The Use of Nest Boxes by the Hellbender Salamander in Western North Carolina

by

Arianne Messerman

Advised by Dr. John Poulsen

April 2014

Master's Project submitted in partial fulfillment of the

requirements for the Master of Environmental Management degree in

the Nicholas School of the Environment of Duke University

2014

Abstract

The hellbender salamander (*Cryptobranchus alleganiensis*) is a unique, large-bodied amphibian that serves as an excellent water quality indicator species in Western North Carolina. This animal has suffered substantial population declines over the past four decades throughout its range. Increased stream siltation largely attributed to human development fills the concave undersides of large rocks, consequently destroying hellbender breeding habitat. Habitat degradation has contributed to reductions in North Carolinian populations to such a degree that the species is now considered of Special Concern in the state. In order to restore hellbender population sizes under current land use conditions, researchers have recently begun developing artificial nest boxes that exclude sediment and promote increased reproduction. To identify the short-term efficacy of these shelters as substitutes for natural hellbender habitat in Western North Carolina, I constructed and placed 54 boxes across five river sites throughout the region. Following summer nest box installment, I examined each shelter through the breeding season for hellbender inhabitation and to determine the quality of water passing through the structures. Additionally, I created a maximum entropy species distribution model and conducted a spatial connectivity analysis for the hellbenders of Western North Carolina to identify ideal locations for nest boxes installation in the future. Although no hellbenders have yet been detected in the artificial shelters, additional structural improvements and time may reveal nest boxes to be useful conservation tools for this iconic species of Special Concern.

Introduction

The Appalachian Mountains of Western North Carolina are known to possess one of the greatest diversities of salamanders in the world (Dodd 2004). At least 31 species have been recorded in the Great Smoky Mountains National Park alone (Freake & Lindquist 2008), including North America's largest salamander, the hellbender (*Cryptobranchus alleganiensis*) (Nickerson *et* *al*., 2002). The hellbender is a completely aquatic, carnivorous species found in cool mountain streams throughout the Appalachian and Ozark Mountains (Nickerson & Mays 1973; Petranka 1998). These salamanders are harmless to humans, and their aquatic nature, sensitivity to pollutants and long lifespan make them particularly useful indicators of stream quality (Olson *et al*., 2012; Welsh & Ollivier 1998).

The hellbender has been listed as a species of Special Concern in North Carolina, as its populations have declined drastically in recent decades (Nickerson & Mays 1973; Wheeler *et al*., 2003). The primary cause of decline is thought to be an increase in human development in many watersheds where hellbenders exist, which leads to higher rates of sediment runoff into local streams (Wheeler *et al*., 2003). The influx of particulate matter reduces stream dissolved oxygen content, potentially reducing the fitness of these fully aquatic salamanders (Harlan & Wilkinson 1981; Ringler & Hall 1975). Additionally, increased sedimentation fills in concave, protected undersides of large rocks that are required for successful hellbender reproduction (Briggler & Ackerson 2012; Browne *et al*., 2012). The loss of nesting habitat has been implicated in breeding failure in many extant hellbender populations (Nickerson *et al.,* 2002; Wheeler *et al*., 2003). The persistence of hellbenders is further threatened by habitat loss and degradation from damming operations, increases in stream acidity and pollution, as well as direct harvesting for herpetological specimen or because they are wrongly perceived as predators of game fish (Beane *et al*., 2010; Freake & Lindquist 2008; Nickerson *et al*., 2002; Nickerson & Briggler 2007; Wheeler *et al*., 2003).

In order to conserve hellbenders in the face of these threats, new approaches are being developed to encourage the growth of known populations. Briggler and Ackerson (2012) demonstrated that artificial nest boxes placed in hellbender-inhabited streams of Missouri have provided sediment-free shelter and nesting sites for hellbender populations with notoriously low reproductive rates associated with habitat loss. Both captive and wild hellbenders occupied these "boot-shaped" nest boxes, suggesting that the structures adequately mimicked natural nest conditions. Briggler and Ackerson's (2012) study also revealed that male hellbenders were capable of effectively defending eggs from within nest boxes. The maintenance of this aggressive male behavior is important in protecting vulnerable eggs from predators, such as other adult salamanders and carnivorous fish (Smith 1907).

Following Briggler and Ackerson's (2012) initial work, I conducted a study to determine the short-term efficacy of using artificial nest boxes to increase available hellbender breeding habitat in the streams of Western North Carolina, with the long-term goal of increasing hellbender populations. More specifically, I built nest boxes based on the designs of Briggler and Ackerson (2012), and monitored these shelters through the hellbender breeding season of 2013. The results of this pilot field study contribute to our understanding of hellbender microhabitat preferences, as well as nest box construction and utility within North Carolina.

In addition to collecting data on the inhabitation of newly installed nest boxes, I set out to determine the best locations to place these shelters in the future. If nest boxes prove to be useful for augmenting hellbender reproduction, managers should install them at sites that will encourage increases in animal abundance and connectivity between extant populations. To identify such locations, I first predicted hellbender habitat and non-habitat within Western North Carolinian stream sites by generating a species distribution model (SDM) using presence-only data from the North Carolina Wildlife Resources Commission's and North Carolina Natural Heritage Program's surveys and records. Maximum Entropy Theory and the corresponding MaxEnt software have frequently been shown to make accurate predictions of habitat and non-habitat from similar records and environmental variable datasets (Elith *et al*., 2006). I therefore used MaxEnt to predict locations where hellbenders are likely to exist in Western North Carolina.

The resulting habitat predictions, combined with dispersal data and expert opinion, allowed me to perform a spatial connectivity analysis using Geographic Information System (GIS) software to identify the best location for future nest box placement. This analysis systematically revealed stepping-stone locations between known hellbender populations across which these salamanders were predicted to be able to disperse. By connecting extant populations with the provision of shelter and nesting habitat at the proposed sites, hellbender populations would hypothetically be able to expand and more frequently exchange genetic material. The occasional sharing of genes between populations leads to increased genetic diversity resulting in greater opportunities for adaptive evolution and a reduced risk of genetic drift (Spielman 2004). The connectivity analysis not only provided a map of recommended nest box installment sites, but also indicated the order in which sites should be installed based on their proximity to known hellbender populations. In this way, the connectivity analysis should serve as a user-friendly and instructive tool for environmental managers concerned with the long-term preservation of hellbenders in Western North Carolina.

Methods

Nest Box Construction

I collaborated with the environmental not-for-profit Wild South and Warren Wilson College (WWC) to design and construct 54 "boot-shaped" nest boxes. We modified the design of Briggler and Ackerson (2012) by using wooden molds and 5500 psi Maximizer Multi-Project Concrete Mix. Nest box construction entailed an eight-step process that was completed over the course of a month (Figure 1). We took care to create long tunnel entrances in accordance with the design of Briggler and Ackerson (2012). We also mixed all concrete with charcoal cement color so that the boxes would blend into surrounding stream habitat. Although the high-quality concrete we used was relatively non-toxic, we placed each shelter in stream sites at least three weeks prior to breeding season to ensure that any unbound chemicals would be washed away before inhabitation by salamanders (Paul Bobbitt *pers. comm.*).

