Responses, Movements, and Survival of Relocated Box Turtles During Construction of the Intercounty Connector Highway in Maryland

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A comparison was done of three relocation techniques to mitigate the impacts of construction of a major highway on eastern box turtles (*Terrapene c. carolina*) in Maryland. Movement patterns, home range size, and the survival of turtles from three treatment groups between 2008 to 2011 were compared: turtles native to the study site and not moved during the study (native turtles), turtles native to the site but that were removed from the limit of disturbance (LOD) of the highway and relocated within 500 m of their original location (on-site relocations), and turtles that came from areas at least 5 km from the study area and that were relocated within 250 m of the LOD (off-site turtles). Turtles in the off-site group had larger home ranges and moved longer distances than either on-site or native turtles. However, off-site–relocated turtles did not make permanent movements off the study area nor have difficulty finding suitable sites for overwintering. No statistically significant differences in survival were found in the three treatment groups. However, overall survival rates were <65% over the study's 3 years, considerably lower than rates seen in other box turtle populations, perhaps because of an emerging pathogen, *Ranavirus,* causing numerous deaths of box turtles. Recommendations for best practices emphasized the need for more effective wildlife exclusion fences to be built and maintained earlier in construction. Failure to create and maintain such a barrier led to numerous trespass events, where turtles moved onto the LOD and would likely have been killed without researchers' intervention.

Large-scale construction projects necessarily result in major modifications to the habitat within and adjacent to the project footprint. Given that habitat for native wildlife species has been shrinking rapidly for decades in the United States, there is widespread interest in testing measures that mitigate the impacts on wildlife species from transportation projects (*1–3*). One of the most commonly used mitigation measures is variously termed relocations or translocations, where animals or plants are removed from the direct path of the construction footprint and are released either adjacent to the construction area (on-site relocations) or well away from the construction zone [off-site relocations; (*4, 5*)]. Relocations have been both popular and successful for many mammalian and avian species, such as turkeys and white-tailed deer, but much less successful for other vertebrates, especially turtles and snakes (*5–10*). Indeed, the very large majority of published relocations of reptiles has not resulted in viable populations, a key determinant of success of any relocation project [(*8, 10*); see Ashton and Burke (*11*) for a recent exception].

Part of the reason for the pessimistic conclusion is the absence of well-designed, long-term studies on relocations of reptiles. One of the major criticisms by critics of relocations is that most studies of reptile relocations were not designed as ecological experiments but rather as ad hoc mitigation measures (*8, 10*). In addition, most relocation studies have followed animals for brief periods (usually less than 1 year and often less than 3 months), periods clearly inadequate given the long generation time of many reptiles (*8, 10*). To date, the authors are familiar with only two studies actually designed to test the effectiveness of turtle relocations under experimental conditions, both on gopher tortoises (*Gopherus polyphemus*). Both studies suggest that the standard practice of off-site releases without confining turtles in an enclosure or pen first has a low probability of success (*11, 12*).

An alternative to traditional off-site relocation programs is to move animals that are threatened by highway construction or other development activities to an area within their natural home range but outside the direct limit of disturbance (LOD). Referred to as onsite relocations, these programs largely obviate concerns about mixing genetic lineages, disease transmission, and lack of suitable habitat that are often issues with moving animals longer distances (*13–15*). However, results of on-site relocations for reptiles are mixed; studies with gopher tortoises in Mississippi (*13*) and canebrake rattlesnakes (*Crotalus horridus*) in North Carolina (*16*) both showed promising results, but experiments in Arizona with gila monsters (*Heloderma suspectum*) (*15*) and eastern diamondback rattlesnakes (*Crotalus adamanteus*) (*14*) suggested that even individuals moved to areas relatively close to their normal home ranges showed greater daily movements and experienced higher mortality rates than did individuals not subjected to relocation. Thus, the understanding of the effectiveness of on-site versus off-site relocations remains quite limited.

