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RH: Starking-Szymanski et al. • Habitat Use of Headstarted Blanding's Turtles

# Movement and Habitat Use of Headstarted Blanding's Turtles in Michigan

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<sup>1</sup> This is the author manuscript accepted for publication and has undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi:<u>10.1002/jwmg.21530</u>

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ABSTRACT Captive breeding or headstarting is a management option to increase population numbers in threatened and endangered animals. The success of these programs depends on increasing population numbers while maintaining fitness within populations that contain some captive-reared individuals. As part of an ongoing conservation project with Shiawassee National Wildlife Refuge (SNWR) in Saginaw, Michigan, USA, the Detroit Zoological Society (DZS) headstarted Blanding's turtles (Emydoidea blandingii) to 18 months of age. To determine survival, movements, and habitat use of these headstarted Blanding's turtles, we externally attached radio-transmitters to 24 turtles and released them at 4 sites in release groups of 6 along a transect in a wetland within SNWR during June 2014. We located turtles weekly during the active season (May-Sep) and every 2 weeks during the inactive season (Oct-Apr) for 18 months, starting immediately after release and ending November 2015. We calculated straight-line distances between release sites and home range centers over the study period. We examined habitat use of the turtles in relation to habitat availability. To predict movement of headstarted Blanding's turtles away from release sites, we used geographically weighted regression (GWR) with measured habitat factors and release sites as independent variables. There were differences in home range sizes across release groups but not between years. Headstarted juvenile Blanding's turtles in this study have home range sizes similar to those measured in other studies for wild-hatched juveniles but smaller home ranges than those measured for wild-hatched adults. Our study showed that headstarted Blanding's turtles used habitats with muskrat (Ondatra zibethica) dens and cattails (Typha spp.) more than proportionally available, and used open water, willows (Salix spp.), and lowland forest less than proportionally available. The GWR model was able to predict habitat characteristics (water depth and temperature, duckweed [Lemna minor], cattails, muskrat dens and fields, buttonbush [Cephalanthus occidentalis]) that influenced the movement of turtles away from the release sites. The habitat coefficient's influence

on movement varied in relation to the current location of the turtle and as habitat characteristics increased or decreased. Turtle distance from release sites decreased as water depth, water temperature, and duckweed increased, and increased as cattails, muskrat dens and fields, and buttonbush increased. Habitat in the small spatial extent at release sites may affect movement of headstarted Blanding's turtles. Furthermore, this study uses novel methodologies for assessing headstart programs that can aid future conservation and management efforts by providing information on habitat use and movement patterns of headstarted Blanding's turtles after release.

**KEY WORDS** Blanding's turtle, *Emydoidea blandingii*, geographically weighted regression, habitat analysis, headstarting, home range, national wildlife refuge, space use, turtle.

Wildlife populations are decreasing across the globe (World Wildlife Fund 2016), and wildlife biologists and managers have attempted to fight this decline. Popular strategies to prevent the extinction of declining populations include captive breeding, or headstarting (i.e., collecting wild-born young animals and raising them for a certain time to bypass a vulnerable life stage before releasing them into their habitat; Jones and Sievert 2012). However, available research suggests the loss of fitness in captivity can be rapid, its magnitude probably increasing with the duration in captivity (Fraser 2008). Ideally, captive-bred and headstarted individuals maintain wild characteristics (i.e., habitat use and behavior) like wild-born counterparts to avoid human-induced changes to the population while increasing population numbers (Mitrus 2008). Thus, part of determining the success of the use of captive breeding or headstarting programs for populations is evaluating what factors increase the survival of captive-reared individuals, and whether

survival of captive-reared individuals differ from survival of wild individuals, which may affect fitness within populations that use these programs.

Little research has addressed under what conditions an increase in population abundance due to captive rearing might offset fitness reductions induced in captivity (Fraser 2008). Many herpetofauna conservation programs use headstarting because many members of this group have a type III survivorship curve, characterized by high egg and hatchling mortality rates, with those surviving this stage experiencing longevity (Pearl and Miner 1935, Heppel et al. 1996, Burke 2015). Headstart programs have the potential to increase population numbers of herpetofauna by collecting individuals at the egg or hatchling stage, rearing them in captivity to survive these vulnerable life stages, and then releasing them into the wild.

Analyzing space use after release can help determine success of headstarting programs (Mignet 2014). Comparing the habitat use and movements of headstarted individuals to wild-born counterparts can inform conservationists to differences between the groups (Escobar et al. 2010). By carefully choosing release site locations within the landscape to limit mortality, greater survival of headstarted reptiles is achieved (Reading et al. 2013). Habitat choice by hatchlings and juveniles can result in death if they find themselves in dry uplands that leave them vulnerable to predation (Hagen et al. 2009, Starking-Szymanski 2016). Analyzing space use of surviving headstarted individuals can evaluate whether their home ranges overlap with wild-hatched animals for future reproduction opportunities and propagation of the species, which will inform biologists of the potential success of headstart programs.

A headstarting program at the Shiawassee National Wildlife Refuge (SNWR) for Blanding's turtles (*Emydoidea blandingii*) has begun to increase abundance of this species of special concern in Michigan, USA. To date, SNWR has received no reports of

juvenile wild-born Blanding's turtles despite multiple trapping attempts in the refuge (S. F. Kahl, SNWR, personal communication). Researchers have had difficulty locating juvenile Blanding's turtles and have reported populations skewed towards large, older adults (Ruane et al. 2008); Congdon et al. (1993) has reported a lack of recruitment in Blanding's turtle populations in Michigan. The success of the headstarting program at SNWR depends on high survivability of headstarted individuals during captive rearing and the fitness of headstarted individuals once released into the wild.

