
EXPERIMENTAL EVALUATION OF CAPTIVE-REARING PRACTICES TO IMPROVE SUCCESS OF SNAKE REINTRODUCTIONS

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Abstract.—The use of captive animals for population re-establishment or augmentation can be an important part of conservation efforts, but practitioners need experimentally derived evidence to guide the best strategies and inform whether such practices could be successful. Here, we examined how several manipulations to captive-rearing practices influence the performance of the Common Watersnake, *Nerodia sipedon sipedon*, during their first year in the wild. Following release, snakes that had experienced a period of enrichment during captivity to better simulate natural environments did not differ from conspecifics reared in more simplistic conditions on any measure of post-release behavior or performance. Moreover, captive snakes in both treatments exhibited habitat use, movement, thermoregulatory, and seasonal activity behaviors largely indistinguishable from resident conspecifics at the release site, and ultimately performed similarly in maintenance of body condition and survivorship. These results are in contrast to earlier releases and suggest that using older and larger individuals that have undergone a period of simulated winter dormancy may improve success during the early phase of establishment. However, captive snakes grew only one third as fast as wild native snakes, suggesting they experienced difficulties foraging in the wild. Further studies testing the effectiveness of translocation programs using captive animals as a management tool are urgently needed, but our findings do point to some success.

Key Words.—captive breeding; Common Watersnake; enrichment; head-starting; repatriation; restoration; *Nerodia sipedon*; translocation

INTRODUCTION

Many efforts have been made to remedy biodiversity declines, including the restoration of habitats to support the rebound of local biota. However, the return of target species to restored patches may not easily or quickly follow, as recolonization depends upon several factors including proximity to remnant populations, the dispersal capacity of the species, and the suitability of intervening habitats (MacArthur and Wilson 1963; Fahrig and Merriam 1994). When natural recolonization is not likely to occur at the desired pace, or is constrained by barriers, conservation professionals may deliberately translocate animals from remnant locations for population re-establishment or augmentation (IUCN 2012). The need for immediate action has accelerated the practice of moving animals for conservation and management purposes in recent years (Seddon et al. 2007), often before even preliminary examinations of its viability as a management tool (Fisher and Lindenmayer 2000; Seddon et al. 2007; Armstrong and Seddon 2008). Due to low success rates, high expenditures, risks of disease transmission, and failure to specifically address the causes of population declines, such manipulative practices are seen as somewhat controversial and in need of rigorous experimental testing to inform such management decisions (Griffith et al. 1989; Dodd and

Seigel 1991; Viggers et al. 1993; Ricciardi and Simberloff 2009).

Wetland habitats and associated fauna are among the most threatened ecosystems in the world (Richter et al. 1997). The strong selection pressures that wetlands impose on biota may severely limit the natural recolonization potential to restored sites. Aquatic reptiles face significant mechanical and physiological challenges to overland movements (Seymour 1982), and even those that are capable of more extensive terrestrial habitat use and dispersal typically maintain associations with a familiar complex of wetlands in a relatively small area (Roe et al. 2003; 2004; Roe and Georges 2007). Aquatic reptiles thus present unique challenges for population recovery, making them a potential candidate for translocations due to these isolating mechanisms. Several wetland-dependent snakes have undergone extensive range contractions and population declines, including Midwestern populations of the Plain-bellied Watersnake, *Nerodia erythrogaster* (Attum et al. 2009; Roe et al. 2013). However, little research has been conducted to test the effectiveness of translocation strategies in snakes to inform such conservation efforts (Kingsbury and Attum 2009). Most commonly, animals are simply captured from one site and immediately released into another (Madsen et al. 1999; Reinert and Rupert 1999; Butler et al. 2005), but several

manipulations may improve the likelihood of establishment, some of which rely upon captive bred source animals (Griffiths and Pavajeau 2008).

Captive breeding programs offer many potential advantages over direct translocation from the wild, including the ability to raise large stocks for release at target times and life stages while limiting stress on donor populations. The practice of head-starting, where captives are maintained at accelerated growth rates throughout an early part of their life cycle, can yield individuals of large size that are presumably less vulnerable to predation, closer to reproductive maturity, and thus more likely of establishing a population (Pritchard 1979). Reptiles are generally well-suited for captive programs due to their high fecundity and relative ease of maintenance (King and Stanford 2006; Germano and Bishop 2009; Santos et al. 2009). However, to be successful, released animals must demonstrate competency in the wild such that they can select appropriate resources, respond correctly to seasonal environmental cues, avoid predators, and ultimately grow, survive, and reproduce (Alberts 2007). Captive-reared animals generally perform poorly following release in the wild (Griffith et al. 1998), which could in part be linked to conditions experienced in captivity (Almi and Burghardt 2006; Aubret et al. 2007; Aubret and Shine 2008; DeGregario et al. 2013). The few published studies that have examined performance of captive-reared snakes in the wild have given mixed results on whether such a program could prove successful (King et al. 2004; King and Stanford 2006; Roe et al. 2010; Harvey et al. 2014; Sacerdote-Velat et al. 2014), suggesting that success may depend upon species, captive husbandry methods, release timing, release site suitability, or interactions among these or other factors.

