

12-2019

Ohio Conservation Plan, Revised 2019, for the Plains Gartersnake, *Thamnophis radix*

Norman Reichenbach
Liberty University, nreichen@liberty.edu

Follow this and additional works at: https://digitalcommons.liberty.edu/bio_chem_fac_pubs



Part of the [Biology Commons](#)

Recommended Citation

Reichenbach, Norman, "Ohio Conservation Plan, Revised 2019, for the Plains Gartersnake, *Thamnophis radix*" (2019). *Faculty Publications and Presentations*. 173.
https://digitalcommons.liberty.edu/bio_chem_fac_pubs/173

This Article is brought to you for free and open access by the Department of Biology and Chemistry at Scholars Crossing. It has been accepted for inclusion in Faculty Publications and Presentations by an authorized administrator of Scholars Crossing. For more information, please contact scholarlycommunication@liberty.edu.



Ohio Conservation Plan

Plains Gartersnake, *Thamnophis radix*

Revised 2019

This plan outlines strategies and methods used in an ongoing study initiated in 1999 to restore a self-sustaining population of the Plains Gartersnake (*Thamnophis radix*) in Ohio. Restoring a self-sustaining population would require increases in the current population to where the ratios of *T. radix* to *T. sirtalis* are from 1:1 to 1:12.2 in multiple locations in Killdeer Plains Wildlife Area (KPWA). This range of ratios would be similar to what was seen between 1978-80 by Reichenbach and Dalrymple (1986) at one site in KPWA and then more recently (2002 to 2009) by Wynn and Reichenbach (2018) at two sites.



The plan was originally developed in 2010 by a team of enthusiastic conservationists representing, the Division of Wildlife (ODW), the Columbus and Cleveland Zoos, Westerville North High School Field Study Class, Liberty University, Northern Illinois University, and the University of Tennessee (Reichenbach et al., 2010). A thorough review of the plan will be made in five years with revisions and updates as needed.

TAXONOMY AND DESCRIPTION

The Plains Gartersnake, *Thamnophis radix*, is in the Order Squamata, Suborder Serpentes, Family Colubridae. Conant et al. (1945:62) provide a good composite description of individuals found in Ohio: "Middorsal stripe bright orange yellow, occupying the median row of scales and adjoining fractions of the adjacent rows. Lateral stripe bright yellow; situated on scale rows 3 and 4. Dorsal ground color dark chocolate brown. A double row of round black spots on each side of the body between the stripes, these approximately 1 to 1 ½ scales in length and about 2 to 2 ½ scales in height; the spots often run together and thus obscure the ground color. A row of similar dark spots between the lateral stripe and the ventrals. Belly light greenish grey, each ventral with a conspicuous black spot at each end; sutures between the ventrals often irregularly bordered with black. There is a tendency in some specimens for spots on adjacent ventrals to run together. Similar, but indistinct, markings on the under side of the tail. Top of head and occipital region black or very dark brown, except for a pair of bright yellow parietal spots. Lower labials, chin and throat uniform pale yellow; sutures between lower labials edged with black in some specimens. Upper labials yellow, their posterior edges broadly bordered with black, especially toward the rear of the head. There are yellow or yellowish areas on the nasals, preoculars and lower postoculars."

The best field characteristics include the bright orange dorsal stripe and the extensive black posterior edges on the yellow upper labials. These characteristics easily distinguish *T. radix* from *T. sirtalis*, which in Ohio is the most commonly found gartersnake in areas with *T. radix*. The lateral stripe for *T. sirtalis* is also confined to scale rows 2 and 3 while for *T. radix* the lateral stripe is on scale rows 3 and 4. Other species which might be confused with the Plains Gartersnake include a) Ribbon snakes (*T. sauritus*), which also have lateral stripes on scale rows 3 and 4, but they are distinctly slender with long tails (more than ¼ or more of their total length (TL) and b) Butler's Gartersnakes (*T. butleri*) which have a lateral stripe (on neck) on row 3 and adjacent halves of rows 2 and 4 and proportionately, their head is very small (Conant and Collins, 1998).

U.S. DISTRIBUTION

Thamnophis radix occurs from south central Canada (Alberta, Saskatchewan, and Manitoba), south through the Great Plains to northeastern New Mexico, northern Texas and Oklahoma, and eastward through southern Wisconsin, northern and central Illinois and northern Indiana, with disjunct populations in north-central Ohio, Missouri and Illinois (Rossman et al., 1996; Walley et al., 2003).



HISTORICAL & CURRENT DISTRIBUTION IN OHIO

The disjunct population was not confirmed in Ohio until 1945 (Conant et al., 1945) though Ditmars (1907; 1936) included western Ohio as part of the range for *T. radix*. Conant et al. (1945) speculated that it was possible that Ditmars received *T. radix* from Ohio early in his career. Many specimens were being sent to him for identification from all over the country. This species was actually recorded in Ohio in 1931 as an aberrant *T. sirtalis* collected 2 miles SW of Upper Sandusky, Wyandot County (Conant, 1951). It was noted as having a lateral stripe on the third and fourth scale rows but since *T. radix* was totally unexpected so far east from its (then) known distribution, this specimen was originally recorded as an aberrant *T. sirtalis* (Conant, 1938). This disjunct population in Ohio is a remnant of a much broader eastward range expansion of this species which is associated with the Prairie Peninsula concept of Transeau (1935; Smith, 1957).

The only recent records for this species (since 1978) are for KPWA in Wyandot County. In 2007 Doug Wynn surveyed several historical sites without finding any *T. radix*. Appropriate habitat for *T. radix* was noted at one site in Crawford County on private property since there were tall grasses adjacent to the Little Scioto River. In Marion County, a site in Grand Prairie Township, might have Plains Gartersnakes since a cemetery was nearby and contained some fencerow-type habitats. Two sites located at the edge of Big Island Wildlife Area also contained suitable habitat (Wynn and Reichenbach, 2007).

GENETIC DISTINCTIVENESS OF *THAMNOPHIS RADIX* IN OHIO

The genetic distinctiveness of the Ohio population of the Plains Gartersnake was studied by a group of faculty and students at The University of Tennessee. Both mitochondrial ND2 gene and 4 microsatellite loci were examined from 9 - 12 animals from 4 populations (Burghardt et al., 2001).

Adult *T. radix* samples were obtained from animals in Ohio, northeastern Indiana (adjacent to Cook County, IL and Lake Michigan), Dekalb County, IL, and Nebraska. *Thamnophis radix* tail tips were obtained from Ohio, Nebraska and Indiana populations. Blood samples were collected from those from Northern Illinois. Standard molecular and statistical methods were employed as detailed in the 2001 report. ND2 data are also now available from other populations of *T. radix* throughout the country as well as the closely related, evolutionarily derived *T. butleri* (Rossman et al., 1996) from the Toledo area of Ohio, Michigan, and Wisconsin. Except as noted below, these new data do not alter the general conclusions reached in the 2001 report.

Results and Discussion

ND2 sequences showed little variation within and across populations, and there was none found at all in the 10 Ohio Plains Gartersnakes analyzed. However, there was a fixed base pair (haplotype) difference that separated the Ohio animals from all other Plains Gartersnakes. Based on comparing across all the populations, the Illinois population was most similar to the Ohio population as compared to Indiana and Nebraska. Furthermore, the only population specific fixed allele was found in the Ohio animals. Interestingly, our more recent work on Butler's Gartersnake, which historically was found in the counties surrounding KPWA, showed that the fixed haplotype difference was also found in *T. butleri* from the Toledo area. These animals are larger and more *T. radix* like in color (but not pattern) than Michigan *T. butleri* from populations less than 50 miles away. Unfortunately, extensive surveys of areas around KPWA from which *T. butleri* were collected in the 1930s to 1970s have not yielded any animals and thus they must be considered extirpated. Given the close relationship between the two species, hybridization may have occurred between the species as has been amply documented in SE Wisconsin (Fitzpatrick et al., 2008).

The microsatellites, as expected, showed much more variability within and between populations. The number of alleles and genotypes differed by population. Although there is no significant pattern, the total number of different genotypes and alleles across loci were both lowest among the Ohio animals, in spite of the relatively large sample size. Without going into details, there was significant genic and genotype differentiation among the four populations at all four loci both individually and combined. At two loci, Ohio animals were statistically distinct from all other populations. Thus, even more so than the sequence data, the microsatellite results suggest that the Ohio population does differ genetically from the others.

In addition, low levels of inbreeding as measured by Fis values were found. Population differentiation is indicated by Fst scores. Comparing Ohio with each population and locus it was found that the combined Fst for the Ohio and Illinois comparison is the lowest, suggesting that Ohio snakes are more similar to Illinois than the Indiana population (which was, however, rather close to Lake Michigan and not inland as were the Ohio and Illinois

populations). In short, the Ohio population is distinct from all the other three populations in both mitochondrial sequence and microsatellite genetic data.

NATURAL HISTORY

Conant et al. (1945) described the habitat where *T. radix* is found in Ohio as the single most extensive wet prairie area in this state. The original prairie has been converted to very productive farmland, restricting the former prairie vegetation to limited areas. Most of the original specimens noted in Conant et al. (1945) were not found in typical prairie land, but rather they were in close proximity to prairie swales and streams. At KPWA they are found in low-lying grassland areas often bordering ponds. In the spring, these grassland areas, are often inundated with water. By midsummer, the water table often recedes as much as several meters below the surface and the soil becomes dry, cracked and very hard (Dalrymple and Reichenbach, 1981). Three adult *T. radix* implanted with transmitters that were tracked from mid-June to late August 2007 moved throughout grassland habitat and never entered the nearby forested areas (Wynn and Reichenbach, 2007). Terrestrial crayfish, *Fallicambarus fodiens*, burrows and cracks in the soil are used for daily refuges (Dalrymple and Reichenbach, 1984; Reichenbach and Dalrymple, 1986; Wynn and Reichenbach 2007).



Crayfish burrows are also used as overwintering sites at KPWA (Dalrymple and Reichenbach, 1984; Reichenbach and Dalrymple, 1986) and in Manitoba, juvenile *T. radix* used an ant hill as an overwintering site (Criddle, 1937).

The activity pattern for *T. radix* in Ohio spans primarily from April through October (Dalrymple and Reichenbach, 1981; Reichenbach and Dalrymple, 1986). This pattern is seen generally for this species (Wright and Wright, 1957). The Plains Gartersnake is almost exclusively diurnal. Dalrymple and Reichenbach (1984) and Reichenbach and Dalrymple (1986) found most of the gartersnakes between 1100-1600 hours during the spring and autumn while in the summer, the activity pattern was bimodal with one peak in the morning and one in late afternoon. During the summer midday temperatures often exceeded 34°C which forced the snakes to retreat to crayfish burrows.



Distances moved between recaptures were generally less than 76 m for time intervals from

several months to over a year (Reichenbach and Dalrymple, 1986). In KPWA, three adult snakes implanted with transmitters and tracked from mid-June to late-August, 2007 showed a home range style of movement throughout an area that averaged 3626 m² (range 1499 to 5717 m²). These same snakes moved on average 5.6 m/day (range 2.8 to 9.1 m/day) (Wynn and Reichenbach, 2008). Siebert and Hagen (1947) found that most individuals moved less than 2 m/day. One snake, captured 4 times, was last seen only 11 m from its original capture location. It had apparently traveled a semicircular arc.

Mating activity occurs primarily in April and May (Reichenbach and Dalrymple, 1986; Ernst and Barbour, 1989, and Stanford and King, 2004) though fall mating may occur (Pope, 1944, Stanford and King, 2004). Sexual maturity appears to be reached at 350 to 370 mm SVL for males and 380-400 mm SVL for females (Stanford and King, 2004) which would correspond to snakes that are 2 years old (Seibert and Hagen, 1947; Gregory, 1977 and Stanford and King, 2004). Spermiogenesis is pronounced in late summer and early fall. The sperm are stored in the ductus deferens during over wintering and are used the following spring (Cieslak, 1945).

Males find females by following their sex pheromone (Kubie et al., 1978b; Ford and Schofield, 1984). In Y-maze experiments, where male *T. radix* were offered a choice between *T. radix* and *T. sirtalis* pheromone trails, the males discriminated and preferred those of their own females (Ford and Schofield, 1984). It is thought that shedding potentiates the release of a sexual pheromone from the dorsal skin of the female (Kubie et al., 1978a). One or more males may court a female (Ernst and Barbour, 1989) and in Missouri small mating balls of 4-6 males per female have been seen (Rossman et al., 1996). After a male mates with a female a copulatory plug may be deposited in the female's cloaca which exerts an inhibitory effect on the courtship activity of other males (Ross and Crews, 1977).

Parturition occurs in late July through early September (Wright and Wright, 1957) after a nine-week gestation period (Cieslak, 1945). Average clutch size ranges from 9 to 29.5 (Smith, 1961; Gregory, 1977; Seigel and Fitch, 1985; Stanford and King, 2004). In Ohio, the average clutch size in 1980 from eight clutches was 15.2 (Reichenbach and Dalrymple, 1986) and more recently 12.5 from 17 clutches from females collected between 1999 and 2001 (Badgley, Quinn and Reichenbach, unpublished data). Clutch size is dependent upon female SVL (Reichenbach and Dalrymple, 1986; Stanford and King, 2004) with average female fertility increasing from 6.4 in one year olds to 21 in six year olds (Stanford and King, 2004). Mean neonate size and mass were recorded as 142 mm SVL (n=51) and 1.9 g (n=199) respectively for females collected in KPWA from 1999-2001 (Badgley, Quinn and Reichenbach, unpublished data). Neonate mass was the same (1.9 g) as that found previously in 1980 at KPWA (Reichenbach and Dalrymple, 1986). In Illinois the average neonate SVL was 138 mm (n=557) (Stanford, 2002).

Growth has been accurately described using the von Bertalanffy growth model for *T. radix* in Illinois (Stanford and King, 2004). They found that males differed from females in asymptotic size (male and female asymptotes were 502 and 582 mm SVL, respectively) but not in the rate at which they approached this size. In Illinois, year class SVLs from males were as follows (average SVL in mm followed by year class in parentheses): 171.1 (0, newborn snakes during the August to October time period), 289.1 (1), 397.4 (2), 442.2 (3), 466.4 (4), 490.3 (5) and

495 (6) and for females: 196.6 (0), 315.8 (1), 460.9 (2), 513.5 (3), 543.3 (4), 596.2 (5) and 605.2 (6) (Stanford, 2002). Seibert and Hagan (1947) found that *T. radix* grew at a rate of 1.6 mm/day to 406-457 mm TL during their first year (20 May to 9 September) and 559-610 mm TL during their second year (1.3 mm/day). In Ohio, growth rates for snake age classes 1+ averaged 0.65 mm/day in 1978-79 and 0.52 mm/day in 1980.