We monitored the survival and space use of headstarted Blanding's turtles in the SNWR over 18 months to quantify the relationship between survival and habitat factors for these individuals. First, we hypothesized that headstarted Blanding's turtles would have higher survival rates when released in locations with preferred habitat characteristics. Second, we hypothesized that microhabitat characteristics within a preferred land cover type (emergent wetlands) would influence headstarted Blanding's turtles movements away from release site. In addition, we determined home ranges for these headstarted individuals to compare them with habitat use of wild-born individuals from other studies.

# STUDY AREA

The study area for this research was SNWR, Saginaw, Michigan. The SNWR is a 3,885-ha wetland complex that is part of the National Wildlife Refuge System (Fig. 1), is within Michigan's largest watershed, and is centered at the convergence of 4 major rivers. It is part of the larger Saginaw Lowland and varies in elevation 167–212 m above mean sea level. Refuge staff manage and conserve all native habitats and native species within the refuge. Emergent marshes, shrub marsh, forested wetlands, river flood plains, and forested uplands are some of the most critical land cover types within SNWR for the Blanding's turtle. The United States Fish and

Wildlife (USFWS) staff actively manage emergent marsh pools by controlling vegetation and water levels at the refuge (Spencer et al. 2001). The surrounding landscape includes agricultural fields and suburban neighborhoods. The climate of central Michigan is humid continental (Peel et al. 2007), with colder winters (29-yr record mean of  $-8^{\circ}$  C in Jan), mild summers (29-yr record mean of 27° C in Jul), and averaging 83.8 cm annual rainfall (Saginaw, MI Weather Station Data). Nesting season occurs May–June; beginning in 2010 SNWR staff and volunteers located adult female Blanding's turtles traversing terrestrial habitats for nesting in SNWR. Our study occurred June 2014 through November 2016. Other species that reside in the refuge include mammals such as raccoons (*Procyon lotor*) that prey on turtle eggs and hatchlings within the SNWR (S. F. Kahl, personal communication), amphibians (e.g., leopard frogs [*Lithobates pipiens*]), many types of reptiles including the Blanding's turtle, and many wading birds that use the refuge as a migration stopover or summer residence (e.g., great blue heron [*Ardea herodias*]).

## **METHODS**

Biologists from SNWR and the Detroit Zoological Society (DZS) transported adult females (determined to be gravid via palpitation) to the DZS's facility and x-rayed them for egg enumeration. These gravid females received an injection of oxytocin (10 U/kg intramuscular) from DZS veterinarians to induce oviposition. A second injection of oxytocin was administered at the same dosage if oviposition did not occur within 2 hours. After ovipositing, refuge staff or volunteers released the adult females at their collection site in SNWR. Staff from the DZS incubated the Blanding's turtle eggs laid by these females to attempt to produce a 30:70 male to female ratio using temperature-dependent sex determination that occurs in turtles (Gutzke and Packard 1987). To achieve this ratio, incubation of 30% of the eggs was at 28.3°C (males) and incubation of the remaining 70% was at 30.8°C (females; Gutzke and

Packard 1987). However, sex of the hatchling Blanding's turtles was not known for certain because secondary sexual characteristics do not develop until sexual maturity at 14–20 years of age. The DZS staff raised these hatchling Blanding's turtles in captivity at the DZS's facilities until turtles either obtained a carapace length of 10.16 cm, or for a duration of 18 months.

In 2014, DZS staff randomly selected 24 turtles from those that were >1 year old and had carapace lengths >10.16 cm. These 24 turtles received 12-mm passive integrated transponder (PIT) tags (12.5 × 2.12 mm, 115 mg; Biomark, Boise, ID, USA) via injection into the body cavity by DZS veterinarians. We then notched each turtle's carapace (Cagle 1939), and used the PIT tags and carapace notches to identify all 24 turtles individually. We fitted all 24 turtles with R1680 radio transmitters (3.6 g; Advanced Telemetry Systems, Isanti, MN, USA) by attaching the transmitters to the carapace externally using Devcon clear coat epoxy (ITW Polymers Adhesives North America, Danvers, MA, USA) and PC Marine hand moldable all-purpose epoxy putty (Protective Coating Company, Allentown, PA, USA). Beginning mass for all headstarted Blanding's turtles was 91–150 g. We fitted the 8 turtles with the lowest added percent mass from transmitters (2–5% g) with Thermochron iButtons (3.3 g; iButtonLink, Whitewater, WI, USA) attached to the carapace with the same 2 previously mentioned epoxies for a concurrent study on thermoregulation. We kept total mass of transmitters, iButtons, and epoxies at <10% of each turtle's body mass to avoid negative effects on movement and survival on the headstarted turtles. Although this is higher than the 5% recommended in the most recent studies, Forsythe et al. (2004) added tracking devices that weighed up to 13% on eastern box turtles (Terrapene carolina carolina) with no ill effects. We recaptured turtles in fall 2014, spring 2015, and fall 2015 to remove and replace the transmitters or iButtons. We completed this research in accordance with University Committee for the Use and Care of Animals from the University of Michigan-Flint (protocol numbers PRO00006431,

PRO00003978, PRO00005752). This research complied with all applicable state of Michigan land use and scientific collection permits issued by the Michigan Department of Natural Resources and in accordance with the USFWS from SNWR.

