies have not changed, refining these techniques, combining them with other methodologies, considering study design, and using advanced analytical methods allow increasingly insightful perspectives on the ecology and conservation of snakes.

For focal animal studies to be effective and meaningful, investigators must (1) develop thoroughly the question(s) of interest and understand how their results can be applied to our understanding of ecology and/or effective conservation efforts; (2) consider carefully what technique(s) are most appropriate to address their question(s); (3) consider how their study will be designed to maximize inferential capability; and (4) consider the inherent limitations of their study, such as sample size, expenses, and required time and effort.

Collection and Selection of Animals

Because the results of focal animal studies are often extrapolated from a small number of individuals to the entire population or even species, the means by which animals are collected and selected for study are extremely important. When the study species is secretive, researchers often have no alternative than to use any and all animals that become available through trapping or incidental captures. In such cases, researchers should be aware of, and attempt to correct for, any biases inherent in the animal selection and how those biases affect their results. For example, if all animals were collected on roads, investigators might infer a far greater use of roadside habitats than if they had a sample truly representative of the population.

Radiotelemetric Studies

The miniaturization of radiotransmitters and the development of surgical techniques to implant radiotransmitters (Reinert and Cundall 1982) have allowed insights into the details of snake ecology unimagined 25 years ago. The basic techniques of radiotelemetry in snakes have been described elsewhere (Reinert and Cundall 1982; Reinert 1992; Ujvári and Korsós 2000; Millspaugh and Marzluff 2001). Here we discuss novel or often-overlooked issues that should be considered when conducting radiotelemetric studies. We recommend that anyone wishing to use radiotelemetry seek hands-on assistance from a snake ecologist experienced in the technique before and during the initial stages of his or her study.

A Few Considerations

The intensive nature and cost of radiotelemetric studies often limit the number of animals that can be sampled. Within the constraints of the study, however, as many snakes as possible should be studied because, in nearly all analyses, each snake represents a single data value. Moreover, in comparisons among groups (e.g., sexes, species, or treatments), the number of animals is divided among groups, thus limiting the ability to discern effects. Combined with the large interindividual variability often observed in radiotelemetric studies (Millspaugh and Marzluff 2001), statistical power is often limited.

In many cases, snake researchers miss the opportunity to gain insights into the ecology of their animals because they do not take time for careful observation. When snake ecologists radio-track an animal, they often just record the geographic coordinates and other information and then move as quickly as possible to tracking the next animal. Often, researchers radio-track their animals at the same time each day, further limiting their ability to observe the full spectrum of activity and behaviors afforded by radiotelemetric studies. Relocating animals at different times of day (e.g., at night) may be less convenient, but it may provide unique insights into the ecology of the study species.

Surgical Considerations

Radiotelemetric studies of snakes have been conducted using transmitters that were force-fed (Fitch and Shirer 1971; Lutterschmidt and Reinert 1990), implanted subcutaneously (Anderka and Weatherhead 1983), or attached externally (Ciofi and Chelazzi 1991). Intraperitoneal implantation of radiotransmitters (Reinert and Cundall 1982), however, allows for the long-term monitoring of individual snakes with minimal disruption of normal physiological processes (e.g., digestion) and behaviors and is currently the method used by most snake ecologists.

Most snake ecologists use gas anesthesia and have found that isofluorane generally works more quickly than others (e.g., halothane) and causes less liver damage (at least in humans; Goldfarb et al. 1989). Generally, inhalation of anesthesia is induced passively by placing the snake's head in a chamber or tube (Hardy and Greene 2000). We have found that using a refurbished anesthesia machine connected to an endotracheal tube and intubating (i.e., placing the tube directly into the glottis) the snakes allows oxygen to be administered during anesthesia and facilitates direct inhalation, resulting in shorter induction times. We have successfully used this technique with ratsnakes (*Pantherophis [Elaphe]*), kingsnakes (*Lampropeltis*), Timber Rattlesnakes (*Crotalus horridus*), and *Python molurus bivittatus*. Propofol has been used by some veterinarians as a form of short-term anesthesia in reptiles. Propofol can be injected directly into the heart (or caudal vein) in snakes and causes rapid and complete anesthesia in many species (Anderson et al. 1999). We have used propofol to anesthetize ratsnakes before the application of gas anesthesia, and it appeared to reduce the stress associated with intubation, allowing the immediate initiation of surgery.

Transmitter Expulsion

It is not uncommon for researchers to find a radiotransmitter but no snake in the field when locating their animals and to assume that the snake died or was depredated. During a radiotelemetric study of pythons, radiotransmitters were found, often within snake fecal material, suggesting that the snakes expelled radiotransmitters implanted intraperioneally (Pearson and

Shine 2002). Dissection of a dead subject revealed a radiotransmitter that was partially incorporated into the stomach. Such expulsion of radiotransmitters from the peritoneal cavity through the gut wall is apparently accomplished through the same physiological mechanism as seen in fish (Chisholm and Hubert 1985) and in PIT tag expulsion in snakes (Roark and Dorcas 2000). We concur with Pearson and Shine (2002) that investigators finding a radiotransmitter but no snake remains should exercise caution in assuming the death of their study animal.

Automated Radiotelemetry

The majority of snake radiotelemetric studies have been conducted in a similar manner—the investigator determines the position of the snake at specified intervals by manually tracking the animal. Today, the use of radiotransmitters outfitted with global positioning systems (GPSs) allows for the real-time automated tracking of animals ranging from whales to turtles (Rogers 2001). Unfortunately, the small size of most snakes and the need to implant radiotransmitters currently prohibits the use of automated GPS in snake studies. Systems have been developed, however, that allow the tracking of animals automatically using a series of directional antennas. Such a system has been used in Panama to follow the movements of various avian and mammalian species (Wikelski et al. 2007), and we see no reason why such a system could not be used for snakes.

Automated monitoring of body temperature (T_b), especially when combined with simultaneous measurements of environmental temperatures, can provide substantial insight into the habitat use and activity patterns of snakes (Peterson et al. 1993). Automated systems (Fast-Data System, Telonics, Mesa, Ariz.) have been used to continually monitor the T_b values of Rubber Boas (*Charina bottae*) in southeastern Idaho and have documented nocturnal activity at low temperatures (Dorcas and Peterson 1998). One system (Lotek—SRX-400 with W21 event logging) has been used for several years to automatically monitor the T_b values of *Crotalus horridus* in Arkansas (S. Beaupre, pers. comm.). This system uses directional antennas that record signal strength as well as temperature and in certain circumstances (e.g., flat, relatively uniform terrain) might be used to estimate the locations of snakes.

Analysis of Radiotelemetry Data

Numerous methods for the analysis of spatial data collected via radiotelemetry have been developed (White and Garrott 1990; Reinert 1992, 1993). Snake researchers frequently evaluate habitat use and home range size of snakes using geographical information systems (GISs; e.g., ArcGIS from ESRI, Redlands, Calif.). Several publications on the analysis of radiotelemetric data (e.g., Millspaugh and Marzluff 2001) and software applications allow relatively easy calculation of spatial parameters (Hooge and

Eichenlaub 2000), but we remind researchers that their question(s) should drive the choice of analytical methods. All too often, snake researchers measure the home ranges of snakes without a thorough understanding of how the analytical technique used (e.g., minimum convex polygon, MCP, or kernel) might influence their conclusions. For example, snake ecologists might calculate a home range for an animal that migrates annually from one area to another. The calculation of a MCP home range for that animal might show a much larger area than is actually used by the animal and include large areas of unsuitable habitat.

Snake ecologists often evaluate habitat use or habitat selection using data generated from radiotelemetric studies. It is important to understand that the analytical methods for the determination of habitat selection must involve the determination of habitats available to the snake (Reinert 1992, 1993).

Automated Cameras

The use of automated photography can provide insights into the ecology of many secretive animals, including snakes. Automated 35-mm film cameras, triggered by the removal of a rat carcass resting on a mechanical switch, have been used to film scavenging *Crotalus horridus* (DeVault and Rhodes 2002). Digital cameras used in this manner increase the image capacity of these systems and, because film developing is not required, greatly reduces costs of operation (Guyer et al. 1997).

Automatically controlled still and video cameras can document predation by various predators, including snakes (e.g., Renfrew and Ribic 2003; Peterson et al. 2004). Most researchers use time-lapse video (2–5 frames/s) that allows recording for a relatively long time (Weatherhead and Blouin-Demers 2004b; Clark 2006). Setting video cameras, positioned at places of high snake activity (e.g., hibernacula), to record based on triggering stimuli such as a switch or the breaking of a light beam should be possible and may allow the deployment of a system without maintenance for longer periods of time.

