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Effects of handling, marking, and recapturing pine snakes (*Pituophis m. melanoleucus*) from the New Jersey Pine Barrens

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Many bioindicators are developed without attention to the effects on the animals themselves. We examined whether the disruption, handling, and recapturing of northern pine snakes (Pituophis m. melanoleucus), New Jersey Pine Barrens, 1976 to 2008) negatively impacted their behavior and survival, information that is critical to the usefulness of snakes as bioindicators. We found: 1) There was no difference in the rate of re-sighting of pine snakes as a function of whether they were branded or fitted with passive integrated transponder devices (PIT tags) and/or radio-transmitters, 2) Marked females continued to use the same nest site in successive years (65 %), 3) The number of nests that hatched successfully did not differ markedly as a function of remaining intact (72 %) or being opened (62 %), 4) Hatching rate of eggs in the laboratory was higher than for those in the field, 5) Excavated hibernacula that we studied were only abandoned due to predators, land development, and habitat management, 6) Nearly half of the snakes handled while hibernating were found once or more in successive years in the reconstructed hibernacula, and 7) The three snakes that were captured the most in hibernacula were found in 13, 17, and 18 different years. Thus, northern pine snakes can be useful bioindicators because the researchers themselves are not impacting their behavior or survival, and they can be used to indicate overall health of the food chains of which they are part

Keywords: snakes, hibernation behavior, hibernacula, hibernation sites as indicators, longevity, PIT tags, Pine Snakes

Introduction

One of the key features of bioindicators is understanding whether researchers themselves (or others) affect the behavior of species such that ecosystem structure and functions are disrupted. If researchers affect behavior and population dynamics of a species, then it is unsuitable as a bioindicator. This is particularly true with species of special concern that live in suburban or developed areas with high human densities. While much attention has been devoted to examining the effects of people on wildlife in reserves or wilderness areas (Knight and Gutzwiller 1995), there is relatively little research in areas that are heavily impacted by people (Reese 1997), nor on those species used as bioindicators. Studies in human-dominated ecosystems mainly deal with fragmentation (Dowd 1992; Jokimaki and Suhonen 1993), pedestrian and car effects on foraging and breeding (Keller 1991; Carney and Sydeman 1999; Fernandez-Juricic 2000), human effects

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on turtles (Garber and Burger 1995; Gibbs and Shriver 2002), and on human disturbance to snakes in rock piles (Goldingay and Newell 2000). Except for the study of researcher effects on birds (Erwin 1989; Burger 1991c; Burger and Gochfeld 1994; Carney and Sydeman 1999; Rodgers and Smith 2005), there are few studies on the effects of specific research practices on other groups of animals. Moreover, most of the studies on the effects of human disturbance deal with short-term effects, and not with observable effects on longer-term behavior, reproductive success, or fitness (but see Weatherhead and Blouin-Demers 2004; Burger and others 2007), all of which affect a species' usefulness as a bioindicator.

Several authors have examined the effects of researchers on stress in animals (Elsey and others 1990; Kreger and Mench 1993; Langkilde and Shine 2006), but seldom are researchers able to examine longer-term effects of their activities. Understanding the effects of researcher activities is also important for reasons of conservation, for obtaining accurate information on the biology and ecology of vertebrates, and to comply with federal, scientific, societal, and university guidelines on the treatment of animals (Animal Behavior Society 2003). Without understanding the long-term needs of vertebrates (such as hibernaculum sites), it is impossible to conserve the habitats they require. Similarly, without understanding how habitat requirements affect bioindicators, they cannot be used effectively.

In many studies on reptiles in human-dominated ecosystems, adverse effects have been attributed to general human disturbance (Parent and Weatherhead 2000; Burger 2001; Moore and Siegel 2006), and not to a specific research activity that can be modified to reduce effects while still allowing their use as bioindicators or of scientific inquiry. Partly this results from the range of techniques employed by researchers, and the difficulty of isolating one effect from another, particularly for animals that are not readily observed. The use of passive integrated transponder devices (PIT tags) and radio-telemetry may be the exception, particularly for amphibians (Perret and Joly 2002; McAllister and others 2004) and snakes (Reinert 1992; Weatherhead and Blouin-Demers 2004; Anderson and Talcott 2006). Weatherhead and Blouin-Demers (2004) reported significant long-term effects for snakes, but concluded that radio-telemetry was still warranted because of the benefits realized by researchers in studies of such secretive animals as snakes.

Further, bioindicators are most useful when the indicator species is at the top of its food chain (and thus indicative of community health and structure), when the indicator species can be followed for many years, and when the activities of the researchers themselves do not influence behavior, accessibility, and the parameter being examined (see Vol. 1 of *Environmental Bioindicators*). In bioindicator development, however, it is often an assumption that researchers (and others) do not impact the biology and behavior of the indicator species. This aspect is seldom studied explicitly over a long period of time (the time needed for a useful bioindicator of temporal trends).

In this investigation, we use data from studies conducted over 33 years of northern pine snakes (*Pituophis m. melanoleucus*) in the New Jersey Pine Barrens to examine the effects of our handling and marking methods in the laboratory and the field. We were particularly interested in 1) whether branding, placing PIT tags in snakes, or radio-tagging them changed re-sighting and recapture rates, 2) whether handling females that were engaged in nesting interrupted their behavior, 3) whether handling females during nesting resulted in abandoning these sites in future years, 4) whether digging up hibernacula resulted in abandonment of those hibernacula, 5) whether individual snakes continued to use hibernacula that were dug up, and 6) whether incubating eggs in the laboratory increased or decreased reproductive success. These aspects were examined to assess whether our handling affected the behavior and population dynamics of the species in a way that would render them less useful as bioindicators of trophic level stability, population dynamics or species viability.