Based on geographic information system (GIS) analysis of a previous study on adult Blanding's turtles in Michigan, and in consultation with USFWS biologists from SNWR, we chose Grefe pool as the release location for the headstarted turtles (Congdon et al. 1993, 2011; Fig. 1). On 17 June 2014, we chose the 24 turtles at random to be released at 1 of 4 release sites (n = 6 at each site) evenly spaced along a transect in Grefe pool (Fig. 1). The different microhabitat conditions at the point of turtle release were open water (site 1), sparse cattails (<25% cattails within a 1-m quadrant) with duckweed (site 2), willows with duckweed (site 3), and dense cattails (100% cattails within a 1-m quadrant, site 4).

We tracked turtles from June 2014 through November 2015, approximately once/week during the active seasons (May–Sep) and once every other week during the inactive season (Oct–Apr). We determined survival by monitoring turtle movements; if an individual had not moved for >3 tracking events then we located the turtle and visually assessed if mortality had occurred. After we located a turtle via radio-telemetry, we placed a 1-m<sup>2</sup> floating quadrat with the turtle approximately in the center and recorded global positioning system coordinates using a Trimble field computer (GeoExplorer 6000 series, Trimble Navigation Limited, Sunnyvale, CA, USA). The habitat factors we recorded within the quadrant were water depth, ice thickness (when applicable), substrate depth, water temperature, air temperature, above water vegetation type, and vegetation dominance. We measured water depth using a Keson metric tape with a Hayward fishing weight (2.72 kg) attached. We measured ice thickness with a clear metric ruler. We measured substrate depth by placing the Hayward fishing weight (2.72 kg) on the end of the Keson Metric measuring tape and measuring how

far the weight sank into the substrate once released. We identified emergent vegetation and estimated dominant of vegetation using methods similar to Millar and Blouin-Demers (2011): 0% vegetation, 25% vegetation, 50% vegetation, 75% vegetation, or 100% vegetation. We recorded air and surface water temperature at each turtle relocation with a liquid-in-glass field thermometer with a temperature range –30 to 50°C (Weksler Glass Thermometer, Charlottesville, VA, USA.). We used the GIS software ArcMap (version 10.3, Environmental Systems Research Institute, Redlands, CA, USA) to create maps of turtle locations, identify homes ranges (ha) of individual turtles using the minimum bounding geometry tool, and determine movement patterns by turtles. We performed additional statistics using R, packages base, Ime4, and sjstats (Bates et al. 2015, R Core Team 2016, Lüdecke 2018).

To calculate the distances moved by each turtle between relocations, we calculated straight-line distances (m) between each turtle point in sequential order. We found mean daily movement pattern distances by dividing the distance between relocations by the number of days between each tracking and relocation event. Although this is an underestimation of movement, previous studies reported this to be a viable way to compare distances moved between animals (Millspargh and Marzluff 2002, Millar and Blouin-Demers 2011, Jaegar et al. 2012, Anthonysamy et al. 2013). We used mixed effects models to analyze cumulative straight-line distances between years and release groups with turtle as a random effect and calculated confidence intervals for the intercepts to reveal a positive or negative biological effect by checking for an overlap of zero (Bates et al. 2015, Lüdecke 2018). If confidence intervals did not overlap zero, we used the Cohens  $f^2$  effect sizes to derive biological relevance, with Cohen's  $f^2$  local effect sizes measured as small ( $f^2 \ge 0.02$ ), medium ( $f^2 \ge 0.15$ ), and large effects ( $f^2 \ge 0.35$ ; Cohen 1988).

We calculated minimum convex polygon (MCP) home ranges (ha) in ArcMap. We used mixed effects models to analyze home range area (ha) between years and release groups with pairwise *t*-tests to show mean differences and calculated confidence intervals for the intercepts and Cohens  $f^2$  effect sizes for models (Bates et al. 2015, Lüdecke 2018). We calculated the center for each home range and measured the distance to the release sites.

We constructed a population range (ha) using the MCP method in ArcMap, similar to the home ranges by creating an MCP around the location points of all of the 24 turtles. We used orthophotos, satellite imagery, and ground truthing to create land cover polygons within ArcMap, and measured the area of each land cover type to determine availability within the population range. Aerial orthophotographs collected and incorporated into a GIS is an approach for carrying out visual and spatial analysis of Blanding's turtles and their habitat (Maktav et al. 2000, Barker and King 2012). We relocated turtles in and around muskrat (*Ondatra zibethica*) dens frequently, which led us to calculate the area of muskrat dens in ArcMap by placing a circular polygon with a diameter of 1 m around each den point location recorded in the field or obtained via orthophotos taken in 2014. We used Chi-square goodness-of-fit tests with Bonferroni z-stat confidence intervals to determine if observed habitat use differed from expected habitat use (Neu et al. 1974, Byers et al. 1984). This test detects selectivity of resources by animals (Alldredge and Griswold 2006).