Here, we examine how variation in translocation strategies using captive stocks influences behavior and numerous performance endpoints in the Common Watersnake, *Nerodia sipedon sipedon*. In a previous study, we found captive-reared *N. s. sipedon* not given enriched environments behaved abnormally and suffered poor growth and survivorship in the wild (Roe et al. 2010). The present study was designed to test whether enrichment of captive rearing conditions to better mimic natural environments improves their success in the wild, using resident snakes at the release site as a benchmark for comparison. It is not our intention to gauge the ultimate long-term success of a repatriation attempt (Seddon 1999), but instead to test individual responses in the early stages (up to one year) following release. By elucidating potential causal mechanisms behind successes or failures in the early stages of establishment using a surrogate species, such experimental studies can inform decisions on how to best proceed with actual repatriation attempts for species of conservation concern.

MATERIALS AND METHODS

Study site.—We conducted the study from July 2007 to June 2010 in northeast Indiana, USA, with field components at Douglas Woods Nature Reserve managed by The Nature Conservancy. The reserve encompasses approximately 500 ha of mixed terrestrial and aquatic habitats, including numerous lentic wetlands and lotic riverine habitats. The core terrestrial habitat is hardwood forest with several peripheral areas replanted with trees for reforestation. Isolated wetlands are interspersed throughout the core forested area and replantings, several of which have been recently created or restored in ongoing restoration efforts (Roe et al. 2010).

For the purposes of quantifying habitat use patterns, we divided aquatic macrohabitats into three classes: (1) open wetland: lentic waterbodies with mostly open canopy dominated by herbaceous emergent vegetation; (2) forested wetland: lentic waterbodies dominated by trees and/or shrubs; and (3) river: an area of flowing water confined to the channel. Analogously, we divided upland habitats into two classes including upland forest, which were areas dominated by tree canopy cover of primarily mixed hardwood forests, and shrubland, which were open areas dominated by grasses, forbs, or shrubs. We digitized habitat maps from aerial photographs using ArcMap 10.1 (ESRI 2012).

Capture, maintenance, and radiotransmitter implantation.—In July 2007, we captured seven pregnant *N. s. sipedon* from a site approximately 5 km north of the study area. Snakes gave birth in the laboratory at Indiana-Purdue University in Fort Wayne, Indiana. We retained 60 of the neonates (born 9–17 August 2007) for captivity. For their first 18 mo, we housed captive snakes individually in small plastic tubs (20 × 65 × 13 cm) stored on a metal shelving rack with access to a hide plate and water bowl (Fig. 1). A DBS – 1000 digital thermostat (Helix Control Systems, Vista, California, USA) maintained heat tape at about 30° C from below at one end of the tubs, room temperature was 25° C, relative humidity typically remained between 30 and 60%, and lights were on a 12L:12D cycle. We fed snakes several live Fathead Minnows (*Pimephales promelas*) 2–3 times per week by placing fish in the water bowls with an aquarium net, and we cleaned tubs once per week. During the first winter, we kept snakes active under the conditions described above. During the second winter (November 2008–February 2009), we did not feed snakes and we maintained room temperature at 12–15° C with the heat tape turned off, and we cleaned tubs and refilled water as needed.

Starting 15 February 2009, we turned heat plates on and resumed normal husbandry protocols. We then

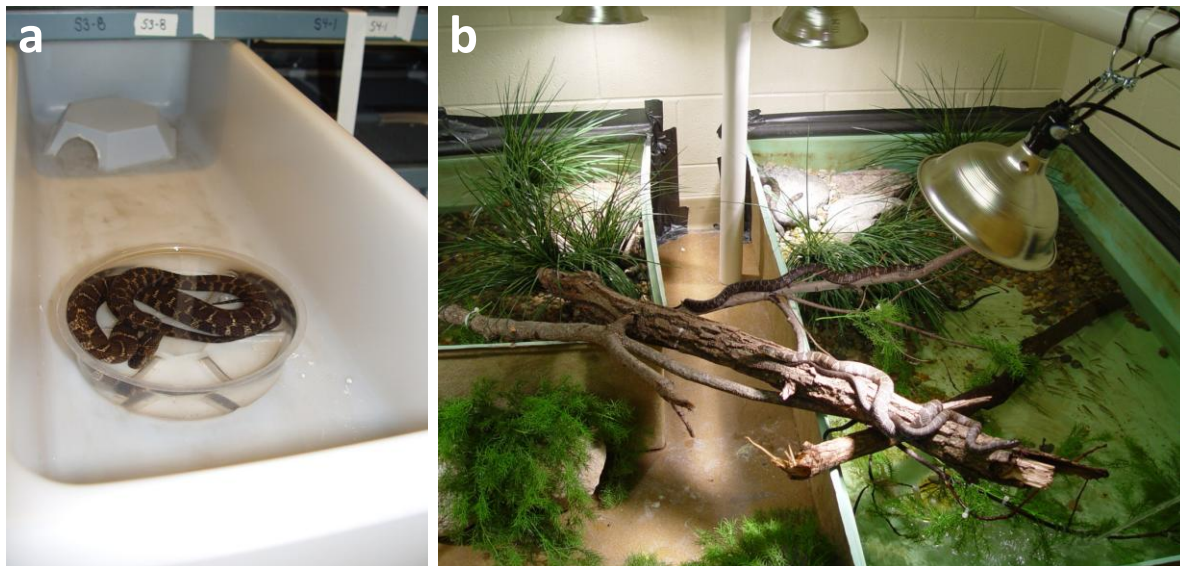


FIGURE 1. Captive conditions under which juvenile *Nerodia sipedon sipedon* were raised included (a) individual tubs with hidebox and water bowl, and (b) communal tanks enriched with conditions to better mimic complex natural environments. (Photographed by John Roe).