Materials and Methods

Study Areas

All studies were conducted under appropriate permits issued by the New Jersey Department of Environmental Protection and were approved by the Rutgers University Institutional Animal Care and Use Committee. We studied the behavior of pine snakes in the New Jersey Pine Barrens in Burlington, Cumberland and Ocean counties. The nesting areas, hibernacula, and radio-tracked pine snakes we studied were located in Bass River State Forest, on Nature Conservancy property, on private land, and on other state lands (Wildlife Management Areas = WMA). The types and degree of habitat management on these areas differ. Nesting areas and hibernacula are generally located in relatively exposed sandy areas dominated by pitch pine (*Pinus rigida*) and various oak tree species (*Quercus* spp.) (Zappalorti and Burger 1985; Burger and Zappalorti 1986, 1988a, 1989). Exact locations are not given because of the threat of poaching; pine snakes nest near hibernacula, and in some years, nearly 40 % of nests were poached (Burger *et al.* 1992).

General Protocol for Handling Snakes and Gravid Females

Snakes encountered within our study areas were captured, searched for markings (either brands or PIT tags), marked, and released where found. Depending upon the year and the study, snakes were marked (after 1988 PIT tags were used) or fitted with radio-transmitters. Initially radio-transmitters were placed in snakes by a veterinarian, but after 1983, staff were taught the appropriate surgical procedures (Reinert and Cundall 1982; Reinert 1992; Reinert and Zappalorti 1988a, 1988b), and thereafter surgical implantation of radio-transmitters was done at our laboratory. There were no survival differences in snakes implanted by veterinarians compared to those implanted by scientists.

Known pine snake nesting areas were searched from mid-May through July between 1976 and 1993 for gravid females and nesting burrows. Each known nesting area had from 3 to 18 marked females living in the vicinity, as determined by long-term observations and radio-telemetry (Zappalorti and others 1983; Burger and Gochfeld 1985; Burger and Zappalorti 1992). Generally gravid females nest in openings or clearings within the pitch pine and blackjack oak (*Quercus marylandica*) forest uplands. Nesting habitats included railroad bed embankments, sandy shoulders of paved or dirt roads, old fields, and natural forest clearings. Nest burrows can be recognized by the snake's trail in the sand, a distinct sand fan, small hole, and diagnostic dump piles opposite the burrow opening.

Our protocol was to locate, capture, and mark any gravid pine snakes at a nesting area, or to capture digging females when they left their nests in the heat of the day to rest under logs or in shade. In some cases, when nests appeared completed, we carefully dug up the nests by hand, and found females within them (these were also marked and released). Excavated nests were either rebuilt with clear plastic or glass as a roof, and the egg mass was left intact, or the eggs were removed to our laboratory. Eggs were removed partly as a conservation measure (the species is threatened in New Jersey, and nests are frequently poached or predated), and to complete experiments on the effects of incubation temperature on development and behavior (see Burger and Zappalorti 1988b; Burger 1991a, b).

We had several conditions that we compared: 1) completely undisturbed nests, 2) nests that were dug up (eggs measured, nest measurements taken), and rebuilt (with a glass ceiling over the egg chamber), 3) nests where the eggs were removed to the laboratory and allowed to hatch as a complete clutch, and 4) nests where the eggs were removed to the laboratory and divided into three sets for incubation at three different temperatures. Eggs in the laboratory were incubated at low (21-23^{o}C), medium (25-28^{o}C), and high temperatures (30-33^{o}C). These are similar temperatures to those that occur in nature in the New Jersey Pine Barrens, although few nests are at the low temperature throughout incubation (see Burger and others 1987; Burger and Zappalorti 1988b; Burger 1989, 1991a, 1998). In all cases, hatchlings were returned to their natal nest, along with their shed skins and hatched eggshells to duplicate natural conditions at a nest site.

Permanent Marking Techniques for Snakes

Initially, snakes were marked by branding dorsal scales in a specific pattern with a fine-tip soldering iron (Clark 1971). When they became available, we used PIT tags to mark all pine snakes (Elbin and Burger 1994; Gibbons and Andrews 2004). Each PIT tag has a unique 9 or 10 digit number, allowing quick, easy, accurate identification of any marked animal. The PIT tags are small, measuring 14 mm x 2.1 mm and weighing 0.8 grams or less, which allows their use in large neonate snakes (such as pine snakes and timber rattlesnakes). The PIT tags are hermetically sealed in biocompatible glass and have an anti-migratory coating of parylene, enabling them to be injected subcutaneously or intramuscularly using 12 gauge needle. The power requirements of the tags are passive, meaning that they are activated by the electromagnetic field of the portable wand reader. The life expectancy of the PIT tag is estimated to be over 100,000 reads.

Radio-Telemetry

Advanced Telemetry Systems, Inc. R1535 or R1520 transmitter units were used. Transmitters were designed so that their mass represented less than 5% of the snake’s mass. The typical range of the transmitters is 400 to 1000 meters. Paraffin and beeswax potted transmitter units were surgically implanted in the coelomic cavity following the general procedure of Reinert and Cundall (1982), with improvements and modifications (Reinert 1992). Pine snakes with transmitter implants were located in the field once every 48 hour period using a Wildlife Materials International (Model TRX-1000S) receiver, unless weather conditions forced changes to the tracking interval. Equipment problems (either transmitter or receiver) also affected the tracking interval, but attempts to locate each snake were made every 48 hours.