From 2008 to 2012, the Maryland State Highway Administration oversaw the construction of the Intercounty Connector (ICC) in

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south-central Maryland. Also known as Maryland Route 200, this six-lane, 30.26-km (18.8-mi) highway had an impact on many Eastern box turtles (*Terrapene c. carolina*) through elimination of their habitat along the right-of-way, and it had the potential to kill many turtles during construction activities. Although box turtles are not listed as threatened on the state or federal level, this is a species that the public is heavily interested in. Starting in fall 2007, the Maryland State Highway Administration funded a research program to determine the effectiveness of on-site versus off-site relocations for box turtles, to prevent direct mortality from construction activities. Here, experimental data are reported to determine whether on-site relocations of box turtles are an effective management option for resource managers and to determine what factors enhance or reduce the probability of success. Specific goals of the research were to (*a*) determine whether turtles relocated on-site had different home range sizes and movement patterns than turtles relocated off site or those turtles not relocated; (*b*) determine whether there were differences in annual survival among turtles relocated on site, off site, or not relocated; and (*c*) on the basis of the foregoing data, provide a series of specific best management practices that future projects can use to better manage impacted wildlife populations while minimizing costs in both time and resources to the project. Data from this research should improve the scientific basis of management decisions associated with construction projects such as the ICC and allow future project managers to expend public dollars on mitigation measures that have a higher probability of success.

MATERIALS AND METHODS

Terminology

For the purposes of this study, box turtles fell into three treatment categories:

1. Native turtles—individual turtles originally found near (but not on) the LOD and not moved during the study except to prevent immediate mortality from road construction activities (see later).
2. On-site turtles—turtles originally captured on the LOD and moved <500 m (0.31 mi) from their original capture locations.
3. Off-site turtles—turtles whose original capture locality was at least 5 km (3.1 mi) from the study area and that were relocated onto the study site within 500 m of the LOD (the same location as for the on-site turtles). These off-site turtles were generally from other portions of the highway construction area where no remaining habitat was available to place the individuals.

Study Area

The work was confined to a single unit within the ICC, the area known as the North Branch Stream Valley Unit (NBSVU) No. 2. The major portion of research work was confined to the area between Muncaster Mill Road to the south and the ICC LOD to the north (Figure 1). In the figure, the LOD for the ICC is shown by the filled polygon, and