To test our hypothesis that microhabitat characteristics would influence headstarted Blanding's turtles movements away from release site we conducted global (i.e., ordinary least squares [OLS]) and spatial regression (i.e., geographically weighted regression [GWR]) analyses. We did this to visualize and explore how habitat variables influenced the distance turtles traveled away from their release sites (i.e., the dependent variable). We predict that turtles released in a preferred cover type (e.g., cattails) will move shorter

distances than turtles released in non-preferred cover types (e.g., open water). The OLS served as a base model in this study; we chose the GWR model as the primary model because it accounts for spatial effects (i.e., spatial auto-correlation; Fotheringham et al. 1998, 2000; McNew et al. 2013) and has been used in previous biological modeling research (Kimsey et al. 2008). We used ArcMap for all regression analyses (i.e., OLS and GWR). The explanatory factors considered were the microhabitat factors collected at each turtle location and the release groups. Microhabitat factors initially consisted of 14 factors, including water depth (cm), water temperature (°C), substrate depth (cm), air temperature (°C), proportion of cover for duckweed, other floating vegetation, cattails up, cattails down (muskrat dens, push-ups, and fields), willow, grass, log, grapevine, buttonbush, and ice (mm). We used dummy variables for the 4 release groups. We assessed possible model correlates using exploratory global regression analysis in ArcGIS. We added all possible independent variables simultaneously to the model. The procedure resulted in traditional measures of model robustness metrics: adjusted  $R^2$ , corrected Akaike's Information Criterion (AIC<sub>c</sub>), variance inflation factor (VIF), and OLS model *t*-test *P*-values (*P* < 0.05). We calculated model estimates, confidence intervals, and Cohen's  $t^2$  effect sizes to determine the best independent variables for explaining turtle travel distance. Variables best explaining turtle travel distance were those with confidence intervals not overlapping zero, thus revealing a positive or negative biological effect.

We developed the OLS model to examine universal relations between turtle distance from release sites and the selected independent variables. This traditional approach is a baseline model because it assumes spatial stationarity among selected variables. After we developed the OLS model, we tested the standardized residuals for autocorrelation using global Moran's *I* index in ArcGIS software. The metric is frequently used to test for spatial autocorrelation (Rosenshein and Scott 2001) and has been used in past

ecological studies (Dormann et al. 2007). The index produces 1 statistic for each variable and ranges from -1 to 1. Positive values indicate positive spatial autocorrelation and negative values highlight an inverse spatial relationship (Burt et al. 2009). We built off these outputs to create a spatially explicit GWR model.

Because spatial autocorrelation is a known factor when assessing environmental and biological relationships (Legendre 1993), we accounted for it by constructing a GWR model in ArcGIS software using the same variables as in the best fit OLS model. The GWR model is a local model because it applies a regression equation to each sample (Charlton and Fotheringham 2009, Fotheringham 2009). Moreover, the mathematical expression for GWR is similar to the OLS in that local parameters take the place of global parameters (Fotheringham et al. 2002). Also engrained in this model is the accountability of distance. The equation allows for a spatial shift in parameters based on local influences, which results in a better fitting model. We used  $AIC_c$  to assess the strength of the model, with a lower  $AIC_c$  indicating a better fit, and compared it to the OLS output.

The GWR equation can be expressed as:

$$\mathbf{y}_i = \boldsymbol{\beta}_0(\boldsymbol{u}_i, \boldsymbol{v}_i) + \sum_{k=1}^{p} \boldsymbol{\beta}_k(\boldsymbol{u}_i, \boldsymbol{v}_i) \boldsymbol{x}_{ik} + \boldsymbol{\epsilon}_i,$$

where  $\mathbf{y}_i$  is the dependent variable (distance to release site) at location *i*,  $\boldsymbol{\beta}_0(\boldsymbol{u}_i, \boldsymbol{v}_i)$  is the intercept at location *i*,  $\boldsymbol{\beta}_k(\boldsymbol{u}_i, \boldsymbol{v}_i)$  is the estimated *k*th parameter at location *i*,  $\boldsymbol{x}_{ik}$  is the independent variable of the *k*th parameter at location *i*, and  $\boldsymbol{\epsilon}_i$  is the error term at location *i*. The GWR model assumes that the error term is independent and identically distributed (Zhao and Park 2004). **RESULTS**  We collected 899 data points on 24 headstarted Blanding's turtles. All 24 turtles survived through spring 2015, past their first hibernation. During the 2015 tracking season, researchers were unable to locate 8 turtles: 5 turtle transmitters were shed with scutes, researchers lost signals for 2 turtle transmitters, and 1 transmitter signal stopped moving under a muskrat den because the turtle died or shed its transmitter. One confirmed death from predation occurred where a turtle was located in a vernal pool that dried during summer 2015. We found mammal teeth marks on the transmitter and turtle's carapace. The overall survival of the headstarted Blanding's turtles released in SNWR was 63–96%. The best-case survival estimate (96%) assumes that every turtle except for the one that was confirmed dead survived, and those who were not located simply lost their transmitters (n = 8) when their scutes shed in the spring or summer the year after release. The worst-case survival estimate (63%) assumes that every turtle that was not located died, and that only turtles that we tracked until the end of the study survived. The best-case survival estimates were 83%, 100%, 100%, and 100% for release groups 1–4, respectively. The worst-case survival estimates were 33%, 100%, 67%, and 50% for release groups 1–4, respectively. We tracked the initial 24 turtles for 310 days and tracked the remaining 15 turtles an additional 205 days (length of the study was 515 days).

The mean daily movement for the headstarted Blanding's turtles was 26.38 m. The mean cumulative straight-line movement distance for the headstarted Blanding's turtles over the course of the study was  $1,005.0 \pm 511.6$  m (SD). The cumulative mean straight-line movement for 2014 was 476.4 ± 280.0 m and for 2015 was  $513.0 \pm 368.3$  m (Fig. 2). The model with individual turtle as a random effect indicated individual turtles did not affect straight-line movements. There was no effect of year ( $F_{1, 40} = 0.156, 95\%$  CI = -153.54-226.61) when comparing mean cumulative straight-line distances between release groups. Mean cumulative straight-line

movements did not differ among release groups ( $F_{3, 20} = 3.850, 95\%$  CI = -345.601-20.769). Additionally, the interaction between year and release group showed no difference in mean cumulative straight-line movement by turtles ( $F_{3, 20} = 0.046, 95\%$  CI = -184.49-149.13).

