Estimating Population Parameters for the Northern Population of Copperbelly Water Snake (*Nerodia erythrogaster neglecta*) to Inform Conservation and Monitoring

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EXECUTIVE SUMMARY

The northern population segment of the Copperbelly Water Snake (*Nerodia erythrogaster neglecta*) is listed as a federally threatened species under the U.S. Endangered Species Act (U.S. Fish and Wildlife Service [USFWS] 2008). This species is known from a small number of locations in south-central Michigan, northwestern Ohio, and northeastern Indiana, and is listed as state endangered in these states (USFWS 2008). Conservation and recovery efforts for this species require estimating and monitoring population size, status, and trends. A statistically robust and efficient long-term monitoring program is needed to facilitate efforts to conserve the Copperbelly Water Snake, but developing such a program for a species that occurs in low densities and when resources are limited can be challenging. Estimating population size also is difficult when detection of a species is imperfect. In recent years, statistical tools, such as occupancy modeling, have been developed to estimate population parameters (e.g., occupancy, abundance) using repeated survey data that incorporate detection probabilities and do not require the capture or identification of individual animals. Occupancy modeling may be a useful approach for long-term monitoring efforts because it allows the estimation of population parameters that could be tracked over time, without the need for more intensive studies, and adjusts estimates for detection probabilities less than one. We analyzed existing data sets using several occupancy models to evaluate the usefulness of these techniques and inform the development of a long-term monitoring program for the Copperbelly Water Snake.

We analyzed Copperbelly Water Snake data from surveys conducted in 2005 and 2006 at three extant areas in south-central Michigan and northwestern Ohio. Observers documented presence/absence and number of copperbellies observed during multiple visits to 105 wetlands in 2005, and a subset of those wetlands (n=31) in 2006. We used single-season occupancy models developed by MacKenzie et al. (2002), Royle and Nichols (2003), and Royle (2004) to estimate occupancy, probability of detection, and animal density and total abundance. We also utilized the multiple-season model developed by MacKenzie et al. (2003) to estimate occupancy, detection probability, colonization probability, and extinction probability. We used occupancy and detection probability results from our models and guidance from MacKenzie and Royle (2005) to estimate number of study sites, survey visits per site, and total surveys needed to achieve different levels of precision for a monitoring program. Model assumptions, sampling designs, and other sampling considerations also were examined.

The occupancy models estimated low levels of Copperbelly Water Snake site occupancy and moderate detection probabilities. The single-season models generated site occupancy estimates that ranged from 0.17 to 0.31, and detection probabilities that ranged from 0.40 to 0.59. The multiple-season models generated site occupancy estimates that ranged from 0.19 to 0.25, and a detection probability of 0.59. Using these preliminary occupancy and detection probability estimates and recommendations from MacKenzie and Royle (2005), we believe a standard repeat survey design would be most appropriate for a Copperbelly Water Snake monitoring program. We estimate between 110 and 230 sites would be needed to achieve moderate levels of precision, and suggest surveying the maximum number of sites possible each year. We also suggest conducting three visits per site. Additional monitoring recommendations were provided.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

The U.S. Fish and Wildlife Service (USFWS) recognizes two distinct population segments (northern and southern) of Copperbelly Water Snake (*Nerodia erythrogaster neglecta*) and listed the northern population segment as a federally threatened species under the U.S. Endangered Species Act of 1973 (USFWS 2008). This population is known from a small number of locations in south-central Michigan, northwestern Ohio, and northeastern Indiana (USFWS 2008). Copperbelly Water Snake is also listed as state endangered in Michigan, Ohio, and Indiana. This species uses a variety of wetlands, including shrub swamps, emergent marshes, and the margins of open water areas, which are usually characterized by open canopies, shallow water, and short dense vegetation (USFWS 2008). Copperbelly Water Snakes also use uplands for foraging, aestivating, hibernating, and traveling among wetlands, and they are known to use uplands more often than Northern Water Snakes (*Nerodia sipedon sipedon*; Roe et al. 2004, USFWS 2008). Habitat loss, fragmentation, and degradation are viewed as the primary threats to the Copperbelly Water Snake (USFWS 1997, 2008).