randomly divided captive snakes into two groups, one to remain in the simplistic tubs (tub snakes, hereafter), and the other to be raised in more naturalistic conditions (enriched snakes, hereafter) for the remaining four months of captivity. The enriched enclosures consisted of two tanks ($90 \times 170 \times 30$ cm) filled with shallow water to a depth of 14 cm. The tanks included plastic aquarium plants, rocks, branches, numerous overhead basking lamps, and were stocked with Fathead Minnows at all times (Fig. 1). The enriched housing provided an environment where snakes could swim, thermoregulate by basking, and actively forage at all times of day among naturalistic structures. Room conditions (temperature, humidity, and light cycle) were similar to the snakes raised in simplistic tubs.

At the end of the 22-mo captive period, we systematically selected individuals for release in the wild to minimize initial size and sex ratio variation between groups, resulting in 12 (seven males and five non-pregnant females, with representatives from six of the seven adult females) snakes from the tub treatment and 10 (four males and six pregnant females, with representatives from five of the seven adult females) from the enriched treatment. The smaller sample size for the enriched group is a consequence of wide size variation among individuals that likely arose from competition in the communal tanks, leaving fewer snakes of comparable size to the tub treatment. Additionally, we captured 15 (six pregnant females, six non-pregnant females, three males) native snakes (resident snakes, hereafter) from the release site. Of the 15 resident snakes, we tracked eight in the 2008–2009 season only, three in the 2009–2010 season only, and

four during both seasons (2008–2010). We surgically implanted radio-transmitters (models SB-2T and SI-2T, 5–9 g; Holohil Systems Ltd., Carp, Ontario, Canada) while snakes were under anesthesia using a technique described in Roe et al. (2003). After a one week recovery period in the lab, we released resident snakes at their point of capture, along-side an approximately equivalent number of snakes from the captive-reared groups. Size at release was 51.2 ± 3.7 cm (mean \pm standard deviation) snout-to-vent length (SVL) for tub snakes, 52.3 ± 6.8 cm SVL for enriched snakes, and 55.8 ± 7.1 cm SVL for resident snakes.

Field data collection.—Using radiotelemetry (Yagi antenna and R-1000 receiver, Communications Specialists Inc., Orange, California, USA), we located snakes at least once per week from May to September, every two weeks in October and November, once per month from December to February, and again at two-week intervals in March and April. At each location, we determined coordinate position using GPS and plotted these on habitat maps using ArcMap 10.1. We also attempted to make a visual observation to confirm status as alive, injured, or dead. If a snake was confirmed dead after an extended period without visual confirmation in the same location, we presumed it had been dead since the last confirmed visual observation or movement.

We assessed several variables to describe the behavior and performance of each snake. We calculated the sizes of 100% minimum convex polygons (MCP) using all active season locations and one overwintering location of ArcMap 10.1. We measured movements as the

Euclidian distance between two sequential locations and transformed distance into estimated daily rates. We counted the number of distinct wetlands used by each individual, as well as the frequency of off-site excursions, defined as any movement outside of the nature reserve borders. We recorded and converted transmitter pulses to temperature using calibrations provided by the manufacturer. We estimated the date of overwintering ingress as the final day of confirmed surface activity in autumn, and overwintering egress as the first date of known spring surface activity. We periodically captured and measured (SVL and mass) snakes using fabric tape and an electronic balance. Although the fabric tape was not checked against standard measurement instruments, the same tape was used to measure all snakes throughout the study so that all treatments were subject to the same potential error.

Data analyses.—We performed statistical analyses with SPSS 17.0 (SPSS 2007) and the program MARK 5.0 (White and Burnham 1999). Where appropriate, we examined the assumptions of homogeneity of variances and normality and when data failed to meet assumptions, they were transformed to approximate normal distributions or equal variances. If transformation did not resolve assumption violations, appropriate non-parametric tests were used. We used an $\alpha = 0.05$ unless otherwise stated. The Bonferroni method was used to adjust statistical significance for multiple-related comparisons to reduce the probability of Type 1 errors. Values are reported as mean \pm SE unless otherwise stated. To bolster sample sizes, we pooled sexes and reproductive status within treatment groups, and included resident snakes from both 2008–2009 and 2009–2010 seasons in the analyses. For snakes tracked in both seasons, we used only the 2009–2010 season in analyses.