Longevity, based upon the von Bertalanffy growth model, was estimated to be from 6-7 years (Stanford and King, 2004). Captive born *T. radix* in colonies at the Columbus Zoo and Aquarium (CZA) and Cleveland Metroparks Zoo (CMZ) have lived up to 12 years 10 months for males and 9 years 11 months for females. Longevity for captive born *T. radix* which died from a variety of causes other than accidental death averaged 5 years (n=6) and 4 years (n=4) for males and females, respectively (Johantgen, Becka and Reichenbach, unpublished data).

Population sizes for *T. radix* are some of the highest recorded for gartersnakes. Seibert (1950) using the Hayne method estimated 845/ha (342/acre) in a disturbed site near Chicago. In Illinois, Stanford and King (2004) estimates ranged from 24 to 65/ha using the Schumacher-Eschmeyer and Jolly-Seber methods, respectively. Bauerle (1972) estimated 320/ha in Colorado. In Ohio, estimates from 1978-1980 ranged from 52-123/ha for one site at KPWA (Reichenbach and Dalrymple, 1986; using the Schumacher-Eschmeyer method).

In Ohio, the diet of the Plains Gartersnake consisted primarily of earthworms and frogs and occasionally toads and leeches (Dalrymple and Reichenbach, 1981). Elsewhere, Plains Gartersnakes have been found to eat similar items as noted for Ohio snakes as well as slugs, fish, salamanders, shrews and mice (Rossman et al., 1996). In Missouri, *T. radix* showed strong seasonal variation with snakes primarily eating frogs in the summer and worms in the spring and fall (Rossman et al., 1996). Ballinger et al. (1979) saw 30-40 adult *T. radix* feeding on tiger salamander larvae in a pond. Cebula (1983) recorded a *T. radix* regurgitating a nestling bird, possibly an eastern meadowlark. Neonate *T. radix* ate earthworms, fish and frogs but rejected grasshoppers (Reichenbach and Dalrymple, 1986). Neonatal Plains Gartersnakes from Illinois and Ohio responded to prey chemical cues for earthworms, amphibians and fish (Burghardt, 1967, 1969; Burghardt and Williams, in prep.). Young snakes can also learn to capture different prey types including fish (Halloy and Burghardt, 1990; Burghardt and Krause, 1999) and thus predatory experience during headstarting may be a factor in survival of released snakes.

Chemical prey trails are followed primarily by use of the tongue which brings odorant molecules to the vomeronasal system (Kubie and Halpern, 1979). Visual cues are also used to detect prey (Chiszar et al., 1981).

Predators of the Plains Gartersnake include red-shouldered hawks and other birds of prey, predatory mammals (foxes, coyotes, skunks, minks and domestic cats) and ophiophagous snakes (Ernst and Barbour, 1989). In addition to predators, cars and mowing may also be substantial forms of mortality. Of 56 snakes found dead on the roads at KPWA during a six hour period in fall, 1979, 10 were *T. radix* and of 39 snakes found dead after mowing operations, 17 were *T. radix* (Dalrymple and Reichenbach, 1984). Yaussy (2003) found four dead and one injured *T. radix* out of 469 snakes found on roads at KPWA during the fall, 2003. In addition to road mortality, improperly timed and duration of management activities such as

prescribed burning are also potential threats. In many cases the actual management outcomes are compatible with the habitat requirements of herpetofauna, however large field burning or mowing activities may be deleterious if they occur during the animal's active season (generally mid-March through October). Wynn (1995, 1996, 1997) found one dead *T. radix* as well as dead Smooth Greensnakes, Dekay's Brownsnakes and an Eastern Massasauga after a prescribed burn at KPWA. Since management practices such as short grass cutting (mower decks <6 inches off the ground) and burning are considered mortality factors for the Plains Gartersnake, the Division of Wildlife modified or stopped plowing, mowing and burning at selected sites (see Management Practices).

Mortality was estimated to be from 8 to 12% per month for newborns and 1.4 to 2.9% for adults (Reichenbach and Dalrymple, 1986). Annual survival estimated for *T. radix* in Illinois increased from 0.17 and 0.16 for male and female neonates (age-class 0) to 0.42 and 0.41 for male and female age-class 1 snakes, respectively. Annual survival for age-class 2+ snakes ranged from 0.35 to 0.52 (survival estimates are weighted averages from the 12 best-fit models from the Program MARK) (Stanford and King, 2004).

The Plains Gartersnake is one of the more mild tempered members of the genus *Thamnophis* (Rossman et al., 1996). Its first defense mechanism is to flee when encountered which is a common strategy used by striped snakes (Jackson et al., 1976). Fitch (1941) noted for *T. sirtalis* that the longitudinally striped pattern disguises motion as the snake moves through grass or brush with only part of its body visible. It may seem to shrink and vanish before the eyes of the observer, who may not be aware that it is in motion or at least may not detect the direction and rate of motion, as he would if there were transverse markings. The longitudinally striped pattern on *T. radix* has a similar effect at KPWA where *T. radix* can simply vanish in the tall grass. Its most common defense mechanism upon capture is secretion of musk or defecation though some individuals will bite (personal observation; Rossman et al., 1996; Ernst and Barbour, 1989).

Newborn *T. radix* that were subjected to a variety of threatening stimuli in the lab would first attempt to crawl away from the investigator until high levels of lactate were attained (i.e. they were exhausted). Thereafter they would adopt one of a variety of antipredator displays including hiding their head under one or more loops of the body, tail waving and closed- or open- mouth attacks (Arnold and Bennett, 1984). Developmental factors are also involved in these antipredator responses (Herzog et al., 1992).

STATE STATUS

The Plains Gartersnake has been a State of Ohio endangered species since being designated as such on August 31, 1974.

POPULATION AUGMENTATION

Plains Gartersnake (*Thamnophis radix*) Husbandry and Captive Propagation

Gartersnake colonies at each zoo are maintained in states of permanent quarantine to prevent pathogen transfer between the colony and the cosmopolitan animal collection. This is achieved through the use of small dedicated isolation buildings and standard quarantine protocols. Fecal samples are checked regularly.

Housing

Cleveland Metroparks Zoo (CMZ):

Adult snakes are housed together (same sex groups after the breeding season, mid-summer until winter and in mixed sex breeding groups from March-July). The snakes are housed in Vision® brand reptile enclosures (36"x 28"x 18"). Substrate consists of several inches of Carefresh® natural paper bedding and sphagnum moss. Occasionally soil (peat/compost mix) is provided in a shallow container as substrate. Cage furnishings include hide-boxes (with and without moist sphagnum moss), flat rocks, branches, large water bowls, and live plants (pothos and ferns). Paper towel rolls, pvc tubes, and boxes are also provided as hides and enrichment. Soiled substrate is removed twice weekly, at which time clean water bowls with fresh water are



provided. Total replacement of substrate and disinfection of the enclosure is performed three times per year, or as needed.

Neonates are housed communally in standard 10 gallon or 20 gallon glass aquaria. Carefresh® natural paper bedding is used as substrate and damp sphagnum moss is provided inside plastic hides. Multiple

shallow water dishes are provided with rocks on the bottom to prevent drowning. Neonates are separated into 32oz deli cups with lids, to eat while their enclosures are spot cleaned. Juvenile snakes are housed individually in clear plastic storage boxes (7" x 13" x 3.5"). The boxes fit into a multi-tier storage rack that is specially designed for housing reptiles. The bottom of each box is lined Carefresh® natural paper bedding. At one end, a small plastic hide is provided for cover along with a shallow water dish. Small holes in the side of each box provide ventilation. The young snakes are transferred from the box to either a 32oz deli cup with lid, or other appropriately sized Rubbermaid® container to eat while their enclosure is spot cleaned. A total cleaning and disinfection is performed weekly.

Columbus Zoo and Aquarium (CZA):

Adult Breeders are held in large Vision® brand reptile enclosures (47"x27.5"x27.5"). Non-breeding snakes, juveniles, or isolated individuals are housed in standard 20-gallon aquaria or Zoo Med® brand enclosures (17.5"x17.5"x18"). Substrate consists of aspen bedding deep enough for snakes to bury in, usually about 3 inches. Isolated individuals can be kept on paper towels or dimple paper. All snakes are given hides ranging from plastic boxes to cork bark. Large water bowls are provided to allow soaking although these snakes rarely soak. Soiled substrate is removed daily and fresh water is provided at least twice weekly. Total breakdown and disinfection of the habitat is performed every 3-6 months as needed, as well as directly after parturition.

Neonate and juvenile snakes are housed individually in clear plastic storage boxes (7" x 13" x 3.5"). The boxes fit into a multi-tier storage rack that is specially designed for housing reptiles.



The bottom of each box is lined with damp paper towels. At one end, sheet moss or a crumpled paper towel is provided for cover along with a shallow water bowl (Syracuse watch glasses are ideal for this application). Boxes are changed and disinfected as needed, usually three to four times per week. Paper towels are changed as soiled, usually daily.

It should be noted these snakes are adept at escaping from their enclosures and care should be taken to determine appropriate housing for the snake based on size. Weather stripping and other fillers can be used to seal gaps in vision caging.

Temperature & Photoperiod (CMZ/CZA)

Temperature and humidity within the garter snake buildings tend to fluctuate with outdoor conditions daily and seasonally. For normal maintenance of adult and juvenile snakes, ambient daytime temperature is kept at a range of 70-80°F and may be allowed to drop

to around 65°F at night. General ambient temperature of the *T. radix* building at CZA is 72-76 degrees. During daylight hours, a 50-75 watt UVA heat lamp warms a region of the adult and juvenile enclosure(s) to 85-95°F. Full spectrum lighting is provided to all adult and juvenile snakes. As the plastic neonate/juvenile boxes are enclosed by a walled rack which are only open on one side, they are illuminated only by ambient room light. Electronic timers are utilized on enclosure and building interior lights to approximate the local natural photoperiod.

Diet (CMZ/CZA)

Adult snakes are individually offered food twice weekly, with two to three days between feedings. Food items may be offered on a glass Petri dish in the snake's enclosure or the snake may be placed into a lidded, ten-gallon Rubbermaid® waste receptacle for feeding.

Food items offered include neonate pinky mice (frozen/thawed) and earthworms. Generally an adult male radix will consume 4 to 6 earthworms and 2 to 3 neonate mice per feeding. Female radix will consume 5 pinkies and 6-7 earthworms in a single feeding. Gravid females however are often ravenous and will readily consume as many as 8 neonate mice per feeding and 10 earthworms. Adult snakes show a preference for earthworms. *T. radix* may slow down or stop feeding in late fall due to photoperiod changes and other environmental cues that stimulate the brumation process.

Juvenile snakes are offered food twice weekly, with two to three days between feedings. Food items regularly offered include earthworms and chopped neonate mice. Juvenile snakes are fed a mixture of chopped earthworm and mouse (approximately 1:1 by volume) which they consume readily (to satiation).

Neonate snakes are offered food three times a week, with one to two days in between feedings. Food items include chopped trout worms or diced earthworm and chopped pinkies. Generally, neonates are started on earthworms and offered pinkies as they start to consume prey readily. Some neonates will initially refuse to consume earthworms but will readily accept a diet of guppy fry, chopped smelt and/or tadpoles. Once they have begun eating reliably, earthworms and eventually mice may be incorporated into their diet. An increased size of prey item is offered to match the needs of the snake. Typically, neonates will eat consistently and put on sufficient mass by two to three months of age, at which time feedings may be reduced to twice a week.

Fish, guppy fry, smelt, and tadpoles have been offered in the past with good response from the snakes but may carry mycobacterium and are no longer offered as food items at CZA unless absolutely needed.

Brumation & Breeding

During the winter months, captive adult snakes are put through a period of brumation in order to induce reproductive behavior in the spring. Light cycles are closely followed. During this time the adult snakes often reduce food consumption or stop feeding altogether. Any snakes that have continued to feed are fasted for at least 14 days to allow their digestive tracts to clear. Zoo Veterinarians are contacted to conduct general physicals on all snakes pre-brumation. This is done to assess health and determine if animals are fit to brumate. Heat lamps in enclosures are turned off 2-3 weeks prior to the start date.

CZA

Adult snakes are moved into brumation enclosures on January 1st. Brumation enclosures consist of standard 20-gallon glass aquariums with screen lids or Rubbermaid® containers with holes drilled in the lids. Three to six snakes can be housed per enclosure, but males and females should be kept separate to prevent breeding during brumation. A thick layer of moistened sheet moss is provided as substrate. This may overlay a 3-6-inch base of cypress mulch which helps to maintain humidity. Light-weight water bowls are placed on top of the moss and are large enough for the snakes to soak in. The brumation enclosures are placed inside a modified household refrigerator or biological incubator (Percival Scientific) at an initial temperature matching ambient temperatures of the room. The temperature is subsequently lowered 3°F per day until 55°F is achieved. This temperature is maintained for approximately 90 days. No light is



provided in brumation. Generally, the snakes are pulled from brumation (at ambient room temperature) on April 1st.

After adult snakes are pulled from brumation, males and females are immediately housed together in large Vision® enclosures. Larger breeding groups are separated into smaller ratios of 3:3 if possible. A heat lamp is provided and snakes will spend the first 2 weeks warming up and gaining energy. The first meal is offered 7 days post brumation and 2 times weekly until end of breeding season. Breeding generally starts within 2-3 weeks. Neonates are born in late June/ early July. Female's weights are monitored bi-monthly to assess for gravidity. Once it is determined the females are gravid, ultrasounds are conducted monthly to help monitor dystocia in females. Once females are gravid, males are removed until the following breeding season. Late season breeding has occurred and resulted in infertile slugs passed in brumation and a missed breeding season.

CMZ

Brumation enclosures consist of Rubbermaid® containers with holes drilled in the lids. A thick layer of Carefresh® natural paper bedding and sphagnum moss is provided as substrate. Large water bowls are placed on top of the substrate. The brumation enclosures are placed inside a biological incubator (Thermo Scientific) at an initial temperature of 65-70° F. The temperature is subsequently lowered 2.5 to 5 degrees per day until 52/53°F is achieved. The humidity inside the snake enclosures maintains ~85% with the substrate remaining dry. The snakes brumate from December-late March. The snakes are gradually warmed in the incubator to room temperature then placed in their breeding groups. The photoperiod and use of heat lamps resume as normal during this time.

Immediately after brumation, all males are housed together with all of the females until it becomes evident that females are gravid. Gravid females are then housed together, separate from the males. Gravid females are kept separate from the males until the following breeding season. When the females show evidence that they are close to giving birth, they are placed in separate enclosures. Once the females give birth, they are separated from the neonates.