Automated Monitoring of PIT-tagged Snakes

Automated systems for monitoring animals implanted with PIT tags have been used in studies of fish (Prentice et al. 1990), voles (Harper and Batzli 1996), and bats (Kunz 2001). In some situations, automated monitoring of PIT-tagged snakes could provide considerable insights into snake activity patterns. To monitor PIT-tagged animals, a reader must be placed in an opening or area through which the animal is expected to move. An automated system that reads PIT tags was used to monitor the movements of Desert Tortoises (*Gopherus agassizii*) when they were diverted under highways through culverts (Boarman et al. 1998). Each time a tortoise passed over the reader's detecting coil, the system recorded the PIT-tag number, time of day, date, and duration of time the PIT tag was within reading distance of the coil. Similar systems could be used to monitor snake movements at communal hibernacula (e.g., Prior and Weatherhead 1996) or snakes passing through openings in drift fences (Gibbons and Semlitsch 1982).

Snake Thermal Ecology

Because temperature affects nearly every aspect of their biology, understanding thermal biology allows us to achieve a more complete understanding of snake ecology (Peterson et al. 1993; Weatherhead and Madsen, Chapter 5). When combined with studies of the effects of temperature, measurements of snake temperatures can be used to estimate the effectiveness of locomotion, prey capture, or digestion and can provide insight into the energetics limitations of snakes in various environments (Beaupre 1995b; Dorcas et al. 1997). For snake thermal ecology studies, proper measurement of snake T_b values and the thermal environment is essential.

When conducting field studies, measuring only air and/or substrate temperatures provides an inaccurate representation of the thermal environments available to snakes (Peterson et al. 1993). Fortunately, it is relatively easy to construct biophysical models for most species of snakes from copper tubing (Peterson 1982). Automated monitoring of these "snake models" using a datalogger provides an integrated and more accurate measurement of the thermal environment (i.e., operative temperature) available to snakes (Bakken and Gates 1975; Peterson et al. 1993).

Traditionally, the temperatures of snakes and other reptiles were measured by capturing an animal and inserting a quick-reading thermometer into its cloaca. In addition, measurements of the thermal environment usually consisted of air and possibly substrate temperatures (Dorcas and Peterson 1997). We now know that cloacal temperature measurements result in a biased sampling of snake T_b values and that measuring air or substrate temperatures provides an inadequate characterization of snakes' thermal environments (Peterson et al. 1993). Automated monitoring of both snake and environmental temperatures provides detailed and unbiased measurements that allow a more accurate understanding of snake thermal ecology and can provide insights into the activity and habitat use of snakes (Peterson and Dorcas 1992, 1994).

Automated monitoring of snake T_b values has primarily been conducted using temperature-sensitive radiotransmitters in conjunction with an automated receiving system (Peterson et al. 1993; Beaupre and Beaupre 1994). Such systems are costly and often require considerable maintenance. In addition, when snakes move out of the range of the system, no data are collected. The recent miniaturization of single-channel temperature dataloggers allows the automated collection of T_b values without a receiving station. We have used miniature dataloggers (Tidbits; Onset, Bourne, Mass.) to automatically monitor the T_b values of Eastern Diamondback Rattlesnakes (*Crotalus adamanteus*) and *Python molurus bivittatus* while tracking their movements using radiotelemetry. Dataloggers were programmed and coated with plastic tool dip (PlastiDip) before implantation into the snake's body cavity. After a period of time (e.g., months), the dataloggers were removed surgically and the data downloaded. It may be possible for snakes to expel implanted dataloggers through their gut in a manner similar to that described for radiotransmitters and PIT tags (Roark and Dorcas 2000; Pearson and Shine 2002).

An examination of T_b plots for *P. molurus bivittatus* in Everglades National Park allowed us to determine that, in November, snakes apparently remained in the water to stay warm at night and then emerged to bask when environmental temperatures were favorable (Fig. 1.2). This information would have been difficult to obtain without automated data acquisition because the snakes were in remote areas of the park, reachable only by helicopter.

Considerably smaller temperature dataloggers (iButton Thermochron; Dallas Semiconductor, Dallas, Tex.) that are inexpensive (approximately US$28) and hold more than 8000 date/time-stamped readings have been used successfully in Eastern Racers (*Coluber constrictor;* Green 2005). Other researchers have used these dataloggers successfully on various species of turtles (Grayson and Dorcas 2004; Harden et al. 2007).

Temperature-sensitive PIT tags have been used in the laboratory to examine thermoregulation in Corn Snakes (Roark and Dorcas 2000) and Rubber Boas (Zhang et al. 2008). The development of PIT readers (Blomquist et al. 2008) that can collect data from distances of greater than 25 cm might allow investigations into the thermal ecology and movements of small snakes in natural or semi-natural conditions.

Energetics

Understanding snake energetics can reveal aspects of snake ecology critical for conservation and management (Beaupre 2002). Because metabolic rate is dependent on temperature, incorporating T_b often allows more realistic models to be developed. For example, we modeled the energetics of *Crotalus adamanteus* using data on thermal dependency of metabolic rate and, thus, could predict the food required to meet the resting energetic demands of various-size snakes (Dorcas et al. 2004). When we incorporated the T_b values from free-ranging snakes (collected using implanted microdataloggers) into the model, we determined that only two prey items (30% of snake body mass) per year were required to meet resting metabolic demands (Fig. 1.3). Modeling the effects of landscape structure and prey abundances on energy acquisition and allocation in snakes allowed predictions of the effects

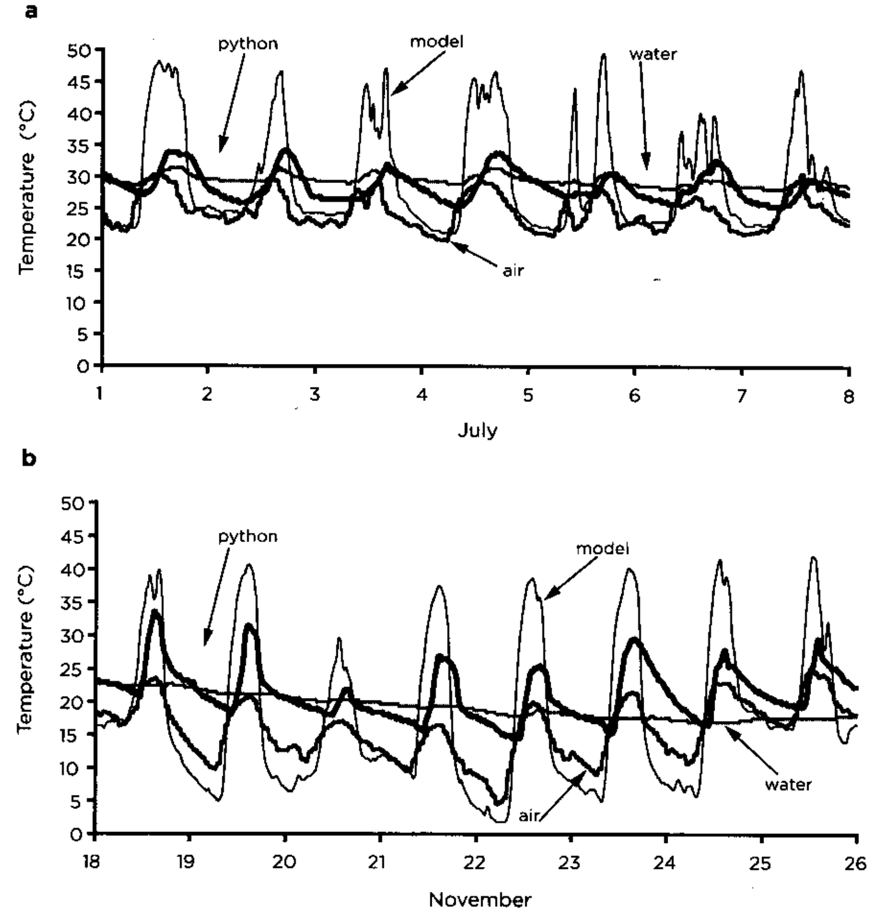

Fig. 1.2. Body temperature variation of an invasive 4.5-m female Burmese Python (*Python molurus bivittatus*) measured using a surgically implanted microdatalogger while being radiotracked in Everglades National Park. (a) July (b) November. Snake model (operative environmental temperatures), water, and air temperatures were measured using other dataloggers or obtained from a nearby weather station. Note that during July, body temperature was higher and less variable than during November. In contrast, during relatively cold weather in November the snake's body temperature approached or exceeded 30 °C each day, apparently by remaining in the relatively warmer water and by basking when possible.

of landscape manipulation (e.g., forestry practices) on energetics of snakes (Beaupre 2002).