Protocol for Hibernacula Studies

In late February to mid-March, depending upon weather and snow cover, we dug up the same hibernacula each year from 1976 to 2008, although systematic studies started in 1981. We removed all snakes for identification, marking, measuring and weighing, then replaced them into their reconstructed hibernacula the same day. At each hibernaculum, we carefully uncovered the opening, and dug with shovels until we reached about 0.8 m depth, or the top of the constructed chamber. Once the roof of the chamber was uncovered, and any snakes were removed, we proceeded with intense care to locate tunnels leading to hibernating snakes. We probed the tunnels with a narrow rod that served as a guide so that we did not lose track of the tunnels. All shoveling was done very slowly, often with hand trowels. In our 25 years of work with the hibernating snakes, no snakes were injured.

In one year we found a shrew actually eating a pine snake, as well as several others that had been partially eaten. Evidence that hibernacula were sometimes abandoned because of the presence of predators included: 1). Excavation patterns of predators (large dump piles at the entrance of large tunnels into the hibernaculum (too big to have been dug by the snakes), 2) Presence of fox or skunk hair in the burrows, 3) Presence of extensive cone shavings of red squirrels).

Once all snakes were removed we rebuilt the hibernaculum by constructing a main underground chamber using cement blocks for walls, plywood or sheet metal for a roof, and hollowed cement blocks laid side to side to create a permanent tubular entrance to the surface. The blocks were offset slightly to ensure that the hole was small enough to prevent red foxes (*Vulpes vulpes*), raccoons (*Procyon lotor*), or striped skunks (*Mephitis mephitis*) from entering. We camouflaged the entrance with leaves, twigs and branches to deter human poachers. After our initial construction, we usually dug up the hibernaculum from the top of the chamber without disrupting the entranceway, so that all snake chemical scent trails leading to it remained intact. Each year we excavated 7 to 17 hibernacula. The number of snakes in each hibernaculum varied from zero to 33 individuals.

Statistical Analysis

Differences among treatment types (marking types, handling methods) were examined using Contingency Tables, and we considered a P value of 0.05 or lower as significant.

Results

We examined the relocation rate of snakes that were branded, PIT-tagged, or radio-tracked during our studies (Table 1), and found that there were no significant differences between snakes that were branded and those that received PIT tags. The objective of radio-tracking was to learn about their seasonal habitat use and habitat selection of free roaming snakes, so these data were not compared to the snakes that were not radio-tagged. We implanted radio-transmitters in 40 snakes without any injuries (Table 1).

Table 1. Physical effect of handling snakes in the field (after Burger and Zappalorti 1988, 1989, and unpublished data).

	Branded	PIT Tagged	Radio Inserted
Year of study	1976-1991	1988-present	1983-2004
Treated	124	340	40
Re-sighted and healthy[a]	62 (50%)	198 (58%)	40 (100%)[c]
Resighted and appeared injured by treatment	1 (1%)	0 (0%)	0 (0%)
X^2(p)[b]	2.50 (NS)		

a. At least 4 months later.
b. Comparing re-sighting between branded and PIT-tagged snakes.
c. Ultimately, 5 died from predation, 3 were killed on the road, and 5 were lost when the transmitter failed.

We examined the behavior of nesting females as a function of treatment (Table 2). There were no differences in the number of females that continued digging nests (that day or on successive days) as a function of being observed only, handled and branded, or handled and fitted with PIT tags. Handling and marking females could have influenced their choice of a nest site the following year. However, we found that a high percentage of all disrupted females continued to use the same nest site the next year and in subsequent years (65%). Most of these nesting females were found at the same sites in consecutive years (N = 30, out of 46). At some nest sites, we found a clutch every year, but not always the female, although when we did find the female it was the same female (N=14). Thus, when we did find a female, she was always in the same nest (Burger and Zappalorti 1992). These data indicate that handling and marking females does not deter them from continuing to dig their nests, or from coming back to the same exact location in the following years.

Our most potentially disruptive research method involved removing pine snake eggs from the wild, incubating them in the laboratory, and subsequently releasing hatchlings back into the wild. These experiments were conducted to describe hatchling behavior, examine the effects of chemoreception (of odors from predators and food), and examine the effects of incubating temperature on hatchling behavior. There was no difference in the hatching rate of nests that

Table 2. Effects of handling nesting females on subsequent behavior for all digging females observed (after Burger and Zappalorti 1991,1992, unpublished data). NS= not significant

	Number	Number continued activity[a]	Percent
Nesting females (digging nests)			
Females not handled	42	39	93%
Female handled (branded)[a]	89	78	88%
Female handled (PIT-tagged)[a]	16	13	81%
X^2(p)	0.10(NS)	0.94(NS)	
Nest Site Philopatry			
Nesting females using the same site in consecutive years		30	
Nesting females using the same site in non-consecutive years (same exact site used up to 11 years[b])		14	
Nesting females using a site only once		2	

a. Activity may have ceased for that day, but female returned later to finish digging and egg-laying.
b. Clutches were laid in the each same place each year, but we did not find the female with the clutch. Whenever we did find the female, it was only the same female in each nest site.

were observed, but not dug up, and those where we opened the nest to count and measure eggs (but no eggs were removed, Table 3). That is, the top panel of Table 3 shows that there was no difference in the percent of nests having some snakes hatch between nests that were observed and not opened (72% hatched at least some snakes) and those that were opened (62% of nests hatched at least some snakes).

Another method of examining success is to examine the percentage of eggs that hatch (Table 3, bottom panel). For eggs incubated in the laboratory, there was a significant difference in hatching rates as a function of incubation temperature; eggs incubated at low incubation temperatures had significantly lower rates (55%) than those hatched at higher temperatures (92% and 95%, after Burger *et al*. 1987, 1992, and unpublished data). Once we clearly established that hatching rate was significantly lower (and there were significant behavioral deficits in the hatchlings), we discontinued incubating eggs at these experimentally lower temperatures.