Morbidity and Mortality

Plains Gartersnakes are generally hardy in captivity. Mortality factors observed include bacterial infections and neoplasia, failure to thrive, and stillborns. Gout has occurred in several cases and it is unknown if this was from primary renal disease, or dehydration. Many deaths are associated with brumation, or leaving brumation. Also, death or illness has been associated with husbandry events such as furnace air conditioning failure. Commonly affected organs include liver, lung, gastrointestinal tract, skin, and kidney. The causes of mortality are relatively consistent between CMZ and CZA. In 2006, there was a cluster of deaths (eight captive born *T. radix*) due to mycobacteria. These were generally neonates. Guppy feeder fish were examined and were also found to have mycobacteriosis. Of the few mycobacteria samples that were cultured and speciated (snakes and fish), there was not a consistent culprit. The cause of this outbreak is still undetermined. To date, unaffected snakes housed in the same enclosure have not developed mycobacteriosis.

In 2019 one wild caught snake died from *Ophidiomyces ophiodiicola* at CZA. All snakes from both colonies were tested for *O. ophiodiicola*, all of which tested negative. As a precautionary measure, release of captive bred snakes from CZA was halted in 2019 and swabs from 11 snakes of four species from KPWA were tested for *O. ophiodiicola* (Common Gartersnake (0

of 3 positive), Plains Gartersnake (1 of 3 positive), Smooth Greensnake (3 of 4 positive), Eastern Massasauga (1 of 1 positive); Greg Lipps, pers. comm. 7/13/2019).

Reproductive output of captive breeding colonies

A captive breeding colony was established in 1999 at the CZA. Thirty-one *T. radix* captured at KPWA were taken to the CZA since at that time it was unknown how many *T. radix* still existed in Ohio. Of the 31 individuals taken to the zoo, four gave birth to 45 neonates. All 31 adult snakes were then released at their capture sites as well as 17 neonates. The rest of the neonates were retained by the CZA with plans that half would be headstarted and the other half would be used to establish a captive breeding colony (Wynn and Reichenbach, 1999). Because 50% of the retained neonates died during their first year in captivity, all the surviving snakes were retained for the captive breeding program and none were released as headstarted snakes. A second captive breeding colony was established at CMZ in 2001 using both captive born neonates from CZA as well as neonates produced from wild *T. radix* collected from KPWA.

The number of live neonates produced per year by the captive breeding colony averaged 45 (range 0--112) and has totaled to 725 from 2001 to 2018 (Table 1). Initial neonate survival rates in captivity were 40--50% when we were establishing the breeding colony using snakes produced by wild caught gravid Plains Gartersnakes from KPWA. As we began to headstart neonates produced by the breeding colonies, husbandry methods were refined and survival rates improved. Survival rates ranged from 63.2 to 73.1% (2004 to 2006) and since 2006, have risen, for most years, over 90%. While the majority of the snakes in the breeding colonies started out as neonates produced by wild caught gravid snakes from KPWA, we have periodically added wild adult snakes from KPWA in order to prevent inbreeding. From the 725 live snakes produced by the captive breeding colonies, we have released 662 snakes. The difference between these two numbers is primarily due to mortality experienced during headstarting.

Table 1. Summary reproductive output of the *Thamnophis radix* breeding colony at CMZ and CZA.

Year	Number born alive	Number stillborn	Total	% born alive	Number females producing live neonates
2001	3	3	6	50.0	1
2002	47	5	52	90.4	4
2003	0	0	0	--	--
2004	38	21	59	64.4	4
2005	67	9	76	88.2	4
2006	65	3	68	95.6	3
2007	0	2	2	--	--
2008	37	15	52	71.2	2
2009	14	0	14	100	1
2010	37	0	37	100	3
2011	53	0	53	100	3
2012	25	0	25	100	1
2013	93	9	102	91.1	4
2014	70	3	73	95.9	3
2015	56	0	56	100	4
2016	20	0	20	100	1

2017	64	5	69	92.7	4
2018	112	3	115	97.4	8
Total	725	75	803		

Tagging and Release of Neonates

In the wild, Plains Gartersnakes are typically born during the month of July. The release of the captive born *T. radix* neonates from CZA and CMZ are timed with the wild births. This is done to increase the survival rate of the released snakes, as predator satiation may occur. Prior to neonate release to KPWA, the snakes are tagged with Coated Wire Tags (CWT; Northwest Marine Technology). The neonates are physically restrained, as the tags (0.5 - 2 mm) are injected subcutaneously in the upper third of the body, using a single-shot or multiple shot tag injector.



The needle is sterilized between injections. The tags are read using a T-wand detector. This wand only detects if the wire is present or absent. There are no identification numbers on the tags. As the snakes are caught in the wild, they are scanned to determine if they are recaptures. A secondary marking method has also been used in combination with CWT, such as cauterization.

POPULATION VIABILITY ANALYSIS FOR *THAMNOPHIS RADIX* AT KPWA

In 2003, a Population Viability Analysis was conducted on the population of *T. radix* at KPWA (Stanford, 2003). Population Viability Analysis (PVA) is a broadly defined term that utilizes a variety of quantitative methods to assess the future status of a population. Over the past 20 years, the methods encompassing PVA have helped to provide insight into population dynamics, refine management strategies and direct future research (Mills and Lindberg, 2002). Many types of PVA modeling techniques require knowledge about age specific survival, fecundity and initial distributions of each age-class. Fortunately, mean adult survival and fecundity for the KPWA *T. radix* had previously been determined. However, since survivorship estimates for both the neonate and juvenile age-classes of snakes in this population were unable to be calculated from the available data, values previously calculated for a Northern Illinois population of *T. radix* were substituted and utilized in a Leslie matrix model (Stanford, 2002, 2003).

Methods

Preliminary Population Assessment

Initial age class distributions were extrapolated using data from the experimental site at KPWA and supplemented with data from the Northern Illinois population (Stanford, 2003). These distributions along with the estimates of survival and fecundity (Table 1) were entered into a Leslie matrix and modeled for 10 generations to obtain a population projection model of *T.*

radix at KPWA. The corresponding elasticity and sensitivity values, along with the population growth rate (λ) and other population characteristics, were then determined from this initial matrix.

An extinction/decline risk curve was also generated for the current population of *T. radix* at KPWA based on this initial matrix. These curves show the probability that the population will fall below a threshold abundance during the duration of the simulation (Akçakaya et al., 1999).

Table 1. Age specific distributions, fecundities and survivorships that were used to model the KPWA population of *Thamnophis radix*. Values in **bold italics** were supplemented with data from Stanford 2002.

Age-Class	Initial Distribution	Fecundity	Survival
Age 0 (Neonate)	151	0	0.16
Age 1 (Juvenile)	5	0	0.41
Age 2+ (Adult)	24	2.63	0.42 ¹

[1 data from KPWA, 0.42 survival values differs from the 0.41 noted under population monitoring since the 0.41 was for all size classes of *T. radix* at the experimental site at KPWA]

Demographic Perturbation Analysis

Elasticities and sensitivities indicate the parameters that are most likely affecting the current population growth rate (λ). To test whether these "sensitive" parameters actually induced an effect on λ , a Sensitivity Analysis (Mills and Lindberg, 2002; Caswell 2000a, 2000b; Akçakaya et al., 1999) was conducted following the methods described in Stanford 2002. Analyses were conducted in two ways. First, λ was examined after parameter estimates were individually adjusted according to their 95% confidence intervals. Second, λ was examined after each initial parameter estimate was either increased or decreased by 10%. A result was considered significant if λ changed by more than 10% from the initial model.

Management Strategies

Although sensitivities and elasticities are useful tools for determining which parameters have the most effect on the population growth rate, modeling potential management strategies can show the possible effects to the population size itself. Several management strategies were examined that involved the introduction of individuals from each age class or a combination of age classes. The average abundance after 10 generations following the implementation of each management strategy was compared to the average abundance of the population with no management in place. The percent change in population size was then determined. A cost rank (1-5) was also placed on each management strategy for comparative purposes. Risk curves were also generated for each of the management simulations to determine the subsequent change in the population size as it relates to the risk of decline/extinction.

Results/Discussion

Preliminary Population Assessment

Using the most current data available, the population models indicated that *T. radix* was in decline at KPWA ($\lambda = 0.7373$). The predicted average female abundance after 10

generations was only 5.41 snakes (Figure 2). The extinction risk curves showed that the population had an 18.5% chance of going extinct within the next 10 years if no management occurred.

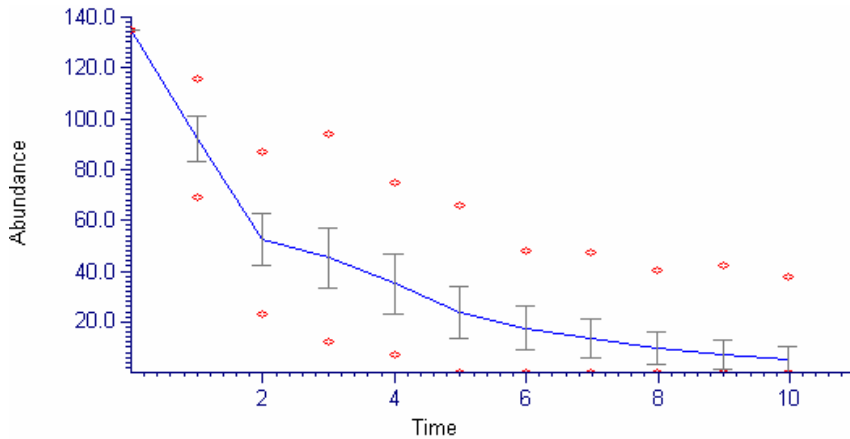


Figure 2. Population projection for *Thamnophis radix* at Killdeer Plains Wildlife Area based on the demographic data available in 2002 ($\lambda = 0.7373$). The population was modeled for 10 generations using the initial distributions, survivorships and fecundities in Table 1. The line represents the abundance mean with ± 1 standard error bars. Symbols appearing below and above the abundance means represent the minimum and maximum abundances, respectively.

Elasticity values were all fairly close, but indicated that adult survival was contributing the most to the observed growth rate. Sensitivities also found a change in neonate survival would have the most effect on changing the population growth rate (Figure 3).

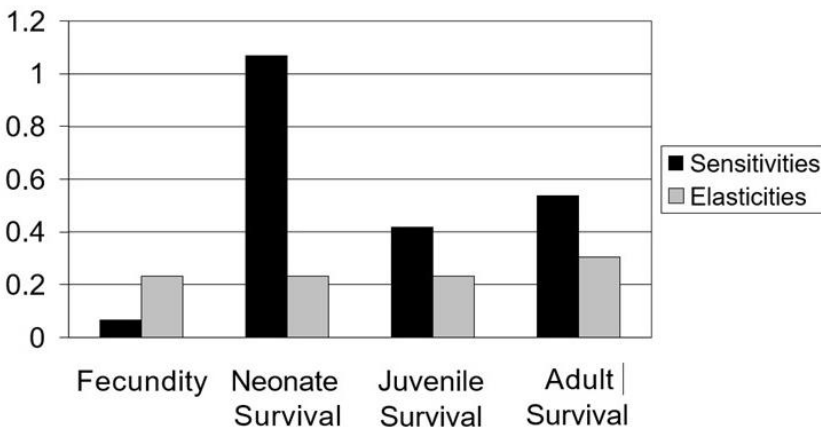


Figure 3. Elasticity and sensitivity values for the Killdeer Plains Wildlife Area population of *Thamnophis radix* based on a Leslie matrix model generated in RAMAS Ecolab using Table 1.

Demographic Perturbation Analysis

Results of the perturbation analyses conducted showed that the majority of the significant effects to the vital statistics occurred when a survival value was changed by the 95% confidence interval (see Tables 4 & 5, Stanford, 2003). Both neonate and adult 95% confidence intervals had significant effects on the population growth rate. Additionally, the upper limit of adult

survival had significant effects on all of the vital statistics. Since both neonate and adult survival induced an effect on the growth rate, it was suggested that both of them would be potential candidates for the focus of management strategies. However, the large confidence intervals of both of these parameters indicated that the estimates themselves were not that reliable.

Management Strategies

Implementation of each of the eight initial management strategies all significantly increased the size of the population in the simulations. A trajectory summary and an extinction/decline curve were also generated for each management strategy in order to visualize the changes to the overall population size from the original model (see Appendix in Stanford, 2003). A cost rank of 1-5 was placed on each strategy based on how "costly" it might be to complete. The

strategy that induced the largest change in population size was the release of 20 adult female snakes (N = 194.42 after 10 generations; 3493% increase, Table 7 Stanford, 2003). The strategy that had the smallest increase in population size was the release of 10 female neonates (N = 23.73 after 10 generations; 339% increase, Table 7 Stanford, 2003).

Conclusions

The results of the analyses indicated that the current adult survival rate was contributing the most to the observed population growth rate, but that a change in this growth rate would most likely be accomplished by changing the neonate survival rate. Management simulations showed that the release of any age class of animals caused an increase to the population size. However, the release of juvenile or adult animals caused larger increases in overall population size after 10 years, than the release of neonates. However, these strategies would also be more costly to implement. The release of larger numbers of neonates would equally increase the overall population size at less of a cost.

CONSERVATION: Population Ecology and Augmentation

Population Ecology: Some History of *Thamnophis radix* at Killdeer Plains Wildlife Area (1978 to 1998)

The first surveys began with studies on the Plains Gartersnake in 1978 (Dalrymple and Reichenbach 1981, 1984, and Reichenbach and Dalrymple, 1986). Survey methods included walking the grasslands as well as road surveys primarily at Killdeer Plains Wildlife Area (KPWA). An ecological study on *T. radix* was conducted at one site where large numbers of this species was seen during the survey work. Weekly trips were made to this particular site at KPWA from March through September from 1978-1980. Snakes were hand collected by walking throughout the 20 ha grassland site. All *T. radix* and *T. sirtalis* collected were marked (ventral scale marking technique; Brown and Parker, 1976), measured (snout-vent length, SVL) and classified as males, females, juveniles or neonates. The densities estimated during those years ranged from 52 to 123 and 45 to 89/ha for *T. radix* and *T. sirtalis*, respectively (using the Schumacher Eschmeyer (1943) mark-recapture methodology) (Reichenbach and Dalrymple, 1986).

From 1981-1993 monitoring of *T. radix* at KPWA was not conducted. Then from 1994-1997 herpetofauna surveys were conducted at KPWA. Cover sheets placed in various locations throughout KPWA was the primary method used to find reptiles. In over 60 trips, only six *T. radix* were seen (Davis et al., 1994; Wynn 1995, 1996, and 1997). In 1998, Reichenbach duplicated his earlier survey methods on the Plains Gartersnake and found five Plains Gartersnakes during 10 trips to the same site he studied 17 years earlier. Earlier, during the 1978-1980 study, five or more *T. radix* would typically be found per trip. It was estimated that the *T. radix* population at this one site had declined by 94% (Reichenbach, 1998).