The total mean MCP home range area for the headstarted Blanding's turtles over the course of the study was  $2.80 \pm 0.95$  ha (Fig. 3). The mean home range for 2014 was  $0.97 \pm 2.4$  ha and for 2015 was  $1.2 \pm 2.1$  ha. Turtle home range area for the first year (2014) and the second year (2015) were not different (Fig. 3;  $F_{1,20} = 0.179, 95\%$  CI = -1.06-1.58). Home range size was different among release groups ( $F_{3,20} = 4.808, 95\%$  CI = -33,733.76 to  $-939.19, f^2 = 0.467$ ). Turtles from release group 1 ( $\overline{x} = 2.9 \pm 3.9$ ) had a mean home range area 3.7 times larger than turtles in release group 2 ( $\overline{x} = 0.61 \pm 1.3$ ), 4.6 times larger than release group 3 ( $\overline{x} = 0.51 \pm 0.37$ ), and 5.6 times larger than release group 4 ( $\overline{x} = 0.43 \pm 0.56$ ). The distance from release site to center home range for all turtles was 184.81 ± 129.60 m (Table 2). Distance between release sites and home range centers did not differ across release groups ( $F_{3,20} = 4.100, 95\%$  CI = -91.04-1.09, Table 2).

We compared habitat use to availability for 6 land cover types (willows, floating vegetation, open water, lowland forest, muskrat den, cattails) within the headstarted Blanding's turtle population MCP range ( $\chi^2_5 = 46,854.06$ , n = 24,  $P \le 0.001$ ; Fig. 4). Out of the 6 land cover types, turtles used open water, lowland forested wetlands, and willows less than their availability, used floating aquatic vegetation in proportion to its availability, and used cattails and muskrat dens more than their availability (Table 3).

We used the highest-ranking OLS model to choose our list of independent variables. We found all positively clustered independent variables in the OLS model indicating spatial autocorrelation. The habitat variables in the model were water depth, water

temperature, duckweed, cattails, muskrat dens (including muskrat disturbed areas with cut down cattails termed muskrat fields), buttonbushes, and release groups. The OLS results indicated an adjusted  $R^2$  value of 0.335. All VIF values were 1.1–1.8, which is below the recommended 7.5 indicating there is no redundancy in explanatory variables.

The GWR model showed marked increase in robustness compared to the OLS model, and several independent variables were related to turtle travel distance (Table 4, Fig. 5). The GWR adjusted  $R^2$  was 0.755, which was greater than the OLS model (adjusted  $R^2$ = 0.329). The AIC<sub>c</sub> for the GWR model was 897.07 units lower than the OLS model AIC<sub>c</sub>, indicating that the GWR model was a better fit. The GWR model results indicate that as turtles moved away from their release sites, the habitat factors encountered would influence the distance turtles moved; if habitat factors with negative coefficients were encountered turtles would continue to move, meanwhile the opposite would happen for habitat factors with positive coefficients. Cohen's  $f^2$  local effect size from the model indicated water depth and release group had the greatest effect on the distance turtles moved from release sites (Table 4). In the GWR model, turtles released in open water (release group 1) moved the greatest distance from their release site, while turtles released in dense cattails (release group 4) moved the least distance from their release site (Table 4). Turtles moved farther from their release sites as they encountered increased water depth, temperature, and duckweed (Table 4). Turtles moved shorter distances from their release sites once they encountered cattails, muskrat down cattails, and buttonbushes. Thus, headstarted Blanding's turtle's home range area was larger when released in non-preferred habitat as turtles moved farther from the release site, and turtles had smaller home range areas when released in preferred habitat as turtle movements remained closer to the release site.

#### DISCUSSION

Three main conclusions emerged from this research. First, survivorship of headstarted turtles was high (100% the first year and 63– 96% the second year). Second, headstarted Blanding's turtles behaved similarly to their wild-born counterparts. The home range comparisons from other studies support this conclusion (Table 1). Third, we found microhabitat use to be an important predictor of movement distance of headstarted Blanding's turtles, and differences in release site habitat contributed to the variation in movement behavior. Because of the high survivorship and similar movement behavior to wild-born hatchlings, we believe that thus far, the headstart program at SNWR has been a success. We suggest further monitoring and population studies to continue to monitor the Blanding's turtle population at SNWR. Future research should look into sex differences, additional age classes, and a continued effort to find wild-born juveniles within SNWR.

We observed high survivorship for the 24 headstarted Blanding's turtles tracked in this study. Congdon et al. (1993) determined that for Blanding's turtle populations to be stable, survivorship >70% for juveniles 1–13 years old is needed with a cohort generation time of 37 years. The survival estimates for the headstarted Blanding's turtles thus far in this study are within the range described by Congdon et al. (1993) if  $\geq 2$  of the headstarted Blanding's turtles that we lost signals for survived (15 surviving turtles, 8 transmitter losses, 1 mortality). An unpublished study reported that a release of headstarted turtles in SNWR in 2012 suffered mortality >50%, with 25% of the mortality occurring within 2 weeks after release due to raccoon predation (A. M. Cleary, affiliation, personal communication,). Release site locations were changed for this study as a result, and survivability of headstarted turtles increased. A balanced percentage of males, females, and juveniles should be found in healthy populations of Blanding's turtles (Valentine National Wildlife Refuge, NE, USA; Bury and Germano 2003). Because trapping and bycatch attempts (volunteer, SNWR

staff, University of Michigan-Flint student search efforts 2008–2015) at SNWR have captured no wild juveniles of this species thus far (M. D. Starking-Szymanski, University of Michigan-Flint, unpublished data), headstarting is likely required for the SNWR population to have any chance of persisting in the long term.