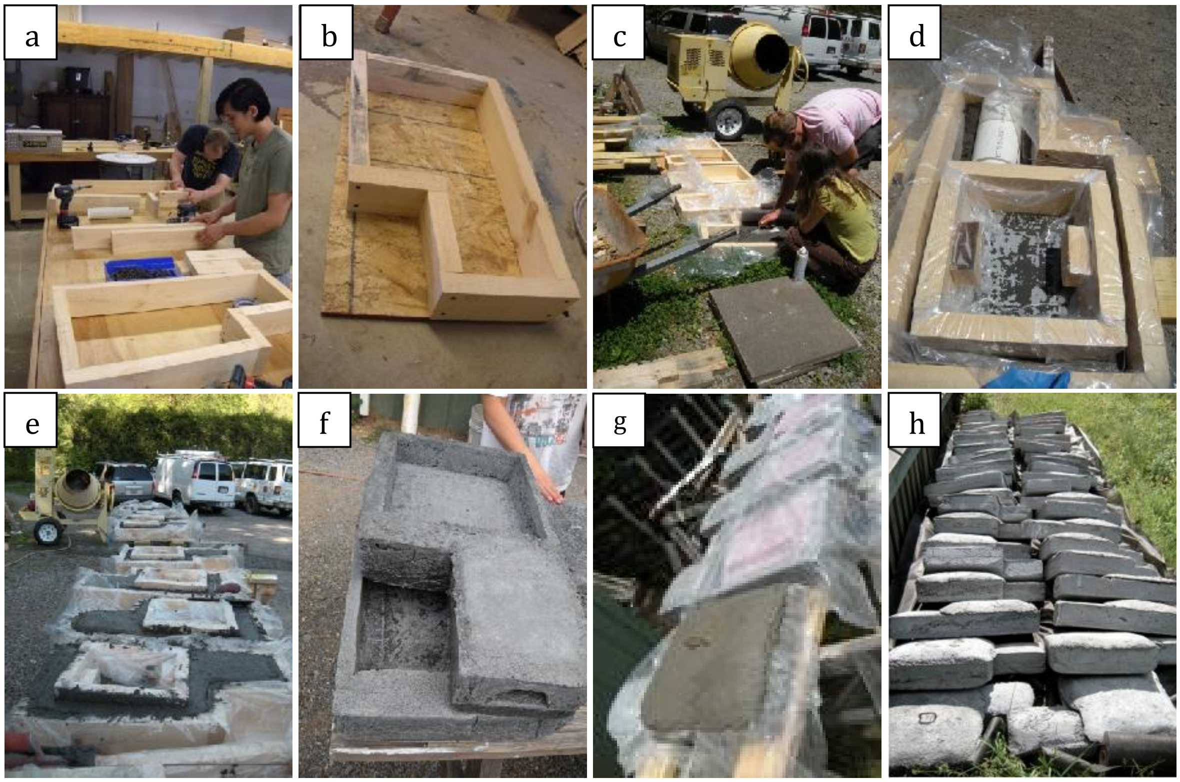

Figure 1. We constructed nest boxes by (a) building wooden molds with 2"x4" walls and (b) a plywood base; (c) pouring concrete for the flooring; (d) inserting two thirds of a 4" PVC pipe, a thin piece of rebar, and an internal wooden frame; (e) pouring concrete to cover the rebar and PVC piping that would serve as walls; (f) removing the hardened concrete from the molds; (g) filling the cavity with insulating foam, and overlaying this foam with painter's plastic and concrete to form a lid that tightly fit each box; and (h) removing all plastic and foam to produce 54 finished structures.

Site Selection, Nest Box Installment and Data Collection

We placed nest boxes in five stream sites located in Western North Carolina. We selected streams with known hellbender abundances so that we could compare the efficacy of the nest boxes across a range of population sizes (unpublished data, Lori A. Williams). Because the hellbender is a species of Special Concern, occurrence locations are kept confidential by the State of North Carolina. Here, we designate the study streams as FC, USM, SMC, TPG, and SLC. The USM, SMC and TPG sites were known to have a relatively large number of sexually mature hellbenders. Adult hellbenders have been recorded at FC and SLC, but no evidence of reproduction has been reported for these populations in recent years. The FC and SLC sites are thought to be representative of many of the declining hellbender populations across Western North Carolina.

We installed all nest boxes by the beginning of August 2013. Boxes were assigned to the various stream sites at random. We used the methods of Briggler and Ackerson (2012) to dig out a small pit for each nest box so that it would lie flush with the stream bottom, set the box into the cavity, and cover it over with stones and debris for stability and camouflage. Typically, males excavate the entrances to nests facing downstream, shielded from the full force of the current (Pfingsten & Downs 1989). Therefore, we positioned nest boxes with entrances facing downstream.

We placed nine shelters at both FC and SLC, and 12 shelters at USM, SMC and TPG. This distribution of nest boxes was chosen after unprecedented rainfall made a sixth stream inaccessible, as flooding and strong currents produced unsafe conditions and increased the probability that nest boxes would be washed away. We distributed the shelters intended for this sixth site across the three sites with the highest observed hellbender abundances (USM, SMC, and TPG) to increase the chances that a hellbender might encounter one of the structures. After installation of the nest boxes, we collected data on stream flow speed, pH, total suspended solids, dissolved oxygen levels, water temperature, air temperature, current time and weather. These data indicated the quality of the water to which nest box inhabitants would be exposed, and could therefore provide insights into the microhabitat preferences of hellbenders. We measured water temperature and pH using a Hanna Instruments HI 98128 pH meter. We sampled suspended solids by collecting one-liter of stream water at the mouth of each tunnel entrance, filtering these samples through Gelman Sciences type A/E glass fiber papers immediately upon return to WWC, drying filter papers in an oven for over 24 hours, and weighing these papers to determine the change in their weight after filtering. We measured dissolved oxygen levels using a YSI model 52 dissolved oxygen meter and model 5739 field probe. Additionally, we collected flow data using a JDC Instruments Flowatch Air or Liquid Flow Measurement Instrument with a 60mm water impeller.

Every three to four weeks following nest box placement, we visited each of the five sites to assess hellbender occupancy of the shelters and collect water quality data. We visually inspected nest boxes for salamander occupancy using a snorkel mask and underwater flashlight. To avoid disturbing natural hellbender nesting habitat and breeding behavior, we were careful not to overturn rocks or upset nest boxes at the study sites. We visited each site throughout the hellbender breeding season from September through November 2013.

Species Distribution Modeling

In order to support future hellbender conservation efforts, I collaborated with the U.S. Fish and Wildlife Service (USFWS), NCWRC, North Carolina Zoo and North Carolina Natural Heritage Program (NCNHP) to produce an SDM for hellbenders in Western North Carolina using MaxEnt software version 3.3.3k (Phillips *et al.,* 2005). MaxEnt constructs models using statistics and machine learning to explain moments in data using the loosest fitting distribution possible (Elith *et al*., 2006). The resulting distribution explains trends in the data on which the model was built, but makes as few assumptions as possible in fitting other datasets. In building such a model, only

Table 1. Environmental variables used to predict the distribution of hellbenders in Western North Carolina**.**

Name	Description	Units	Source
Drainage	Cumulative drainage area averaged over a stream segment	km^2	NHDPlusV2
Flow	Average annual volume of water passing through a stream segment per unit time	ft^3/second	NHDPlusV2
Geology	Geological classification pertaining to the substrate of each stream segment	Categorical	North Carolina Geologic Map Data
Pctbarren	Percent of barren land per catchment of each stream segment	Percent	NLCD2006
Pctcrop	Percent of croplands per catchment of each stream segment	Percent	NLCD2006
Pctdev	Percent of developed lands per catchment of each stream segment	Percent	NLCD2006
Pctforest	Percent of forested lands per catchment of each stream segment	Percent	NLCD2006
Pctimperv	Percent of impervious surface per catchment of each stream segment	Percent	NLCD 2006 Percent Developed Imperviousness
Pctpasture	Percent of pasture lands per catchment of each stream segment	Percent	NLCD2006
Pctshrub	Percent of shrublands per catchment of each stream segment	Percent	NLCD2006
Pctwater	Percent of water per catchment of each stream segment	Percent	NLCD2006
Pctwetland	Percent wetlands per catchment of each stream segment	Percent	NLCD2006
Precip	Average annual precipitation received by a stream segment's catchment	mm	NHDPlusV2
SARP	Southeast Aquatic Resource Partnership measure of streamside development within 100 meters of each stream segment	Percent	NLCD2006
Sinuosity	Degree of deviation in each stream segment's path from the shortest possible path	N/A	NHDPlusV2
Slope	Slope of flowline	cm/cm	NHDPlusV2
Strahler	Strahler's stream order category based on upstream tributary number	Categorical	NHDPlusV2
Temp	Average annual temperature for each stream segment's catchment	˚C	NHDPlusV2
Velocity	Average annual rate of discharge per stream segment	m/s	NHDPlusV2

presence and background data are compared. Because many species datasets only accurately provide presence points, the so-called "presence-only" nature of MaxEnt is extremely appealing to many ecologists (Elith *et al*., 2011). Further, many MaxEnt models have proven to be quite accurate when used in species distribution modeling (Elith *et al*., 2006). In ecological studies, the closer environmental predictor values of a given point are to those of known presence locations, the higher the habitat quality predictions of MaxEnt will be for that site. In this study, we used nineteen environmental variables and a final data set consisting of 868 GPS point locations where hellbenders had been observed within the last ten years from the NCWRC and NCNHP databases. Duplicates had been removed from the merged dataset before being used in MaxEnt.