Table 3. Effects of handling Pine Snake Eggs in the New Jersey Pine Barrens. (after Burger and Gochfeld 1985; Burger and Zappalorti, 1988; Burger, 1989, 1991; Burger *et al*. 1987, 1992, and unpublished data). NS= Not Significant

Condition	Number	Number Hatched (Percent)
Nests in the field (Number of nests)		
Years of study 1976-1993		
Observed but not opened	36	26 (72%)
Observed, opened, no eggs removed	22	141 (62%)
X^2(p)	8	
	0.31(NS)	
Field-hatched clutches (number of eggs)		
Years of study 1976-1990		
Only clutches where some hatched (N= 13)[a]	138	126 (91%)
All clutches (N=42)	380	201 (53%)
Lab-hatched clutches (number of eggs)[b]		
Normal incubation temperatures[c]		
Clutches not separated	120	114 (95%)
Clutches divided	561	516 (92%)
Low incubation temperatures	137	75 (55%)
X^2(p)[d]		29.6 (p<0.0001)
X^2(p)[e]		0.01 (p<0.92 NS)

a. All eggs in a nest (none removed by poachers or predators

b. Does not include any clutches incubated below 24°C.

c. Incubation temperatures below 24°C.

d. Compares field-hatched (only clutches where some hatched) with all lab hatched eggs incubated at normal temperatures.

e. Compare all field hatched nests with eggs from laboratory incubated at normal temperatures.

There are two factors that can affect hatching rates in the field: infertility or predation. There were no differences in the hatching rate for eggs incubated at medium and high temperatures in the laboratory (92 and 95 %), and those incubated in the field where at least some eggs hatched in the nests (91%); the latter field hatching rate is for nests with no eggs lost to predators or poachers. However, most nests (29 of 42 clutches) had some poaching or predation (predators do not always get all eggs). Eggs incubated in the laboratory at normal temperatures (medium to high) had a significantly higher hatching rate than those from all nests in the wild (53%, the differences were largely due to predation and poaching). These data indicate that there was no difference in the hatching rate of wild eggs in a nests with those eggs hatched in the laboratory, if those eggs in the laboratory were incubated at temperatures from 25 to 33^{o}C. Snakes hatched in the laboratory were subsequently returned to their natal nest site.

Table 4. Effects of conducting hibernation studies on Pine Snakes in the New Jersey Pine Barrens (after Burger et al., 1988b; 1992b, 2007; Elbin and Burger, 1994, and unpublished data).

	Number	*Percent*
Hibernacula Studied		
Years studied 1981-2008		
Total number studied	17	
Fate of Hibernaucla		
Number still active[a]	7	53
Number destroyed by development	1	6
Number destroyed by management[b]	1	6
Number destroyed by predators[c]	3	17
Number destroyed by poaching[c]	3	17
Number abandoned for no apparent reason[d]	0	17
Behavior of individual snakes[e]		
Total number of Pine Snakes found	320	
Number found more than once in hibernacula	153	48
Number found three or more times	86	28
Three longest number of different years captured	13,17,18[f]	

a. Contained one or more Pine Snakes during most years of the study, including the last 2 years.
b. Hibernaculum was dished up as part of management for deer production.
c. Destroyed permanently.
d. Not destroyed by development, management or predators.
e. Includes only PIT-tagged snakes tagged prior to 2008.
f. Not always found in consecutive years.

We also examined the behavior of snakes using hibernacula (Table 4). Once we had located a hibernaculum, we continued to check it in successive years, and once abandoned, continued to check it for a number of years to make sure it was not reused. In the early 1990's, we lost a number of our hibernacula to residential development, predators, and management for other wildlife values (deer production). No hibernacula were abandoned due to our digging them up. While individual hibernacula might be used in successive years, snakes have the option of abandoning that site, and going elsewhere. Of the 320 snakes in the hibernacula that were marked with PIT tags, 48 % were found in successive years, and 28 % of all PIT-tagged snakes were found three or more times (Table 4). The three snakes that were captured in the most successive years were captured in 13, 17 and 18 years. The continued use of the hibernacula by snakes, the continued use of the study hibernacula by the same snakes, and the capture of three snakes over 13, 17, and 18 years (not necessarily consecutive) indicates that our digging up of the hibernacula is not dissuading them from using them. In the course of our hibernation studies we handled over 500 pine snakes (including hibernacula not followed for many years), and thus had an opportunity to observe natural injuries or deaths. Of these, 35 were missing part of their tails (7%), 9 had dorsal scars (2%), 6 had hibernation sores or scars on the ventral surface (1%), and 4 were missing an eye (<1%). Mortality resulted from shrew predation (N = 8, 2%), freezing during the winter (N = 6, 1%), death from impact with motor vehicles (N=6, 1%), and death from forest fire (N=6, 1%).

Discussion

Top level predators, such as snakes, are often useful bioindicators because they can provide information on species conditions, contaminant levels, population dynamics, and community stability (Burger 2006). Long-lived snakes are useful because they are relatively sedentary, and the same individuals could be followed for many years. However, a major difficulty with behavioral and population studies of amphibians and reptiles is the daunting task of simply finding them, once or several times (Mazerolle *et al*. 2007). Snakes, such as pine snakes, that spend a great deal of their time under logs, leaves or underground are particularly difficult to study. Even mark-recapture studies are difficult because it is problematic to relocate secretive animals, such as snakes.

We have been particularly fortunate that there are no burrowing mammals in the New Jersey Pine Barrens that do not prey on pine snakes. Thus, New Jersey pine snakes need to dig their own nests and hibernacula (Burger and Zappalorti 1986, 1992; Burger *et al*. 1988, 1992, 2007). This provided us with the opportunity to examine the effects of different research techniques, such as handling and marking, on their behavior and ecology, and makes them a useful bioindicator. PIT tags became available during the period of this study, and greatly enhanced our ability to track individuals accurately and reduced the possibility of tag loss (Bjorndal *et al*. 1996), as has been reported elsewhere (Jemison et al. 1995, Gibbons and Andrews 2004).