The Recovery Plan (USFWS 2008) for the northern population segment of the Copperbelly Water Snake provides a set of criteria to assess delisting or reclassification of the population, which requires estimation of population size. However, estimation of population size is difficult when detection of the species is imperfect. The USFWS and its partners also need information on the species' population status to inform conservation planning and implementation and provide a means to evaluate the success of recovery efforts. A statistically robust and efficient long-term monitoring program is needed to facilitate efforts to conserve the Copperbelly Water Snake, but developing such a program for a species that occurs in low densities and when resources are limited can be challenging. A variety of methods have been employed by the USFWS and its partners (e.g., Michigan Natural Features Inventory [MNFI], Indiana-Purdue University at Fort Wayne [IPFW]), including repeated surveys of wetlands, distance sampling, radio telemetry, and mark-recapture studies. Funding and personnel constraints and low population levels make some of these methods unfeasible for evaluating population status over large spatial and temporal scales.

In recent years, statistical tools have been developed to estimate population parameters (e.g., occupancy, abundance) using repeated survey data that incorporate detection probabilities and do not require the capture or identification of individual animals (e.g., MacKenzie et al. 2002, 2003, Royle and Nichols 2003, Royle 2004). Occupancy modeling may be a useful approach to incorporate into a long-term monitoring program, because it allows the estimation of population parameters that could be tracked over time, without the need for more intensive studies, and adjusts estimates for detection probabilities less than one (i.e., some individuals are present but not detected). The MNFI proposed to analyze existing data sets using several occupancy models to evaluate the usefulness of these techniques and inform the development of a long-term monitoring program. Specifically, we set out to achieve the following objectives: (1) analyze existing Copperbelly Water Snake survey data using occupancy modeling to estimate occupancy and detection probabilities and assist in the evaluation of population estimates and survey/monitoring protocols; (2) provide recommendations based on survey data analysis and relevant literature for establishing a Copperbelly Water Snake monitoring protocol; and (3) work with the USFWS and other partners to develop a Copperbelly Water Snake monitoring protocol.

METHODS

Study Area

We analyzed data gathered in 2005 and 2006 at three extant Copperbelly Water Snake areas in south-central Michigan and northwestern Ohio. Observers surveyed 105 wetlands of a variety of sizes and types. Wetlands ranged from ephemeral to permanent and consisted of inundated shrub swamp, southern wet meadow, emergent marsh, southern floodplain forest, and southern swamp community types (Kost et al. 2006). Lee et al. (2007) provided a detailed description of the study area, and the USFWS (2008) described current and historical distributions of the northern population of Copperbelly Water Snake.

Copperbelly Water Snake Surveys

Observers surveyed wetlands known or likely to harbor Copperbelly Water Snakes between mid-April and mid-July during 2005 and 2006. In 2005, between one and five surveys were conducted per site (0 = 3.0 surveys/site, n = 105), and in 2006, between one and six surveys were conducted per site (0 = 2.8 surveys/site, n = 31). Survey teams (usually two or three individuals, but occasionally one or four) conducted visual surveys by walking routes parallel and immediately adjacent to the wetland shoreline, and in some cases along the wetland edge in shallow water. Visual surveys were conducted by walking slowly along the entire length of the shoreline and surveying the vegetation and open water from one or more fixed locations with binoculars. In a few cases when it was not possible to walk or wade around a portion of a wetland, surveys were only conducted with binoculars from points offering the best view. Surveyors recorded the number of individuals observed by study site (i.e., wetland). Observers only conducted surveys during appropriate weather conditions when snakes were expected to be most visible. A detailed description of the survey methods was provided by Lee et al. (2007).