Models using measures of field-metabolic rates using the doubly-labeled water technique have been developed for several snake species. For example, snake T_b data were combined with metabolic rates measured in the field to compare the consequences of foraging mode in ambush (Sidewinder Rattlesnakes, *Crotalus cerastes*), and active foragers (Coachwhips, *Masticophis*

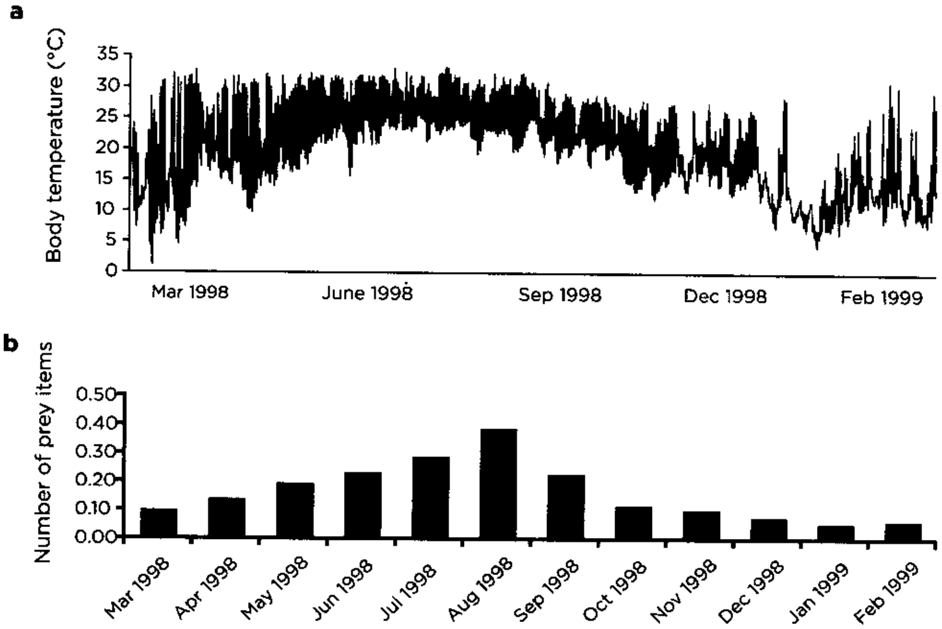

Fig. 1.3. Body temperatures and estimation of number of prey items per month required to sustain resting metabolic rate for an Eastern Diamond-backed Rattlesnake (*Crotalus adamanteus*). (a) Body temperatures obtained from free-ranging snake tracked using radiotelemetry and implanted with a microdatalogger (b) Calculations of the number of prey items required; these assume a 2500-g snake, prey items equivalent to 30% of the snake's body mass with an energy content of 5.9 kJ/g, and 80% assimilation efficiency: $\log_{10}$ SMR = (0.930 × $\log_{10}$ Mass) + (0.044 × Temp) –2.589 (equation from Dorcas et al. 2004). Number of prey items required per year (calculated by summing the values across all months) = 1.96. SMR, standard metabolic rate.

flagellum; Secor and Nagy 1994). The doubly-labeled water technique has also been used to examine the field metabolic rates of Rock Rattlesnakes (*C. lepidus;* Beaupre 1995b, 1996).

Diet and Trophic Structure

Traditionally, the diet of snakes has been determined by dissection of museum specimens (Greene 1986) or by forcing captured snakes to regurgitate a recently ingested meal by manual palpation (Mushinsky and Hebrard 1977; Fitch 1987a). Although these techniques are useful, they do have limitations. Diet analyses based on literature or museum specimens often use individuals spanning broad geographic areas and may be inappropriate for determining the diets of specific snake populations (Rodríquez-Robles 1998), missing subtle but important information, such as spatial or temporal variation in diet (Fitch 1999). Moreover, diet patterns determined by dissection provide only a snapshot of the diet of that individual snake. For species that eat infrequently, dissection or palpation of many snakes may be needed before even a single prey item is recovered. In addition, dissection requires snakes to be killed and palpation can be stressful to the animal as a result of the associated physical manipulation and the potential loss of an important meal. Finally, capture biases may lead to misinterpretations of diet or feeding frequency. For example, after eating a large meal a snake may bask more conspicuously or be less able to escape, resulting in an overrepresentation of large prey taxa in the diet analysis. Small diet items may be underrepresented because they are digested more rapidly or are more difficult to detect by palpation than larger prey items. Using molecular techniques (e.g., DNA and monoclonal antibodies) to identify prey taxa from gut and fecal material (Sheppard and Harwood 2005) may ameliorate some of these biases.

Recently, stable isotope techniques have been proposed as a method for assessing diet and trophic relationships without incurring the biases inherent in traditional gut- or fecal-content analyses (Ehleringer et al. 1986; Gannes et al. 1997, 1998; Bearhop et al. 2004; Schindler and Lubetkin 2004). Stable isotope techniques use variation in the relative amounts of naturally occurring stable isotopes of ecologically important elements (e.g., $C^{13}:C^{12}$ and $N^{15}:N^{14}$) as tracers within living systems (Ehleringer and Rundel 1989; Gannes et al. 1998). Because diet isotopes are often transferred to consumer tissues in conservative or predictable ways, the isotopic composition of food sources can be used to draw inferences about consumer trophic relationships. Although stable isotope techniques have been used for a variety of other taxa, they have only recently been implemented in studies of snake ecology (Pilgrim 2005, 2007).

Using isotopes to investigate trophic dynamics within or among snake populations requires the generation of detailed prey isotope profiles across space and time (e.g., seasons and ontogeny). In addition, prey isotope profiles must be specific to the system in which snake tissues will be sampled. The greatest inference can be drawn when isotope signatures of prey taxa or functional groups are distinct and relatively constant through time. For example, the isotope profiles for amphibian prey taxa available to aquatic snakes at an isolated wetland in South Carolina clustered into functional groups based largely on taxonomy (Fig. 1.4a). In contrast, taxonomy was not a good predictor of isotopic similarity among amphibians at a terrestrial Florida site because the isotopic composition of treefrogs (*Hyla*) encompassed the entire range of both the carbon and nitrogen isotope values observed in the system (Fig. 1.4b). Several authors have reviewed stable isotope techniques and their use in ecological studies (e.g., Gannes et al. 1997, 1998).