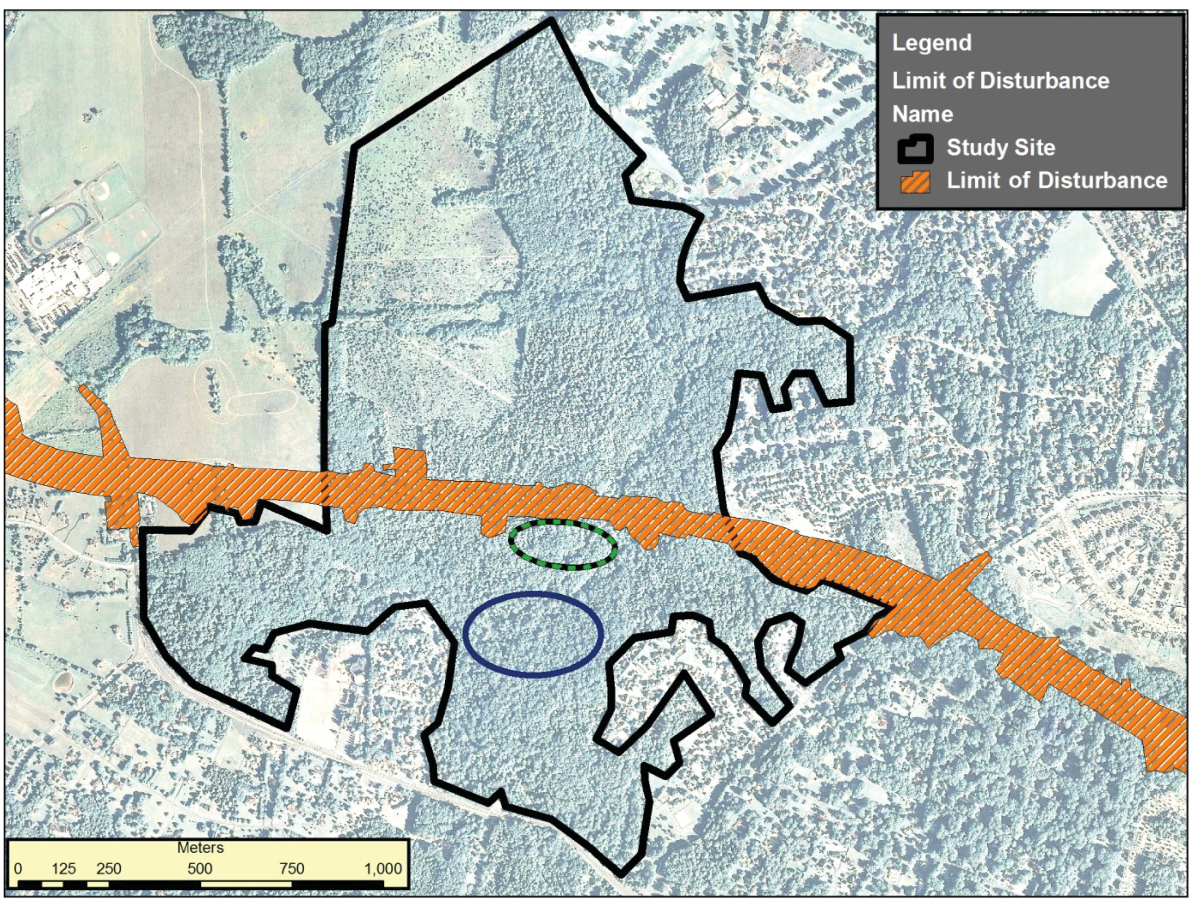

FIGURE 1 Map of North Branch Stream Valley Unit No. 2, study site for box turtle relocation study.

the general study area is outlined in black. The approximate areas where offsite and onsite turtles were released are shown by solid and dashed circles, respectively. Additional work was done north of the ICC LOD in response to turtles moving in that area. This approximately 215-hectare area (531 acres) was composed of three major habitat types on the onset of the study: second growth oak woodlands, a natural wetlands, and open fields located on the ICC LOD (Figure 1). The study site is bounded on the east and west by suburban home development.

Handling and Processing of Turtles

Most box turtles were captured by hand and through the use of trained dogs that are raised to track the scent of turtles (*17*). Both offsite and onsite turtles were moved from their original capture locations into a temporary holding pen for up to 8 weeks before being processed and released at the study site. All turtles were measured for carapace and plastron length to the nearest 1 mm (0.039 in.) with either a tape measure or tree calipers, and body mass was recorded with either a portable balance or Pesola spring scales. Gender was determined by using four characters in combination: presence or absence of plastron depression; position of vent in relation to rear edge of plastron; shape of the rear claws (useful in older adults); and eye color (C. Swarth, personal communication, 2009). All turtles were given an individual mark by notching marginal scutes with a Dremel tool (*18*). Turtles were examined for obvious signs of respiratory disease (nasal discharge, puffy eyes, difficulty in breathing) at each capture. If turtles were apparently close to death, they were sent via overnight shipping to the U.S. Geological Survey's Wildlife Health Center laboratory in Madison, Wisconsin, for necropsy and evaluation of the presence of infectious diseases.

Radiotelemetry

Box turtles were equipped with radio transmitters by epoxying transmitters to the sides of the carapace. Transmitters (Advanced Telemetry Systems, Inc.) weighed a maximum of 10% of turtle body mass, but the large majority weighed 5% or less. Box turtles were located with handheld telemetry receivers about once per week and more often if turtles were making long movements. To avoid disturbance, box turtles were not always located visually, but they had their approximate location determined by triangulation [±5 m (16.4 ft)]. A Geographic Positioning System reading was taken at each location and data were recorded on general landscape features (e.g., type of habitat). Data were plotted in ArcGIS 9, and home range size was determined via the minimum convex polygon methods generated with Hawth's Tools.

Statistical Methods

Data were initially entered into an Excel spreadsheet and then transferred to the software packages JMP (*19*) or by using R programming language for analysis. A combination of simple nonparametric methods and more sophisticated survival analysis (*20*) was used to test hypotheses about differences in survival rates between relocated and nonrelocated individuals, and the differences in survival rate among gender and age classes.

Animal Care Procedures

All procedures were approved by the Institutional Animal Care and Use Committee at Towson University, and state collecting permits were provided by the Maryland Department of Natural Resources and by Montgomery County Parks.