In collaboration with the USFWS, we selected environmental variables with known ecological relevance to hellbenders to predict habitat in the SDM (Table 1). Specifically, we used the National Hydrography Dataset Plus version 2.1 (NHDPlusV2) to assign values of cumulative drainage area, stream flow, velocity, water temperature and Strahler's stream order to each stream segment in North Carolina (Horizon Systems Corp. 2013). Additionally, we generated stream sinuosity from NHDPlusV2 using the ArcToolbox Calculate Sinuosity (ESRI 2011). We also calculated percent land use and land cover layers, with the exception of percent of impervious surface, for each stream segment using the 2006 National Land Cover Database (NLCD2006)(USGS 2013a). The percent of impervious surface surrounding each stream segment was generated from the 2006 National Land Cover Dataset Percent Developed Imperviousness (USGS 2013b). We assigned a geological code to each stream segment from North Carolina Geological Map Data (Nicholson *et al.,* 2005). Lastly, a percent riparian disturbance variable was incorporated by using the methodology described by the Southeast Aquatic Research Partnership (SARP) restricted to North Carolina (Kaeser & Watson 2011). Briefly, we reclassified the categories of developed open space, low intensity development, medium intensity development, high intensity development, barren land, cultivated crops, and hay/pasture from NLCD2006 to a single value. We then calculated the area of this new riparian disturbance class within a 100-meter buffer zone around each NHDPlusV2 stream segment. The tabulated areas were divided by the total stream segment buffer area and multiplied by 100 to generate the percent of riparian disturbance for each stream segment.

The processing of each of the environmental variables was performed in ArcGIS Version 10.1 (ESRI 2013). Before import into MaxEnt, we masked each of the environmental variable layers to the state border and to locations with an elevation greater than 330 meters (equivalent to the Appalachians within North Carolina to which hellbenders are restricted), as determined by a 30-meter resolution digital elevation model (DEM) of the state from the National Elevation Dataset (USGS 2013c). After masking, each variable layer was converted to a 30-meter resolution ASCII file format for use in MaxEnt.

We employed MaxEnt software to construct a reliable model for predicting where hellbender habitat and non-habitat currently exist in Western North Carolina. Further, we were interested in the importance of each environmental variable in predicting hellbender habitat. We therefore programmed MaxEnt to perform clamping, jackknifing, produce plots and run ten-fold cross-validation as described below.

Clamping restricted MaxEnt habitat predictions to locations that had environmental variable values within the range of predictor values observed at known occurrence points. This prevented the model from predicting habitat at sites that were extremely different from any recorded hellbender location in Western North Carolina.

Commands to perform jackknifing and produce plots allowed us to assess the importance of individual habitat variables. Jackknifing builds a model with all variables included but one, and a model with no variables included except for the one that had previously been excluded. This was done for all of the environmental variables used to construct the SDM. Those variables that reduced the predictive gain of the model when removed possessed unique information for model construction that was not accounted for by any other variable. Similarly, those variables that produced models with the most predictive gain in the absence of all other environmental variables were strong predictors of hellbender habitat. Parameterizing MaxEnt to produce plots generated figures of correlations between habitat prediction and singular environmental variable values. These figures demonstrated how shifts in environmental variables impacted the occurrence of hellbender habitat and non-habitat.

Cross-validation determines the accuracy of the model by randomly extracting 10% of the occurrence points and then predicting their values from a model built with the remaining 90% of the data. We ran ten of these sub-sampled models, with each presence record being used in a testing sub-set only once, as ten-fold cross-validation is a commonly employed technique which reduces variance while allowing only a small amount of statistical bias into the model's predictions (Kohavi 1995). By using these methods, the final model representing the averaged cross-validated model more closely estimated the true predictive power of the SDM.

The output of the MaxEnt SDM was an ASCII file that assigned a value from one (high quality hellbender habitat) to zero (non-habitat) to all stream locations in Western North Carolina. Based on these binary predictions, we created a confusion matrix to assess model accuracy in ArcGIS. We performed this post-processing using the averaged threshold values of the ten cross-validated models. The averaged cutoff value chosen was 0.01, and represented the threshold recommended by MaxEnt, which balances training omission, predicted area and cumulative threshold values. This calculated cutoff is commonly used, and has been suggested to produce more accurate predictions of habitat and non-habitat for vertebrates than the other available cutoff values generated by MaxEnt (VanDerWal *et al.*, 2009). To create the aforementioned confusion matrix, we loaded the averaged logistic MaxEnt output into ArcGIS, converting from an ASCII format to a raster layer. We defined these spatial data using the NAD 1983 State Plane NC FIPS 3200 (meters) projected coordinate system. We then applied the averaged threshold value to the data by reclassifying the output file so that predictions greater than or equal to 0.01 would be given a value of one (habitat), while predictions less than 0.01 would be given a value of zero (non-habitat). We randomly generated 868 pseudo-absence points (*n* equal to that of presence points) no closer than 500 meters apart across Western North Carolina using the Create Random Points tool. After appending the presence points to this dataset and creating a feature layer of the output, we used the Select Layer by Location tool to identify the points in the new dataset that intersected stream segments reclassified as habitat. We then utilized the Calculate field tool to convert the ID values of the selected points to one (habitat). We repeated these methods to determine which points overlapped stream segments reclassified as non-habitat. Next, we exported the attributes table of the combined presence and pseudo-absence shapefile to a database table for use in Microsoft Office Excel. In Excel, we calculated the number of presence points located in predicted habitat, the number of presence points located in predicted non-habitat, the number of pseudo-absence points located in habitat, and the number of pseudo-absence points located in non-habitat. We were then able to calculate the total number of accurate predictions made by the model, as well as the specificity (true negative rate) and sensitivity (true positive rate) of the model.

Connectivity Analysis and Site Recommendations

Using the MaxEnt binary habitat/non-habitat prediction layer in ArcGIS, we performed a connectivity analysis designed to identify the best locations to install nest boxes with the purpose of enhancing dispersal between extant hellbender populations. Ideal locations were designated as those that (1) are within the maximum movement distance of 1000 meters for hellbenders (Gates 1985); (2) occur in stream segments predicted to be habitat, so that a salamander could survive and travel through the area; (3) boast an absence of dams, which are thought to inhibit hellbender