Our nesting and hibernating studies are clearly a form of capture-recapture studies, although our other studies were not as formalized. Capture/recapture techniques partly can address detectability (Mazerollie *et al*. 2007; MacKenzie *et al*. 2005). However, unlike almost all other mark-recapture studies with reptiles, we attempted recapture every year for 12 years for nesting females and every year for 23 years for hibernating snakes; although in other years our studies were more directed toward other objectives. We captured and identified any snakes we found during the 33 years of our studies.

Finally, as in all studies of secretive animals, not all snakes were found again (except for those with radio-transmitters). However, 50 - 58 % of those marked and released to the wild for movement studies were observed again (Table 1), and 48 % of snakes marked in hibernacula were found again. While there is no way to determine whether our behavior influenced these snakes that were never found again, our studies over short periods of time (e.g. nesting, Table 2) did not show an appreciable effect.

Handling, Surgical Transmitter Implants, Branding and PIT Tag Use

We did not find any difference in relocation as a function of whether the snakes were branded or fitted with PIT tags. Further, we did not find any case where injection of a PIT tag injured the pine snake. We were not able, however, because of the overall secretiveness of snakes and our inability to individually identify them unless they were branded or pit-tagged, to compare the behavior of completely unmarked snakes to those that were marked, except for nesting females. Nesting females that were handled and fitted with PIT tags did not abandon their nest site. Similarly, Jehle and Hodi (1998) found no effect of PIT tags on recapture rate and body condition in newts (*Triturus dobrogicus*) and toads (*Pelobates fuscus*), and Brown (1997) reported no effects of pit-tagging in Anurans. PIT tags provide a completely reliable and long-term marking method that, at least for pine snakes, has lasted over 20 years, which surely indicates their usefulness for long-term population studies.

Our observations showed that repeated surgical transmitter implants did not have adverse impacts on the health or fitness of the same pine snakes. Several adult pine snakes were re-implanted every year from 2 to 5 years. One adult male whose transmitter batteries died prematurely was lost within our Ocean County study area, but were recaptured 10-years later still carrying the radio-transmitter in its body. The key aspects of surgically implanting radio-transmitters are to follow sterile procedures, make a small incision of only three scale rows, make sure the transmitter remains stationary in the coelomic cavity, and most importantly, to avoid performing transmitter surgery after August 15 to allow enough time for proper recovery and healing before the onset of winter hibernation (Rudolph *et al*. 1998a,b).

Behavior Effects on Nesting Females

Seldom is it possible to examine the effect of particular research methods, especially capture and handling, on immediate or long-term behavior. However, McMann and Paterson (2003) did not find an effect of observer handling or capture time on the territorial displays of a lizard (*Anolis sagrei*), although others have (McFarlane and King 2002). Germano (2007) reported an increase in movement behavior following capture for the endangered Otago skink (*Oligosoma otagense*) in New Zealand.

One important goal of our studies with pine snakes was to provide information on nesting phenology and philopatry (Burger and Zappalorti 1992). We did not find a significant difference between undisturbed females that were observed digging nests, and those that were handled and branded or fitted with PIT tags (Table 2). Higher sample sizes might have indicated a slight effect of fitting them with PIT tags. The failure of females to abandon their digging when disrupted by a human is remarkable, given how easily other reptiles, such as diamond-backed terrapins (*Malaclemys terrapin*) or bog turtles (*Clemmys muhlenbergii*) abandon their nests when disturbed (Burger 1977). This may be due to the vastly greater effort required of a female pine snake to excavate a burrow than a turtle to dig a nest. A diamondback terrapin or bog turtle may take a few minutes to an hour to dig the nest, lay her eggs and cover it, while a female pine snake digs over two to ten days, and may remain in the nest chamber with the eggs for two to seven more days (Burger and Zappalorti 1991). Remarkably, females that were handled and marked did not abandon the site in subsequent years; they continued to use the same exact site (as indicated by hatched eggshells from previous years). Marked females continued to come back to the same exact site, despite being handled and marked the first year, and handled every time they were captured in subsequent years, indicates that these methods were not a deterrent to continued use of their nest sites. One female, for example, used the same site for nine consecutive years (Burger and Zappalorti 1992).

After we clearly showed that snakes from eggs incubated at low incubation temperatures (21-23^0 C) had lower hatching rates and showed behavioral deficits (Burger *et al*. 1987; Burger and Zappalorti 1988a, b; Burger 1989), we stopped these experiments. Incubation temperature not only affected hatching rate and behavior of pine snakes, but it also affected subsequent survival. Only one hatchling from artificial nests (out of 98 released) incubated below 24^o C was found subsequently in hibernacula, while 22 from medium- and high- incubation temperature nests (of 180 released) have been found (Burger 1998; unpublished data).

Behavior Effects on Snakes in Hibernacula

Snakes continued to use the hibernacula we excavated, unless the hibernacula were destroyed by predators, poachers (in which case they were left open), or by management or development (which usually destroyed the habitat). Interestingly, some hibernacula that were destroyed completely by predators were abandoned permanently, while others were reused after varying periods of disuse. Nearly half of the snakes we marked with PIT tags were captured a second time, and nearly a third were captured more than three times. These data do not take into account detectability (see Mazerollie *et al*. 2007), immigration and emigration, or births and deaths, making it remarkable that this many pine snakes were recaptured. There are several reasons why snakes might continue to use a traditional hibernaculum site despite our excavation, including homing fidelity, the continued existence of scent trails leading to the entrance, and safety from predators and poachers.