We used a multivariate analysis of covariance (MANCOVA) to test for the effects of treatment group on movement rate (\log_{10} m/day) and size of area used (\log_{10} MCP), with \log_{10} SVL and number of radio-fixes as covariates. We used analysis of covariance (ANCOVA) to examine differences among treatments in the number of wetlands used, with \log_{10} SVL and number of radio-fixes as covariates. We examined variation among groups in dates of overwintering ingress and egress using a Kruskal-Wallis test, using Julian day counted from 1 January as the dependent variable.

We used Euclidian distance analysis to investigate habitat use and selection. For each snake location, we measured the nearest distance to each habitat type. Additionally, we generated 1000 random points within the study area, defined as an MCP encompassing locations for snakes in all treatment groups and years, and measured nearest distances to each macrohabitat type. For each snake, we calculated the mean distances

to each habitat (u_i) and divided these by mean distances to each habitat type from random points (r_i) for active (April–August) and inactive (September–March) seasons. To assess variation in habitat use, we used MANOVA to compare distance ratios (u_i/r_i) among treatment groups within seasons. To assess habitat selection within each treatment, we compared distance ratios to a matrix of the value one to assess if habitats were selected ($u_i/r_i < 1$), avoided ($u_i/r_i > 1$), or used randomly ($u_i/r_i = 1$; Conner et al. 2003).

To assess differences in body temperature (T_b) among treatments, we compiled T_b measures into monthly mean values for each individual. We then used ANCOVA to test for the effects of treatment on T_b for each month, with mean monthly time of day of location as a covariate. The Bonferroni adjusted level of significance for this series of T_b tests was 0.005. To test for differences in body condition, we compared body condition indices (BCI) among groups at time of release and upon final capture using the method described by Beaupre and Douglas (2009). This involved calculating the residuals of the regression between body mass and SVL of all snakes to estimate BCI, followed by a comparison of residuals among treatments using analysis of variance (ANOVA). To compare growth rates among treatments, we used ANCOVA with change in length (\log_{10} cm/day) as the dependent variable and \log_{10} initial SVL as the covariate.

We estimated survival probabilities using known-fate models in the program MARK, using Akaike Information Criterion (AIC) to rank candidate models. We considered models as having support if AIC values were < 2.0 . We started with a fully-saturated model in which survival probability was dependent on treatment group, time interval, and their interaction, and then proceeded to compare a series of reduced-parameter models. We set time intervals as bi-weekly during the active season and condensed into a single period during overwintering, defined as the time between the final date of autumn ingress and first spring emergence of resident snakes. We included initial SVL as a covariate in all models, and report model averages.

RESULTS

Behavior and activity.—Movement rates and size of area traversed were similar among treatment groups, positively correlated with number of radio-fixes, but independent of body size (treatment: $F_{4,56} = 1.31$, $P = 0.279$; radio-fixes: $F_{4,56} = 10.96$, $P < 0.001$; svl: $F_{2,28} = 2.78$, $P = 0.080$; Table 1, Fig. 2). Individuals of each treatment group typically associated with multiple wetlands, but treatment group and body size were not significant (treatment: $F_{2,29} = 0.34$, $P = 0.718$; svl: $F_{1,29} = 0.288$, $P = 0.596$; Table 1), though number of wetlands used did increase with tracking duration (radio-fixes:

TABLE 1. Movement and spatial variables for resident *Nerodia sipedon sipedon* compared to individuals raised in captivity in simplistic tubs or enriched conditions before release into the wild.

Group	n	Area used MCP ^a (ha)		Wetlands used (n)		Movement distance (m/d)	
		Mean ± 1SE	Range	Mean ± 1SE	Range	Mean ± 1SE	Range
Tub captive	12	2.8 ± 0.6	0.2 – 6.7	2.6 ± 0.2	1 – 3	10.8 ± 1.6	3.5 – 18.3
Enriched captive	10	3.5 ± 1.5	0.1 – 12.4	2.4 ± 0.5	1 – 5	14.0 ± 6.2	4.0 – 68.1
Resident	15	4.7 ± 1.2	0.1 – 15.3	2.3 ± 0.3	1 – 4	15.5 ± 2.7	3.4 – 40.6