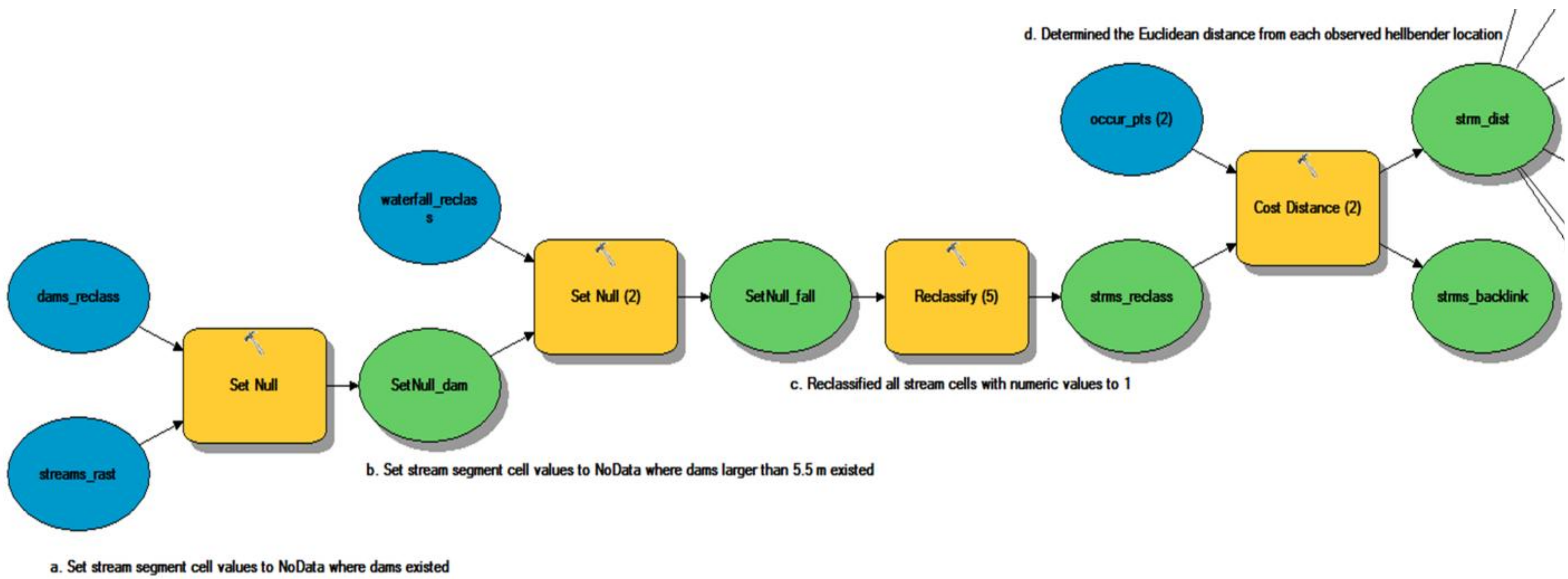

Figure 2. Steps a through d of the geoprocessing model designed to conduct a connectivity analysis to determine locations where hellbender nest boxes could be installed in the future in Western North Carolina.

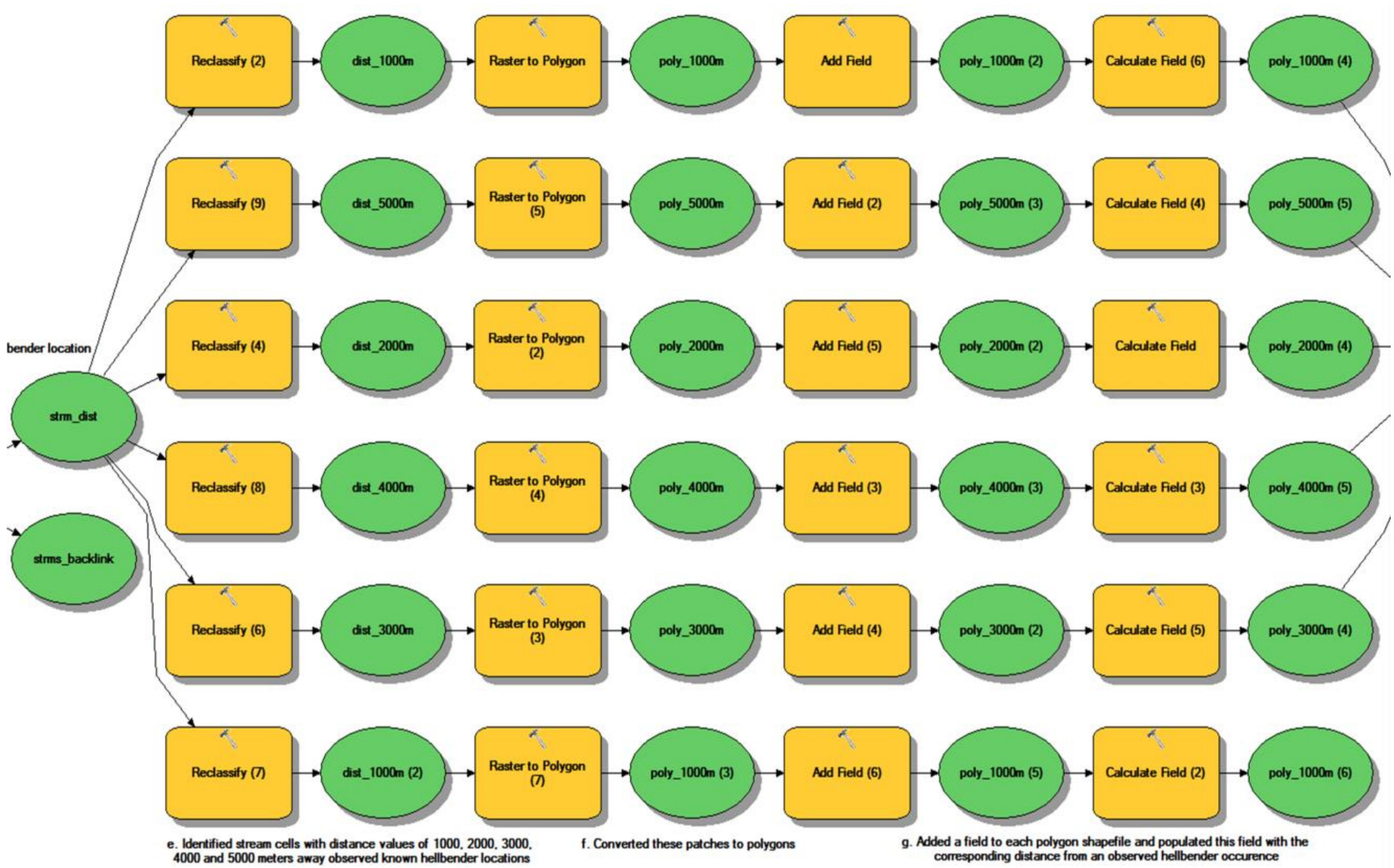

Figure 3. Steps e through g of the geoprocessing model designed to conduct a connectivity analysis to determine locations where hellbender nest boxes could be installed in the future in Western North Carolina.