Further, and perhaps more importantly, we constructed the hibernacula to reduce the potential for predation. By using cement blocks for the entrance, we made it impossible for large predators (e.g., coyote, red fox, striped skunk) to dig down by following the snake trails to the below ground hibernaculum chamber since predators could not fit down the holes in the cement blocks. Secondly, we created a chamber at the appropriate depth that snakes could use in the summer for dens, and once snakes used them during the summer, they could easily return to hibernate. Thirdly, the snakes could always dig side chambers off the main constructed chamber to create individual chambers (and in fact, the snakes did so; see Burger et al. 1988).

While it is reasonable to assume that some snakes would use the excavated hibernacula for the reasons stated above, it is possible that individuals would abandon these sites and go elsewhere to another hibernaculum. It is noteworthy that nearly half of the snakes we handled and marked returned to the hibernaculum at least once. While we have no control (snakes not handled in a hibernacula, and we do not know how many snakes return to one that has not been excavated), we feel that a 48 % return rate is remarkable, given that many of the marked snakes were hatchlings (presumably with a lower survival rate than adults), many were surely old, and many others were poached or preyed upon (Burger *et al*. 1992). Over the years of our study we only found one dead adult pine snake in a hibernaculum (she had failed to go deep enough after a winter warm spell was followed by a rapid freeze), and we found many dead hatchlings (from being crushed by heavy adults, from shrew (*Barina brevicauda)* predation, freezing, and dehydration). A 40-50 g hatchling in a side chamber with a 1000 g adult can die from the weight of the larger snake lying on top of it.

Further, the continued return of some snakes year after year (28 % were captured three or more times and one was captured 18 times in 22 years), indicates that the procedures were not sufficient to deter their continued use of a critical habitat site. The fact that snakes miss years in our excavated hibernacula indicate that there are other suitable dens nearby that we have not yet found.

It was not the purpose of this paper to examine either survival rates or philopatry to particular hibernation or nesting sites, but instead to examine whether the data support a conclusion that our methods are detrimental to the snakes. The lack of abandonment or shifting of nest sites by digging, the lack of shifting nesting sites in consecutive years, the lack of differences in hatching rates for eggs in nature compared to the laboratory (removing the effects of incubation temperature and predators), the lack of abandonment of hibernacula that were excavated (except when disturbed by poachers or predators), and the continued use of hibernacula by snakes indicates that the methods used to study pine snakes are not disrupting their behavior and habitat use. Based upon the results of our long-term studies it is apparent that most northern pine snakes are neither subject to, nor influenced by the, the presence of researchers, although we were always careful and remained far as possible from snakes we were observing.

Conclusions and Implications for Bioindicator Studies

The data presented in this paper provides evidence that the long-term handling of pine snakes for marking, studying nesting females, or studying hibernation behavior has no ill effects. That is, there were few differences in behavior as a function of handling and marking pine snakes, and pine snakes continued to return to the same hibernacula even though they were handled many times over several years. We did not, however, examine the short-term stress pine snakes experienced as a result of our handling and marking them, and several other studies have noted short-term stress effects from handling vertebrates (Elsey *et al*. 1990; Kreger and Mench 1993; Mathies and others 2001; Langkilde and Shine 2006). We suggest, however, that considerably more data are necessary on the longer-term effects of researcher's activities to determine whether research is affecting the survival and reproduction of vertebrates. Further, as Langkilde and Shine (2006) suggest, experimental studies that identify the relative stress effects of different handling methods are needed to provide quantitative, rather than intuitive, information on the best practices to use when handling different vertebrates for experimental purposes. Moreover, roughly half of the snakes marked were not seen in subsequent years, which could be due to our disturbance, but was more likely due to their secretive habits, mortality (especially of hatchlings), and switching among hibernation sites. Overall, the results suggest that endangered or threatened snakes residing in suburban and developed regions, even in protected areas such as the Pine Barrens, can be monitored and studied without undue effects on their behavior and ecology, making them ideal bioindicators.

The assumption that researcher activities do not affect the behavior, ecology, or eco-toxicology of the species being examined is important, because it underlies our ability to associate changes in bioindicator populations to some environmental factor. For example, if researchers' activities affected nesting or breeding behavior of females, then studies of population levels using presence of nesting females would be severely impacted, making it impossible to determine whether decreases in female nesting populations were due to the dependent variable (e.g. housing increases, human disturbances, changes in habitat availability) or to researcher activity.

We suggest that there is a need for careful studies of how researchers affect the behavior of indicator species. While this will add some time to the development of indicators, it is critical to understanding whether the environmental variable the public and public policy makers are interested in is truly affecting the indicator. An analysis, such as that provided above, may well be possible with some other on-going, long-term studies. This information would increase the usefulness of a given species (or a group of species, such as snakes) as bioindicators of species well-being, predator-prey relationships, and for ecotoxicology studies.

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References

Anderson, C.D., Talcott, M., 2006. Clinical practice versus field surgery: a discussion of the regulations and logistics of implanting radiotransmitters in snakes. Wildl. Soc. Bull. 34, 1470-1471.

Animal Behavior Society (and Association for the Study of Animal Behaviour)., 2003. Guidelines for the treatment of animals in behavioral research and teaching. Anim. Behav. 65, 249-255.

Bjorndal, K.A., Bolten, A.B., Laguenux, J.C., Chaves, A**.,** 1996. Probability of tag loss in green turtles nesting at Tortuguero, Costa Rica. J. Herp. 30, 566-571.

Brown, L.J., 1997. An evaluation of some marking and trapping techniques currently used in the study of Anuran population dynamics. J. Herp. 31:410-419.

Burger, J., 1977. Determinants of hatching success in Diamondback Terrapin, *Malaclemys terrapin*. Am. Midl. Natural. 94, 446-464.

Burger, J., 1989. Incubation temperature has long-term effects on behavior of young pine snakes (*Pituophis melanoleucus)*. Behav. Ecol. Sociobiol. 24, 201-207.