Analysis

Up to six surveys were conducted at a given site, but the survey dates varied across sites within a field season (e.g., the first visit to all sites spanned more than a month in 2005). We arranged the data into the following four periods to control for the effect of survey timing and evaluate models in which detection probability varied by period: (1) April 15 to May 10; (2) May 11 to May 31; (3) June 1 to June 20; and (4) June 21 to July 15. We selected survey periods to coincide with typical changes in weather and vegetation conditions that might affect snake activity and visibility. Two or three surveys sometimes fell within one of the post hoc survey periods. In those situations, we considered Copperbelly Water Snake present for the survey period if it was observed during at least one survey and used the maximum number of snakes observed among the surveys when estimating abundance. We did not analyze data from the fourth time period because only about 10% of the sites were surveyed, and snake occupancy of the wetlands may have been impacted by dry wetland conditions. We used models available in PRESENCE 3.1 (http://www.mbr-pwrc.usgs.gov/software/presence.shtml) to estimate population parameters for the northern population of Copperbelly Water Snake. Covariates that might influence occupancy and detection probability were not available at the time of analysis, so we only used simple models lacking covariates. We provide information about the assumptions of the models used in our analyses in the Discussion section.

Single-season Models

We estimated site occupancy (i.e., Ψ, proportion of sites occupied) and probability of detection (*p*) for 2005 and 2006 using the approach described by MacKenzie et al. (2002). For each season, we ran two predefined models in PRESENCE: (1) detection probability constant across surveys, and (2) variable detection probability among surveys. We assessed which of the two models was "best" supported by the data in each year using the Akaike Information Criterion (AIC).

Two recently developed modeling methods (Royle and Nichols 2003, Royle 2004) built upon the single-season model developed by MacKenzie et al. (2002) to allow estimation of animal density and total abundance, in addition to occupancy and detectability. Royle and Nichols (2003) provided a method of abundance estimation using detection-nondetection data, whereas Royle (2004) developed a model to estimate abundance with count data from repeat surveys. We ran both models using 2005 data to provide coarse Copperbelly Water Snake abundance estimates and illustrate alternative approaches to estimating population abundance other than distance sampling. We did not estimate abundance for 2006 because data were only available for a small number of sites (n = 31) compared to 2005 (n = 105).

Multiple-season Models

We analyzed data from both 2005 and 2006 using the model developed by MacKenzie et al. (2003). This model allows estimation of occupancy, detection probability, colonization probability (i.e., probability that an unoccupied site in season one will become occupied in season two), and extinction probability (i.e., probability that an occupied site in season one will become unoccupied in season two). We compared the following four simple multi-season models: (1) occupancy and detection probability constant across seasons and surveys; (2) occupancy varying by season and detection probability constant across seasons and surveys; (3) occupancy and detection probability varying by season; and (4) occupancy varying by season and detection probability varying among all surveys. We assessed which of the models was "best" supported by the data using AIC.

Survey Effort Estimation

MacKenzie and Royle (2005) provided guidance on the optimum number of visits to conduct and sites to survey given levels of occupancy, detectability, and precision. We determined the approximate number of visits (*K*) to conduct according to MacKenzie and Royle (2005) based on occupancy and detectability estimates from our models. We estimated the number of study sites (*s*) required using a standard design (i.e., all sites surveyed *K* times) for the range of occupancy and detection probability estimates obtained from our models and using three levels of standard error (of occupancy) that approximately corresponded to coefficients of variation (CV) of 15%, 20%, and 25%. We then estimated the total number of surveys (i.e., $s \times K$) that would be required given the above range of values for $K = 2$ and 3.