DISCUSSION OF RESULTS

Sample Size

From 2008 to 2011, marking was done of 233 box turtles: 146 males, 83 females, and 4 juveniles of undetermined gender. Of these, 168 were classified as native turtles, 32 were on-site turtles, and 33 were off-site turtles. During the study, 123 turtles were used for radiotelemetry during the study: 58 native turtles, 32 on-site turtles, and 33 off-site turtles.

Differences in Movement Patterns and Home Range Sizes

A common finding in studies of offsite relocations is that the home range size and daily movements of animals brought into unfamiliar habitats are much larger than those of native animals (*5, 21–23*). In extreme cases, animals relocated into unfamiliar habitats may wander extensively, and they may never be incorporated into the existing populations because of failure to mate or to find necessary resources.

As shown in Table 1, off-site turtles in the study showed significantly larger home range sizes than did either on-site or nature box turtles, but there was no difference in home range size between native and on-site turtles. Differences among the groups were highly significant (analysis of variation, $F = 7.13$, df = 2,49, $P = .002$). A Tukey's honestly significant difference showed no difference between the native ($n = 18$) and on-site groups ($n = 20$), but both of those groups were significantly lower than the off-site ($n = 14$) group. Only animals with a minimum of 30 locations and tracked from the beginning of the study were used for the analysis. Indeed, turtles in the off-site group had average home range sizes that were 3.4 times those of native turtles and 2.8 times those of on-site turtles (Table 1 and Figure 2). These findings are similar to those seen in other studies of off-site relocations (*5, 21–23*). Unexpected in the study was that no evidence was found that the off-site turtles made a directional movement back toward their original collecting locality, nor evidence that they moved off the study site for any length of time. Instead, aside from those off-site turtles that died from disease issues (see later), virtually all of these turtles were able to overwinter successfully and

TABLE 1 Average Home Range Size, in Hectares, for On-Site, Off-Site, and Native Box Turtles, Calculated with Minimum Convex Polygons

Group	Average (ha)	Lower 95% CI (ha)	Upper 95% CI (ha)	Range (ha)
Native (*n* = 20)	4.31	2.72	6.83	0.68–32.42
On-site (*n* = 18)	5.16	3.33	7.98	1.30–41.62
Off-site (*n* = 14)	14.71	8.73	24.78	3.58–153.33

NOTE: CI = confidence interval.