Current range at KPWA, inbreeding assessment, establishment of captive breeding colonies and evaluation of coded binary tags for marking neonates (1999 to 2001)

An intensive survey for the Plains Gartersnake began during the 1999 season. Combined efforts by the Columbus Zoo and Aquarium (CZA), the Ohio Division of Wildlife (ODW), Norm

Reichenbach and a research class from Westerville North High School resulted in 41 Plains Gartersnakes being found. Snakes were found by walking the grasslands similar to what was done during the 1978-80 study in addition to using the cover sheets placed during the KPWA herpetofauna studies from 1994-1997. Some of the captured snakes were used to establish a captive breeding colony at CZA (for details see Section of Reproductive output of captive breeding colonies).

In the seasons following 1999, only a few additional adult Plains Gartersnakes were permanently retained in captivity in order to augment the captive breeding colonies. In addition, some gravid females were temporarily held in captivity until they gave birth in order to gain a better knowledge of their reproductive biology.

The following is a chronological account of the Plains Gartersnake restoration program at KPWA. Key points of this narrative are summarized in Table 1.

Table 1. Summary of key information from the Plains Gartersnake restoration program compiled on an annual basis.

Year	Reference Site	Experimental Site	Captive Plains Gartersnake released ^a	Notes on Released Captive born Plains Gartersnakes	Comments
1999	--	14			Establishment of breeding colony at Columbus Zoo.
2000	--	25			Establishment of breeding colony at Cleveland Zoo.
2001	19	35	3 N/HR	not marked	All N/HR released at site near experimental site.
2002	15	11	47 N/HR	CWT marked; 0 recaptured.	All N/HR released at site near experimental site.
2003	7	11	0		all captured Common Gartersnakes at experimental site were removed to assess potential for interspecific competition with Plains Gartersnake.
2004	6	7	0		all captured Common Gartersnakes at experimental site were removed.
2005	10	7	24 HS/HR	PIT tagged; 1 recaptured 1 month after release.	Shift to HS captive born snakes; all captured Common Gartersnakes at experimental site were removed.
2006	2	5	35 HS/HR	PIT tagged; 9 with transmitters; moved up to 100 m in less than 8 days.	all captured Common Gartersnakes at experimental site were removed.

2007	28	4	36 HS/HR	PIT tagged; 4 with transmitters; moved up to 130 m in 7 days.	all captured Common Gartersnakes at experimental site were removed.
2008	7	1	3 ^b HS/HR	PIT tagged; 3 with transmitters; moved up to 294 m in 23 days.	all captured Common Gartersnakes at experimental site were removed.
2009	3	0	21 HS/SR; 11 HS/HR	PIT tagged; 9 SR and 6 HR with transmitters; 71 and 66% survival rates for SR and HR respectively; median tracking time 29 and 26 days for SR and HR, respectively. 12 SR and 5 HR with PIT tags only; 2 SR and 0 HR were recaptured in 2009 and 1 HR was recaptured in 2010.	Shift to SR of HS snakes; Common Gartersnakes removals stopped since no effect of interspecific competition noted (i.e. increase in Plains Gartersnake numbers).
2010	--	0	11 HS/SR	PIT tagged; all with transmitters; 62.5% survival rate; median tracking time 47 days.	illegal snake collecting activity noted; cover sheets removed from reference site.
2011	--	0	20 HS/SR	PIT tagged; all with transmitters; 45% survival rate; median tracking time 52 days.	losses of transmittered snakes noted to be near ponds/reservoirs; possible mortality from fish/bullfrog predation?
2012	--	0	42 HS/SR	PIT tagged; 2 recaptured in 2013	
2013	--	0	22 HS/SR; 32 N/HR	HS PIT tagged; N marked by cauterization; 0 recaptured.	
2014	--	0	47 ^c HS/SR	PIT tagged and cauterized; 1 recaptured in 2014 at new site and another one recaptured in 2015 (lacked pit tag but showed cauterization marks).	moved release location to site without ponds/reservoirs nearby; shift to N/HR since HS/SR did not increase Plains Gartersnake population at experimental site.
2015	--	6 ^d	54 HS/SR; 40 N/HR	HS PIT tagged and N cauterized	7 new sites used in coordination with E. Massasauga work at KPWA, <i>T. radix</i> found at 2 of these sites; shift underway to releasing N/HR instead of HS/SR.
2016	--	3 ^d	10 HS/HR; 20 N/HR	HS PIT tagged; N marked by CWT and cauterization; 3 HS/HR recaptured (two from 2016 and one from 2015) and 4N/HR from	<i>T. radix</i> found at 2 of the 7 sites used in coordination with E. Massasauga work at KPWA.

				2016 recaptured in 2017.	
2017	--	15 ^d	62 N/HR	3N/HR released in 2016 were recaptured in 2017; 2 HS PIT tagged released in 2016 recaptured in 2017 and 1 HS PIT tagged released in 2015 was recaptured in 2017.	<i>T. radix</i> found at 3 of the 7 sites used in coordination with E. Massasauga work at KPWA.
2018	--	16 ^d	112 N/HR	No recaptures of captive-born snakes.	<i>T. radix</i> found at 2 of the 7 sites used in coordination with E. Massasauga work at KPWA.

a N=neonate, HS=headstarted snake, HR=hard release, SR=soft release

b snakes implanted were born in 2006 and retained in captivity for 2 years

c of the 47 snakes soft released, 10 escaped from enclosures at the original experimental site and the other 37 were released in the new site that did not have a pond or reservoir nearby

d Plains Gartersnake numbers are only for PIT tagged snakes at the new experimental site

2000--2001: Our goals during these two years were to a) evaluate binary coded wire tags (CWT, Northwest Marine Technology, Inc.) as a method for marking neonate Plains Gartersnakes, b) determine if inbreeding was a problem with the Plains Gartersnake at KPWA through comparing this species reproductive output to that of the Common Gartersnake and by comparing allelic diversity of the population at KPWA with populations of the Plains Gartersnake from areas where it is not isolated, c) determine the range of the Plains Gartersnake at KPWA using presence/absence information from 27 sites throughout KPWA plus road surveys, d) begin the determination of this species population demographics using mark/recapture methodology (population size, survival and ratios of Plains to Common Gartersnakes) at our two "best" sites, and e) establish another breeding colony at the Cleveland Metroparks Zoo (CMZ) using neonates produced by captured gravid snakes from KPWA. During these years the recovery team expanded to include Hugh Quinn from the CMZ and Gordon Burghardt from the University of Tennessee.

Six Plains Gartersnakes born in August 2000 from wild-caught KPWA gartersnakes were implanted with CWT about one week after they were born. These snakes were compared to five controls. Retention of the CWT was 100% and the tag appeared to be safe for use with Plains Gartersnake neonates since it did not increase mortality or decrease growth rates relative to control snakes (Wynn and Reichenbach 2001).

Our inbreeding assessment indicated that a decline in this species reproductive output was not evident since their output was similar to the Common Gartersnake, as was determined previously in the 1978--1980 study (Reichenbach and Dalrymple 1986). The total mass of neonates produced when adjusted for SVL indicated no significant differences between the species (Wynn and Reichenbach 2001). In addition, the findings from the molecular genetics on the Plains Gartersnake indicated that Ohio snakes were most similar to Illinois Plains Gartersnakes and that there was no consistent evidence for inbreeding of this species in Ohio relative to populations in three other locations (Illinois, Indiana and Nebraska; Burghardt, 2001

and “Genetic distinctiveness of *Thamnophis radix* in Ohio” section of this conservation plan).

While inbreeding effects were not evident with regard to reproductive output and molecular genetics, we did see the following three conservation challenges for *T. radix*:

1) range contraction was noted based upon data from field surveys (Wynn and Reichenbach 2001) and extensive surveys of the roads throughout KPWA conducted in the fall and spring of 2003 and 2004 (Yaussy 2003). *Thamnophis radix* was present at 14 of 27 sites surveyed in KPWA. The north to south range for *T. radix* at KPWA (3.2 km or 2 mi) remained the same relative to its range in 1978-80 while the east to west range (6.8 km or 4.2 mi) contracted eastward by about 3.2 km (2 miles) (Fig. 1).

2) Common Gartersnake was much more common than the Plains Gartersnake; ratios at multiple sites averaged 1 Plains Gartersnake to 10 Common Gartersnakes whereas at one site from 1978--1980 the ratio was approximately 1:1 (Wynn and Reichenbach 2001), and

3) Evidence for higher recruitment as seen by a larger number of Common Gartersnake young-of-the-year captured relative to the Plains Gartersnake (Fig. 2) (Wynn and Reichenbach 2002).

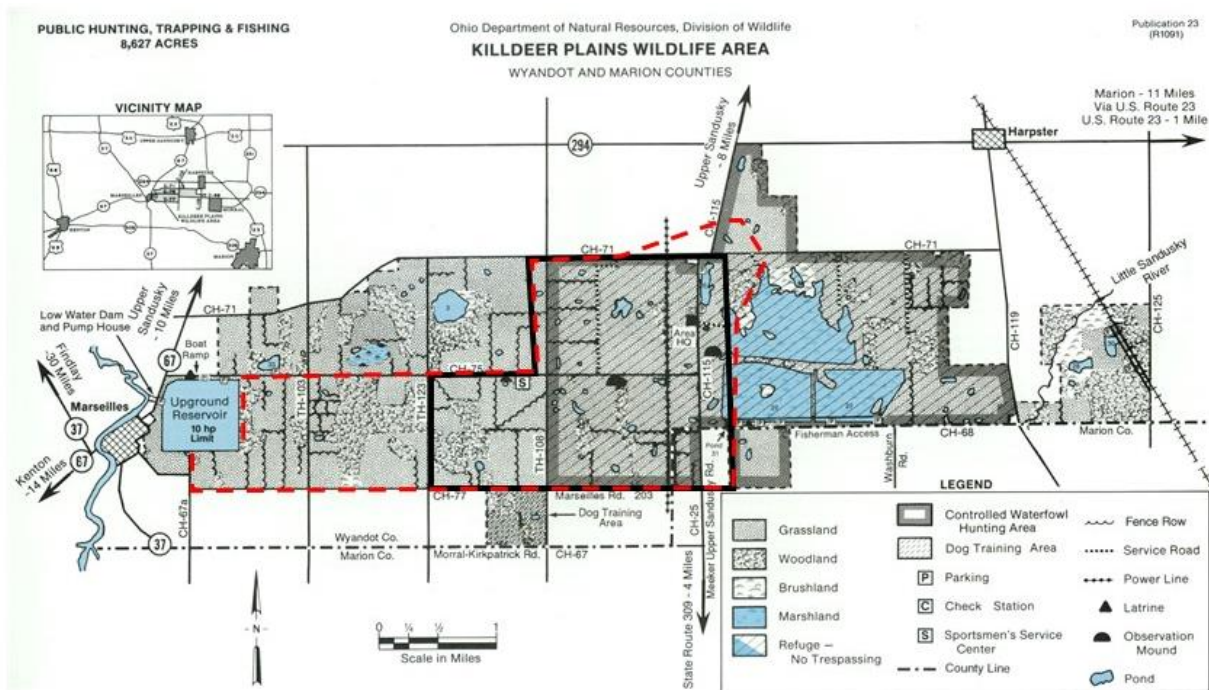


Figure 1. Range of *Thamnophis radix* at KPWA (dashed line polygon represents the distribution in 1978-80 and the solid line polygon represents the current distribution).

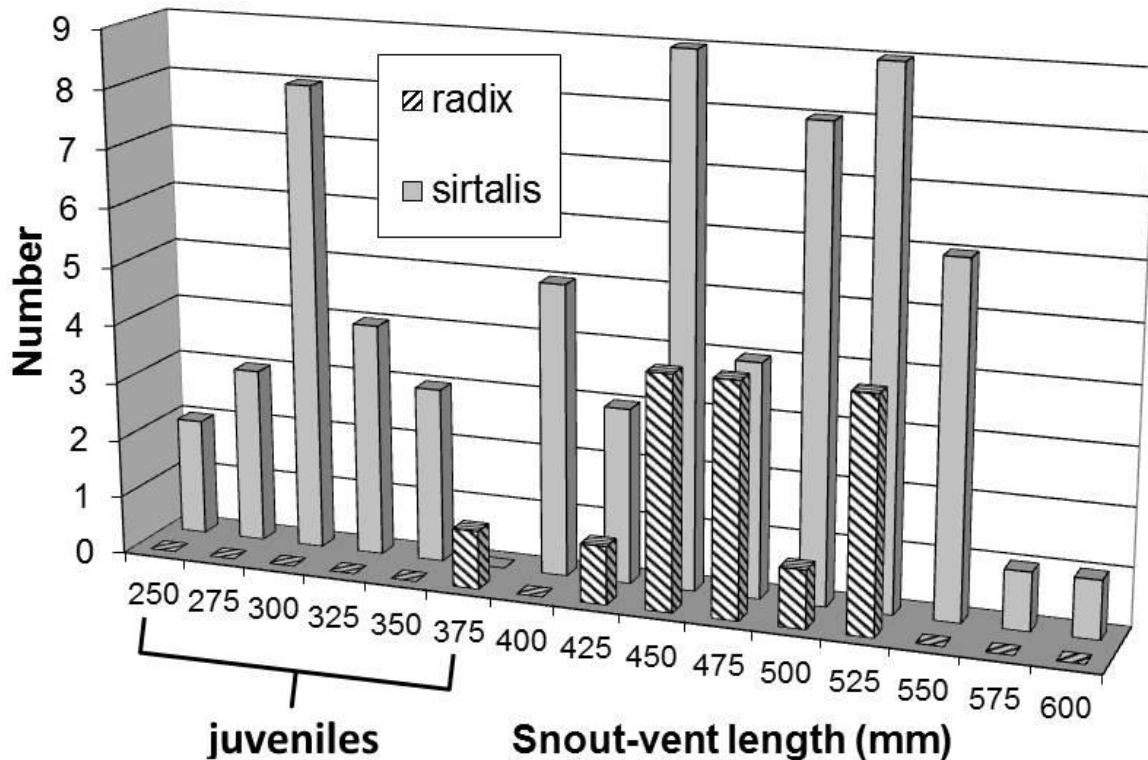


Figure 2. Snout-vent lengths (mm) for Plains and Common Gartersnakes collected during one year at one of the “best” sites at KPWA.

Headstarting captive born snakes, hard releases, population viability analysis, and demographics from two sites (2002 to 2008)

2002--2003: During these two years we focused on how to improve Plains Gartersnake recruitment. At this time 50 neonates produced by the breeding colony had been released in the vicinity of the 1978--1980 study area (Reichenbach and Dalrymple 1986). Three were released in 2001 (these were the first three captive born neonates produced from neonates retained from KPWA Plains Gartersnakes in 1999) and 47 in 2002. All the neonates in 2002 were batch marked with the CWT. Several trips to the release site were made in order to find some of the released neonates, but none were found.

Using demographic data from KPWA and Illinois along with a population matrix model, Kristin Stanford (a new member of the recovery team and at that time she was a graduate student at Northern Illinois University) determined that the best life stage to release at KPWA would be juvenile Plains Gartersnake (Stanford, 2003 and “Population Viability Analysis for *Thamnophis radix* at KPWA” section in this conservation plan). Consequently, we planned to start releasing

snakes produced by the captive breeding colonies that had been headstarted over the winter instead of releasing neonates as was done in 2001 and 2002.