RESULTS

Single-season Models

Using several occupancy models, we estimated low levels of Copperbelly Water Snake site occupancy and moderate detection probabilities. Two models, one with constant occupancy and detection probabilities and the second containing abundance-induced heterogeneity in detection probability (Royle and Nichols 2003), were similarly supported by the 2005 data (Table 1). Burnham and Anderson (2002) stated that models with AIC differences less than two have substantial empirical support. In 2005, naïve occupancy was 0.14, whereas both of the best-approximating models estimated occupancy at 0.17 (SE=0.04). Detection probability was similar for the two best-supported 2005 models, with an estimate of 0.58 (SE=0.10) for the constant occupancy and detectability model and 0.55 (SE=0.11) for the abundance-induced heterogeneity model (Table 2). The repeated-count model (Royle 2004) produced a greater occupancy estimate (0.31, SE=0.07) and lower probability of detection (0.40, SE=0.09) than the other 2005 models. In 2006, the model assuming constant occupancy and detectability was again the best-approximating model. Naïve occupancy in 2006 was 0.19; model-estimated occupancy was 0.31 (SE=0.15) and probability of detection was 0.46 (SE=0.21).

Similar to the low estimates of occupancy, we obtained low abundance estimates using the Royle and Nichols (2003) and Royle (2004) models (Table 2). We estimated average Copperbelly Water Snake abundance at 0.19 (SE=0.05) snakes per site using the abundance-induced heterogeneity model and 0.38 (SE=0.10) snakes per site with the repeated-count model. Total abundance for the sites surveyed was estimated at 19.7 (SE=5.7) by the abundance-induced heterogeneity model and 39.4 (SE=10.1) by the repeated-count model.

Table 1. Summary of single-season models used to estimate occupancy (Ψ) and detection probability (*p*) for Copperbelly Water Snake detection-nondetection data from 2005-2006 at sites in Michigan and Ohio.

Model	ΔAIC	AIC Weight	No. Parameters
2005 (n=105)			
Ψ (.), *p* (.)	0.00	0.4937	2
Ψ (.), *p* (abundance-induced heterogeneity)[1]	0.24	0.4378	2
Ψ (.), *p* (survey-specific)	3.95	0.1219	4
2006 (n=31)[2]			
Ψ (.), *p* (.)	0.00	0.7320	2
Ψ (.), *p* (survey-specific)	2.01	0.2680	4

[1]Royle and Nichols (2003) estimator.
[2]Michigan sites only.

Table 2. Observed and model-estimated Copperbelly Water Snake population parameters, standard errors (SE), and lower and upper 95% confidence limits (LCL and UCL) for single-season models fit to 2005-2006 survey data from Michigan and Ohio.

	Occupancy					Detection Probability				Total Abundance			
Model	Naïve	Est. Ψ	SE	LCL	UCL	*p*	SE	LCL	UCL	*N*	SE	LCL	UCL
2005 (n=105)													
Single-season Occupancy[1]	0.14	0.17	0.04	0.08	0.26	0.58	0.10	0.37	0.78	NA[2]	NA	NA	NA
Abundance-induced Heterogeneity[3]	0.14	0.17	0.04	0.08	0.26	0.55	0.11	0.33	0.77	19.7	5.6	8.6	30.9
N-Mixture Repeated Count[4]	0.14	0.31	0.07	0.18	0.44	0.40	0.09	0.23	0.58	39.4	10.1	19.7	59.2
2006 (n=31) [5]													
Single-season Occupancy[1]	0.19	0.31	0.15	0.00	0.62	0.46	0.21	0.03	0.90	NA[2]	NA	NA	NA

[1]MacKenzie et al. (2002) model.
[2]Parameter is not estimated by the model.
[3]Royle and Nichols (2003) estimator.
[4]Royle (2004) model.
[5]Michigan sites only.

Multiple-season Models

We found the model with constant occupancy and detection probability among years and surveys to be the best-approximating model of those examined (Table 3). The second best-approximating model (occupancy varying by season and constant detection probability) had an AIC difference less than two, indicating it was also supported by the data (Burnham and Anderson 2002). Of the sites available for multi-year analysis, Copperbelly Water Snake was observed during at least one survey in 0.15 of the sites in 2005 and 0.19 of the sites in 2006. The model best supported by the data provided an occupancy estimate of 0.22 (SE=0.07) for both years, whereas we obtained occupancy estimates of 0. 19 (SE=0.08) for 2005 and 0.25 (SE=0.10) for 2006 from the second best-approximating model (Table 4). Both models produced the same detection probability estimate of 0.59 (SE=0.13). The best-approximating model estimated the probability of colonization at 0.17 (SE=0.08) and extinction probability at 0.62 (SE=0.21).