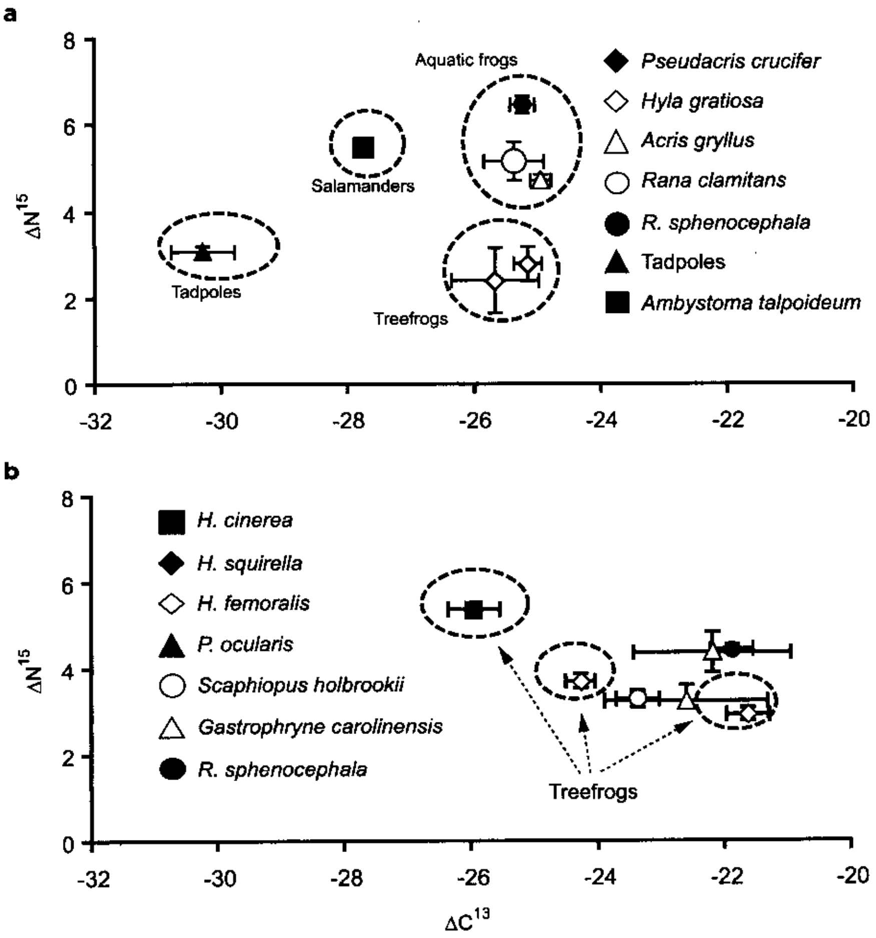

Fig. 1.4. Prey isotope profiles for two ecosystems in the southeastern United States. (a) Amphibian prey taxa available to aquatic snakes (collected in aquatic minnow traps) at Ellenton Bay, Aiken Co., South Carolina, in 2005–2006 (b) Amphibian prey taxa available to terrestrial snakes (collected in terrestrial drift fences) at a site in Volusia Co., Florida, in 2001–2002. Axes represent isotopic composition (carbon and nitrogen) of prey in delta values (proportion of heavy to light isotope in a sample, relative to a standard). Note that in (a) prey functional groups cluster by isotopic composition, whereas in (b) prey taxonomy is a poor predictor of isotope similarity. (Data for [b] adapted from Pilgrim 2005)

Population Studies

The goals of most monitoring efforts, and of many applied ecology studies, lie at the population level and include investigations of demography, site occupancy, population size or density, vital rates (i.e., survival, recruitment, immigration, and emigration), and mechanisms underlying population change. Unfortunately, the accurate estimation of population parameters often requires large investments of time and resources (but see Seigel and Mullin, Chapter 11). Moreover, investigators studying snake population often struggle with low precision in parameter estimates as a result of low recapture rates, high variation in capture rates caused by environmental stocasticity, and unaddressed sources of bias that can cloud results. However, recent advances in efficacy and standardization of collection methodology, in analytical techniques, and in our understanding of snake ecology are paving the way for a new generation of carefully executed, question-driven, snake population studies. In this section, we first discuss conceptual advances in design of snake population studies; then, we detail important analytical advances in methods for studying snake populations at multiple levels of intensity or scale.

Definitions

First, let us define a few terms that are used extensively in this section and discuss how each concept relates to this section.

Species detection probability (p in the presence/absence literature). The probability of encountering any one individual of a given species with a given unit of effort, provided that the species is present. Thus, detection probability is influenced by both abundance and ease of capture/observation. Detection probability is a key parameter in presence/absence monitoring.

Capture probability (p in the mark-recapture literature). The probability of capturing one particular individual of a given species with a given unit of effort. Thus, capture probability is unrelated to population density and is a function only of how easy it is to capture each individual. Capture probability is the primary consideration in mark-recapture studies because it also describes the probability of recapture (although capture and recapture probabilities may differ as a result of trap responses). Generally, mark-recapture analyses lose power when capture probabilities are low.

Heterogeneity Variability in capture probability among individuals, resulting in some individuals being more catchable than others.

Temporary emigration A situation in which a portion of the population is not available for capture during some sampling intervals (Kendall et al. 1997; Bailey et al. 2004a). Individuals may be unavailable for capture due to behavior (e.g., ecdysis, inactivity, or reproduction) or because they are using habitats or geographic areas that are either outside the sampling area or are not sampled effectively by the capture method.

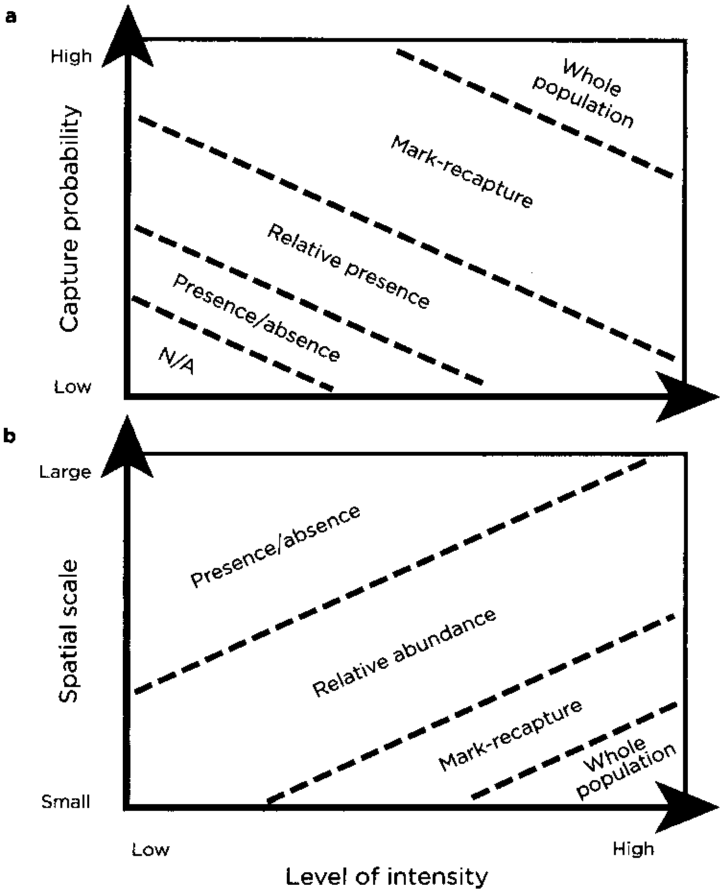

Fig. 1.5. Population-monitoring techniques most appropriate for various combinations of study intensity (time, resources, etc.). (a) Capture probability of the target species (b) Spatial scale of inference. The dashed lines indicate that boundaries between the methods are not rigid and that situations exist in which multiple methods may be applicable. N/A indicates that combinations of detectability and effort that will not yield meaningful results.

Design of Snake Population Studies

Defining a Question

The first step in designing a snake population study is to clearly define the question(s) of interest. All too often snake studies are initiated with little foresight, and ultimately inconsistencies in the sampling methodology preclude useful results. Defining explicit questions will determine the spatial and temporal scale, level of intensity, capture method, and analytical techniques necessary to complete the study given the time and resources available (Fig. 1.5).

Next we introduce several major categories of snake population studies in order of increasing intensity.

1. *Presence/absence, occupancy, or inventory.* Determining occupancy, whether or not a species occurs at a given site or set of sites, is the simplest form of population assessment. Determining occupancy may be an initial step for investigating a snake population or it may be the ultimate goal if the study area is large or the species is particularly intractable (Fig. 1.5). In its simplest form, inventory involves using unstandardized effort and a variety of (often haphazard) techniques with the goal of having the highest probability of documenting the species of interest. Alternatively, and especially for rare or elusive species, standardizing effort at some cost to capture rate is advisable because, with standardized effort, species detection probabilities can be calculated and a likelihood of species absence can be estimated for sites where the species was not found (discussed later in the chapter).

2. *Snapshot population assessment.* Snapshot population assessments seek to understand population characteristics at a single time and are, by definition, short in duration. In fact, lengthening duration can obscure results because of population change over the course of the study. The intensity necessary to complete a snapshot assessment varies depending on the specific question(s) of interest. For example, if the goal is to assess population demography (e.g., size, age, or sex structure) at one time, only enough effort is needed to obtain an adequate sample size using relatively unbiased methods (or methods for which the biases are understood). Alternatively, estimating population size (or density) at a given time requires mark-recapture methods, probably with high-intensity sampling to obtain adequate recapture rates. A snapshot has the potential, however, to miss information if temporary emigration exists. Furthermore, yearly variation in snake populations can be considerable (e.g., Seigel and Fitch 1985; Seigel et al. 1995; Willson et al. 2005), and a short duration study might not be representative of the population in most years.