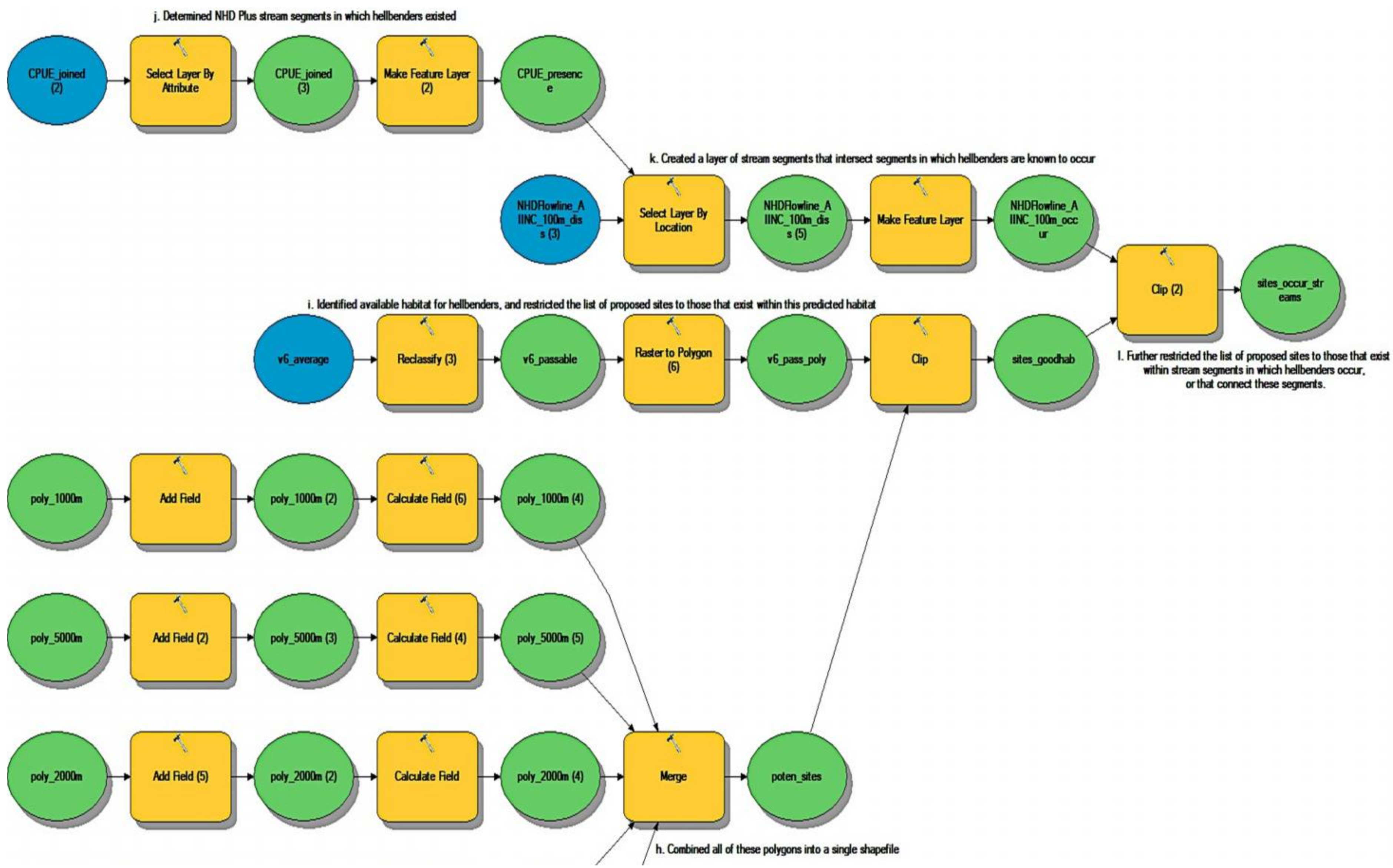

Figure 4. Steps h through l of the geoprocessing model designed to conduct a connectivity analysis to determine locations where hellbender nest boxes could be installed in the future in Western North Carolina.

dispersal (Dr. John Groves and Lori A. Williams *pers. comm.*); (4) are characterized by an absence of waterfalls greater than 5.5 meters tall, which are thought to be impassable for hellbenders (Dr. John Groves and Lori A. Williams *pers. comm.*); and (5) lie between two known hellbender populations.

To determine stream sites that met the above-designated criteria, we first obtained dam point locations (NCDENR 2013) and well-known waterfall point locations (Mitchell 2013) for Western North Carolina. Although the waterfall data available were not comprehensive, they did allow us to identify many of the waterfalls in the region that stand greater than 5.5 meters tall. With these files, we created a geoprocessing model in ArcMap and created a polygon shapefile from all of the stream segments of a raster layer (Figure 2). We used the SetNull tool to set portions of stream segments overlapping dams and waterfalls (buffered by 200 meters) in the stream polygon layer. This action indicated to the software that dispersal distance could not be calculated past one of these barriers. After converting the stream polygon layer back to a raster file and reclassifying the stream values to one, we calculated the cost distance from each hellbender occurrence record across the stream layer. In effect, this step calculated the Euclidean distance away from presence records within the confines of the streams. We then identified patches between 900 and 1100 meters away from a known hellbender location, and generated a polygon shapefile to mark these sites (Figure 3). We repeated site selection every 1000 meters, out to a value of 5100 meters. We added data indicating the distance away from a population for each of the proposed site polygons, combined these features into a single shapefile, and clipped the combined site polygon layer exclusively to the stream segments predicted to be hellbender habitat (again using a cutoff threshold value of 0.01[Figure 4]). To further refine the number of proposed sites to stream segments that lie between known hellbender populations, we then clipped the proposed sites to those intersecting with stream segments that contained recent hellbender observations, as well as those in stream segments that intersected occupied streams. Using the Editor toolbar in ArcGIS, I manually removed any remaining patches that did not lie between two observation locations, as

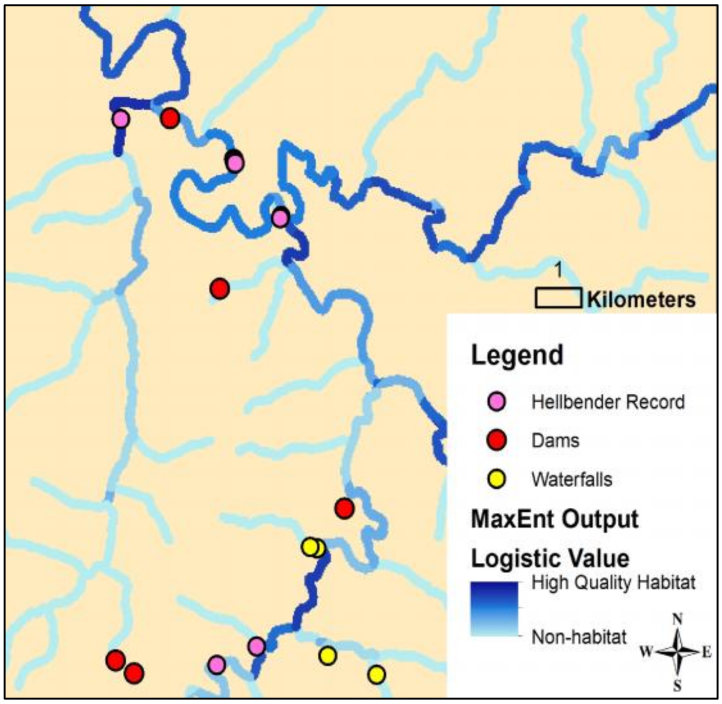

Figure 5. A visualization of the logistic predictions of hellbender habitat (one) and non-habitat (zero) produced by the MaxEnt model for an anonymous stream in Western North Carolina.

well as those that existed in stream segments where a dam or large waterfall separated known hellbender populations. Lastly, I cleaned these data by removing any small polygon fragments that were created during the analysis by deleting those that had an area of less than 600 meters^2.

Results

Nest Boxes

No hellbenders were observed within any of the installed nest boxes between August and December of 2013. Western North Carolina received 13.34 cm of rain from June through August 2013, compared to the hundred-year average of 7.72 cm for that same time period (National Climatic Data Center 2014). Following this record-breaking rainfall, all but three of the nest boxes remained intact. The shelters that were lost had been placed at FC early in the summer, before stream flow was at its peak. Some of the entry tunnels on the remaining boxes became buried in sediment and leaf litter over time. By the conclusion of the initial study period, small fish and large crayfish were observed inside several of the nest boxes.

Table 2. The percent contribution of each environmental variable to the MaxEnt hellbender distribution model.

Variable	Percent contribution	Permutation importance
drainage	31.9	21.8
geology	14.9	3.4
flow	12.6	4.2
strahler	10.7	5.4
pctbarren	6	1
precip	5.7	14
sarp	3.3	10.2
slope	3.1	16.8
temp	2.7	7.7
velocity	2.1	2
pctshrub	1.2	1.2
pctforest	1.2	3.7
pctwater	1.2	2
pctwetland	0.9	0.4
pctimperv	0.9	3.2
pctdev	0.6	1.3
pctcrop	0.4	0.8
sinuosity	0.4	0.4
pctpasture	0.2	0.4

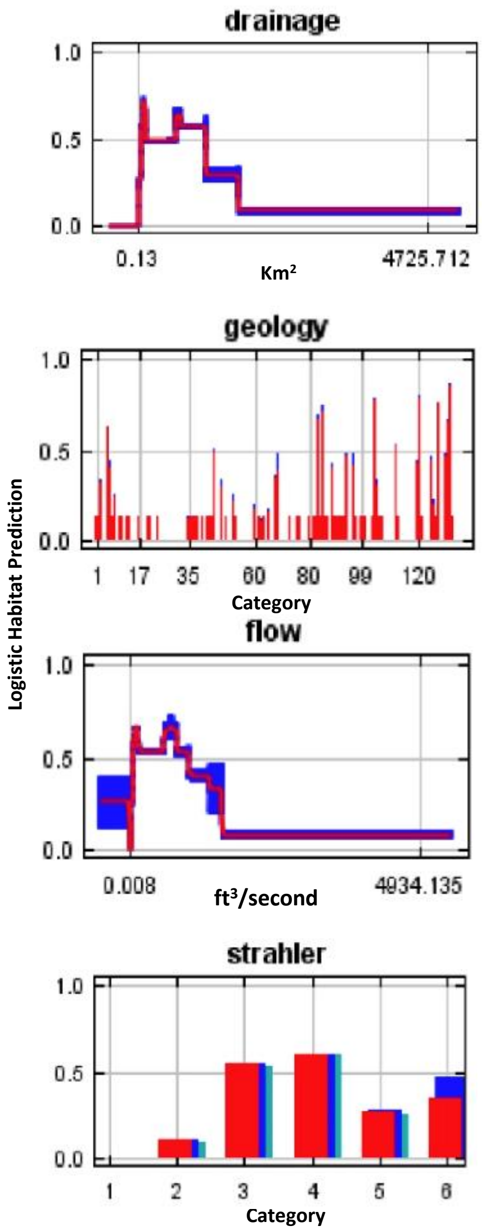

Figure 6. Average response curves (red) of habitat predictions over variable values for the top-contributing environmental predictors for the ten cross-validated models produced by MaxEnt with +/- standard deviation values (blue).