Table 3. Summary of multi-season models used to estimate Copperbelly Water Snake occupancy (Ψ) and probabilities of detection (*p*), extinction (ε), and colonization (γ) during 2005-2006 at sites in Michigan and Ohio.

Model[1]	ΔAIC	AIC Weight	No. Parameters
Ψ (.), γ, ε, *p* (.)	0.00	0.6050	3
Ψ (season), γ, ε, *p* (.)	1.73	0.2547	4
Ψ (season), γ, ε, *p* (season)	3.13	0.1265	5
Ψ (season), γ, ε, *p* (survey-specific)	8.96	0.0069	9

[1]MacKenzie et al. (2003) multi-season occupancy model.

Table 4. Observed and model-estimated Copperbelly Water Snake occupancy (Ψ) and probabilities of detection (*p*), extinction (ε), and colonization (γ) during 2005-2006 at sites in Michigan and Ohio.

	Occupancy									Detection Probability				Colonization Probability				Extinction Probability			
		2005				2006															
Model	Obs.[1]	Ψ	SE	LCL	UCL	Ψ	SE	LCL	UCL	*p*	SE	LCL	UCL	γ	SE	LCL	UCL	ε	SE	LCL	UCL
Ψ (.), γ, ε, *p* (.)	0.23	0.22	0.07	0.07	0.36	0.22	0.07	0.07	0.36	0.59	0.13	0.33	0.81	0.17	0.08	0.07	0.38	0.62	0.21	0.21	1.04
Ψ (season), γ, ε, *p* (.)	0.23	0.19	0.08	0.03	0.35	0.25	0.10	0.05	0.44	0.59	0.13	0.33	0.81	0.21	0.11	-0.01	0.43	0.57	0.26	0.04	1.10

[1]Observed or naïve occupancy.

Survey Effort Estimation

Using the range of occupancy (0.17 – 0.31) and detection probability (0.40 – 0.59) estimates, we determined that the optimum number of surveys to conduct at each site would range from two to four according to the recommendations of MacKenzie and Royle (2005). The number of survey visits required per site increases as occupancy increases and detectability decreases (MacKenzie and Royle 2005).

The estimated number of sites that would be needed in a Copperbelly Water Snake monitoring program increased as levels of occupancy, detection probability, and standard error decreased (Figure 1). We estimated occupancy at nearly 0.20 and detection probability between 0.50 and 0.60, so a monitoring program that uses three surveys per site would require approximately 110-130 sites to achieve a coefficient of variation (CV) of about 20% or 200-230 sites for a CV of approximately 15%. Compared to conducting three visits per site, more sites would be needed to achieve the same level of precision under a design using two visits per site (Figure 2). Depending on estimated detection probability, we would need to survey between 1.1 ($p = 0.8$) and 2.1 ($p = 0.40$) times as many sites when conducting two compared to three visits per site. Using the same parameter values as above but conducting only two visits per site, we estimate about 155-225 sites would be needed for a 20% CV on occupancy and approximately 275-400 sites for 15% CV. However, when considering the total number of surveys that would need to be done in a given season (i.e., $s \times K$), a design using two visits per site appears to be more efficient when detection probability is greater than about 0.55, regardless of occupancy and precision level (Figure 3).