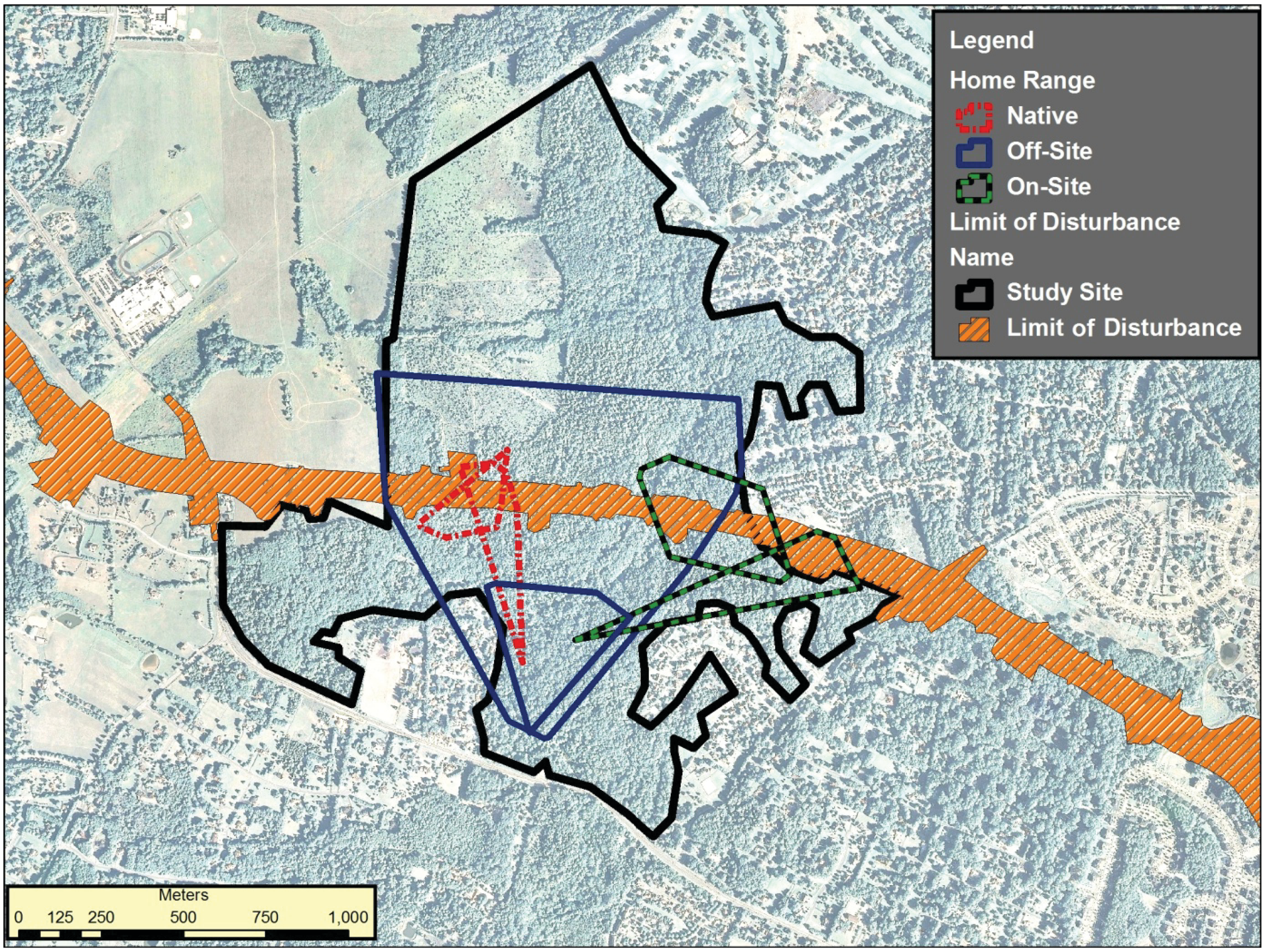

FIGURE 2 Representative home range sizes for off-site, on-site, and native box turtles, calculated with minimum convex polygons.

within the same area as the native NBSVU population did. Whether this outcome stemmed from the fact that these off-site turtles were allowed to hibernate at the study site before their first activity season or whether the habitat at NBSVU was more suitable than their original site is unknown. The authors are not aware of any similar result for an off-site box turtle relocation, so this represents something of a novel finding. However, it is possible that some off-site turtles made short-term movements off the NBSVU site between radio locations, movements not detected during the study.

Annual Survival and Sources of Mortality

The research involved following 95 box turtles from their emergence from hibernation in April 2008 through their entrance to hibernation in November 2011. Two additional turtles not included from April of 2008 to October 2008 were tracked from April 2009, and seven more turtles were tracked from September 2009 until November 2011. Another turtle that had not been located at the beginning of the study was found in October 2010 and was then tracked until November 2011. An additional 18 turtles found near the permanent exclusion fencing adjacent to the ICC were added in June 2011 and tracked until November 2011. Because of the brief amount of time to follow this last group of turtles, they were not included in any analyses but were used to determine potential trespass events.

Kaplan–Meier survivorship curves for the three groups of turtles are shown in Figure 3. The overall mortality rate among turtles tracked for at least 1 year was 29.5% (31 of 105 turtles). The mortality rate of off-site turtles was 27.3% (9 of 33 turtles), compared with 34.4% (11 of 32 turtles) for on-site relocated turtles, and 27.5% (11 of 40 turtles) for the native turtles. There are no significant differences in mortality rate among the groups (contingency table analysis, Pearson's $\chi^2 = 0.521$, df = 2, $P = .771$). Causes of mortality included direct killing by machinery (two turtles); burial by road construction activities (one turtle); road mortality (one turtle); and disease or unknown causes (27 turtles). The overall rate of mortality of these turtles was surprisingly high, resulting in most part from the unexpected outbreak of *Ranavirus* among the turtles. Details of this outbreak, its potential causes, and effects on the study will be discussed later.