Species Distribution Model

The MaxEnt SDM produced a map of stream segments in Western North Carolina, with each location being assigned a predictive logistic value between zero (non-habitat) and one (high-quality habitat) (Figure 5). A predictive model's area under the receiver operating characteristic curve (or AUC) provides an estimation of that model's accuracy in classifying locations as habitat or non-habitat, with a potential value of one indicating perfect accuracy and a potential value of 0.5 being no better than random. The averaged AUC from the ten cross-validated hellbender models demonstrated high predictive accuracy, with a value of 0.968 and a standard deviation of 0.003. The averaged SDM revealed cumulative drainage area to contribute most substantially to the model, followed by geology, flow, and then Strahler's stream order (Table 2). None of the other variables used contributed more than 6% to the model. From the response curves generated by the software, it was clear that smaller cumulative drainage areas, geologic substrates from the Toxaway Gneiss, Boyd Gap formation, Grandfather Mountain formation or Rock of Brevard fault zone, low to moderate flow levels, and a Strahler's stream order value of three or higher were important qualities of predicted hellbender habitat (Figure 6). Metamorphic

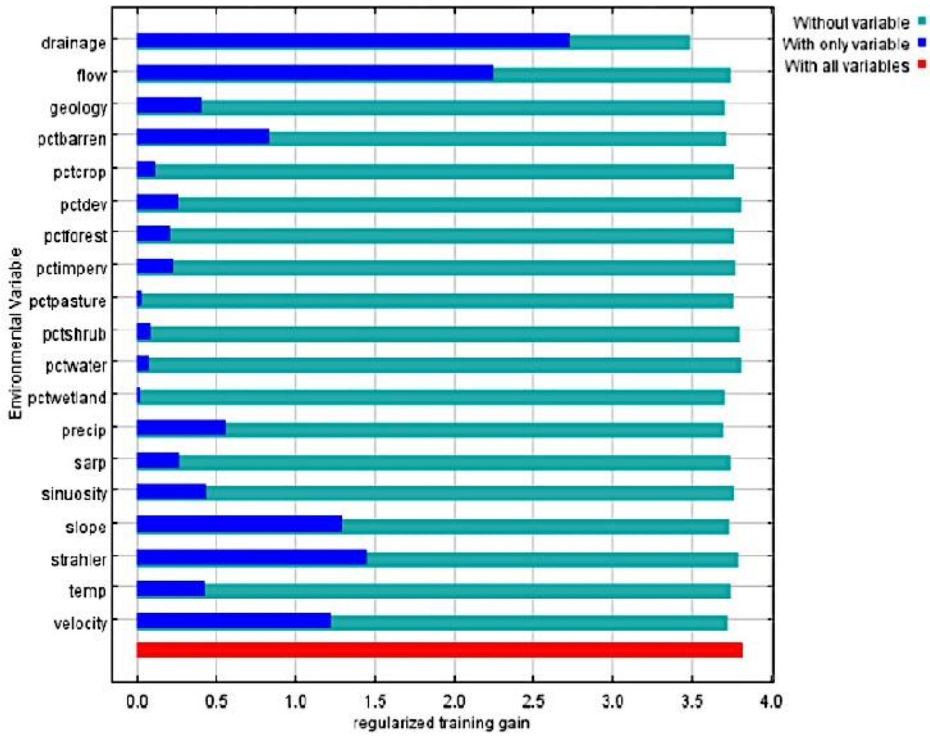

Figure 7. The averaged results of jackknifing for all of the environmental variables used to construct the ten cross-validated hellbender species distribution models produced in MaxEnt software.

and slate rock types characterize the geologic substrates important in predicting hellbender habitat. The response curves also provided some indication that hellbenders might prefer streams with velocity values between 1 and 2 m/s, precipitation values between 60 and 80 mm, temperature values between 49 and 54°C, and stream segments that were less impacted by human development. When each predictor variable was excluded from the model in turn during jackknifing, cumulative drainage area contributed the most predictive gain to the model (Figure 7). Similarly, when a model was made containing only a single environmental variable, the model using cumulative drainage area showed the greatest gain, closely followed by the model including flow.

Post-processing of the MaxEnt SDM thresholded to a value of 0.01 indicated high accuracy in predicting hellbender habitat and non-habitat (Table 3). The proportion of hellbender occurrence points that were accurately predicted to be habitat was 99.19%. The proportion of pseudo-absence points that were accurately predicted to be non-habitat was 79.15%. The overall proportion of accurate predictions made by the SDM was 89.17%.

Table 3. A confusion matrix indicating the accuracy of the MaxEnt species distribution model in predicting hellbender habitat and non-habitat based on presence and pseudo-absence points.

		Data	
		Habitat	Non-habitat
Predictions	Habitat	861	181
	Non-habitat	7	687
	Sensitivity = 99.19%		
	Specificity = 79.15%		
	Total True Predictions = 89.17%		

Connectivity Analysis

The connectivity analyses in ArcGIS produced a map of 356 proposed sites for future nest box placement in Western North Carolina (Figure 8). The numbers of proposed sites that were within 1000, 2000, 3000, 4000 and 5000 meters of a hellbender observation point were 172, 88, 51, 27 and 18, respectively. If the use of these installation sites effectively connects known hellbender populations across Western North Carolina, 57% of the recently described hellbender occurrence locations would become better connected to known neighboring hellbender populations.

Discussion

Nest Boxes

The lack of hellbender inhabitation of nest boxes in 2013 may have been due to flaws in the design of the structures, an abundance of quality habitat at my study sites, or the short time period over which the shelters were in place. Alternatively, hellbenders may not have occupied the shelters because these animals were no longer present at the study sites, or because unusually high water levels prevented nesting.

The nest boxes may have failed to offer the habitat conditions preferred by hellbenders. Potential deficiencies of our structures may have included low water flow within the nest box cavities, too much sediment within the shelters, or an interior compartment that was too small for large adults. Evidence for these deficiencies comes from low flow readings at the mouths of the nest boxes, small piles of sediment found in tunnel openings and suggestions made by initial prototype creator, Dr. Jeffrey Briggler. However, the unusually high precipitation made it difficult to assess whether the absence of hellbenders within the nest boxes was due to shortcomings of our design or