In 2003, neonates were not produced from the captive breeding colony, and hence headstarting captive born snakes had to wait for another year. In 2004, 38 neonates were produced by one breeding colony. The breeding colonies in Columbus and Cleveland were primarily under Pete Johantgen's and Kristy Becka's care, respectively, both new members of the recovery team. The 38 neonates produced in 2004 were headstarted over the winter. Neonate growth over the winter showed mass increases from 2--3 to 12--18 g and SVL increases from 13--16 to 24--31 cm. Survival rates for headstarted snakes released to KPWA were expected to increase from 16% (for free-ranging snakes in their first year) to possibly 40% ("best case" scenario survival rate for second year head-started snakes) (King and Stanford, 2006). On June 14, 2005, 24 headstarted snakes (Fig. 3) were hard released at Site 12 (14 of the 38 live neonates produced in 2004 died during headstarting). One of these headstarted snakes was recaptured one month after release and it showed increase in length and mass.



Figure 3. Example of a headstarted Plains Gartersnake just before being released.

In 2003, one of our two “best sites” (monitored since 1999) was designated as our “experimental site” where interventions would occur such as releases of headstarted snakes and the other “best” site (monitored since 2001) was designated as our “reference site”. “Best sites” designation was based upon these sites having the highest number of Plains Gartersnakes relative to the other sites we were monitoring at KPWA.

2003--2008: From 2003 to 2008, we continued work on improving recruitment including an evaluation of the effectiveness of releasing headstarted Plains Gartersnakes. In addition to headstarting, in 2003, at our experimental site, we began removing a potential competitor, the Common Gartersnake. We recognized that this was not a management option, but rather a field experiment to see if the Plains Gartersnake was being negatively impacted by interspecific competition with the Common Gartersnake. Our goal was to reduce the number of Common Gartersnakes at the experimental site and then see if this would increase the number of the Plains Gartersnakes at this site.

The following is a summary of some of the demographic data collected at our two “best sites” (reference and experimental site).

At the reference site, 97 Plains Gartersnakes (82 with PIT tags) were collected over nine years (2001 to 2009). At this site, 21% of snakes marked were recaptured during the same year they were marked (17 out of 82) and then between years the recapture rate declined to 3.7% (3 out of 82). The average number of Plains Gartersnakes found annually at the reference site was 11 and ranged from 2 to 28 (Fig. 4). The ratios of Plains to Common Gartersnake were between 1:1.3 to 1:12.7 except in 2006 where the ratio was 1:34.5.

Several years into our study we suspected the cover sheets at this site were being hit illegally by snake collectors who were likely searching for Eastern Massasaugas, *Sistrurus catenatus*. In 2010 we documented this illegal activity using a motion activated camera placed at this site. At this time cover sheets were removed from the reference site and most other sites at KPWA due to these illegal collection activities.

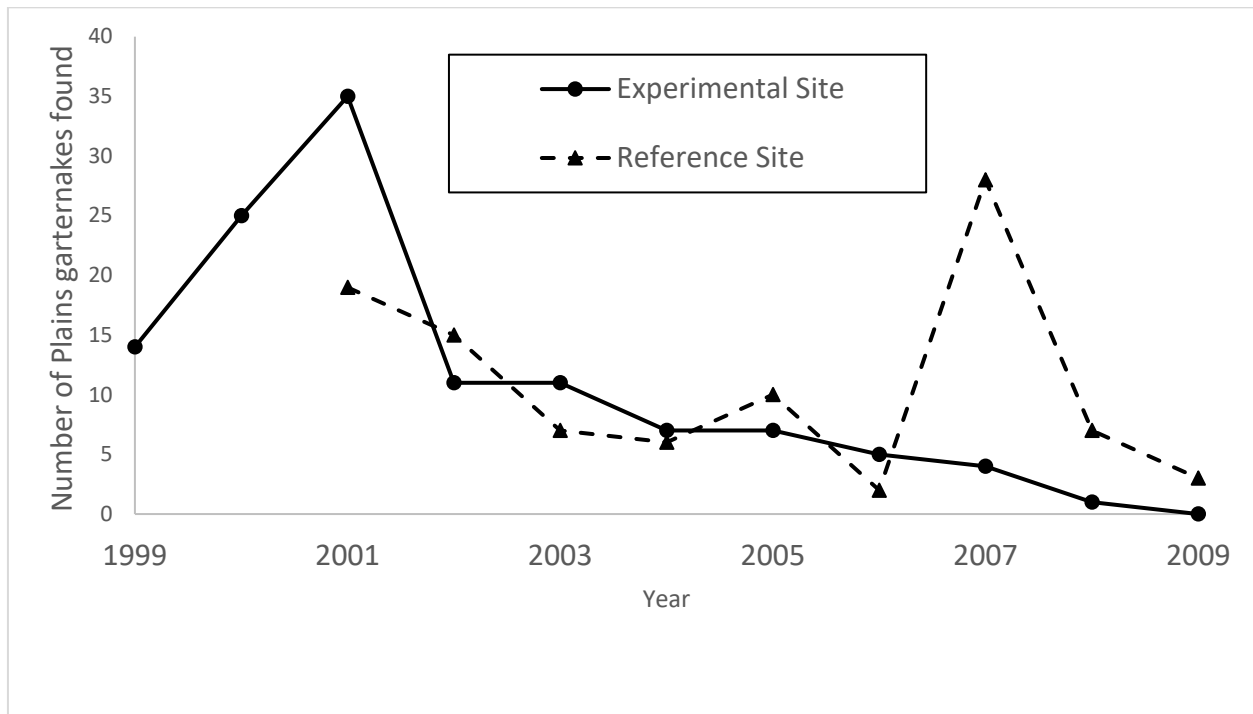


Figure 4. Number of Plains Gartersnakes captured at the KPWA experimental and reference sites.

At the experimental site, 120 Plains Gartersnakes were captured over a period of 11 years (1999 to 2009). Of these snakes, 10 have been recaptured at least once during the year they were tagged (9.3%) and nine have been recaptured in the years beyond the year they were initially tagged (8.4%). For the experimental site, during the years where recaptures were available between years, survival and recapture rates as well as population sizes were estimated. The survival and recapture rates ranged from 0.36 to 0.41 and 0.24 to 0.42, respectively, using a model in Program MARK that assumed constant survival and recapture rates. Using the same model as noted for Program Mark (Jolly-Seber Model D), the population estimates for the adult Plains Gartersnakes were 100, 145, 44 and 45 snakes (estimates for Years 2000, 2001, 2002 and 2003, respectively) with an average of 84 (0, 204, 95% confidence intervals). The average number of Plains Gartersnakes found annually at the experimental site was 11 and ranged from 0 to 35 (Fig. 4). The ratios of *T. radix* to *T. sirtalis*, for years when *T. radix* was found at this site, ranged from 1:4.9 to 1:12.6 except in 2008 when the ratio was 1:36.

At this experimental site, we successfully reduced the number of the Common Gartersnakes, a potential competitor of the Plains Gartersnake. The initial numbers removed were 93 and 88 in 2003 and 2004, respectively, and then they ranged from 26 to 42 from 2005 to 2008. The drop in numbers of Common Gartersnakes removed did not correspond to an expected increase in Plains Gartersnakes if interspecific competition was occurring (Fig. 5). Consequently, removals were stopped in 2009.

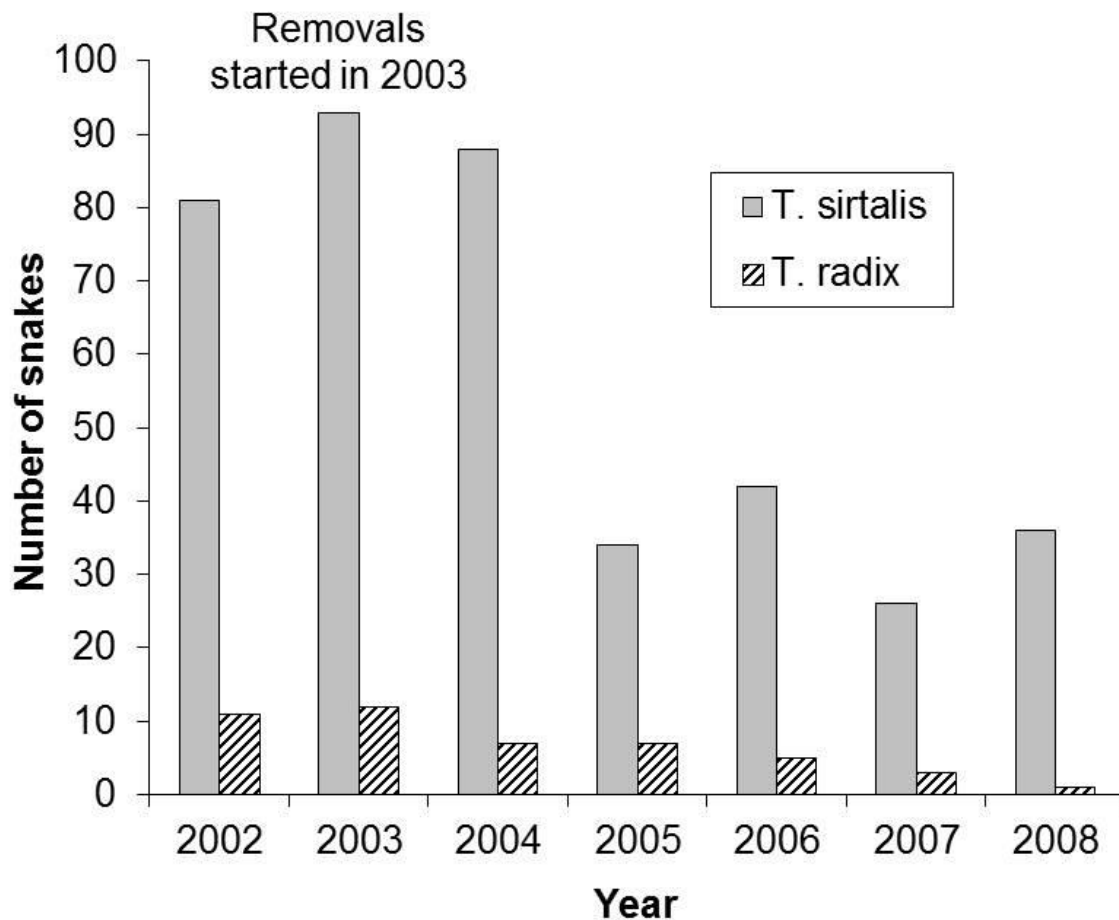


Figure 5. Number of Plains Gartersnakes (*T. radix*) and Common Gartersnakes (*T. sirtalis*) captured at the experimental site. Removal of the Common Gartersnake began in 2003 and continued through 2008.

As noted above, the number of Plains Gartersnakes recaptured the year subsequent to when it was marked was low (3.7% and 8.4% at the reference and experimental site, respectively). This probability was examined in relationship to the number of snakes collected and tagged in a given year for data collected from 1999 to 2008. When 11--15 snakes were marked, recaptures in the subsequent year occurred 25% of the time. If 16 or more snakes were marked, recaptures in the subsequent year occurred 100% of the time. When 10 or fewer snakes were marked in a given year, there was never a recapture in the subsequent year. Considering that the annual survival rates for Plains Gartersnakes ranged from 0.36 to 0.41 at KPWA, if 10 or fewer snakes were marked in a given year, then in the subsequent year only four snakes might still be alive and available for recapture. As seen in Figure 4, there are multiple years when 10 or fewer snakes were captured at a particular site and hence recaptures would be very unlikely.

Assessing the impact of releasing headstarted snakes to augment declining populations of Plains Gartersnakes at KPWA was an ongoing process in the restoration program. For our assessment, we used data collected on movement patterns, growth and survival rates of headstarted snakes we released at KPWA. Initially, hard released headstarted snakes were

only PIT tagged and then the release location was surveyed in order to recapture released animals. Releases of 24, 35 and 36 headstarted snakes born in 2004, 2005 and 2006 were done in 2005, 2006 and 2007, respectively (Table 1). As noted above, only one of these headstarted snakes was recaptured (one snake was found about one month after it was released in 2005). We expected to recapture a higher number of headstarted snakes since our mark/recapture data on wild Plains Gartersnakes showed that when we marked 16 or more snakes in a given year, then there was a 100% success rate in recapturing one or more of these snakes in the subsequent year (Wynn and Reichenbach 2008). So either the survival rates for headstarted snakes were lower than those noted for Plains Gartersnakes (0.36 to 0.41 for adults at the experimental site, Wynn and Reichenbach, 2002, 2003, or 0.4 for “best-case” scenario for second year headstarted Plains Gartersnakes in Illinois, King and Stanford, 2006) or some other factor was adversely affecting recapture rates of headstarted snakes at KPWA. Consequently, in 2006, we started using telemetry to assess our headstarting program. Nine headstarted snakes (average mass was 17.5g; range 16--18 g) were implanted with transmitters from Wildlife Materials SOPR-2011 (approximate dimensions were 0.9 x 0.5 x 0.4 cm and mass 0.6--0.8g) and hard released at our experimental site. The life expectancy for these transmitters was only between 6--8 days. Monitoring of the snakes was initially daily and then every 3--4 days. We were able to confirm that four survived at least four days, one, five days and one, eight days. Movements were up to 100 m from the release site.

In 2007 four headstarted Plains Gartersnakes were implanted with the same transmitter model as was used in 2006. Following hard release on August 4, 2007 at our experimental site the snakes were located approximately twice a day. The snakes were visually located twice in order to confirm that they were actually alive. These observations occurred three and eight days after release. By August 7, 2007 the snakes moved from 2--15 m and all remained near the pond where they were released. By August 11, 2007 three had moved from 30 to 50 m while one moved approximately 130 m. This was the last time the snakes were seen and signals from the transmitters were lost after this date. In 2008, three snakes were implanted with the same transmitter models used in previous years and one snake moved up to 294 m in 23 days.

Telemetry data were now available for 16 hard released headstarted snakes (9 in 2006, 4 in 2007 and 3 in 2008). Overall these data indicated that the snakes survived over the lifespan of the transmitter (average of 7.3 days; range 2--24 days) and that many randomly dispersed to distances of up to 294 m from the release site. Because many were dispersing randomly, it was difficult to find them once the transmitters ceased to function since many were moving outside of our monitoring sites where cover sheets were available to find snakes. The high rate of movement of the headstarted snakes and the lack of recaptures from annual releases of 16 or more snakes in 2005, 2006 and 2007 (where recaptures were expected), caused us to reconsider, for 2009, the hard release protocol used for headstarted snakes.