^aMinimum convex polygon

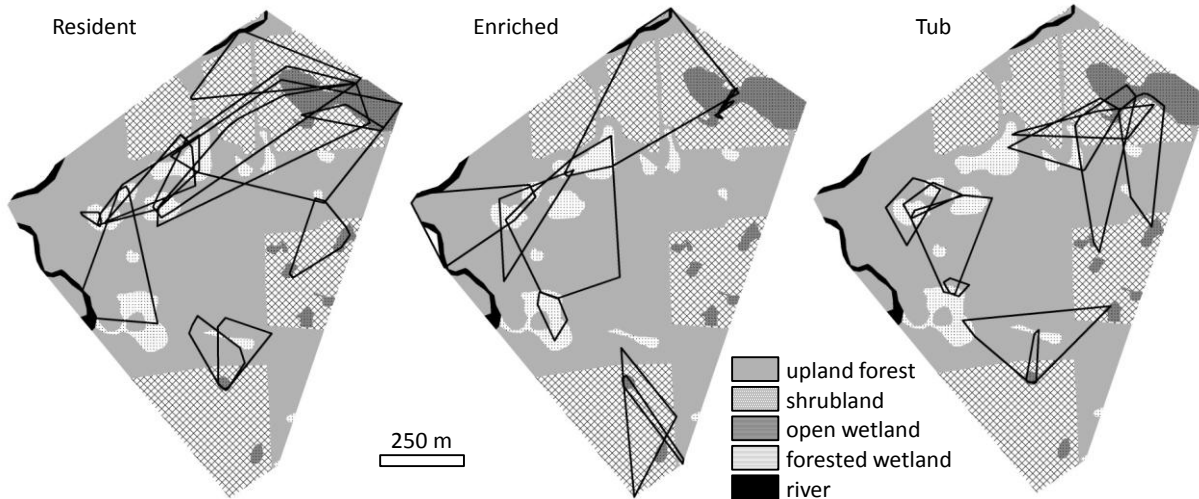


FIGURE 2. Areas used (100% minimum convex polygons) by free-ranging native *Nerodia sipedon sipedon* compared to those released into the wild after being raised in simplistic tubs or enriched conditions during captivity.

$F_{1,29} = 11.86, P = 0.002$). Snakes in all treatment groups remained within the boundaries of the nature reserve.

Treatment groups exhibited largely similar habitat use to one another in both active and inactive seasons (active season: $F_{10,56} = 1.24, P = 0.287$; inactive season: $F_{10,46} = 0.98, P = 0.471$; Fig. 3). However, examination of habitat selection for each treatment group independently revealed some differences among groups in selection and avoidance of particular habitat types. For the active season, tub snakes avoided upland forest and selected forested wetlands ($F_{1,22} = 4.53, P = 0.045$; $F_{1,22} = 8.57, P = 0.008$). Resident snakes avoided upland forest ($F_{1,26} = 7.82, P = 0.010$) but used all other habitats according to availability ($F_{1,26} < 3.35, P > 0.079$). Enriched snakes did not exhibit strong selection or avoidance of any habitats ($F_{1,16} < 3.94, P > 0.065$). During the inactive season, tub and resident snakes selected forested wetlands ($F_{1,22} = 111.44, P < 0.001$; $F_{1,20} = 62.88, P < 0.001$), enriched snakes selected upland forest ($F_{1,12} = 27.11, P = 0.001$), but all other habitats were used in accordance with their availability for the three treatment groups (tub: $F_{1,22} < 2.17, P > 0.155$; enriched: $F_{1,12} < 1.76, P > 0.210$; resident: $F_{1,20} < 2.05, P > 0.168$).

Treatment groups exhibited similar overall patterns in seasonal T_b fluctuation (Fig. 4). The only differences in T_b among groups were confined to the first month post-release, when tub snakes had T_b approximately $3^\circ C$ lower than enriched and resident groups in July ($F_{2,39} = 5.49, P = 0.008$). Snakes were not tracked with sufficient frequency between November and March to document variation in overwintering temperatures.

Tub snakes ($n = 10$) entered overwintering retreats on 15 October ± 6.0 d, followed by enriched snakes ($n = 7$) on 26 October ± 7.4 d, and resident snakes ($n = 9$) on 5 November ± 2.5 d. All tub and enriched snakes that entered overwintering emerged alive, whereas two residents never emerged and were presumed dead. The date of hibernation egress for residents was 19 March ± 0.5 d, followed by enriched and tub snakes on 25 March ± 3.6 d and 30 March ± 3.8 d, respectively. There was no significant difference in the dates of overwintering ingress or spring egress among treatment groups ($\chi^2 = 4.57, df = 5, P = 0.470$).

Performance.—Initial BCIs (upon release) were similar among treatment groups ($F_{2,34} = 0.976, P =$

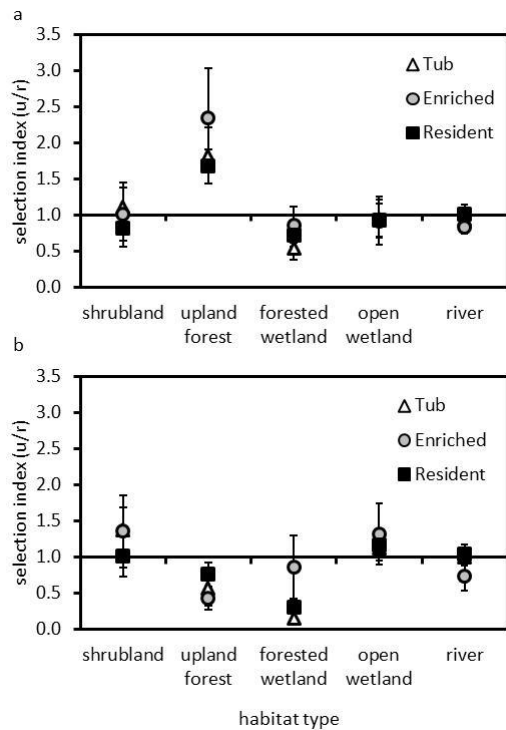


FIGURE 3. Mean macrohabitat selection indices for free-ranging *Nerodia sipedon sipedon* following lab rearing in simplistic tubs or more complex enriched conditions compared to wild native snakes during a) active (April-August) and b) inactive (September-March) seasons. Note that indices (u/r) < 1 indicate preference, > 1 indicate avoidance, and = 1 indicate random use of habitats. Values are mean (symbols) ± 1 SE error bars.