The headstarted Blanding's turtles in this study moved an average of 1,005 m in straight-line distance between relocations for the duration of the study. Turtles did not move differently between their first and second active seasons, similar to findings from Anthonysamy et al. (2013) where adult and juvenile Blanding's turtles did not differ in distances moved between years. We saw wide variability of cumulative and weekly movement distances in the headstarted Blanding's turtles (568.8–2,972.3 m) over the duration of the study. In Maine, Blanding's turtles migrate long distances between wetland types because of differences in seasonal needs (Beaudry et al. 2009). Adult Blanding's turtles studied by Congdon et al. (2011) in Michigan moved a variety of distances, primarily for mating and nesting; most nests were within 1,000 m away from the gravid females' resident wetland. This is similar to the 1,005-m mean distance moved by the headstarted Blanding's turtles in our study, and similarities might link to nesting dispersal distances.

For the first 18 months after release, turtle distances from release site to center home range were not different among release groups. We believe this shows the importance of release locations for headstarted turtles; turtles will remain near release sites. In the GWR analysis, distance from the release site was affected by the release site. This shows cumulatively how even though the mean center home range did not vary, home ranges were somewhat larger for some release sites presumably due to unfavorable land cover. Congdon et al. (2011) reported that 75% of adult Blanding's turtles stayed in the same resident wetland over a 19-year study, showing that Blanding's turtles have site fidelity. Newton and Herman (2009) also reported that Blanding's turtles have site fidelity, and turtles

raised in captivity overwintered with wild Blanding's turtles, indicating that headstarted turtles used the same habitats as their wild counterparts.

We used the MCP method because one of the goals of this study was to compare headstart home ranges to the home ranges of their wild counterparts. Recent methods like Brownian bridges or local convex hull (LoCoH) consider time and space when computing animal home ranges, and are more accurate at representing space use of animals (Getz et al. 2007, Horne et al. 2007). These methods, however, required 1 week between relocations, which we were unable to achieve in our study. Several studies have reported on Blanding's turtles home ranges using the MCP method, even though MCP can be an over estimation of home range size (Table 1). Additionally, Row and Blouin-Demers (2006) reported kernel home ranges to be unreliable home range estimators for herpetofauna because they overestimate area and because selection of a smoothing factor is subjective. The measured MCP home ranges for the headstarted Blanding's turtles indicated a difference between release groups; however, with no difference between years. Release group 1 had the largest mean group home range (2.9 ha) and release group 4 had the smallest mean group home range (0.4 ha). We can infer from this that the differences in these home range sizes were due to the variation of microhabitats found at the release sites. The mean MCP home range of Blanding's turtles in our study was 2.8 ha, and MCP home ranges of juvenile Blanding's turtles measured in other studies ranged from 1.3 ha to 12.8 ha (Piepgras and Lang 2000, Kasuga et al. 2007, Innes et al. 2008, Anthonysamy et al. 2013). Juvenile Blanding's turtle home ranges are smaller on average than adult Blanding's turtles; previous research supports this in studies with wild-born juveniles (Table 1). More broadly, our finding is additional evidence that the headstart program is not interfering with the juvenile turtles' behaviors and movements in the SNWR. However, we were unable to find and

compare the headstarted turtles to wild-born Blanding's turtles at SNWR because no wild-born Blanding's turtles have been captured or observed in SNWR over multiple years of sampling, thus leading to the headstart program.

We found microhabitat features to be an important predictor of habitat use of headstarted Blanding's turtles. Headstarted Blanding's turtles at SNWR avoided open water, which is similar to studies on adults (Hamernick 2000, Millar and Blouin-Demers 2011). Millar and Blouin-Demers (2012) reported a low density of open water fit in a habitat suitability model for Blanding's turtle. We found that headstarted Blanding's turtles also avoided willows and lowland forested wetlands. When we released group 1 turtles in open water, they used lowland forested wetlands. Within the lowland forest, we found these turtles most often near buttonbush or cattail stands. Hartwig and Kiviat (2007) reported that subadult and adult Blanding's turtles were associated with buttonbush in New York, USA. Headstarted Blanding's turtles used cattails and muskrat dens in a greater proportion than their availability. We released groups 2 and 4 in cattails; they had the shortest measured straight-line distance to mean center home range among release groups. We found headstarted Blanding's turtles basking on top of muskrat dens (Starking-Szymanski 2016), and tracked to muskrat dens where turtles seemed to be residing inside or under the dens. Previous data within SNWR reported that Blanding's turtles prefer emergent wetlands that contain cattails and muskrat dens (A. M. Cleary, unpublished data). Contrastingly, other research has not documented this, but other studies have not separated and analyzed land cover types separately as we have.

The results of GWR analysis showed improvements over the OLS model, and may be the first attempt at using this method for examining contributing factors on turtle travel distances from release sites. The independent variables in the GWR model (water depth and temperature, duckweed, cattails, muskrat dens and fields, buttonbush, and release sites) were able to explain 75.5% of the

variation in distance from release sites by headstarted Blanding's turtles. Taken together, the land cover types at the release sites and subsequent relocations influence the distances that head-started Blanding's turtles will travel away from their release site. Fortin et al. (2012) used logistic regression and reported that landscape composition had a weak impact on movement and home range size differences of Blanding's turtles. They reasoned that low variability of land uses within the home ranges diminished any explanatory power. We predicted distances of headstarted Blanding's turtles from release sites and compared these to home ranges and space use using microhabitat factors (dominant vegetation within a quadrat at each turtle relocation), instead of home range size and broad wetland categories. This gave us the ability to analyze the influences of variables on movement with a local geographic regression approach on animal space use.