Shift toward soft releases of headstarted snakes and comparison of movement patterns and survival rates of soft and hard released snakes (2009 to 2014)

In 2009, our release protocol was modified so that the headstarted snakes could gradually adjust to the environmental conditions at KPWA (soft release protocol). The goal was to increase the likelihood that headstarted Plains Gartersnakes would remain near the release

site where we would have the opportunity to recapture them in order to assess their survival and growth rates. We also started breeding snakes in the captive colonies earlier (in March rather than May with birthing occurring in May rather than July/August). This provided more time for the headstarted snakes to grow while in captivity and hence they would be larger at the time of release. This in turn would allow us to implant larger transmitters with longer life expectancy.

Our plan was implemented as follows: On June 19, 2009, 22 headstarted snakes born in 2008 (12 males and 10 females) which were large enough to be pit-tagged, were split between two outdoor enclosures at the experimental site. The enclosures were each approximately 2 x 1 x 1 m. Each enclosure had a top, bottom and sides made of wire mesh with a small enough mesh size to prevent escape by the snakes. Soil to a depth of approximately 30 cm was removed along with the vegetation and then each enclosure was placed in their respective hole. The sides of each enclosure were 1 m in height and hence approximately 70 cm of the enclosure was above the soil surface. The soil removed was returned to fill the bottom of the enclosures and small boxes with tubes leading to the boxes were buried in the soil to simulate the crayfish burrows used by garter snakes as retreats at KPWA (Fig. 6). Three pieces of cover sheets were also placed in each enclosure to provide sites for thermoregulation as well as to introduce the snakes to this type of cover object. Worms were placed in the enclosures on a weekly basis for food and the snake's general behavior was observed on days they were fed. The vegetation in the enclosures was also periodically watered and a large water bowl buried in the enclosure was filled with water whenever the enclosure was visited.



Figure 6. Enclosure for soft releases of headstarted Plains Gartersnakes at KPWA.

A total of 21 headstarted Plains Gartersnake were soft released in 2009 (one snake accidentally died in the enclosure during the acclimation period). The soft released snakes were retained in the two enclosures from 17 to 43 days (median days 43). Of the soft released snakes, nine were implanted with transmitters. Depending on the size of the snake, either Holohil BD-2 transmitters (0.9g, transmitter life expectancy 42 days) or Holohil BD-2N transmitters (0.51g, transmitter life expectancy 21 days) were implanted. Six were implanted with transmitters and released after being in the enclosures for 29 days and three were implanted and released after 43 days in the enclosures. Thirteen snakes removed from the enclosures on June 19, 2009 for potential use in telemetry all showed mass increase. Mass increase averaged 0.19 g/day (range 0.08--0.52 g/day) with masses averaging 32.5 g (range 18--60 g). These masses were higher than for snakes previously released with transmitters because of the earlier breeding that we were now doing with our snakes in the breeding colonies as noted above.

A total of eleven snakes were hard released in 2009. Six of these were implanted with transmitters and were released after being in the enclosures for 3 days (similar protocol used for soft released snakes implanted with transmitters where the snakes were placed in the enclosures at KPWA for a few days post-surgery).

The data on telemetered snakes released in 2009 are shown in Table 2 and their final tracked location is shown in Figure 7.

Table 2. Summary of headstarted Plains Gartersnakes implanted with transmitters in 2009.

transmitter frequency	Release protocol	Sex	Expected transmitter life (days)	days tracked	survived	return to enclosure	hr100 ^a (m ²)	hr90 ^b (m ²)	median velocity (m/day)
150.920	hard	F	42	26	No	no	120	39	2.77
150.820	hard	F	42	31	Yes	yes	185	185	0.00
150.398	hard	F	21	26	Yes	no	3517	902	2.69
150.459	hard	M	21	24	Yes	yes	93	69	3.36
150.597	hard	M	21	26	No	no	416	327	4.22
150.714	hard	M	21	24	Yes	yes	33	33	0.84
				26^c	66.7%^c	50.0%^c	152^c	127^c	2.73^c
150.739	soft	F	42	24	No	yes	83	45	0.79
150.798	soft	F	42	24	Yes	no	5600	5065	5.76
150.436	soft	F	21	29	Yes	no	787	238	3.74
150.858	soft	F	42	62	Yes	yes	4714	4115	1.70
150.958	soft	F	42	55	No	yes	94	3	0.00
150.759	soft	M	42	24	Yes	yes	16	14	1.38
150.779	soft	M	42	31	Yes	no	1846	1271	0.00
150.677	soft	M	21	4 ^d					
150.880	soft	F	42	7 ^d					
				29^c	71.4%^c	57.1%^c	787^c	238^c	1.38^c

a) home range using minimum convex polygon method with 100% of the location data used

- b) home range using minimum convex polygon method with 90% of the location data used
- c) medians or %'s
- d) transmitters considered to have failed since their expected life was 21 days for 150.677 and 42 for 150.880. Data for these two snakes were not used in any of the summary statistics for soft released snakes



Legend

● Final locations for 2009	Low level urban	Streams and canals
Killdeer Plains Habitat	Cropland	Lakes
Mowed edge/Roads	Shrub & Brush, Old field	Forested wetland
Dike	Wooded herbaceous	Non-forested wetland
Grassland	Deciduous forest	
	Evergreen	

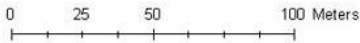


Figure 7. Final tracked location for hard and soft releases of headstarted Plains Gartersnakes released at KPWA in 2009. The white dot is the location for the soft release enclosures and is also the release location for the hard released snakes. The numbers by the red dots are several digits associated with the snakes' transmitter frequencies.

Six hard released snakes implanted with transmitters were tracked a median time of 26 days in 2009. Sixty-six percent of the snakes survived during the time they were tracked and 50% of them returned to the vicinity of the enclosure (see Figure 8 for an example of a movement pattern scored as a "return to enclosure" and Figure 9 for a snake that did not return to the enclosure). Median home ranges were 152 and 127 m² when 100% and 90% of the locational

data points were used, respectively. The median velocity was 2.73 m/day.

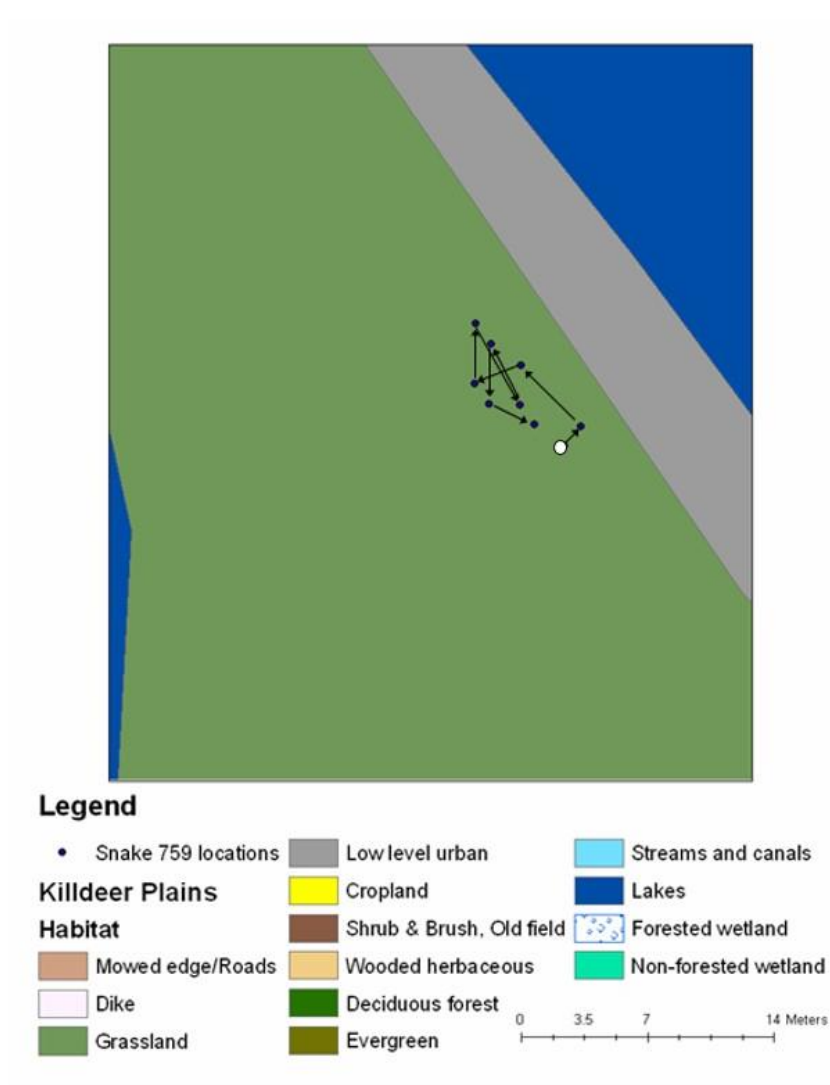


Figure 8. Example of a soft released Plains Gartersnakes released at KPWA (transmitter frequency 150.759) which was scored as a snake that returned to the enclosure. The white dot is the location for the soft release enclosures.

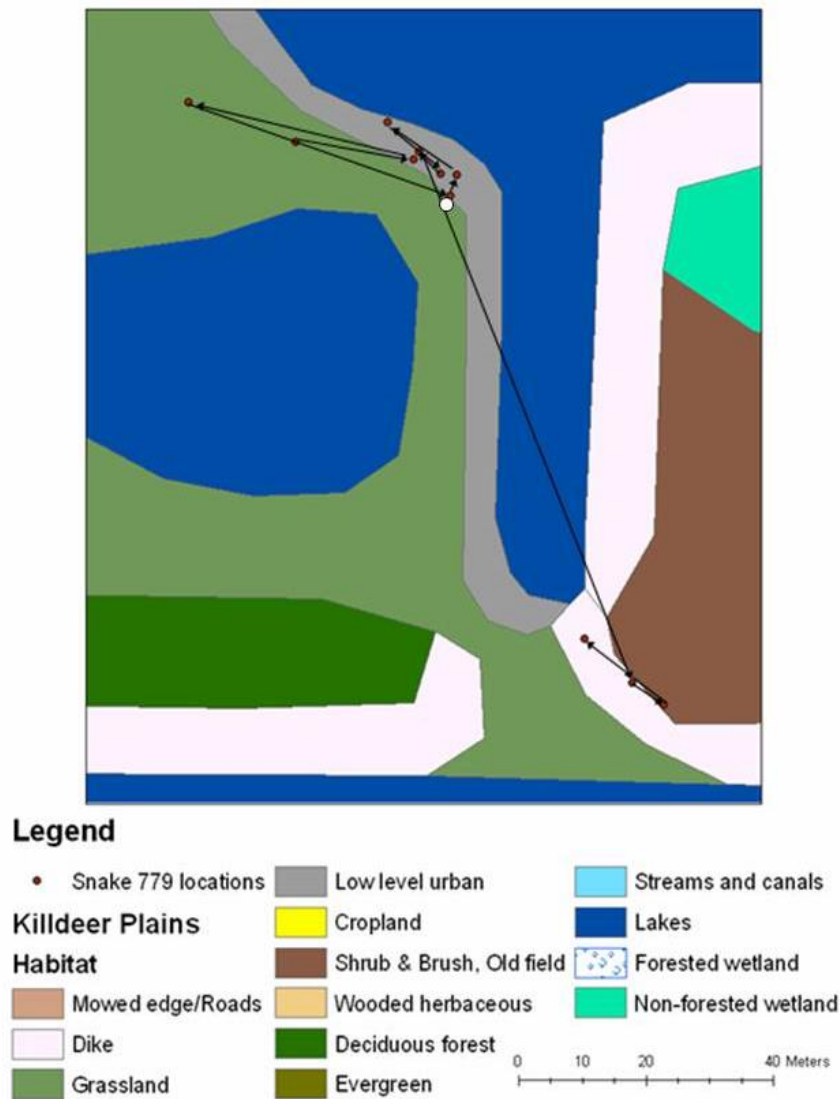


Figure 9. Example of a soft released Plains Gartersnakes released at KPWA (transmitter frequency 150.779) which was scored as a snake that did not return to the enclosure. The white dot is the location for the soft release enclosures.

Of the nine soft released snakes implanted with transmitters in 2009, two had transmitters fail within one week of release (Table 2). The remaining snakes were monitored for a median time of 29 days. Seventy-one percent of the snakes survived during the time they were tracked and 57.1% of them returned to the vicinity of the enclosure. Median home ranges were 787 and 238 m² when 100% and 90% of the locational data points were used, respectively. The median velocity was 1.38 m/day.

The rest of the headstarted snakes released in 2009 were only PIT tagged. Five of these snakes were hard released and 12 were soft released. None of the hard released snakes were recaptured in 2009. Of the 12 snakes that were soft released, two were recaptured for a recovery rate of 16.7%. One snake, released on August 1, 2009 with a mass of 28 g, was

recaptured on August 4, 2009 with a mass of 30 g. This snake had moved 88 m from the north enclosure. The other recaptured snake was also released on August 1, 2009. It was recaptured twice (August 8 and 10, 2009). Both times it was found under cover sheets adjacent to the enclosure it had been in. Its mass on release was 19 g and upon both recaptures, it was 18 g.

With the overall higher survival rates, recapture rates, lower median rates of movement and higher % return to enclosures for soft versus hard released snakes, we decided to only do soft releases of headstarted Plains Gartersnakes. This was implemented from 2010 to 2014. In 2013, because more snakes were produced by the breeding colonies than could be headstarted by the zoos, hard releases of neonates was also done. More telemetry was done through 2011 (Table 1) and we noted that a common location where transmitters were found without the snake (=mortality) were ponds and reservoirs near the release location. It was possible that predation by fish, bullfrogs, and other aquatic predators may have occurred. We therefore shifted our release site in 2014 to one without nearby ponds or reservoirs.

In 2014, the soft release enclosures were retained at our original experimental site for security purposes and 10 of the 47 headstarted snakes escaped during the acclimation period due to warping of one of the enclosures used to contain the snakes. The remaining 37 headstarts were released at another site distant from any ponds or reservoirs. One of these snakes was recaptured in 2014 and another one was found on April 28, 2015. This snake was in good condition. The snake lacked a PIT tag but a cautery mark we had given some headstarted snakes was visible.

While soft released headstarted snakes have been periodically found, capture rates were low and there was no apparent increase in the Plains Gartersnake population at the experimental site even after releasing a total of 267 headstarted snakes since 2005 (Table 1). We had initially thought the problem was because we were releasing neonates where survival rates were only 16% (Stanford, 2003). We shifted to headstarting because release of juveniles was projected to cause larger increases in overall population size compared to release of neonates (see "Population Viability Analysis" in this conservation plan). Survival rate for headstarted snakes was expected to increase to possibly 40% ("best case" scenario survival rate for second year headstarted snakes) (King and Stanford, 2006). We then shifted from the hard to soft release protocols since we found that hard released, headstarted snakes were moving beyond the cover sheets in our monitoring sites where encountering them was unlikely. But even after shifting to the soft release protocol, we still had low capture rates of headstarted snakes and we did not see an increase in *T. radix* population size at our experimental site. It seemed like the soft released, headstarted snakes were not surviving at the projected 40% rate possibly due to higher rates of predation and/or poorer foraging/thermoregulatory ability of snakes headstarted in captivity. Therefore, we recommended shifting from soft releases of headstarted snakes to hard releases of large numbers of neonates (see "Population Viability Analysis" in this conservation plan). In 2015, we began the transition from releasing headstarted snakes to releasing large numbers of neonates.