Of the 31 known mortalities, 22 were males (22 of 68 total males or 32.35%); and 9 were females (9 out of 37 total females or 24.32%), but these differences were not significantly different (contingency table analysis, Pearson's $\chi^2 = 0.742$, df = 2, $P = .39$). With use of a Kaplan–Meier estimator, it was found that the overall annual survival rate of females was 0.897 per year, and that of males was

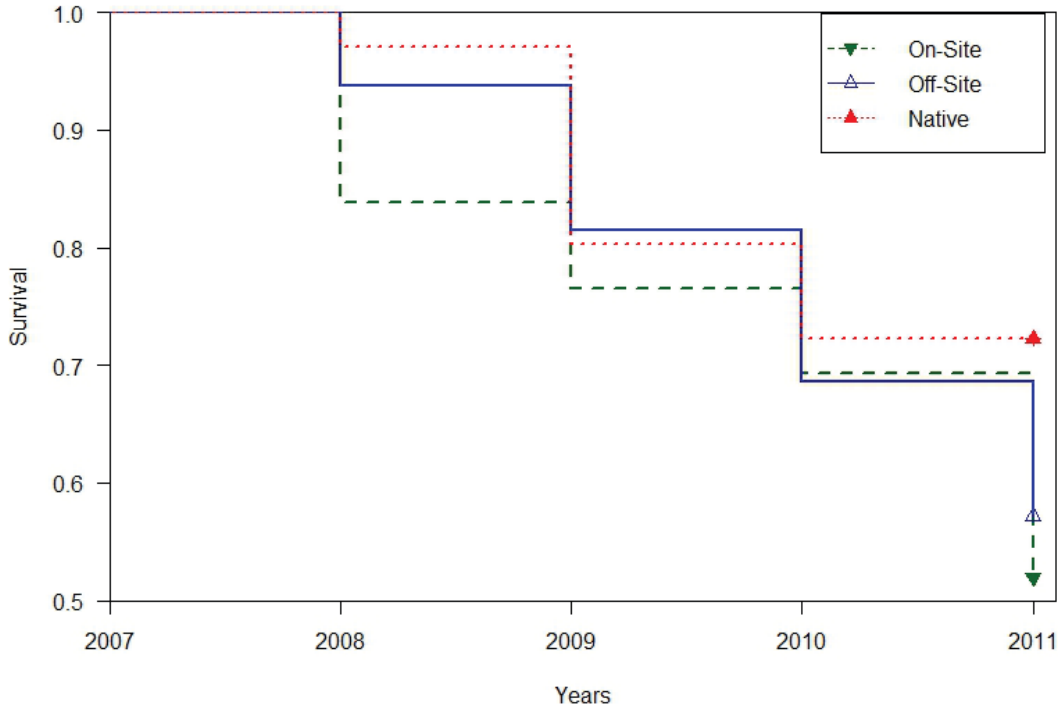

FIGURE 3 Kaplan–Meier survivorship curves by relocation group; triangular markers represent final survival estimates of 0.723 for native group, 0.520 for on-site group, and 0.572 for off-site group.

0.886 per year. Thus, the data show no apparent effect of gender on annual survivorship rates.

Very limited data are available on gender-based differences in survivorship for box turtles. Dodd et al. found no differences in annual survival rates between male and female Florida box turtles: 0.886 for males and 0.871 for females (*24*). Nazdrowicz et al. reported annual survival estimates ranging from 0.813 to 0.977 in four fragmented habitats in Delaware (*25*), and Currylow et al. found overall annual survival rates of 0.95 to 0.98 at a natural site in Indiana (*26*). Neither of the latter papers reported gender-specific results. Data from other turtle species show variable results. For example, Litzgus reported much higher survival rates for female spotted turtles (*Clemmys guttata*), with females having estimated longevities almost twice that of males (*27*). By contrast, Steen and Gibbs suggested that male-skewed sex ratios in two common turtle species (painted turtles, *Chrysemys picta,* and snapping turtles, *Chelydra serpentina*) were the result of decreased annual survival of female turtles resulting from road mortality (*28*).

No association was found between temporal factors and survivorship for box turtles in this study. During the winters of 2008 to 2009 and of 2009 to 2010, a focal group of 30 turtles (10 from each relocation group) was used to assess habitat selection and overwinter survival. Most turtles overwintered on the slopes of the upland areas adjacent to the LOD, but some spent the winter in habitats that one would describe as suboptimal, including in wetlands that are subject to seasonal flooding.