3. *Monitoring population trends over time.* Studies that monitor trends in populations over time may be conducted with or without in-depth snapshot studies, and the dichotomy between the two is critical in terms of study design. In short, when monitoring a snake population over time, the researcher must ask, is it necessary to determine the population size (or density)? Alternatively, is it sufficient to determine only if the population is growing, declining, or stable? If the goal is simply to assess stability, and especially if the area of interest is large, then an unbiased index of relative abundance may be sufficient. But if the study area is small, funding is sufficient, and the species has a relatively high capture probability, mark-recapture methods may be used to estimate the population size and quantify vital rates. In these cases, however, open or robust design models must be used because the assumption of population closure is violated (discussed later in the chapter).

4. *Monitoring to assess mechanisms for population change.* Both the commitments of time and of resources necessary to complete a study increase when the goals involve assessing mechanisms for changes in population size or structure. In addition to intensive mark-recapture, such studies probably also involve monitoring immigration and emigration rates or focal animal studies that assess movements, reproduction, and sources of mortality. Especially important when designing a study to assess mechanisms for population change is a careful consideration of biotic and abiotic factors (e.g., weather, habitat, prey availability, and predator abundance) that should be monitored in conjunction with animal monitoring.

Level of Intensity and Spatial Scale

Before beginning any snake population study, careful consideration must be given to the scope or scale of the question(s) in light of available resources and tractability of the species (Fig. 1.5). For studies addressing questions on a small spatial scale, mark-recapture is a viable option, provided the species has a relatively high capture probability (Fig. 1.5). For species with low capture probability, however, mark-recapture may not be possible, even on relatively small spatial scales (discussed later in this chapter).

When questions concern large spatial scales, and especially for intractable species, animals may be monitored across the entire area using low-intensity approaches such as occupancy monitoring or indices of relative abundance. Alternatively, one or, ideally, several subpopulations can be studied intensively. Data on subpopulations can be used in conjunction with more limited data on larger populations to address questions on larger scales (see section on Relative Abundance Indices).

Defining the Population of Interest

Explicitly defining a target population for study is a critical step in the design of any population study. We define a population from the biological perspective as a group of individuals in which movement within the group is greater than movement into or out of the group. In some cases, a clear delineation of biological populations is possible and those populations are of a size that can be studied manageably as a unit. Examples of such defined populations include those existing on islands (e.g., King and Lawson 2001; Sun et al. 2001; Bonnet et al. 2002b; Pearson et al. 2002), species with extremely small geographic ranges (e.g., Webb and Shine 1997b; Holycross and Goldberg 2001; Prival et al. 2002), and those centered around naturally patchy habitats such as isolated wetlands (e.g., Winne et al. 2005; Willson et al. 2006; Winne 2006b) or suitable hibernacula (e.g., Diller and Wallace 2002; Weatherhead et al. 2002). In many cases, however, it is impossible to study an entire biological population because the population is too geographically widespread to examine with the desired level of intensity. In such cases, it is necessary to define a study population, which is defined by arbitrary boundaries and thus does not represent a true biological population. Population size, in this case, applies only to the arbitrary study area and represents density per unit area.

Defining the size of the study population must include a consideration of the scale at which results will be interpreted (e.g., unit of land, population, region, or range of species) in light of the resources available and the tractability of the species. When limited resources necessitate the definition of an arbitrary study population, it is important that the study population be as representative of the biological population of interest as possible. In practice, this may mean that the study population should not be situated in the area where snakes are most abundant but, rather, in habitat that is typical of the entire area of interest. In a study of either an entire biological population or an arbitrary study population, knowledge of immigration and emigration rates can aid in the interpretation of results. These rates can be quantified using capture techniques that intercept animals entering or leaving the study area (e.g., drift fences; Dodd 1993a; Willson et al. 2006; Winne et al. 2006b), by following individuals using radiotelemetry, or using genetic techniques (see King, Chapter 3). Regardless of the question of interest, conducting pilot studies to assess individual capture probability will help determine the size of the study population that can reasonably be studied given the available resources.

Exceptional situations exist in which a combination of sampling efficiency and tractability of the snake species allows the researcher to capture nearly all the individuals in a population. Although the communal denning of high-latitude snake populations may be the most familiar example of such a situation, similar opportunities also exist in other habitats. For example, dredging was used to thoroughly sample uniform mats of Water Hyacinth (*Eichhornia crassipes*) at Rainy Slough, Florida (Godley 1980). By assuming that all snakes within the hyacinth mats were captured, densities of Striped Crayfish Snakes (*Regina alleni*) and Black Swampsnakes (*Seminatrix pygaea*) could be directly calculated.

Another promising direction in studies of snake population ecology is the use of closed or experimental snake populations. Other fields have benefited from the use of mesocosms, penned populations, or the experimental manipulation of field populations. For example, numerous studies have used field enclosures (e.g., Wilbur and Collins 1973; Wilbur 1976; Todd and Rothermel 2006) or laboratory mesocosms (e.g., Morin 1981; Semlitsch and Gibbons 1985; Semlitsch 1987a, 1987b) to investigate mechanisms driving population dynamics in amphibians. Few similar studies have been conducted with snakes, however, perhaps as a result of the remarkable escape abilities of many snake species. Studies on *B. irregularis* have shown that even large arboreal species can be confined by relatively simple barriers (Rodda et al. 2007a). Such barriers were used to successfully enclose a 5-ha *B. irregularis* population with no evidence of trespass after 4 years (Rodda et al.

2007b). Many small, sedentary, aquatic, or litter-dwelling snakes are ideal for similar closed population studies. Such studies not only facilitate population monitoring by eliminating immigration and emigration but also allow experimental manipulations (e.g., food availability) that can be replicated at the population level.

Designing a Sampling Scheme

The temporal pattern of data collection largely reflects the analytical technique that will be used. The first concern in this regard is the duration of the study. For a snapshot assessment, a short duration study with high intensity is preferable. The timing of sampling within a study of longer duration also reflects the analytical technique used. For most long-term studies, short-duration, high-intensity sampling is preferred to continuous sampling, and such "pulsed" designs are necessary to meet the assumptions of most mark-recapture analyses. Because the main concern in relative abundance assessment is bias, successive sampling occasions should be timed to minimize differences in behavior and are best conducted in similar seasons and/or under similar environmental conditions.

Likewise, the spatial pattern of sampling will reflect the goals of the study. In mark-recapture analyses, it is preferable to sample as large a subset of population as possible during each interval to reduce heterogeneity. For relative abundance assessments, the entire population need not be sampled, but a sampling scheme that maximizes comparability is preferred. Studies wishing simply to confirm species presence often benefit from focusing on optimal habitat; but a standardized effort is needed to infer species absence with statistical confidence.

Choosing a Capture Method

The first consideration when selecting a capture method is how effective that method is for sampling the target species. It is important to remember, however, that obtaining a high total number of captures is not always the most important goal. In many cases (e.g., relative abundance assessments) the primary concern is repeatability of the samples, even at the expense of total captures. In such cases, passive capture methods are preferable because they minimize bias (Table 1.1).

Understanding potential capture bias is critical because many capture methods underrepresent certain segments of the population. For example, although arboreal funnel traps are highly effective for capturing large *B. irregularis*, small individuals can be detected only in visual surveys (Rodda et al. 2007b). Likewise, aquatic funnel traps differ in their usefulness for sampling aquatic snakes (Willson et al., 2008). Understanding such capture biases is an underappreciated component of studying snake populations. In many cases, comparing samples collected using multiple methods (e.g., Prior et al. 2001; Rodda et al. 2007b; Willson et al., 2008) or using laboratory or enclosed populations to test assumptions of equal catchability can yield invaluable insights for the design and interpretation of snake population studies.