0.387). We were able to recapture twelve resident, eleven tub, and seven enriched snakes for growth measurements. Growth rates measured as change in SVL were positive for all groups, but highest for residents compared to both captive-reared groups ($F_{2,24} = 3.83$, $P = 0.036$; Fig. 5), with snakes from tub and enriched groups growing 0–0.049 cm/d and 0–0.047 cm/d, respectively, and residents 0–0.111 cm/d. Initial SVL was not correlated with growth rates for the size range of animals examined ($F_{1,24} = 0.47$, $P = 0.830$). BCIs at final capture were similar among treatment groups ($F_{2,28} = 0.692$, $P = 0.509$).

Survival probability did not vary over time intervals or among treatment groups (Fig. 6, Table 2). Annual survival rate was 46.2% (34.4–61.6%) for resident snakes, 63.5% (50.4–79.6%) for tub snakes, and 50.2% (35.5–70.7%) for enriched snakes. Seven snakes were depredated (mammals and snapping turtles), two died during winter, and the cause of death was undetermined for the remaining two individuals.

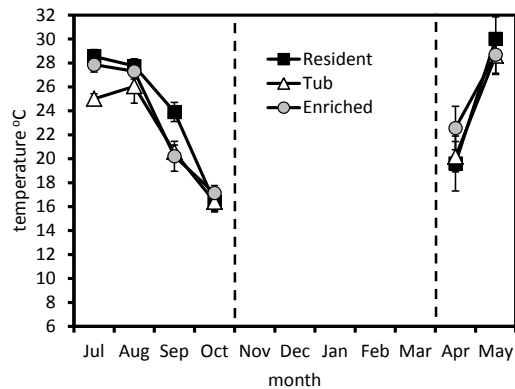


FIGURE 4. Monthly day-time body temperatures (T_b) of free-ranging resident *Nerodia sipedon sipedon* compared to those released into the wild after being raised in simplistic tubs or enriched conditions during captivity. The dashed lines indicate the approximate overwintering period when temperatures were not measured. Values are mean (symbols) ± 1 SE error bars.

DISCUSSION

The value of captive-reared animals as sources for translocation depends on the performance of animals following release, yet few studies have examined the mechanisms responsible for their success or failure in snakes. Comparisons across studies reveal variation in the performance of captive-reared snakes following release, making it difficult to generalize about the potential of this practice as a management tool (King et al. 2004; King and Stanford 2006; Roe et al. 2010; Harvey et al. 2014; Sacerdote-Velat et al. 2014). We were able to compare the responses of snakes kept under different captive conditions in the early stages of release while controlling for several potentially confounding sources of variation, including species, site of origin, release site quality, and release methodology. To our knowledge, this is the first experimental examination of snake responses to enrichment of captive environments following release in the wild.

The differing conditions experienced in captivity did not significantly influence responses of snakes up to one year following release. Individuals raised in enriched conditions exhibited habitat use, movement patterns, and seasonal activity behaviors largely indistinguishable from those reared in simplistic un-enriched environments, and ultimately performed similarly in growth, body condition, and survivorship. Tub captives had lower body temperatures in the first month post-release, but attained similar body temperatures to enriched snakes for every month afterwards. As T_b measures were constrained to point sampling at telemetry observations, our ability to compare thermal biology among treatments is limited (Taylor et al. 2004).

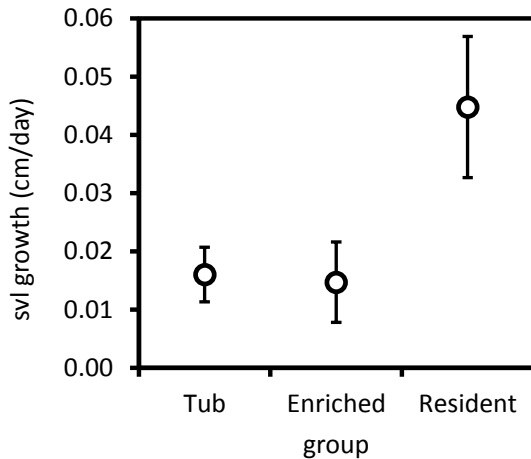


FIGURE 5. Growth rates for free-ranging resident *Nerodia sipedon* ($n = 12$) compared to those released into the wild after being raised in simplistic tubs ($n = 11$) or enriched ($n = 7$) conditions during captivity. Values are mean (symbols) ± 1 SE error bars.