By releasing headstarted Blanding's turtles at 4 different release sites with varying microhabitat features, measuring habitat factors at relocations, and then examining how these habitat factors related to distance from release site, we tested for the biological importance of habitats as suggested by Alldredge et al. (2006). In our study, we randomly released turtles at release site 1 into open water, with the only vegetation nearby being willows; headstarted Blanding's turtles used open water and willow less than available. Five out of 6 turtles from release site 1 left the site and never returned. This behavior does not match juvenile movement patterns from other studies. Juvenile Blanding's turtles from another study traveled less often than adults and >60% of them never moved out or between their resident wetland (Piepgrass and Lang 2000). Headstarted turtles released at site 1 had a high rate of movement out of the original wetland, which could be because the cover type at the release site was open water. Headstarted turtles released at this site had to cross a road, which is a behavior adult and juvenile Blanding's turtles often avoid (Proulx et al. 2014), and their aversion to open

water was likely an influencing factor. Edge et al. (2010) reported that fine-scale habitat preference detection may not be possible in high-quality landscapes, but we were able to detect a preference of certain land cover types even though SNWR is a complex of wetlands. Juvenile Blanding's turtles released in preferred land cover types had smaller home range sizes overall. Release locations for headstarted Blanding's turtles benefit the turtles most when they are in preferred land cover types, and it is possible to use microhabitat variables to predict turtle movement.

# MANAGEMENT IMPLICATIONS

Given the results of our study, biologists should release headstarted Blanding's turtles in their preferred habitat of dense cattails with plenty of muskrat dens for basking and refuge if available. We found that juvenile Blanding's turtles could be headstarted to 18 months of age while still behaving similarly to their wild-born counterparts. Using spatially explicit models helped to increase the predictive power of space use by these headstarted Blanding's turtles. Researchers should continue to use these models to study headstart program success because of the late maturity and longevity of Blanding's turtles. As technology increases and transmitter weight decreases, newer methods to depict home ranges of turtles will improve. By releasing turtles in a location with microhabitats suited to their needs, biologists can minimize the dangers from predation and improve management techniques that increase and stabilize populations of turtles, the most threatened vertebrate group in the world (Buhlmann et al. 2009).

#### ACKNOWLEDGMENTS

We thank the USFWS, Michigan Department of Natural Resources, S. F. Kahl, E. M. Dunton, and other employees at Shiawassee National Wildlife Refuge for all their support and assistance. We also thank T. H. Kaufman for taking orthophotos of the study area for this project. We thank J. A. Jundt and other employees at DZS for help with this research. We thank M. J. Boyer, E. S. Kiser, M. K. Schott, and H. M. Smith for field assistance. We thank the turtle crew who went above and beyond the call of duty in helping with this research and whom without this project could not have happened: W. D. Gibala, M. J. Smith, S. R. Davis, K. M. B. Hughes, J. L. Taylor, and M. M. Warda. We thank L. W. Atherton, A. C. Louis, D. P. Viele, and others from University of Michigan-Flint for support and assistance. We thank M. Goode and 2 anonymous reviewers for providing useful comments on an earlier version of this manuscript. We thank the Biology Department (Graduate Student Research Fund and Murchie Memorial Research Fund), College of Arts and Sciences, E. A. Kingsley and Office of Research and Sponsored Programs ("Doc" Studier Research Memorial Fund and Undergraduate Research Opportunity Program), Office of Graduate Programs (Graduate Student Research Assistantship), and the Fran Frazier Student Travel Scholarship for supporting this study.

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Associate Editor: Matt Goode.

Fig.1. Pools within Shiawassee National Wildlife Refuge (SWNR), Michigan, USA, June 2014 to November, used by Blanding's turtles. Release sites in Grefe Pool are shown. Shiawassee National Wildlife Refuge shown in inset map in Michigan.

*Fig.* 2. Straight-line distances (m) between relocations (weekly Apr–Oct, biweekly Nov–Mar) that each headstarted Blanding's turtle moved for 2014 and 2015 in Shiawassee National Wildlife Refuge, Michigan, USA.

*Fig.* 3. Area (ha) of minimum convex polygon home ranges for 2014 and 2015 for each headstarted Blanding's turtle in Shiawassee National Wildlife Refuge, Michigan, USA.

*Fig.* 4. Minimum convex polygon home ranges of 24 headstarted Blanding's turtles in Shiawassee National Wildlife Refuge, Michigan, USA, June 2014 to November 2015 over 18 months. Home ranges are in black. Available land cover types are shown within the population range with release sites of the turtles.

*Fig.* 5. The geographically weighted regression (GWR) adjusted local  $R^2$  values at each Blanding's turtle point Shiawassee National Wildlife Refuge, Michigan, USA, June 2014 to November 2015. The independent variables (IV) used are water depth, water temperature, cattails, muskrat downed or disturbed cattails, buttonbush, and release groups. Points represent local  $R^2$  values. Black points are areas where the IV predict the distance away from release site well (higher  $R^2$  value) with the highest  $R^2$  value at 0.877,

white points are less explained by the IV (lower  $R^2$  value) with the lowest  $R^2$  at 0.511. Additional factors may be responsible for headstarted Blanding's turtle movement at locations with lower values within Shiawassee National Wildlife Refuge. We show  $R^2$ standard deviations from high to low within the turtle population range.