Dealing with Low Capture Rates

Mark-recapture, the most in-depth method for studying populations, requires relatively high individual capture probability (to produce adequate recaptures) to provide useful estimates of population parameters. Although the secretive nature of many snakes leads to low capture probability, this problem can be ameliorated in several ways:

1. *Increase effort or decrease spatial scale.* It is often tempting to try to maximize the number of individual snakes captured by employing low-intensity techniques across a large study area. Increasing effort and/or decreasing the area sampled may reduce the overall capture rate, but it increases individual capture probability, thus improving the precision of parameter estimates (see Fig. 1.5).
2. *Incorporate temporary emigration.* In populations in which individuals exhibit temporary emigration, capture probability may be underestimated because all individuals are not available for capture during all samplings (Kendall et al. 1997; Bailey et al. 2004a). Testing for and/or estimating temporary emigration can improve parameter estimation by ensuring that capture probability estimates include only animals available for capture during each sampling event (Bailey et al. 2004a). Temporary emigration, however, can only be addressed using robust design models (Kendall et al. 1997).
3. *Use a monitoring technique that does not depend on recapturing individuals.* Mark-recapture analyses depend on obtaining adequate numbers of recaptures, but presence/absence designs, indices of relative abundance, and distance sampling do not require recaptures. These methods are sensitive only to species detection probability, which is a rate of capture over an arbitrary sampling unit. By increasing the effort of the sampling unit, sufficiently high detection probabilities can be generated for nearly any species. Note, however, that indices of relative abundance are sensitive to bias and should be interpreted with caution.
4. *Study a surrogate population.* In some cases, individual snakes are sufficiently intractable that mark-recapture is ineffective (Fig. 1.5a). Likewise, the spatial scale of interest might be so small that presence/absence monitoring is inappropriate (Fig. 1.5b). In such cases, the most satisfactory option may be to study a different population of the same species in hopes of gaining insights that could help manage the focal population (see Seigel and Mullin, Chapter 11).

Presence/Absence Monitoring

Presence/absence monitoring uses relatively low-intensity sampling to investigate patterns of distribution, generally on fairly large spatial scales. Recent improvements in analytical techniques have made quantitative presence absence monitoring a useful tool that can be applied on scales too large fo other forms of monitoring (Fig. 1.5b). In addition, presence/absence may b the most appropriate method for monitoring species for which low captur probability makes mark-recapture infeasible (Fig. 1.5a).

Traditional Methods for Presence/Absence Monitoring

Presence/absence data have been the impetus for many snake conservatio efforts. For example, declines in the Southern Hog-nosed Snake (*Heterodon simus*) were detected through a comparison of the historical distribution (largely from museum records) and the distribution in recent report (Tuberville et al. 2000). Traditionally, studies of this type have relied o haphazard observations made over long time scales. Such anecdotal report are difficult to interpret, however, because the lack of standardized effor makes it impossible to calculate detection probability, thus making it impossible to confirm species absence with statistical confidence from nondetection data (Kery 2002; Bailey et al. 2004b).

Advances in Presence/Absence Monitoring

Analytical software. Recently, the applicability of presence/absence monitoring has been enhanced by advances in analytical techniques and software that makes those techniques accessible to the public. The software program PRESENCE (http://www.mbr-pwrc.usgs.gov/software/) uses likelihood-based techniques (developed by MacKenzie et al. 2002) to estimate site occupancy and species detection probability. PRESENCE requires presence/absence data from repeated sampling occasions (consisting of any standardized unit of effort) and allows for the inclusion of site (e.g., habitat characteristics) and sampling (e.g., climatic conditions, season) covariates that can improve precision of parameter estimates. PRESENCE has recently been used to model factors influencing the distribution and species detection probability of amphibians (Bailey et al. 2004b; Gooch et al. 2006) and at least one group of snakes (Luiselli 2006).

Inferring species absence. A related, but alternative question involves determining the confidence of species absence at sites where the species has not been detected. One study used repeated visits to 87 sites to calculate the species detection probability for three common European snakes, the Aspic Viper (*Vipera aspis*), Smooth Snake (*Coronella austriaca*), and Grass Snake (*Natrix natrix;* Kery 2002). By calculating the detection probability at sites where each species was known to occur, the researcher determined the number of unsuccessful visits necessary to declare the absence of each species with statistical confidence.

Relative Abundance Indices

Traditional Methods for Assessment of Relative Abundance

Relative abundance indices are generally rates of capture standardized for effort or time (e.g., captures per trap-night or sightings per kilometer; see sources in Parker and Plummer 1987). Relative abundance data, although often easy to collect, must be interpreted with caution. The key assumption in a comparison of relative abundance is that individual capture probability is relatively constant and thus that overall capture rate reflects population density. In other words, any comparison of relative abundance assumes a consistent (generally assumed to be positive and linear) relationship between density and capture rate. In reality, however, capture rate reflects a combination of factors, including population density, behavior (activity levels, habitat use, etc.), and individual capture probability. Thus, when using indices of relative abundance, care must be taken to ensure that the sampling method used is as repeatable and unbiased as possible, even at the expense of increased overall captures. In addition, because individual capture probability often varies among species and among populations, relative abundance indices generally provide poor indicators of community composition or differences in density between sites, unless they are used in conjunction with detection probability estimates obtained using mark-recapture.

Advances in Relative Abundance Assessment

Methods for assessing relative abundance are straightforward (generally consisting of standardized sampling events repeated across the spatial or temporal scale of interest; Parker and Plummer 1987), and recent progress has been made in the assessment of bias and testing of the critical assumption that capture rate correlates directly with population density. The efficacy of visual surveys as abundance indicators has been tested for introduced *Boiga irregularis* on Guam (Rodda et al. 2005). When the sighting rate was correlated with population density (estimated using mark-recapture), no correlation existed between snake relative abundance (sighting rate) and population density (Fig. 1.6a), demonstrating that not all capture methods yield useful indices of relative abundance. Conversely, capture rate (hand captures) correlated strongly with population density across 11 insular populations of the Lake Erie Watersnake, *Nerodia sipedon insularum* (Fig. 1.6b; King et al. 2006a). This correlation allowed for the estimation of population density at 19 additional sites where there were insufficient data to provide mark-recapture population estimates.

Distance Sampling

Distance sampling uses data on species detection probability to estimate density from VES without relying on mark-recapture (Buckland et al. 2001,

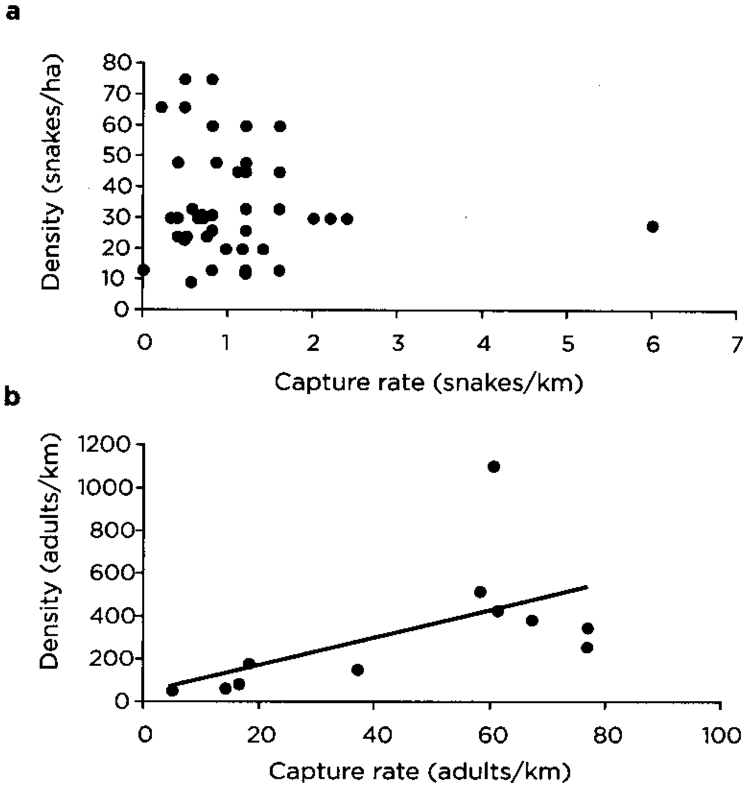

Fig. 1.6. Tests of the efficacy of relative abundance indices (sighting rate) for predicting population density (estimated via mark-recapture). (a) Brown Treesnakes (*Bioga irregularis*) on Guam (b) Lake Erie Watersnakes (*Nerodia sipedon insularum*) on islands in Lake Erie, United States. For *B. irregularis,* there is no relationship between sighting rate and population density ($R^2 = .0005$; not significant), whereas for *N. sipedon* there is a significant relationship between ln(Sighting rate) and ln(Population density) ($R^2 = .851$; $p = .001$). Removing outliers from either analysis does not change the results. (Data for [a] adapted from Rodda et al. 2005, with permission; data for [b] adapted from King et al. 2006a, with permission)

2004). Essentially, distance sampling works by measuring the distance at which animals are observed from a transect line or observation point. The distribution of captures around the line is then used to calculate a detection function assuming total (100%) detection along the transect and declining detection probability at increasing distances from the transect. One limitation of distance sampling is the need for a relatively large number of observations (a minimum of 60–80 observations from 10–20 transects; Buckland et al. 2001). to generate meaningful density estimates. In addition, many snake species may violate the assumption of 100% detection along the transect, although distance sampling models that relax this assumption have been proposed (Buckland et al. 2004). Distance sampling could be used in some snakes that are easily observed using VES and has been used to investigate habitat associations of sympatric vipers (*Bitis*) in West Africa (Luiselli 2006). However, a study designed to validate the efficacy of distance sampling for reptiles found that distance sampling underestimated densities of *B. irregularis* by 700% (Rodda and Campbell 2002).