However, sampling methods were consistent among treatments and our measures were within the preferred T_b range of 25–30° C for *N. s. sipedon* (Brown and Weatherhead 2000) during most active season months (May–August). Snakes raised in captive conditions can maintain several behavioral and physiological capacities similar to wild counterparts (Marmie et al. 1990; Chiszar et al. 1993), but may also develop differences in habitat preference, locomotor performance, exploration, learning, and foraging depending upon captive environments (Almli and Burghardt 2006; Aubret et al. 2007; Aubret and Shine 2008; DeGregorio et al. 2013). A potential reason for the lack of differences between captive treatments here is the relatively short duration of enrichment. Snakes in both captive groups experienced un-enriched environments for the first 18 mo, giving the enriched group only the final four months experience in a more naturalistic captive environment. Important avenues of development may have already occurred such that any phenotypically plastic traits could not be further modified (Herzog et al 1992; Madsen and Shine 2000; Aubret and Shine 2008). Future studies should initiate manipulations in captive environments at an earlier life stage (perhaps even from birth or hatching) to more fully explore the role of environmental enrichment on post-release behavior and performance.

Regardless of whether captive environments measurably shape the phenotype of *N. s. sipedon*, snakes from both captive environments behaved and exhibited similar survivorship to wild-caught natives. These results are largely in contrast to our previous findings, where captive-reared *N. s. sipedon* released at the same site exhibited abnormal habitat use, reduced movement

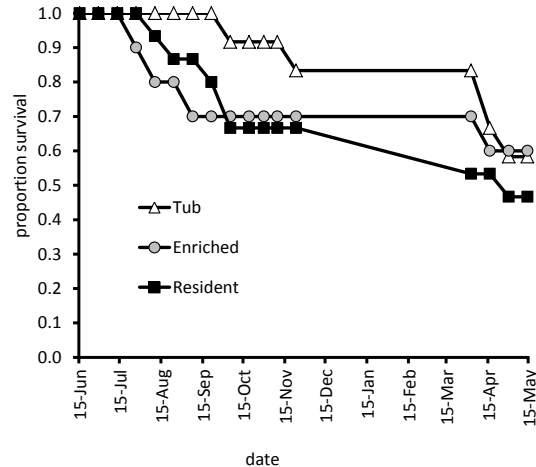


FIGURE 6. Proportion of resident *Nerodia sipedon* surviving over time compared to those released into the wild after being raised in simplistic tubs or enriched conditions during captivity. Snake status (alive or dead) was assessed at bi-weekly intervals except during the overwintering period (November–March), when status could not be consistently confirmed for snakes in underground retreats. Note that survivorship values are not derived from our estimates generated by known-fate models in the program MARK.

and activity, inability to maintain preferred body temperature, early spring emergence from overwintering refuges, and ultimately low survivorship (Roe et al. 2010). There are several potential explanations for the differences observed between studies. First, captive snakes in the current study (second release cohort, hereafter) were one year older and larger (+ 4.4 cm SVL and + 33.6 g) than those from the prior study (first release cohort, hereafter). While we have little knowledge of ontogenetic and size-related shifts in behavior in wild *N. s. sipedon*, survivorship increases with age and size up to maturity at 2–4 y, or about 55 cm SVL (Brown and Weatherhead 1999). Annual survivorship estimates in the wild for captive *N. s. sipedon* in the second release cohort were 3–4 times higher than the first release cohort, and within the range reported for similar-sized *N. s. sipedon* elsewhere (Brown and Weatherhead 1999). King and Stanford (2006) also found an age and size effect on survivorship in *Thamnophis radix*, which suggests head-starting programs may benefit from releasing animals of older age and/or larger size. However, the optimum timing of release will likely vary by life-history traits (i.e., growth rates, age and size of maturity, survivorship curves; Parker and Plummer 1987) and need to be determined on a species-specific basis.

Another possibility for the conflicting results between the first and second release cohorts involves the inadvertent selection of individuals that performed well in captivity, but that were not well suited for survival in

TABLE 2. Models of survivorship probability (S) for resident *Nerodia sipedon sipedon* compared to individuals captive-reared in simplistic tubs or enriched conditions prior to release. All models include initial body size (snout-to-vent length) as a covariate.

Model	AICc	Δ AICc	Weight	n	Deviance
$S(\cdot)$	142.9	0.0	0.84	2	138.9
$S(\text{treatment})$	146.2	3.3	0.16	4	138.1
$S(\text{time})$	157.3	14.4	0.00	18	119.8
$S(\text{treatment} \times \text{time})$	215.5	72.6	0.00	52	98.6

the wild. Due to constraints of transmitter size, captive individuals in the first release cohort (Roe et al. 2010) were selected in part for their larger body size and healthy condition, but traits advantageous in captivity may not translate to success in the wild. For instance, animal temperament traits such as exploration, boldness, tameness, activity, and aggression vary among individuals and may influence responses to captivity (McDougall et al. 2006), including in snakes (Burghardt and Lane 1995; Chiszar et al. 1995). Captive environments and husbandry practices can lead to the unintended and rapid artificial selection for traits potentially maladaptive in the wild in a population maintained in captivity for multiple generations (Heath et al. 2003; Araki et al. 2008; Connolly and Cree 2008), but important behavioral changes have been observed in individuals after just a few weeks in captivity in snakes (DeGregorio et al. 2013). Whether or not traits making individuals more conducive to rearing in the captive environment were linked to the performance of an individual after release in our snakes remains speculative without further exploration, though we suggest that careful consideration be given to releasing animals representing the natural spectrum of genotypes and phenotypes from a population (McDougall et al. 2006).