Shift toward hard releases of captive born neonates and population monitoring at seven new sites (2015 to present)

For the protocol of hard releasing neonates, we wanted the births of captive born Plains Gartersnakes to coincide with those of wild Plains Gartersnakes at KPWA (late July to early August; Reichenbach and Dalrymple, 1986). In addition, we planned to produce larger numbers of neonates than when we were headstarting snakes because survival rates for hard released captive born neonates was expected to be only 16% (Stanford, 2003). Releasing large numbers of neonates would also increase the probability of recapturing released snakes to assess survival rates and movement patterns. A simulation of releases of 50, 100, 150 and 200 hard released neonates using a Leslie matrix population model showed that adding only 50 neonates promoted moderate growth in a Plains Gartersnake population at KPWA (Wynn and Reichenbach, 2016). In order to produce more neonates, the captive breeding colony was expanded. The primary marking method planned for hard released neonates was the CWT method with cauterization being used if we wanted to double mark neonates or have a mark visible in the field.

In 2015, we shifted the sites being monitored for Plains Gartersnakes in order to coincide with the seven sites being used for Eastern Massasauga surveys. A total of 12 Plains Gartersnakes were found in 2015 at these new sites, most of which were found at one site. We designated this one site as our “new” experimental site where we would release captive born Plains Gartersnakes. The area where cover sheets were placed was also distant from ponds or reservoirs. Fifty-four headstarted snakes (marked with PIT tags) and 40 neonates (marked with CWT) were hard released at the new experimental site in 2015 as we began our shift toward releasing primarily neonates. None of these snakes were recaptured in 2015. In 2016, 10 headstarted snakes and 20 neonates were hard released at the new experimental site. During this same year, we found 27 wild Plains Gartersnakes (only three were pit tagged) in addition to one headstarted snake released in 2015 and two headstarted snakes released in 2016. The headstarted snakes released in 2016 were retained in captivity primarily to see how long cauterized marks remained easily readable (marks were easily readable after 1 year, Wynn and Reichenbach 2016).

Of the snakes captured in 2017, four had CWT. These snakes grew from an average birth weight of 2 g in 2016 to 21.6 g. It is possible one of the CWT tagged snakes was the same snake since CWT tags do not have unique numbers associated with them (snake found on May 17, 2017 and June 6, 2017 may be the same animal due to similarity in size and location where it was found). Recaptures of 3-4 CWT tagged snakes was encouraging since only 20 neonates were released at the “new” experimental site in 2016. The recovery rate was then either 3/20 (0.15) or 4/20 (0.25) which was similar to survival rates recorded for free-ranging Plains Gartersnakes in their first year (0.16; Stanford 2003).

In 2018 a total of 47 *T. radix* were captured or seen, most of which were found at the new experimental site (total includes recaptures of wild and captive-born snakes). The number of snakes that had PIT tags or were PIT tagged in 2018 was 17 (16 at one site and one at another site). Of the total snakes seen or captured, three were recaptures of wild snakes marked in 2017. No captive born snakes were recaptured in 2018. While we still did not have adequate numbers of recaptures between years to calculate population estimates and survival rates using the Jolly-Seber method, we did have enough recaptures within the 2018 field season at

the experimental site to use the Schnabel method to estimate a population size which was determined to be 22 (95% confidence intervals from 14 to 53).

There were 112 live neonates produced in 2018 by eight female snakes. All were tagged with CWT on July 24, 2018 and were hard released at the experimental site July 31, 2018. None of these neonates were recaptured in 2018.

In 2018, there were 2553 sightings of the Common Gartersnake. The majority were seen at the experimental site (n=1089). The ratio of total number of *T. radix* to *T. sirtalis* at the two sites where *T. radix* was found ranged from 1:25.3 to 1:52. When considering the ratio for only days where *T. radix* was found at the experimental site (23 collection days), the average ratio was 1:17.6.

Historically we had a 1:1 ratio of *T. radix* to *T. sirtalis* at one location (Reichenbach and Dalrymple, 1986). We have never seen that ratio since the 1978-80 study. Our original experimental and reference sites yielded average *T. radix* to *T. sirtalis* ratios of 1:12.2 and 1:9.3, from 2002 to 2009, respectively. We have found *T. radix* at three of our seven sites used in conjunction with Eastern Massasauga surveys and our current experimental site is now our highest yielding site for *T. radix* (1 *T. radix* to 17.6 *T. sirtalis*). Our original goal was to have some sites at KPWA with the 1:1 ratio, but this may not be the typical ratio across all of KPWA since historically that ratio was recorded at only one location. A more realistic goal is to have multiple sites with ratios of *T. radix* to *T. sirtalis* ranging from 1:1 to 1:12.2.

We are continuing to hard release neonate Plains Gartersnakes produced by the captive breeding colonies as well as monitor populations of wild Plains Gartersnakes at KPWA. The evaluation of the Plains Gartersnake population augmentation process (currently being hard releases of neonate snakes) is a work in progress just as the conservation of Plains Gartersnakes in Ohio is an ongoing project. The Plains Gartersnake has been in Ohio since at least 1931 (Conant 1951) and was likely there long before this time. It has persisted in KPWA with *T. radix* being present in 14 of 27 sites (52%) surveyed from 2001 to 2009, and more recently (2015 to 2018), three of seven sites (43%). Our goal is that viable populations of this species would remain a part of the snake community at KPWA.

MANAGEMENT PRACTICES

Current Knowledge & Understanding

From the 1970's to the mid 1990's, Killdeer Plains Wildlife Area was managed to benefit the reintroduction of Canada geese in Ohio. During this time period, extensive areas of the wildlife area were mowed for goose pasture or placed into row crop agriculture to benefit resident and migratory Canada geese. Beginning in the mid 1990's, management strategy within the Division of Wildlife shifted to more of a habitat management strategy. Killdeer Plains was designated as a grassland management area. In addition, the number of Canada geese had increased dramatically in the state which greatly decreased the need for goose management areas. Also our knowledge of Plains Gartersnakes and massasauga rattlesnakes increased greatly during this time period. Thus beginning in the mid 1990's the amount of mowing greatly decreased and is now conducted to maintain open habitats. In addition, guidelines for mowing were established to minimize effects on Plains Gartersnakes and massasauga rattlesnakes since mowing can be a mortality factor for the snakes (see Natural History).

Based upon the Biological Opinion from the USFWS for Eastern Massasauga Rattlesnakes and recommendations from Dalrymple and Reichenbach (1984) for the Plains Gartersnake, mowing operations are conducted in the following manner: If listed snakes are present, mowing should be conducted after November 1st in the fall (Johnson, 2000) and in the late winter-early spring before the soil temperature at 30 cm has exceeded that at 60 cm for 10 consecutive days (Hileman, 2016). If mowing is conducted after the soil temperature at 30 cm has exceeded that at 60 cm for 10 consecutive days in the spring and before November 1, raise mower decks to a height no lower than 9 inches and ideally keep the mower blades above 12 inches, or if shorter turf grass in administrative areas (campgrounds, roadsides, etc.) must be maintained, do so by mowing during the hottest part of mid-day when Eastern Massasaugas are least likely to be present (Clemency, 2018).

Controlled burning, which also can be a mortality factor for the Plains Gartersnake and Eastern Massasauga rattlesnake, is an essential habitat management tool for restoring and maintaining habitat for both snake species. It is used to prepare, enhance and maintain cool and warm season grass fields. Burning in snake sensitive areas is conducted after November 1st (Johnson, 2000) and before the soil temperature at 30 cm has exceeded that at 60 cm for 10 consecutive days (Hileman, 2016). Snakes should not be active during this time period therefore no restrictions on ignition technique or fire intensity are implemented. If there is concern that the snakes might be above ground, then firing techniques are utilized to facilitate the opportunity for snakes to move to safety.

OHIO CONSERVATION PARTNERS ROLES & RESPONSIBILITIES

Division of Wildlife, Ohio Department of Natural Resources

The primary role of the Division of Wildlife has been to facilitate ongoing survey, inventory, captive-rearing, release, and outreach efforts by the conservation team. In addition, the Division owns the Killdeer Plains Wildlife Area and actively manages the site for the benefit of this and other grassland-dependent species which inhabit the 9,230 acre area. In recent years, the Division has coordinated snake surveys in the spring utilizing Division staff and conservation partners to facilitate needed data collection. The Division supports the population survey work and some of the captive-rearing efforts by using revenues from the Wildlife Diversity and Endangered Species Fund or U.S. Fish and Wildlife Service State Wildlife Grants.

Westerville North High School Field Studies Class

Surveys and mark-recapture studies at selected sites were initiated in 1999 to determine the current range and population size of *T. radix* at KPWA. Telemetry has also been used extensively to assess the effectiveness of using headstarted snakes to augment *T. radix* populations at KPWA. All components of the field work have been coordinated by herpetologist Doug Wynn with the help of students from his Westerville North High School Field Studies Class.

Columbus Zoo & Aquarium and the Cleveland MetroParks Zoo

The Columbus Zoo and Aquarium initially developed the protocols for breeding and rearing Plains Gartersnakes in captivity with input and assistance from the Cleveland MetroParks Zoo and Northern Illinois University. This information has been used to produce captive-born snakes with sufficient genetic diversity for head-starting and release back into the wild. The

zoos have been active members of the conservation team and have undertaken much of the captive rearing and implantation of transmitters in headstarted snakes with little or no financial support from the Division.

Liberty University

Norman Reichenbach, Professor at Liberty University in Virginia, did research on the population biology of the Plains Gartersnake in the late 1970s at KPWA. He returned to his KPWA study site in 1998 and determined that the Plains Gartersnake population had declined significantly. He works extensively with Doug Wynn on the study design and analysis of the field and captive snake data and remains an active partner in the project.

Northern Illinois University

A population viability analysis conducted by Northern Illinois University Research Associate Kristin Stanford suggested that holding neonates (newborn) snakes in captivity for their first year (referred to as “headstarting”) would improve their survival rate when released into the wild. This new approach was then implemented at the zoos in 2004. Ms. Stanford remains active in the conservation team.

University of Tennessee

Ecologist and evolutionary biologist, Professor Gordon M. Burghardt at The University of Tennessee has worked to examine the genetic diversity of the Ohio gartersakes compared to the diversity found in the robust Midwestern population. In addition, Dr. Burghardt also conducted comparative developmental studies of neonatal Plains Gartersnakes and their potential competitor, the Common Gartersnake, in his laboratory to help determine the nature of any competition that does occur among young snakes, which are very hard to study in the field.

Ohio State University

Gregory Lipps, Amphibian & Reptile Conservation Coordinator, Ohio Biodiversity Conservation Partnership, has been involved in annual censuses of Plains Gartersnakes at KPWA and in the conservation planning for Eastern Massasauga. OSU has been testing a camera trapping system for snake detection and conducting habitat assessments throughout Ohio, including KPWA. Greg’s focus generally is in building partnerships to develop and implement conservation strategies for Ohio herps.

THE ROLE OF PRIVATE LANDOWNERS

While it is believed the entire population of the Plains Gartersnake is on the state-owned Killdeer Plains Wildlife Area, if snakes are discovered on privately-owned lands the Division of Wildlife will work with the landowners to alleviate any concerns they may have and encourage measures to conserve the species on their property.

OUTREACH AND EDUCATIONAL OPPORTUNITIES

In 1951, noted Ohio herpetologist Roger Conant stated, “We have learned only recently that this snake is a part of Ohio fauna. The presence of this western snake in the prairies of Ohio, so far east of any other known colony, would seem to constitute one of the most

remarkable examples of prairie relict yet recorded.” Even today while this species may not be well-known by the general public, it has garnered support from herpetologists and others interested in its conservation. As a result, media interest in the species remains high. The snake has been the subject of several WildOhio magazine and video program segments and has been featured in several newspaper articles. The Columbus and Cleveland Zoos have spotlighted the species in outreach programs and educational materials and the Division of Wildlife has materials available on their website. The snake and its conservation has been part of the curriculum for students who participate in the Westerville North High School Field Studies Class as well as the Ohio State University Stone Lab Herpetology Class. As additional opportunities to showcase this species arise the conservation partners will do so.

RESEARCH NEEDS

- 1) Determine *T. radix* demographics (survival rates, recapture rates, population size, ratios of *T. radix* to *T. sirtalis*) at multiple sites.
- 2) Estimate survival rates for captive born *T. radix* neonates hard released at KPWA.
- 3) Develop monitoring plan that increases the likelihood for recaptures of marked *T. radix*, potentially through increased use of “radix blitzes”, in order to estimate and detect changes in the demographic parameters noted under points 1 and 2.
- 4) Monitor *T. radix* and *T. sirtalis* at KPWA for snake fungal disease (*Ophidiomyces ophiodiicola*) using both skin swabs and scoring system (Baker et al., 2019).
- 5) Determine when *Ophidiomyces ophiodiicola* may have first been present at KPWA and compare its prevalence in *T. radix* and *T. sirtalis* using specimens and samples from specimens collected historically.
- 6) Evaluate the extent of predation by bullfrogs in KPWA on *T. radix* and *T. sirtalis*.
- 7) Survey of the Crawford and Marion County Historical Sites plus areas in KPWA that are distant from the current range for *T. radix*.
- 8) Collect habitat data on sites with and without *T. radix* at KPWA to determine key habitat parameters associated with occupied sites.
- 9) Use information under point 8 to determine potential future repatriation sites and management actions that could increase suitable habitat.
- 10) Measure genetic heterozygosity of *T. radix* at KPWA and compare to that found in 2001.
- 11) Create grass corridors between sites with *T. radix* and monitor corridor use with camera traps.
- 12) Correlate cover board monitoring of *T. radix* populations with data recorded from camera traps.

LITERATURE CITED

Akcakaya, H. R., M. A. Burgman, and L. R. Ginzburg. 1999. Applied Population Ecology. Sinauer Associates, Inc., Sunderland, Massachusetts.

Arnold, S.J. and A.F. Bennett. 1984. Behavioural variation in natural populations. 3. Antipredator display in the Garter Snake *Thamnophis radix*. Animal Behaviour 32: 1108-1118.

Baker, S., E. Hayes, M. Gramhofer, K. Stanford, S. Bailey, M. Christman, K. Conley, S. Frasca Jr, R. Ossiboff, D. Lobato, and M. Allender. 2019. Case Definition and Diagnostic Testing for Snake Fungal Disease. Herpetological Review 50:279–285.