Mark-Recapture Studies

Since Henry Fitch's pioneering snake population studies in Kansas (reviewed in Fitch 1999), mark-recapture has been the favored approach for high-intensity monitoring of snake populations. Executing a snake mark-recapture study, however, demands more than simply capturing and marking as many snakes as possible. Both the analytical technique used and the required sampling design vary depending on the question(s) of interest, and a consideration of study design is necessary to ensure these question(s) can be evaluated. Moreover, the combination of sampling intensity and spatial scale must produce recapture rates sufficient to make population estimation possible. To some extent, as a result of low recapture rates, many previous studies used population demography (e.g., size distribution, proportion reproductive, and body condition) or relative abundance indices rather than direct population estimates when assessing population status over time (e.g., Shine and Madsen 1997; Madsen and Shine 2000a Lacki et al. 2005; Madsen et al. 2006; Willson et al. 2006; Winne et al. 2007). Given careful design consideration, however, meaningful mark-recapture studies are possible for many snake species.

Traditional Methods for Mark-Recapture

Closed population models. Closed population models are the simplest form of mark-recapture analysis and most are derivations of the Lincoln-Peterson estimator (Lincoln 1930; discussed in detail in Pollock et al. 1990). Closed models use two or more samples collected over a short period to estimate population size (Fig. 1.7a) and assume population closure. For this reason, they do not provide estimates of vital rates (e.g., survivorship and population growth rate). Moreover, because closed population studies are necessarily of short duration, they may underestimate the population size if a portion of the population is unavailable for capture during the study.

A major advantage of closed population models is that they do not necessarily assume equal capture probability (Pollock et al. 1990). Closed models are available that account for time-varying capture probability, trap responses (i.e., "trap-happy" or "trap-shy" responses), and heterogeneity in capture probability. Perhaps because of their simplicity, closed models have been used to estimate population size in several snake species including Rough Greensnakes (*Opheodrys aestivus;* Plummer 1997), Tiger Snakes

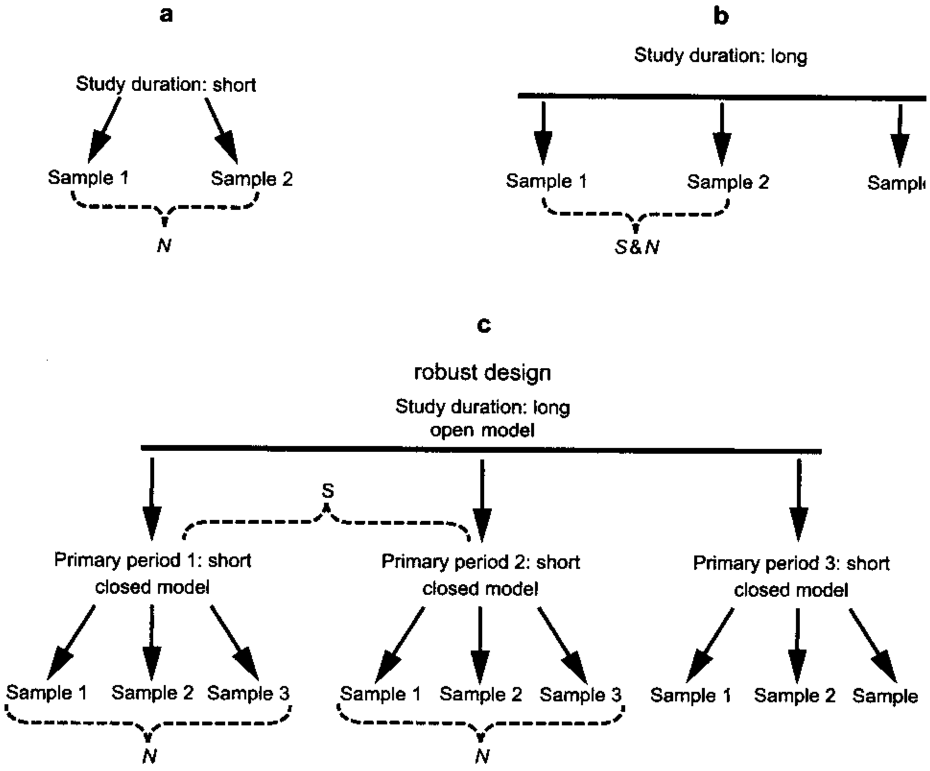

Fig. 1.7. Schematic representation of sampling schemes used in mark-recapture studies. (a) Closed population models (b) Open population models (c) Robust design (mixed population) models. For each design, the intervals over which population size (*N*), survivorship (*S*), or both are estimated are indicated.

(*Notechis scutatus;* Bonnet et al. 2002b) and *V. aspis* (Lourdais et al. 2002), among others (reviewed in Parker and Plummer 1987).

Open population models. Open population models allow the estimation of vital rates (e.g., survivorship and population growth) when populations are open to births, deaths, immigration, and emigration. The most popular open models are based on the Jolly-Seber group of estimators (Seber 1982; Pollock et al. 1990; Lebreton et al. 1992) and require a sampling design comprising at least three samples separated by relatively long intervals, across which population parameters are estimated (see Fig. 1.7b). The major drawback of open models is that they assume constant capture probability and thus cannot account for capture probabilities that vary due to temporal effects, behavioral effects, or heterogeneity in capture probability. For this reason, they generally do not provide particularly robust estimates of population size (Pollock et al. 1990). Despite this, open and closed models produced similar population size estimates for N. *sipedon insularum,* although standard errors were large due to low recapture rates (King et al. 2006a. Open models have been used successfully to estimate survivorship and population size over long time scales in Water Pythons (*Liasis fuscus;* Madsen et al. 2006), and ratsnakes (*Pantherophis;* Weatherhead et al. 2002).

Advances in Mark-Recapture Studies

Analytical software. Recently, mark-recapture analyses have become more accessible and user-friendly through advances in publicly available software packages such as the programs CAPTURE (http://www.mbr-pwrc.usgs.gov/software/; Otis et al. 1978) and MARK (http://www.warnercnr.colostate.edu/~gwhite/mark/mark.htm; White and Burnham 1999). These programs allow the analysis of large mark-recapture data sets using a variety of models (including open, closed, and robust designs) and use maximum likelihood selection procedures to compare multiple competing models using Akiake's information criterion (AIC) or similar methods. Moreover, MARK allows for partitioning of data sets into demographic groups (e.g., sexes and cohorts) and inclusion of both individual (e.g., body length, mass, and age) and sampling (e.g., environmental conditions and effort) covariates, all of which can help partition variance in data sets, thus maximizing the precision of parameter estimates.

Robust design models. Robust designs (Pollock 1982) require a sampling scheme in which the population is sampled several times over short secondary sampling intervals (often successive days) separated by longer primary sampling intervals (Fig. 1.7c). Population size and capture probability are estimated within secondary intervals using closed population models and survivorship is estimated over the longer, open, primary intervals. Thus, the design is robust in the sense that it permits the estimation of both survivorship and population size without violating the assumptions of either open or closed models (Pollock 1982). When using robust design models, all individuals must be available for capture at each sampling event, so if secondary sampling intervals are successive days, animals must be marked and released on the day of capture.

One advantage of robust design models is that they allow the investigator to test for the presence of temporary emigration. Standard open and closed mark-recapture analyses assume that all animals are available for capture during all sampling events and thus may provide imprecise parameter estimates if temporary emigration exists but is not accounted for (Kendall et al. 1997; Bailey et al. 2004a). Although temporary emigration probably exists in many snake populations, to our knowledge no studies have tested for temporary emigration in snake populations.