A third factor that could have contributed to the improved performance of the second release cohort involves the implementation of a simulated overwintering period in captivity. Animals in the first release cohort were not overwintered to maximize growth and early life-stage survival, a practice common to head-starting programs for temperate zone reptiles (Mitrus 2005; King and Stanford 2006). However, snakes brumated in the lab are capable of rapid compensatory growth upon initiating activity (Sacerdote-Velat et al. 2014), and seasonal cycles that include the overwintering period influence reproductive physiology and behavior (Bona-Gallo and Licht 1983; Crews et al. 1988, Sacerdote-Velat et al. 2014), and perhaps other important aspects of snake biology. First release cohorts suffered high mortality during their first winter in the wild (Roe et al. 2010), compared to 100% survival in the second release cohort. The death of several captive snakes of cohort two during the laboratory overwintering

may have resulted in the eventual release of only those hearty enough to survive the challenges of winter conditions. Others have documented high overwintering mortality for captive-reared snakes in the wild (King et al. 2004; Harvey et al. 2014), and such mortalities could be the result of poor body condition, failure to select an appropriate refuge, or timing activity with inappropriate seasonal cues. Management protocols involving the release of captive-reared snakes may improve winter survival by first exposing them to overwintering conditions in captivity.

Despite the many improvements in behavior and performance, growth rates of captive snakes were only one third as high as wild conspecifics. These results suggest that captive animals had difficulties foraging in the wild, a finding common to many other such conservation programs (Jule et al. 2008). However, it is promising that body condition for captive *N. s. sipedon* following release remained similar to wild-caught snakes, and growth rates were positive and on par with snakes native to the site for at least a few individuals. In some cases, responsiveness to prey can decline with time in captivity, suggesting that individuals may lose some abilities to forage successfully in the wild (DeGregorio et al. 2013; but see Chiszar et al. 1993). Additionally, individuals of a population naturally vary in prey recognition, capture, handling, and ingestion (Halloy and Burghardt 1990), and early dietary experience can influence the development of such foraging behaviors in snakes (Burghardt 1992). Due to a diet limited to mostly fish in captivity, it is possible that captive snakes were unprepared for the challenges of foraging on diverse prey items encountered in the wild. *Nerodia s. sipedon* is a dietary generalist, consuming fish and amphibians in approximately equal proportion (Roe et al. 2004) or according to local prey availability (Bowen 2004). With the exception of the river, most available water bodies were shallow and isolated, sustaining abundant populations of amphibian prey but few fish (pers. obs.). Feeding is one of the most difficult challenges of snake husbandry, especially in the early life stages (Burghardt and Layne 1995; Daltry et al. 2001). However, to the extent that it is possible, we suggest that captive-rearing programs for generalist species offer a diverse diet to ensure the development of the full suite of foraging behaviors required for success in the wild.

If captive animals are to be used successfully in conservation programs to re-establish or augment populations, practitioners need information on how to best optimize their limited resources. To that end, our study was successful in highlighting aspects of captive husbandry that may influence the success or failure of animals in the early establishment phase at an unfamiliar site. In the case of *N. s. sipedon*, exposure to more naturalistic captive environments would not have payoffs in improved performance of animals after

release to the wild. Environmental enrichment can be costly compared to more simplistic captive-rearing protocols (Brown and Day 2002; Alberts 2007; Santos et al. 2009), and should only be incorporated into conservation programs if there are demonstrated benefits in animal health, welfare, and performance post-release. On the other hand, releasing older and larger animals that have experienced captivity for additional time, including a period of winter dormancy, may better prepare individuals for life after captivity. Further studies manipulating only one factor at a time will give a clearer indication of whether they warrant incorporation into conservation programs. A rigorous examination of how sex and reproductive status influence the responses of released animals would also be important, as these demographic differences often have a strong influence on snake behavior and vital rates (Brown and Weatherhead 1999; Shine et al. 2001).

Snakes can reach high abundances (Godley 1980) and play important roles in aquatic ecosystems (Dorcas et al. 2012), yet numerous snake species and/or populations are in decline and in need of immediate conservation action (Reading et al. 2010). Whether these findings extend to other species and contribute to improving ex-situ conservation programs more broadly remains to be seen, as to some degree captive-rearing protocols will need to be tailored to the biology of the target species (Daltry et al. 2001). It is encouraging that other head-starting programs have been successful for natricine snakes (King and Stanford 2006), giving promise for translocation efforts supported by captive stocks for imperiled water snakes. The numerous recent examples of invasions by snakes (Dorcas et al. 2012), including *Nerodia* spp. (Rose and Todd 2014), demonstrates that successful translocations are possible. Perhaps lessons learned from such accidental invasions may offer valuable insight into the mechanisms underlying the success or failure of intentional population re-establishments, providing further guidance in optimizing translocation strategies.

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