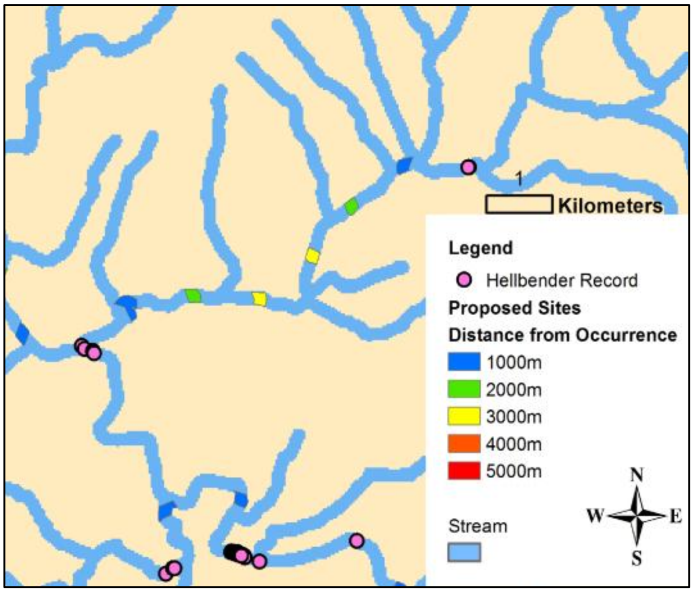

Figure 8. A visualization of proposed artificial hellbender habitat instillation sites as identified through a geospatial connectivity analysis for an anonymous stream in Western North Carolina.

environmental disturbance. If in subsequent breeding seasons no hellbenders are found within the 2013 nest boxes, it will likely be more apparent that the structures themselves are problematic. In such a case, new designs should be created and tested for enhanced performance as hellbender habitat. Still, the appearance of crayfish and fish inside nest boxes in the final month of the 2013 monitoring period suggested that other aquatic species (including the hellbender's primary food source [Nickerson & Mays 1973]) are finding the shelters useful as habitat. Hellbenders may follow suit in coming field seasons, as the nest boxes become weathered like natural components of the ecosystem.

Another potential cause of inhabitation failure was the placement of the majority of the nest boxes in stream sites that already possessed quality nesting rocks. Habitat availability may simply not have been limited for the small to moderately sized hellbender populations at our sites. In such cases, hellbenders may prefer to use natural habitat over newly placed artificial habitat. This hypothesis should be tested in coming field seasons by quantifying the amount of available natural habitat in study stream segments. If hellbenders have more time to discover and inhabit nest boxes, we may find that artificial habitat is indeed useful to this species over longer periods of time, possibly depending on the abundance of quality habitat already available to local hellbender populations.

Conversely, nest boxes may have remained unoccupied because there was not enough time between installation and the beginning of the breeding season for hellbenders to locate and inhabit the structures. It is likely that sexually mature males who had bred in past years simply defended their territory instead of seeking out new breeding habitat (Humphries & Pauley 2005). Further, completing nest box installation by early August left little time for the few newly matured males to both discover and establish territorial nests before the breeding season began in September (Nickerson & Mays 1973). Thus, late installation timing and a high degree of territoriality may have drastically reduced the opportunities for inhabitation of the artificial habitat during the 2013 breeding season. Wild South has secured a ten-year permit and four additional years of funding to continue monitoring the installed nest boxes for durability, internal water quality and occupancy. The results of this ongoing research will illuminate the long-term utility of nest boxes for the conservation of hellbenders in Western North Carolina.

Species Distribution Model

The MaxEnt SDM predicted hellbender habitat and non-habitat with a high degree of accuracy. This model suggested that cumulative drainage area, geology, flow, and Strahler's stream order were the most informative environmental variables in locating hellbender habitat. These results agreed with those of previous studies, which found hellbenders to prefer stream reaches characterized by moderate water levels and flow, with an abundance of large, flat rocks (Humphries & Pauley 2000; Nickerson & Mays 1973). Further, research that showed a negative correlation between human development and hellbender abundance supported the model's findings that high percentages of developed, barren, agricultural and impervious land cover types result in poor hellbender habitat (Wheeler *et al*., 2003).

Even so, our MaxEnt hellbender SDM may be improved in several ways during future assessments. Our model assumed that there was little to no sampling bias in our presence dataset. However, NCWRC surveys on which these points were frequently based are often conducted along stream reaches that are accessible from roads, trails, parking lots and campsites. This inherent spatial sampling bias may have impacted the MaxEnt output (Elith *et al.,* 2011). Future attempts at modeling should work to build a bias grid to compensate for the over-representation of accessible sites in MaxEnt (Elith *et al.,* 2011).

Additionally, the use of the NHDPlusV2 dataset meant that the smallest spatial unit of environmental data was that of a stream segment. The use of this multi-pixel unit meant that fine-scale habitat data within stream segments were lost in our predictions of preferred hellbender habitat. This limitation was largely a product of the current environmental stream GIS data readily available to modelers. As predictor variable data advances and becomes available at finer scales for Western North Carolina, the model presented here should be rerun to enhance our understanding of hellbender microhabitat preferences.

Connectivity Analysis

If artificial nesting habitat proves to be a useful long-term conservation tool for hellbenders in Western North Carolina, managers should concentrate on installing nest boxes at stream sites that will enhance the abundance of, and connectivity between, known populations. My connectivity analysis produced 356 recommendations for stream sites where hellbender nest box installation should occur in the future, which could result in the majority of known hellbender populations experiencing enhanced connectivity within the state. Additionally, the proposed sites are categorized by proximity to known hellbender populations. Installing nest boxes at the closest recommended sites first would encourage movement to the outskirts of the dispersal range of known hellbender occurrence locations, allowing dispersing hellbenders to more easily access increasingly distant natural or artificial habitat sites in the future. By establishing the recommended sites incrementally at greater and greater distances away from the original known populations, hellbenders should be able to disperse between further-removed populations more frequently over time. Therefore, the output of this analysis should be instructive to environmental managers in both where nest boxes should be placed, and in what order sites should be addressed. Moreover, managers may use the map produced to select nest box installation sites based on areas that may be of particular interest to stakeholders. For example, the NCWRC may choose to focus their conservation efforts within streams that pass through state-owned and public lands. In such a scenario, the recommended sites within these specific streams could be extracted from the original ArcMap layer for planning use.

Conclusion

The hellbender salamander is an ancient, charismatic animal, with great value to stream ecosystems and humans as an indicator species. Nevertheless, increased human development, and the resulting sediment in runoff that destroys hellbender breeding habitat, threatens the persistence of these amphibians. To protect hellbenders from the negative effects of sedimentation, it is necessary that environmental managers develop new solutions for providing quality habitat to dwindling populations.

Although nest boxes have successfully served as shelter and nesting habitat for hellbenders in some locations (Briggler & Ackerson 2012), this study indicated that these tools need to be further tested and improved. Changing the design of the structures, placing nest boxes in streams with few quality nest rocks, and allowing more time for hellbenders to occupy the shelters may reveal that nest boxes can enable successful reproduction in known populations. Continued monitoring will be necessary to determine the long-term efficacy of using nest boxes for hellbender conservation in Western North Carolina. These sustained research efforts will also increase our understanding of hellbender habitat preferences, regardless of the ultimate utility of nest boxes as a conservation tool.

The hellbender SDM created in MaxEnt will help to guide both future nest box placement and surveys conducted to identify previously undocumented North Carolinian hellbender populations. Ground truthing will be required to determine the true accuracy of the model. Fortunately, the NCWRC will be using the map of predicted habitat to guide their annual hellbender surveys in the summer of 2014. These efforts will test the accuracy of the model, and the overall usefulness of the MaxEnt output to environmental managers. As additional hellbender presence and fine-scale environmental data become available, the MaxEnt model should be rerun to elucidate the microhabitat preferences of hellbenders.

If nest boxes prove to serve as valuable habitat for hellbenders in Western North Carolina and the MaxEnt SDM is shown to be accurate, then I recommend that environmental managers begin installing nest boxes in the sites that I have proposed. Sites should first be examined for natural habitat quality and the absence of barriers to hellbender dispersal. Managers should then place nest boxes at sites that align with their organization's conservation interests in order from closest to known populations to furthest from these observed hellbenders. By doing so, nearby populations will be encouraged to increase, and dispersal to neighboring populations could be enhanced. The probability of hellbender persistence in the face of sedimentation could thus be improved through population growth and a rise in genetic diversity.