Table 1. Mean home range size for Blanding's turtles across studies. We specify adult males (M), adult non-gravid females (NGF), adult gravid females (GF), and juveniles (J) and present sample size (n) in parentheses. We also show the reference, study location, and method (minimum convex polygon [MCP] or fixed kernel) used to calculate home range size.

Reference	Location	Method	п	<del>x</del> (ha)	SE
Anthonysamy et al. (2013) <sup>a</sup>	IL, USA	МСР	M (4)	22.9	±0.92
			F (9)	17.9	±5.2
			J (4)	5.4	±0.92
Edge et al. (2010)	ON, Canada	МСР	M (5)	57.1	±15.3
			F (16)	61.2	±30.4
Fortin et al. (2012)	QC, Canada	МСР	Pooled (44)	29.7	±32.3

Grgurovic and Sievert (2005)	MA, USA	95% fixed	M (14)	27.5	±0.10
		kernel	F (27)	19.9	±0.07
Hamernick (2000) <sup>a</sup>	MN, USA	МСР	M (8)	94.92	±19.31
	S		F (16)	60.75	±3.05
Innes et al. (2008) <sup>b</sup>	NH, USA	MCP	M (4)	3.7	
			F (6)	3.35	
			J (1)	3.2	
Kasuga et al. (2007) <sup>a</sup>	IL, USA	МСР	M (5)	32.53	±7.45
			NGF (9)	47.64	±3.85
			GF (2)	29.26	±2.48
			J (1)	1.27	
Millar and Blouin-Demers (2011)	ON, Canada	МСР	M (20)	8.5	±1.7

			NGF (5)	7.3	±3.2
			GF (12)	20.3	±3.5
Piepgras and Lang (2000)	MN, USA	МСР	M (6)	7.8	±0.61
			F (13)	7.8	±0.34
			J (6)	5.9	±0.40
Schuler and Thiel (2008)	WI, USA	МСР	M (9)	26.1	
			F (9)	20.7	
This study	MI, USA	МСР	J (24)	2.80	±0.95
a. Other methods along with N	MCP were used in study	, such a kernel	density.		
Median values used.		1			1 1
headstarted Blanding's turtles a	ndard deviation (m) betw at Shiawassee National V	ween home ran Wildlife Refuge	ge center and rel e, Michigan, USA	ease site for A, June 2014	to Novemb
Release site	Distance		SD		

1 (open water)	371	254	
2 (sparse cattails)	148	133	
3 (willows)	213	68	
4 (dense cattails)	150	141	
x	185	130	
			,

Table 3. Habitat availability versus use Chi-square goodness-of-fit test results with Bonferroni *z*-stat confidence intervals for headstarted Blanding's turtles at Shiawassee National Wildlife Refuge, Michigan, USA, June 2014 to November 2015. We show the 95% confidence intervals around proportion of habitat use (observed) compared to habitat available for the headstarted Blanding's turtles.

Habitat variable	Proportion of	Habitat use	Number of	Bonferroni z-stat CI
	area available	compared to	relocations	on proportion of
		available	observed	observed
Open water	0.395	less	4	-0.001 to 0.010

Cattails	0.270	more	489	0.501 to 0.587
Lowland forest	0.190	less	132	0.117 to 0.178
Willows	0.119	less	20	0.009 to 0.035
Floating aquatic	0.025	in proportion	16	0.006 to 0.029
Muskrat dens	0.001	more	238	0.226 to 0.302
		300		

Table 4. Geographically weighted regression analysis of distance moved from release sites for headstarted Blanding's turtles at Shiawassee National Wildlife Refuge, Michigan, USA, June 2014 to November 2015. For each independent variable, we present the variance inflation factor (VIF), mean geographically weighted regression model coeffecient, confidence interval, and Cohen's  $f^2$  effect sizes (small =  $f^2 \ge 0.02$ , medium =  $f^2 \ge 0.15$ , and large =  $f^2 \ge 0.35$  effects; Cohen 1988).

Habitat variables	VIF	Mean 95% CI		Cohen's $f^2$
		coefficient		
Water depth (cm)	1.791	-0.737	-0.786 to -0.688	0.343

Water temp (C°)	1.330	-0.257	-0.308 to -0.205	0.076
Duckweed <sup>a</sup>	1.531	-0.272	-0.287 to -0.256	0.047
Cattails <sup>a</sup>	1.398	0.173	0.149 to 0.196	0.116
Muskrat down cattails <sup>a</sup>	1.723	0.350	0.331 to 0.368	0.051
Buttonbush <sup>a</sup>	1.145	1.197	1.044 to 1.350	0.124
Release group 3 <sup>b</sup>	1.233	127.153	124.703 to 129.603	0.267
Release group 2 <sup>b</sup>	1.339	149.010	137.422 to 160.597	0.430
Release group 1 <sup>b</sup>	1.742	392.117	378.007 to 406.226	0.302

<sup>a</sup>Proportion measured in quadrant at turtle relocations.

<sup>b</sup>Dummy variables (reference is release group 4).

Article Summary: We aimed to determine survival, movements, habitat use in relation to availability, and impact of release sites on 18-month-old headstarted Blanding's turtles by releasing them at 4 different sites in Shiawassee National Wildlife Refuge. We showed high survival of the headstarted Blanding's turtles, reported differences in home range size across release groups, and used geographically weighted regression to explain how turtle distance from release sites varies by measured habitat characteristics; we concluded that habitat characteristics in the small spatial extent at release sites affect movement and space use of headstarted Blanding's turtles.









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Figure 3