- Ballinger, R.E., J.D. Lynch. and P.H. Cole. 1979. Distribution and natural history of amphibians and reptiles in western Nebraska with ecological notes on the herptiles of Arapaho Prairie. *Prairie Naturalist* 11:65-74.
- Bauerle, B.A. 1972. Biological productivity of snakes on the Pawnee Site, 1970-1971. U.S. IBP Grassland Biome Technical Report No. 207. Colorado State University, Fort Collins. 72p.
- Burghardt, G.M. 1967. Chemical-cue preferences of inexperienced snakes: Comparative aspects. *Science* 157:718-721.
- Burghardt, G. M. 1969. Comparative prey-attack studies in newborn snakes of the genus *Thamnophis*. *Behaviour* 33:77-114.
- Burghardt, G. M., J. Albright and G. McCracken. 2001. Genetic distinctiveness of *Thamnophis radix* in Ohio. Unpublished Report Ohio Division of Wildlife, Columbus, Ohio. 24p.
- Burghardt, G. M. and M.A.Krause. 1999. Plasticity of foraging behavior in garter snakes (*Thamnophis sirtalis*) reared on different diets. *Journal of Comparative Psychology* 113:277-285.
- Caswell, H. 2000a. Prospective and retrospective perturbation analyses: their roles in conservation biology. *Ecology* 81(3): 619-627.
- Caswell, H. 2000b. *Matrix Population Models: Construction, Analysis, and Interpretation*. Sinauer Associates, Inc. Sunderland, Massachusetts.
- Cebula, J.J. 1983. A note on the food choices of the Plains Garter Snake. *Bulletin Chicago Herpetological Society* 18:46.
- Chiszar, D., S.V. Taylor, C.W. Radcliffe, H.M. Smith, and B. O'Connell. 1981. Effects of chemical and visual stimuli upon chemosensory searching by garter snakes and rattlesnakes. *Journal of Herpetology* 15:415-424.
- Cieslak, E.S. 1945. Relations between the reproductive cycle and the pituitary gland in the snake *Thamnophis radix*. *Physiological Zoology* 18:299-329.
- Clemency L. 2018. Biological Opinion. Section 7 programmatic consultation on actions undertaken by the U.S. Fish and Wildlife Service related to habitat management for the Eastern Massasauga Rattlesnake (*Sistrurus catenatus*). Chicago, Illinois: U.S. Fish and Wildlife Service. 67p.
- Conant, R. 1938. The Reptiles of Ohio. *American Midland Naturalist* 20:1-200.
- Conant, R. 1951. *The Reptiles of Ohio*. 2nd edition. University of Notre Dame Press, Notre Dame, Indiana. 284p.

- Conant, R. and J.T. Collins. 1998. Reptiles and Amphibians: Eastern and Central North America. 3rd edition, Houghton Mifflin Co., Boston, Massachusetts. 616p.
- Conant, R., E.S. Thomas, and R.L. Rausch. 1945. The Plains Garter Snake *Thamnophis radix* in Ohio. *Copeia* 1945:61-68.
- Criddle, S. 1937. Snakes from an ant hill. *Copeia* 1937:142
- Dalrymple, G.H. and N.G. Reichenbach. 1981. Interactions between the Prairie Garter Snake (*Thamnophis radix*) and the Common Garter Snake (*T. sirtalis*) in Killdeer Plains, Wyandot County, Ohio. Ohio Biological Survey Biological Notes No. 15:244-250.
- Dalrymple, G.H. and N.G. Reichenbach. 1984. Management of an endangered species of snake in Ohio, U.S.A. *Biological Conservation* 30:195-200.
- Ditmars, R.L. 1907. The Reptile Book. Doubleday, Page and Co., New York. 472p.
- Ditmars, R.L. 1936. The Reptiles of North America. A Review of the Crocodiles, Lizards, Snakes, Turtles, and Tortoises Inhabiting the United States and Northern Mexico. Doubleday Doran and Co., New York. 476p.
- Ernst, C.H. and R.W. Barbour. 1989. Snakes of Eastern North America. George Mason University Press, Fairfax, Virginia. 282p.
- Fitch, H.S. 1941. Geographical variation in garter snakes of the species *Thamnophis sirtalis* in the Pacific Coast Region of North America. *American Midland Naturalist* 26:570-592.
- Fitzpatrick, B.M., J.S. Placyk, M. L. Neimiller, G. S. Casper and G. M. Burghardt. 2008. Distinctiveness in the face of gene flow: Hybridization between generalists and specialist gartersnakes. *Molecular Ecology* 17:4107-4117.
- Ford, N.B. and C.W. Schofield. 1984. Species specificity of sex pheromone trails in the Plains Garter Snake, *Thamnophis radix*. *Herpetologica* 40:51-55.
- Gregory, P.T. 1977. Life history observations of three species of snakes in Manitoba. *Canadian Field-Naturalist* 91:19-27
- Halloy, M. and G. M. Burghardt. 1990. Ontogeny of fish capture and ingestion in four species of garter snakes (*Thamnophis*). *Behaviour* 112:299-318.
- Herzog, H. A. Jr., B. B. Bowers and G. M. Burghardt. 1992. Development of antipredator responses in snakes: V. Species differences in ontogenetic trajectories. *Developmental Psychobiology* 25:199-211.
- Hileman, E.T. 2016. Filling in the gaps in demography, phenology, and life history of the eastern massasauga rattlesnake (*Sistrurus catenatus*). Ph.D. Dissertation, Northern Illinois University.
- Jackson, J.F., W. Ingram II and H. W. Campbell. 1976. The dorsal pigmentation pattern of

- snakes as an antipredator strategy: A multivariate approach. *American Naturalist* 110:1029-1053.
- Johnson, G. 2000. Spatial ecology of the eastern massasauga (*Sistrurus c. catenatus*) in a New York peatland. *Journal of Herpetology* 34: 186-192.
- King, R. B. and K. M. Stanford. 2006. Headstarting as a management tool: A case study of the Plains Gartersnake. *Herpetologica* 62:282-292.
- Kubie, J.L., J. Cohen and M. Halpern. 1978a. Shedding enhances the sexual attractiveness of oestradiol treated garter snakes and their untreated penmates. *Animal Behaviour* 26:562-570.
- Kubie, J.L., A. Vagvolgyi, and M. Halpern. 1978b. The roles of the vomeronasal and olfactory systems in the courtship behavior of male snakes. *Journal of Comparative and Physiological Psychology* 92:627-641.
- Kubie, J.L. and M. Halpern. 1979. Chemical senses involved in garter snake prey trialing. *Journal of Comparative and Physiological Psychology* 93:648-667.
- Mills, L. S. and M. S. Lindberg. 2002. Sensitivity Analysis to Evaluate the Consequences of Conservation Actions. Pp 338 – 366. in S.R. Bessinger and D.R. McCullough, editors. *Population Viability Analysis*. The University of Chicago Press, Chicago.
- Pope, C.G. 1944. *Amphibians and Reptiles of the Chicago Area*. Chicago Natural History Museum, Chicago, Illinois.
- Reichenbach, N.G. and G.H. Dalrymple. 1986. Energy use, life histories, and the evaluation of potential competition in two species of garter snakes. *Journal of Herpetology* 20:133-153.
- Reichenbach, N. 1998. Population assessment of the Ohio Endangered Prairie Garter Snake, *Thamnophis radix*, in Killdeer Plains Wildlife Area, Final Report submitted to Ohio Department of Natural Resources, Sept 1998.
- Reichenbach, N., M. Barrie, K. Becka, G. Burghardt, S. Butterworth, C. Caldwell, F. Dierkes, P. Johantgen, K. Stanford and D. Wynn. 2019. Ohio Conservation Plan: Plains Gartersnake, *Thamnophis radix*. Ohio Division of Wildlife, Columbus, Ohio, 27p.
- Ross, P. and D. Crews. 1977. Influence of the seminal plug on mating behaviour in the garter snake. *Nature* 267:344-345.
- Rossman, D.A., N.B. Ford, and R.A. Seigel. 1996. *The Garter Snakes: Evolution and Ecology*. University of Oklahoma Press, Norman. 332p.
- Seibert, H.C. 1950. Population density of snakes in an area near Chicago. *Copeia* 1950:229-230.
- Seibert, H.C. and C.W. Hagen, Jr. 1947. Studies on a population of snakes in Illinois. *Copeia* 1947:6-22.

- Seigel, R.A. and H.S. Fitch. 1985. Annual variation in reproduction in snakes in a fluctuating environment. *Journal of Animal Ecology* 54:497-505.
- Smith, P.W. 1957. An analysis of post-Wisconsin biogeography of the Prairie Peninsula region based on distributional phenomena among terrestrial vertebrate populations. *Ecology* 38:205-218.
- Smith, P.W. 1961. The amphibians and reptiles of Illinois. *Illinois Natural History Survey Bulletin* 28:1- 298.
- Stanford, K.M. 2002. Demography and life history of an urban population of Plains Garter Snakes, *Thamnophis radix*. Unpublished Master's Thesis, Northern Illinois University, Dekalb.
- Stanford, K.M. 2003. Population viability analysis of the eastern plains garter snake (*Thamnophis radix*) at Killdeer Plains Wildlife Area, Wyandot County, Ohio. Unpublished report of the Ohio Department of Natural Resources, Division of Wildlife.
- Stanford, K.M. and R.B. King. 2004. Growth, survival and reproduction in a Northern Illinois population of the Plains Gartersnake, *Thamnophis radix*. *Copeia* 2004:465-478.
- Transeau, E. N. 1935. The prairie peninsula. *Ecology* 16:423-437.
- Walley, H.D., T.L. Wusterbarth and K.M. Stanford. 2003. *Thamnophis radix*. *Catalogue of American Amphibians and Reptiles* 779.1-779.13.
- Wright, A.H. and A.A. Wright. 1957. *Handbook of snakes of the United States and Canada*. Comstock, Ithaca, New York. 1105p.
- Wynn, D. 1995. Biodiversity Survey Model: Killdeer Plains Wildlife Area. Unpublished report of the Ohio Department of Natural Resources, Division of Wildlife.
- Wynn, D. 1996. Biodiversity Survey Model: Killdeer Plains Wildlife Area. Unpublished report of the Ohio Department of Natural Resources, Division of Wildlife.
- Wynn, D. 1997. Biodiversity Survey Model: Killdeer Plains Wildlife Area. Unpublished report of the Ohio Department of Natural Resources, Division of Wildlife.
- Wynn, D. and N. Reichenbach. 1999. A conservation program for the endangered eastern plains garter snake (*Thamnophis radix*) at Killdeer Plains Wildlife Area, Ohio. Unpublished report of the Ohio Department of Natural Resources, Division of Wildlife.
- Wynn, D. and N. Reichenbach. 2001. A continuation of the eastern plains garter snake (*Thamnophis radix*) restoration program at Killdeer Plains Wildlife Area, Wyandot County, Ohio. Unpublished report of the Ohio Department of Natural Resources, Division of Wildlife.
- Wynn, D. and N. Reichenbach. 2002. A continuation of the eastern plains garter snake

(*Thamnophis radix*) restoration program at Killdeer Plains Wildlife Area, Wyandot County, Ohio. Unpublished report of the Ohio Department of Natural Resources, Division of Wildlife.

Wynn, D. and N. Reichenbach. 2015. A continuation of the eastern plains garter snake (*Thamnophis radix*) restoration program at Killdeer Plains Wildlife Area, Wyandot County, Ohio. Unpublished report of the Ohio Department of Natural Resources, Division of Wildlife.

Wynn, D. and N. Reichenbach. 2016. A continuation of the eastern plains garter snake (*Thamnophis radix*) restoration program at Killdeer Plains Wildlife Area, Wyandot County, Ohio. Unpublished report of the Ohio Department of Natural Resources, Division of Wildlife.

Wynn, D. and N. Reichenbach. 2017. A continuation of the eastern plains garter snake (*Thamnophis radix*) restoration program at Killdeer Plains Wildlife Area, Wyandot County, Ohio. Unpublished report of the Ohio Department of Natural Resources, Division of Wildlife.

Wynn, D. and N. Reichenbach. 2018. A continuation of the eastern plains garter snake (*Thamnophis radix*) restoration program at Killdeer Plains Wildlife Area, Wyandot County, Ohio. Unpublished report of the Ohio Department of Natural Resources, Division of Wildlife.

Yaussy, N. 2003. Road mortality of snakes in Killdeer Plains Wildlife Area. Unpublished report of the Ohio Department of Natural Resources, Division of Wildlife

Yeager, C. and G. M. Burghardt. 1991. The effect of food competition on aggregation: Evidence for social recognition in the plains garter snake (*Thamnophis radix*). *Journal of Comparative Psychology* 105:380-86.

PLAN DEVELOPMENT AND SECTION AUTHORS

This plan was developed and written by the following partners for this conservation endeavor:

Division of Wildlife, Ohio Department of Natural Resources

Carolyn Caldwell and Kate Parsons coauthored the following sections: Introduction/Purpose of the Plan, State Status, Ohio Conservation Partners Roles & Responsibilities, The Role of Private Landowners, and Outreach and Educational Opportunities.

Fred Dierkes, Scott Butterworth and Kate Parsons coauthored the Management Practices section.

Westerville North High School Field Studies Class

Doug Wynn coauthored the following sections: Historical & Current Ohio Distribution, Reproductive output of captive breeding colonies and Conservation: Population Ecology and Augmentation.

Columbus Zoo & Aquarium and the Cleveland MetroParks Zoo

Tara Archer, Michael Barrie, Kristy Becka, and Peter Johantgen coauthored the section on Captive *T. radix* Colonies at Cleveland Metroparks Zoo and Columbus Zoo and Aquarium. Others who have contributed greatly to the captive breeding program at these zoos include Dan Badgley, Becky Ellsworth and Hugh Quinn.

Liberty University

Norman Reichenbach was the editor of this plan and authored the following sections: Description/Taxonomy, U.S. Distribution, and Natural History. He also coauthored the following sections: Historical & Current Ohio Distribution, Reproductive output of captive breeding colonies and Conservation: Population Ecology and Augmentation.

Northern Illinois University

Kristin Stanford authored the section on Population Viability Analysis for *Thamnophis radix* at Killdeer Plains.

University of Tennessee

Gordon Burghardt authored the section on the Genetic Distinctiveness of *T. radix* in Ohio.

Ohio State University

Gregory Lipps contributed to the Research Needs section.

Citation format

Reichenbach, N., T. Archer, M. Barrie, K. Becka, G. Burghardt, S. Butterworth, C. Caldwell, F. Dierkes, P. Johantgen, G. Lipps, K. Parsons, K. Stanford and D. Wynn. 2019. Ohio Conservation Plan: Plains Gartersnake, *Thamnophis radix*, Revised 2019. Ohio Division of Wildlife, Columbus, Ohio, 44p.