Acknowledgments

Funding for this project was made possible by two grants awarded to Wild South from the Herpetofauna Foundation and the Foundation for the Carolinas, as well as a generous stipend provided by the Stanback Internship Program. None of the work presented here would have been possible without the endless support of the Wild South staff and interns, the faculty, staff and students of WWC, NCWRC, USFWS, USFS, NCHP, and the North Carolina Zoo. I would like to expressly thank Benjamin Prater, Dr. J.J. Apodaca, Lori A. Williams, Mark Endries, Dr. Dean Urban, John Fay, Sean Cheetham, Anna Grant, Benjamin Colvin, Minshu Deng and Cheri Southwick for their tireless assistance in completing this work. Further, I owe many thanks to the Nicholas School of the

Environment at Duke University for fully supporting my pursuit of this project and career goals. In particular, my advisor, Dr. John Poulsen provided me with incredible guidance and encouragement throughout my time at Duke, and I cannot thank him enough for all of his help.

Literature Cited

Beane, J.C., A.L. Braswell, J.C. Mitchell, W.M. Palmer, and J.R. Harrison III. 2010. Amphibians and Reptiles of the Carolinas and Virginia. The University of North Carolina Press, Chapel Hill, USA, 2: 51.

Briggler, J.T., J.R. and Ackerson. 2012. Construction and Use of Artificial Shelters to supplement Habitat for Hellbenders. Herpetological Review, 43(3): 412-416.

Browne, R.K., H. Li, D. McGinnity, S. Okada, W. Zhenghuan, C.M, Bodinof, K.J. Irwin, A. McMillan, and J.T. Briggler. Survey techniques for giant salamanders of the aquatic Caudata. Amphibian and Reptile Conservation, 5(4): 1-16.

Dodd, C.K., Jr. 2004. The Amphibians of the Great Smoky Mountains National Park. The University of Tennessee Press, Knoxville, Tennessee, USA.

Elith, J., C.H. Graham, R.P. Anderson, *et al*. 2006. Novel methods improve prediction of species' distributions from occurrence data. Ecography, 29:129-151.

Elith, J., S.J. Phillips, T. Hastie, M. Dudik, Y.E. Chee, and C.J. Yates. 2011. A statistical explanation of MaxEnt for ecologists. Diversity and distributions 17: 43-57.

ESRI. 2011. Calculate Sinuosity. Available at <http://www.arcgis.com/home/item.html?id=00e708a448b74810a0e805c4a97f9d46>

ESRI. 2013 ArcGIS Desktop: Release 10.1. Redlands, CA: Environmental Systems Research Institute.

Freake, M.J., and E.D. Lindquist. 2008. Geographic Pattern Analysis of Pesticide Exposure in Salamander Populations in the Great Smoky Mountains National Park. Herpetological Conservation and Biology 3(2): 231-238.

Gates, J. E., Stouffer, R. H., Stauffer, J. R., & Hocutt, C. H. (1985). Dispersal patterns of translocated *Cryptobranchus alleganiensis* in a Maryland stream. *Journal of herpetology*, 436-438.

Harlan, R.A., and R.F. Wilkinson. 1981. The effects of progressive hypoxia and rocking activity on blood oxygen tension for hellbenders, Cryptobranchus alleganiensis. Journal of Herpetology, 383-387.

Horizon Systems Corporation. 2013. National Hydrography Dataset Plus, Version 2.1. Available at <http://www. Horizon-systems.com/nhdplus/>

Humphries, W.J., and T.K. Pauley. 2000. Seasonal changes in nocturnal activity of the hellbender, *Cryptobranchus alleganiensis* in West Virginia. Journal of Herpetology, 34(4): 604-607.

Humphries, W.J., and T.K. Pauley. 2005. Life History of the Hellbender, *Cryptobranchus alleganiensis*, in a West Virginia Stream. The American Midland Naturalist, 154(1):135-142.

Kaeser, A.J., and E. Watson. 2011. A GIS–based Assessment of Land Cover within Stream and River Riparian Buffers of the Southeastern United States. A publication of the Science and Data Committee, Southeast Aquatic Resources Partnership, 1-43.

Kohavi, R. 1995. A study of cross-validation and bootstrap for accuracy estimation and model selection. In *IJCAI*, 14(2): 1137-1145.

Mitchell, J. 2013. North Carolina Waterfalls.info. Available at <http://www.northcarolinawaterfalls.info/map.html>

National Climatic Data Center. 2014. Climatological Rankings: North Carolina, Climate Division 1 and 2 Precipitation Rankings as of August 2013. Available at <http://www.ncdc.noaa.gov/tempandprecip/ranks.php?periods[]=3&parameter=pcp&state=31&div=2&year=2013&month=8&asof=on#ranks-form>. Accessed 3/22/2014.

Nicholson, S.W., C.L. Dicken, M.P. Foose, and J.A.L. Mueller. 2005. Integrated Geologic Map Databases for the United States: Alabama, Florida, Georgia, Mississippi, North Carolina, and South Carolina, Version 1.0. U.S. Geological Survey Open-File Report, 2005-1323. Reston, VA. U.S. Geological Survey. <http://pubs.usgs.gov/of/2005/1323/>

Nickerson, M.A., and C.E. Mays. 1973. The hellbenders: North American ‘‘giant salamanders’’. Publ. Biol. Geol., Milkwaukee Public Museum, 1: 1-106.

Nickerson,M.A., K.L. Krysko, and R.D. Owen. 2002. Ecological status of the hellbender (*Cryptobranchus alleganiensis*) and the mudpuppy (*Necturus maculosus*) salamanders in the Great Smoky Mountains National Park. Journal of the North Carolina Academy of Science, 118(1): 27-34.

Nickerson, M.A. and J.T. Briggler. 2007. Harvesting as a factor in population decline of a long-lived salamander; the Ozark hellbender, *Cryptobranchus alleganiensis bishop* Grobman. Applied Herpetology, 4(3): 207-216.

North Carolina Department of Environment and Natural Resources. 2013. North Carolina Dam Inventory. Available at <http://portal.ncdenr.org/web/lr/dams>

Olson, Z.H., J.T. Briggler, and R.N. Williams. 2012. An eDNA approach to detect eastern hellbenders (*Cryptobranchus a. alleganiensis*) using samples of water. Wildlife Research, *39*(7): 629-636.

Petranka, J. W. 1998. Salamanders of United States and Canada. Smithsonian Institution Press, Washington, D.C., 587.

Pfingsten, R.A., and F.L. Downs. 1989. Salamanders of Ohio. Bulletin of the Ohio Biological Survey, 7(2): 344.

Phillips, S. J., *et al*. 2005. Maxent software for species distribution modeling v. 3.3.3k. <http://www.cs.princeton.edu/~schapire/maxent/>

Ringler, N.H., and J.D. Hall. 1975. Effects of logging on water temperature, and dissolved oxygen in spawning Beds. Transactions of the American Fisheries Society, 104(1): 111-121.

Smith, B.G. 1907. The life history and habits of *Cryptobranchus alleganiensis*. Biological Bulletin, 13: 5-39.

Spielman, D., B.W. Brook, and R. Frankham. 2004. Most species are not driven to extinction before genetic factors impact them. Proceedings of the National Academy of Sciences of the United States of America, 101(42): 15261-15264.

U.S. Geological Survey. 2013a. National Land Cover Dataset 2006. Available at <http://www.mrlc.gov/nlcd_2006.php>

U.S. Geological Survey. 2013b. National Land Cover Dataset 2006 Developed Impervious Surfaces. Available at <http://www.mrlc.gov/nlcd_2006.php>

U.S. Geological Survey. 2013c. National Elevation Dataset. Available at <http://www.ned.usgs.gov/>

VanDerWal, J., L.P. Shoo, C. Graham, and S.E. Williams. 2009. Selecting pseudo-absence data for presence-only distribution modeling: How far should you stray from what you know? Ecological Modelling, 220(4): 589-594.

Welsh, H. H., and L.M. Ollivier. 1998. Stream amphibians as indicators of ecosystem stress: a case study from California's redwoods. Ecological Applications, 8: 1118–1132.

Wheeler, B.A., E. Prosen, A. Mathis, and R.F. Wilkinson. 2003. Population declines of a long-lived salamander: A 20+ year study of Hellbenders, *Crytobranchus alleganiensis*. Biological Conservation 109: 151–156.