Burger, J., 1991a. Effects of incubation temperature on behavior of hatchling pine snakes: implications for reptilian distribution. Behav. Ecol. Sociobiol. 28, 297-303.

Burger, J., 1991b. Response to prey chemical cues by hatchling pine snakes (*Pituophis melanoleucus*: effects of incubation temperature and experience. J. Chem. Ecol. 17, 1069-1078.

Burger, J., 1991c. Foraging behavior and the effect of human disturbance on the Piping Plover (*Charadrius melodus*). J. Coastal Res. 7, 39-52.

Burger, J., 1998. Effects of incubation temperature on hatchling pine snakes: implications for survival. Behav. Ecol. Sociobiol. 43, 11-18.

Burger, J., 2001. The behavioral response of basking Northern water *(Nerodia sipedon)* and Eastern garter *(Thamnophis sirtalis)* snakes to pedestrians in a New Jersey park. Urban Ecosyst. 5, 119-129.

Burger, J., 2006. Bioindicators: A Review of Their Use in the Environmental Literature 1970-2005. Environmental Bioindicators. 1, 136-144.

Burger, J., Gochfeld, M., 1985. Behavioral development: nest emergence of young pine snakes (*Pituophis melanoleucus)*. J. Comp. Psychol. 99, 150-159.

Burger, J., Gochfeld, M., 1994. Predation and effects of humans on island-nesting seabirds. Pages 39-67 in D.N. Nettleship, J. Burger, and M. Gochfeld (Eds). Seabirds on Islands: Threats, Case Studies and Action Plans (pp 39-67). Bird Life International Conservation Series Number 1, Cambridge, England.

Burger, J., Zappalorti, R.T., 1986. Nest site selection by pine snakes *Pituophis melanoleucus* in the New Jersey Pine Barrens. Copeia 1986, 116-121.

Burger, J., Zappalorti, R., 1988a. Habitat use in free-ranging pine snakes *Pituophis melanoleucus* in the New Jersey Pine Barrens. J. Herp. 44, 48-55.

Burger, J., Zappalorti, R.T. 1988b. Effects of incubation temperature on sex ratios in pine snakes: differential vulnerability of males and females. Am. Natural. 132, 492-505.

Burger, J., Zappalorti, R.T., 1989. Habitat use by pine snakes *(Pituophis melanoleucus)* in the New Jersey Pine Barrens: individual and sexual variation. J. Herp. 23, 68-73.

Burger, J., Zappalorti, R.T, 1991. Nesting behavior of pine snakes (*Pituophis m. melanoleucus*) in the New Jersey Pine Barrens. J. Herp. 25, 152-160.

Burger, J., Zappalorti, R.T., 1992. Philopatry and nesting phenology of pine snakes *Pituophis melanoleucus* in the New Jersey Pine Barrens. Behav. Ecol. Sociobiol. 30, 331-336.

Burger, J., Zappalorti, R.T., Gochfeld, M. 1987. Developmental effects of incubation temperature on hatchling pine snakes *Pituophis melanoleucus*. Comparat. Biochem. Physiol. 87A, 727-732.

Burger, J., Zappalorti, R.T., Gochfeld, M., Boarman, W.I., Caffrey, M., Doig, M., Garber, S.D., Lauro, B., Mikovsky, M., Safina, C., Saliva, J. 1988. Hibernacula and summer dens sites of pine snakes (*Pituophis m. melanoleucus*) in the New Jersey Pine Barrens. J. Herp. 22, 425-433.

Burger, J., Zappalorti, R.T., Dowdell, J., Georgiadis, T., Hill, J., Gochfeld, M., 1992. Subterranean predation on pine snakes *(Pituophis melanoleucus)*. J. Herp. 26, 259-263.

Burger, J., Zappalorti, R.T., Gochfeld, M., DeVito, E., 2007. Effects of off-road vehicles on reproductive success of pine snakes (*Pituophis melanoleucus*) in the New Jersey Pine Barrens. Urban Ecosyst. 10, 275-284.

Carney, K.M., Sydeman WJ. 1999. A review of human disturbance effects on nesting colonial waterbirds. Waterbirds 22, 68-79.

Clark, D.R. Jr., 1971. Branding as a marking technique for amphibians and reptiles. Copeia 1,148-151.

Dowd, C., 1992. Effect of development on bird species composition of two urban forested wetlands in Staten Island, New York. J. Field Ornithol. 63,455-461.