Because robust designs use closed analyses to estimate population size, they can account for time-varying capture probability, trap responses, and heterogeneity. There is strong evidence that these factors are important in some snake species, necessitating the use of robust design models for long-term monitoring of population size and vital rates. For example, models including heterogeneity were favored within closed population estimates of population size for *S. pygaea* in an isolated wetland in South Carolina (Fig. 1.8a). This heterogeneity was probably due in part to differences in capture probability among seasons and between sexes (Fig. 1.8b). Unfortunately, heterogeneity is difficult to account for analytically. Indeed, although robust design models that use Pledger's finite mixtures (Norris and Pollock 1996; Pledger 2000) to model heterogeneity are available in the software program MARK, their properties and precision have not been examined in the literature. However, heterogeneity can often be mitigated in several other ways, including:

1. *Design of sampling methodology.* Heterogeneity can result directly from sampling methodology. For example, uneven sampling within the study area or variation in the effectiveness of capture method across habitats can lead to some individuals being captured more often than others. Thus, homogeneous sampling across the entire study area and the use of multiple capture methods can minimize heterogeneity.
2. *Inclusion of individual and sampling covariates.* Heterogeneity can result if demographic subsets of the population have different capture probabilities. For example, nonreproductive female *V. aspis* have lower capture probabilities than reproductive females (Bonnet and Naulleau 1996). In such cases, heterogeneity can be reduced by dividing the population into subgroups (e.g., sexes or cohorts) that are analyzed separately and thus may differ in capture probability. Analyzing the capture probabilities of *S. pygaea* separately by sex reduced the support for models favoring heterogeneity in favor of null models (constant capture probability; Fig. 1.8). Likewise, the inclusion of both individual and sampling covariates can reduce heterogeneity if there are relationships between those covariates and capture probability (Pollock 2002).
3. *Investigation of temporary emigration.* The presence of temporary emigration can lead to apparent heterogeneity in capture probability. Robust design analyses can address temporary emigration, ameliorating the effects of heterogeneity on population estimation.

Although robust design models are not particularly new, they are poorly represented in current herpetological literature. However, several recent studies have used robust design sampling to examine aspects of the biology of woodland salamanders in the Appalachian Mountains of the eastern United States (Bailey et al. 2004a, 2004c, 2004d). To our knowledge, no published studies have yet used robust design analyses to investigate snake population dynamics.

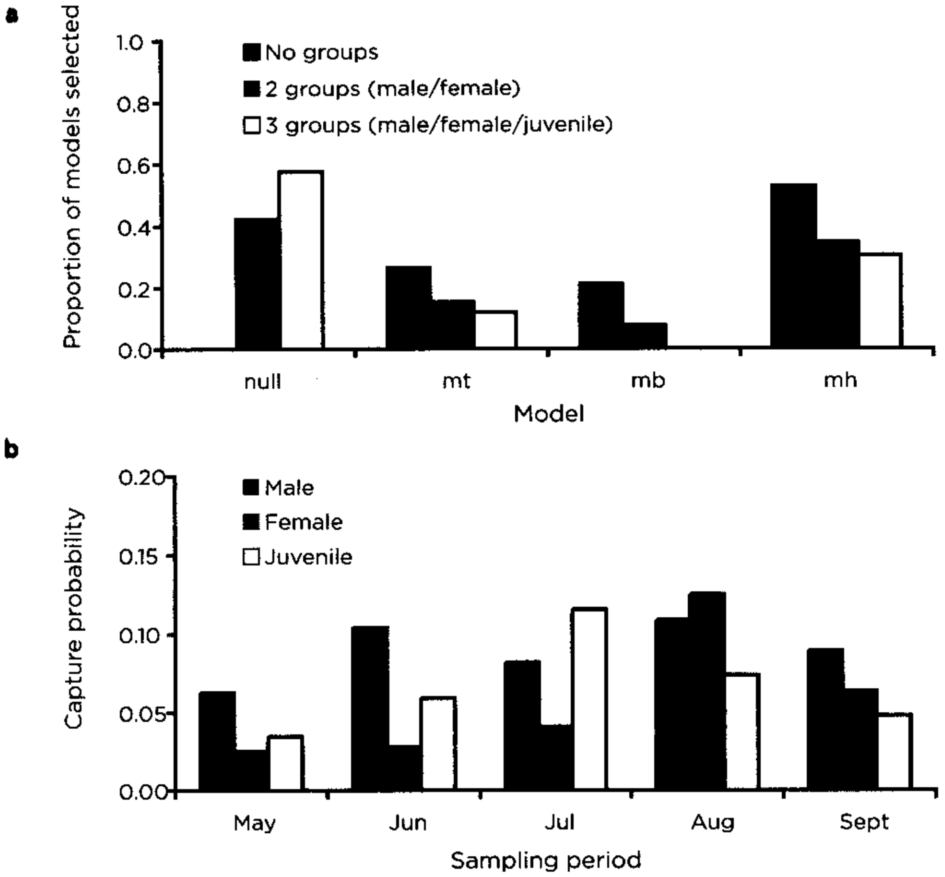

Fig. 1.8. Capture probability (P_c) of Black Swampsnakes (*Seminatrix pygaea*) at Ellenton Bay, Aiken Co., South Carolina, in 2005. (a) Factors influencing P_c in *S. pygaea* as indicated by the proportion of total selected models that included a constant P_c (null model), time-varying P_c (mt), behavioral effects (mb), and heterogeneity in P_c (mh), with data divided in to one, two, or three groups by sex and life stage (b) Variation in P_c across seasons and sexes. Figures based on a total of 1192 captures of 462 individual snakes during monthly 10-day sampling periods between May and September 2005. Model selection was performed within 10-day periods using closed population models in program CAPTURE.

Population Viability Analyses and Population Modeling

With data on population size and vital rates comes the ability to model population trends over time. Indeed, population viability analyses (PVAs), which use various population models to project population trajectories, have been used in the conservation and management of variety of animal taxa (reviewed in Reed et al. 2002). Moreover, many PVA software packages are now available, allowing researchers to conduct a variety of analyses including the calculation of extinction risk, minimum viable population size, and sensitivity analysis (weighing of factors that drive population change). Unfortunately, PVAs and other forms of population modeling have seldom been applied to snakes (but see Ferriere et al. 1996; Altwegg et al. 2005; Row et al. 2007; Shine and Bonnet, Chapter 6), perhaps due to the lack of vital rate estimates for many species.

Another approach to population modeling is to project individual data to populations using individual-based population models (IBMs; DeAngelis et al. 1991). For example, physiological data from laboratory experiments was used to generate a mechanistic IBM of time-energy allocation for the lizard *Sceloporus merriami* (Dunham 1993). This model was combined with environmental data to predict that a rise in mean temperature as small as 2–5 °C would constrain lizard activity enough to drive populations to extinction. IBMs may be particularly amenable to snakes because of the wealth of laboratory and field studies that have investigated snake physiological ecology. For example, detailed physiological data were used to model individual time-energy allocation decisions in *C. horridus* (Beaupre 2002). Individual allocation decisions predicted by the model were extrapolated to the population level, predicting the relative influences of changes in temperature and prey availability on population persistence.

Future Research

In this chapter, we have provided information designed to improve the effectiveness of snake studies. Our hope is that future snake researchers will use this information to address the myriad of ecological questions about snakes that remain. A more complete understanding of snake ecology at the various scales (individual, population, and landscape) will then aid in the development of more effective conservation programs. We hope that the recent methodological advances we describe will both prompt meaningful question-oriented field studies of snakes in the future and also encourage theoretical investigations that seek to understand the unifying factors common to many snake species.

Acknowledgments

For advice and assistance, we thank L. Bailey, S. Beaupre, D. Cundall, V. Cobb, S. Gary, J. Gibbons, M. Pilgrim, S. Price, R. Reed, G. Rodda, B. Todd, and C. Winne. We thank S. Bennett, M. Cherkiss, W. Hopkins, W. Kalinowski, F. Mazzotti, R. Snow, and C. Winne for allowing our use of unpublished data collected collaboratively with M. E. D. or J. D. W. We thank R. King and G. Rodda for supplying raw data for the figures. Support was provided by Duke Power; the Department of Biology at Davidson College; the Savannah River Ecology Laboratory; National Science Foundation grants REU DBI-0139153 and DEB-0347326 to M. E. D.; a National Science Foundation Graduate Research Fellowship to J. D. W.; and the Environmental Remediation Sciences Division of the Office of Biological and Environmental Research, U.S. Department of Energy, through Financial Assistance Award number DE-FC09-96SR18546 to the University of Georgia Research Foundation.