Survivorship during the overwintering period was quite high; during all three hibernation periods (2008 to 2009, 2009 to 2010, and 2010 to 2011), only one turtle (a male from the native group) was known to have died during the winter, and this individual may have entered hibernation with an active infection.

Impact of Construction Activities

Although only three turtles were killed as a direct result of ICC construction activities, this was primarily a consequence of the researchers' presence on this study area, since 31 individual turtles were moved 80 times from the LOD during the construction phase of the project (average of 2.58 removals per individual). Native and on-site turtles were much more likely to trespass on to the LOD than were off-site turtles (13 native turtles trespassed 38 times, 15 on-site turtles trespassed a total of 39 times, and three off-site turtles trespassed a total of three times). That most of these trespass events were from turtles originally from the NBSVU site is not surprising, since the LOD seems to be a focal area for nesting and foraging for this population (unpublished data). This likely stems from the fact that the LOD for the ICC represented one of the few patches of relatively open habitat remaining in the NBSVU park area. Open habitats are often essential for basking and nesting sites for ectothermic vertebrates such as turtles, and the loss of such habitats can result in decreased species diversity (*29, 30*).

The contention is that many, perhaps most, of these trespassing turtles would have been killed during construction without the researchers' intervention. Given the presence of fast-moving construction activity in proximity to very slow-moving turtles, this is a defensible assumption. Thus, only the active intervention of the researchers resulted in such a low mortality rate owing to construction activities. In addition, reports were received that construction workers also removed some turtles from the LOD. Although exact numbers are not available, this suggests that the number of trespass events as noted likely underestimates the number of turtles that actually moved back onto the LOD.

The reasons for the high rate of trespass events were twofold. First, when active construction adjacent to the study area began in

July 2008, the only fence installed at that time was a plastic boundary fence that was not designed to keep turtles from trespassing onto the LOD (Figure 4*a*). Second, when the initial wildlife exclusion fence was installed in July and August 2008, the contractor frequently failed to ensure that the fence was adequately secured to the ground; in many cases, gaps high enough for a box turtle to pass through were commonly seen along the fence line (Figure 4*b*). In addition, the lack of daily maintenance by the local contractor caused gaps from falling tree limbs or direct wind damage to persist for several days at a time, increasing the probability of a trespass event. The lack of maintenance also made gaps caused by vandalism (common in the early part of the project) more likely to result in a trespass event.

Once a permanent wildlife fence was installed in mid-2011, the rate of trespass events fell dramatically but was still not reduced to zero. Although the main portion of the permanent wildlife fence is well designed to prevent trespass events, segments along maintenance gates still allowed two turtles to gain access to the ICC during the summer and fall of 2011. Since these gates are a necessary part of the maintenance plan for the ICC, a new design for the wildlife fence in these areas will prove necessary, as will routine maintenance of the fence.

(a)

(b)

FIGURE 4 Examples of fences and trespass events: (*a*) large gap under ICC boundary fence, showing how easy it was for a turtle to evade the fence barrier, and (*b*) how the initial wildlife exclusion fence was laid over existing coarse woody debris, making an easy pathway for turtles to evade the fence.

Impacts of Ranaviral Disease

Recognition is growing that pathogens in the genus *Ranavirus* (Family Iridoviridae) are important sources of mortality in a variety of amphibians and reptiles (*31, 32*). Because the overall mortality rates were unexpectedly high, as mentioned earlier, and because *Ranavirus* had previously been documented from Maryland (C. Swarth, personal communication, 2009), special attention went to the presence of this disease at the study site.

Signs of *Ranavirus* infection in box turtles were first seen in 2008 and continued through 2011. Turtles infected with *Ranavirus* presented with a variety of signs, including lethargy, puffy eyes, nasal discharges, difficulty in breathing, and skin and mouth lesions. In total, there were 27 cases of confirmed or suspected mortality from *Ranavirus* in the study population. Of 12 turtles sent to the U.S. Geological Survey Wildlife Health Center laboratory for necropsy, 10 were positive for *Ranavirus*, and two were negative. Although some mortality in the turtles may be the result of other causes, most known predators of box turtles are absent from the NBSVU, and most mortality occurred in the summer months (unpublished data), so that was not the result of overwinter mortality.