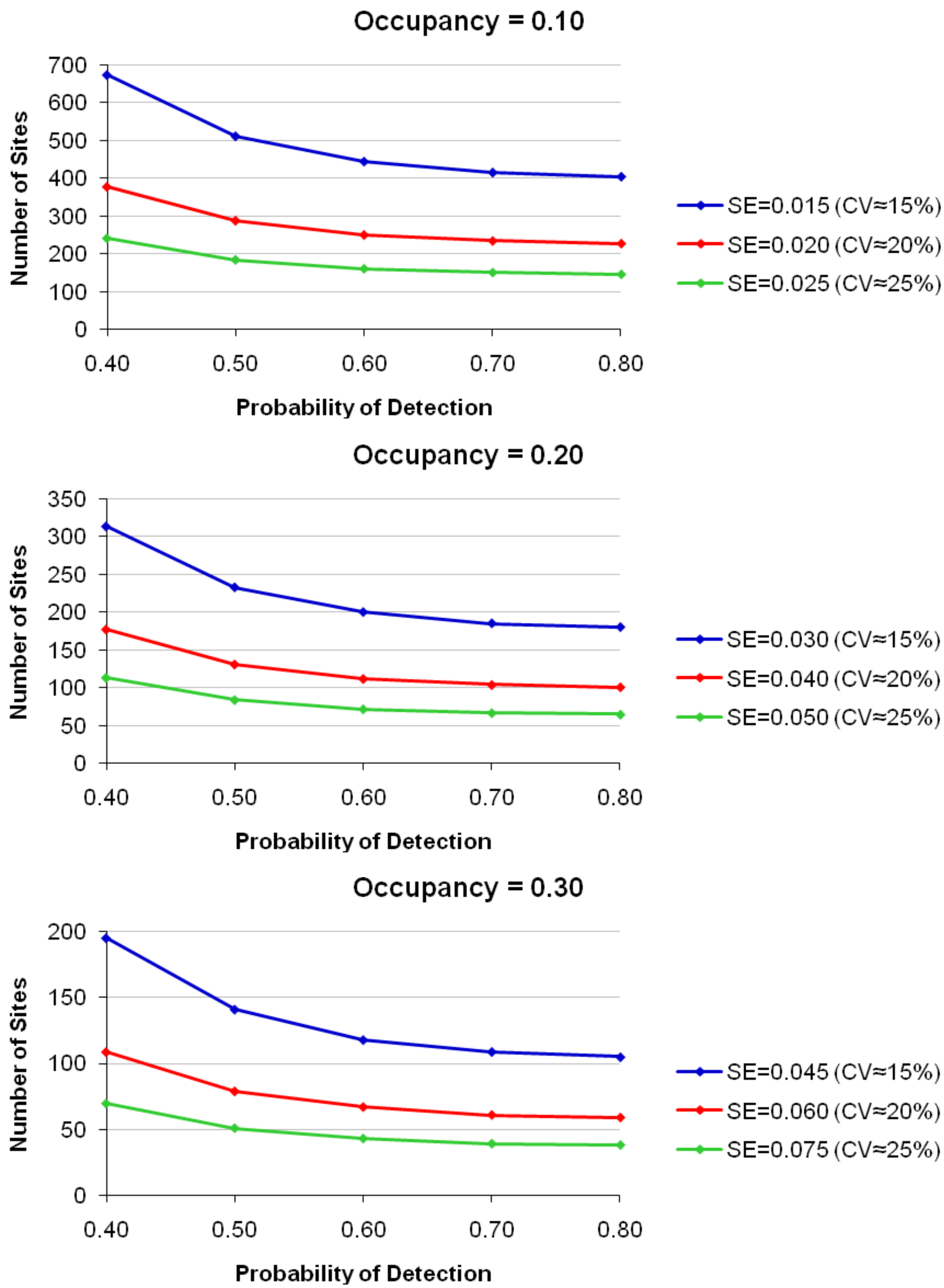

Figure 1. Estimated number of sites that would need to be surveyed in Michigan and Ohio for Copperbelly Water Snake assuming three visits to each site and several levels of occupancy (0.10-0.30), detectability (0.40-0.80), and standard error (SE) of occupancy associated with three coefficients of variation (CV).