Elbin, S.B., Burger, J., 1994. Implantable microchips for individual identification in wild and captive populations. Wildl. Soc. Bull. 22, 677-683.
Elsey, R.M., Joanen, T., McNease, L., Lance, V., 1990. Stress and plasma levels in the American alligator – relationship with stocking density and nesting success. Comp. Biochem. Physiol. 95A, 55-63.
Erwin, R.M., 1989. Responses to human intruders by birds nesting in colonies: experimental results and management guidelines. Colonial Waterbirds 12, 104-108.
Fernandez-Juricic, E., 2000. Local and regional effects of pedestrians on forest birds in a fragmented landscape. Condor 102, 247-255.
Garber, S.D., Burger, J. ,1995. A twenty year study documenting the relationship between turtle decline and human recreation. Ecol. Appl. 5, 1151-1162.
Germano, J.M., 2007. Movements, home range, and capture effect of the endangered Otago skink (*Oligosoma otagense*). J. Herp. 41, 179-186.
Gibbons, J.W., Andrews, K.M., 2004. PIT tagging: simple technology at its best. BioSci. 54, 447-454
Gibbs, J.P., Shriver, W.G,. 2002. Estimating the effects of road mortality on turtle populations. Conserv. Biol. 16, 1647-1652.
Goldingay, R.L., Newell, D.A. 2000. Experimental rock outcrops reveal continuing habitat disturbance for an endangered Australian snake. Conserv. Biol. 14, 1908-1912.
Jehle, R., Hodi, W., 1998. PITs versus patterns: effects of transponders on recapture rate and body condition of Danube crested newts (*Triturus dobrogicus*) and common spadefoot toads (*Pelobates fuscus*). Herpetol. 8, 181-186.
Jemison, S.C., Bishop, L.A., May, P.G., Ferrell, R.M., 1995. The impact of PIT-tags on growth and movement of the rattlesnake, *Sistrurus milarius*. J. Herp. 29, 129-132.
Jokimaki, J., Suhonen, J., 1993. Effects of urbanization on the breeding bird species richness in Finland: a biogeographical comparison. Ornis Fenn. 70, 71-11.
Keller, V.E., 1991. The effect of disturbance from roads on the distribution of feeding sites of geese (*Anser brachyrhynchus, A. anser*), wintering in north-east Scotland. Ardea 79, 228-232.
Knight, B.L., Gutzwiller, K.J., (Eds.). 1995. Wildlife and recreationists, Island PressWashington D.C.
Kreger, M.D., Mench, J.A., 1993. Physiological and behavioral effects on handling and restraint in the ball phython (*Phython regius*) and the blue-tongued skink (*Tiliqua scincoides*). Appl. Anim. Behav. Sci. 38, 323-336.
Langkilde, T., Shine, R., 2006. How much do researchers inflict on their study animals? A case study using a scincid lizard, *Eulamprus heatwolei*. J. Experim.Biol. 209, 1035-1043.
MacKenzie, D.I., Nichols, J.D., Royle, J.A., Pollock, K.H., Hines, J.E., Bailey, L.L. 2005. Occupancy estimation and modeling: inferring patterns and dynamics of species occurrence. Elsevier, San Diego.
MacMann, S., Paterson, A.V., 2003. The relationship between location and displays in a territorial lizard. J. Herp. 37, 186-188.
Mathies, T., Felix, R.A., Lance, V.A., 2001. Effects of trapping and subsequent short-term confinement stress on plasma corticosterone in the brown treesnake (*Boiga irregularis*) on Guam. Gen. Comp. Endocrinol. 124, 106-114.
Mazerolle, M.J., Bailey, L.L., Kendall, W.L., Royle, J.A., Converse, S.J., Nichols, J.D., 2007. Making great leaps forward: accounting for detectability in herpetological field studies. J. Herp. 41, 672-689.
McAllister, K.R., Watson, J.W., Risenhoover, K., McBride, T., 2004. Marking and

radiotelemetry of Oregon spotted frogs (*Rana pretiosa*). NW. Natural. 85, 20-25.
Moore, M.J.C., Seigel, R.A., 2006. No place to nest or bask: effects of human disturbance on the nesting and basking habits of yellow-blotched map turtles (*Graptemys flavimaculata*). Biol. Cons. 136, 386-393.
Parent, C., Weatherhead, P.J., 2000. Behavioral and life history responses of eastern massasauga rattlesnakes *(Sistrurus catenatus catenatus)* to human disturbance. Oecol. 125, 170-178.
Perret, N., Joly, P., 2002. Impacts of tattooing and pit-tagging on survival and fecundity in the alpine newt (*Triturus alpestris*). Herp. 58, 131-138.
Reese, W.E., 1997. Urban ecosystems: the human dimension. Urban Ecosyst.1, 63-75.
Reinert, H.K., 1992. Radio-telemetric field studies of pit vipers: Data acquisition and analysis. In J. A. Campbell and E.D. Brodie, eds. Biology of the Pit Vipers, Selva Press, Tyler, Texas, pp. 185-197.
Reinert, H.K., Cundall, D., 1982. An improved surgical implantation method for radio-tracking snakes. Copeia 1982, 702-705.
Reinert, H.K., Zappalorti, R.T., 1988a. Timber rattlesnakes (*Crotalus horridus*) of the Pine Barrens: their movement patterns and habitat preference. Copeia 1988, 964-978.
Reinert, H.K., Zappalorti, R.T., 1988b. Field observation of the association of adult and neonatal timber rattlesnakes (*Crotalus horridus*), with possible evidence for conspecific trailing. Copeia 1988, 1056-1059.
Rodgers, J.A. Jr, Smith, H.T., 2005. Set-back distances to protect nesting bird colonies from human disturbance in Florida. Cons. Biol. 9, 89-99.
Rudolph, C., Burgdorf, S.J., Conner, R.N., Dickson, J.G., 1998a. The impacts of roads on the timber rattlesnake, (*Crotalus horridus*), in eastern Texas. Proc. Intl. Conf. Wildl. Ecol. Transport. Ft. Myers, Florida. pp. 236-240.
Rudolph, C., Burgdorf, S.J., Schaefer, R.R., Conner, R.N., Zappalorti, R.T., 1998b. Snake mortality associated with late season radio-transmitter implantation. Herp. Rev. 29, 155-156.
Weatherhead, P.J., Blouin-Demers, G., 2004. Long-term effects of radiotelemetry on black ratsnakes. Wildl. Soci. Bull. 32, 900-906.
Zappalorti, R.T., Burger, J., 1985. On the importance of disturbed sites to habitat selection by pine snakes in the Pine Barrens of New Jersey. Environ. Cons. 12, 358-361.
Zappalorti, R.T., Johnson, E.W., Leszczynski, Z., 1983. The ecology of the Northern pine snake (*Pituophis melanoleucus*), (Daudin - Reptilia, Serpentes, Colubridae), in Southern New Jersey, with special notes on habitat and nesting behavior. Bull. Chicago Herp. Soc. 18, 57-72.