No evidence was found that mortality was associated with relocation status. Using only animals that were tracked for the entire study and which had known fates (i.e., excluding animals lost during the study because of transmitter failure or other reasons), mortality rates were 60.0% for off-site relocated turtles (9 of 15); 61.1% for on-site turtles (11 of 18); and 40.7% for the native group (11 of 27). These differences were not statistically significant (Pearson's $\chi^2 = 2.351$, $P = .309$, $n = 60$). This suggests that it is unlikely that the disease was brought into the NBSVU population by the relocated turtles (i.e., they were infected before they were initially captured). If that were the case, one would expect that the off-site turtles would have suffered the highest (and earliest) mortality, since they would have been exposed to *Ranavirus* for some period of time before they were relocated to the study area. Instead, it was found that the on-site relocation group suffered the largest mortality during the first year (Figure 3), and, of the only two known mortalities within the off-site group that year, one was attributed to road mortality.

These results indicate the clear need for comprehensive health examinations for any individuals being moved among populations, to prevent spreading potentially lethal pathogens to otherwise unaffected areas. Also recommended is that the host population be tested for infections before any off-site relocations.

CONCLUSIONS AND RECOMMENDATIONS FOR BEST MANAGEMENT PRACTICES

Data from the research showed no statistically significant differences in the percentage of turtles surviving among the three relocation groups. In addition, although off-site turtles had larger home range sizes than did turtles from the other groups, almost all of these turtles remained on the study area. From this perspective (though limited), the on-site relocation as practiced in this study would have to be deemed a success, for it prevented large-scale mortality among the turtles moved from the ICC footprint, while avoiding the costs and complications of moving turtles to new habitats. However, there are three major caveats to this conclusion that must be considered before on-site relocations can be termed a best practice.

Effectiveness of Barrier Fences

Numerous cases were found of the telemetered turtles trespassing onto the LOD. This was especially true of turtles from the on-site and native relocation groups, both of which showed a much higher probability of trespassing than did turtles relocated from off-site. In one case, the same individual turtle successfully evaded the barrier fences nine times. Given the intensive nature of the construction activity during this period, it is highly likely that many (if not most) of these trespass events would have resulted in mortality to the turtles. If one counted each turtle that trespassed onto the ICC as having even a 50% chance of being killed by construction activities, this would have reduced the overall survival substantially, clearly an unacceptable outcome.

In the study, the actual number of turtles killed by construction activities was relatively low. This resulted from the researchers' ability to relocate telemetered turtles away from the LOD and the fact that construction workers received training alerting them to the presence of these turtles on the LOD. Recommended is that such training be incorporated on any projects involving onsite relocations.

Effects of Ranavirus

The data on survival are at least partially confounded by the sudden onset and impact of *Ranavirus* on the turtles starting in 2008. Given the virtual absence of data on how *Ranavirus* is transmitted among turtles (M. Gray, personal communication, 2012), it cannot be determined whether the off-site–relocated turtles may have been the source of the original infection or whether this outbreak was unrelated to the off-site relocation. Considering the short distances involved between the source and the relocation site, coupled with the lack of any immediate mortality of individuals in this group after their release in spring 2008, such an association may seem unlikely, but this possibility cannot be ruled out. Thus, even though there were no differences in mortality rates among the three groups of turtles, the overall mortality rate may have been affected by the outbreak of *Ranavirus,* making conclusions about the effectiveness of on-site relocations less robust than they would otherwise have been. Given the occurrence of *Ranavirus* in box turtles, it is recommended that all turtles subject to on-site or off-site relocations be subject to a detailed health inspection by a qualified veterinarian or biologist. Animals showing apparent signs of disease should not be relocated but sent for examination by a qualified professional. Similar examinations of the host population should also be conducted.

Limitations of Short-Term Data

Box turtles have a generation time of perhaps 15 to 20 years (*21*), so the 4-year data set represents 20% to 25% of a single generation interval. Whether this time is sufficient to adequately determine the long-term success of on-site relocations is problematic. Thus, while preliminary recommendations can be made for best practices, such recommendations will be subject to revision and modification if longer-term data are collected on this population or other populations of turtles subjected to on-site relocations.

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