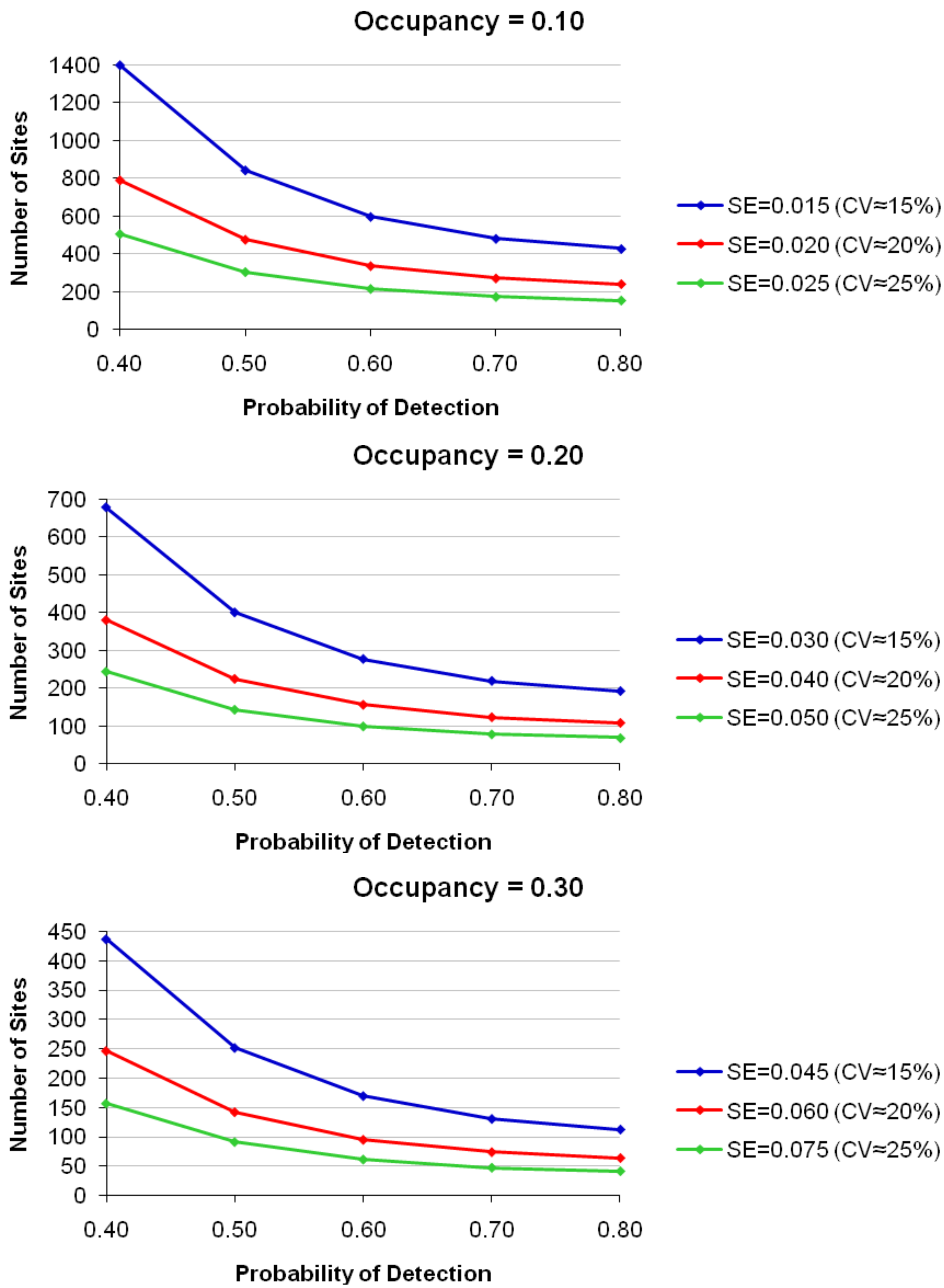

Figure 2. Estimated number of sites that would need to be surveyed in Michigan and Ohio for Copperbelly Water Snake assuming two visits to each site and several levels of occupancy (0.10-0.30), detectability (0.40-0.80), and standard error (SE) of occupancy associated with three coefficients of variation (CV).

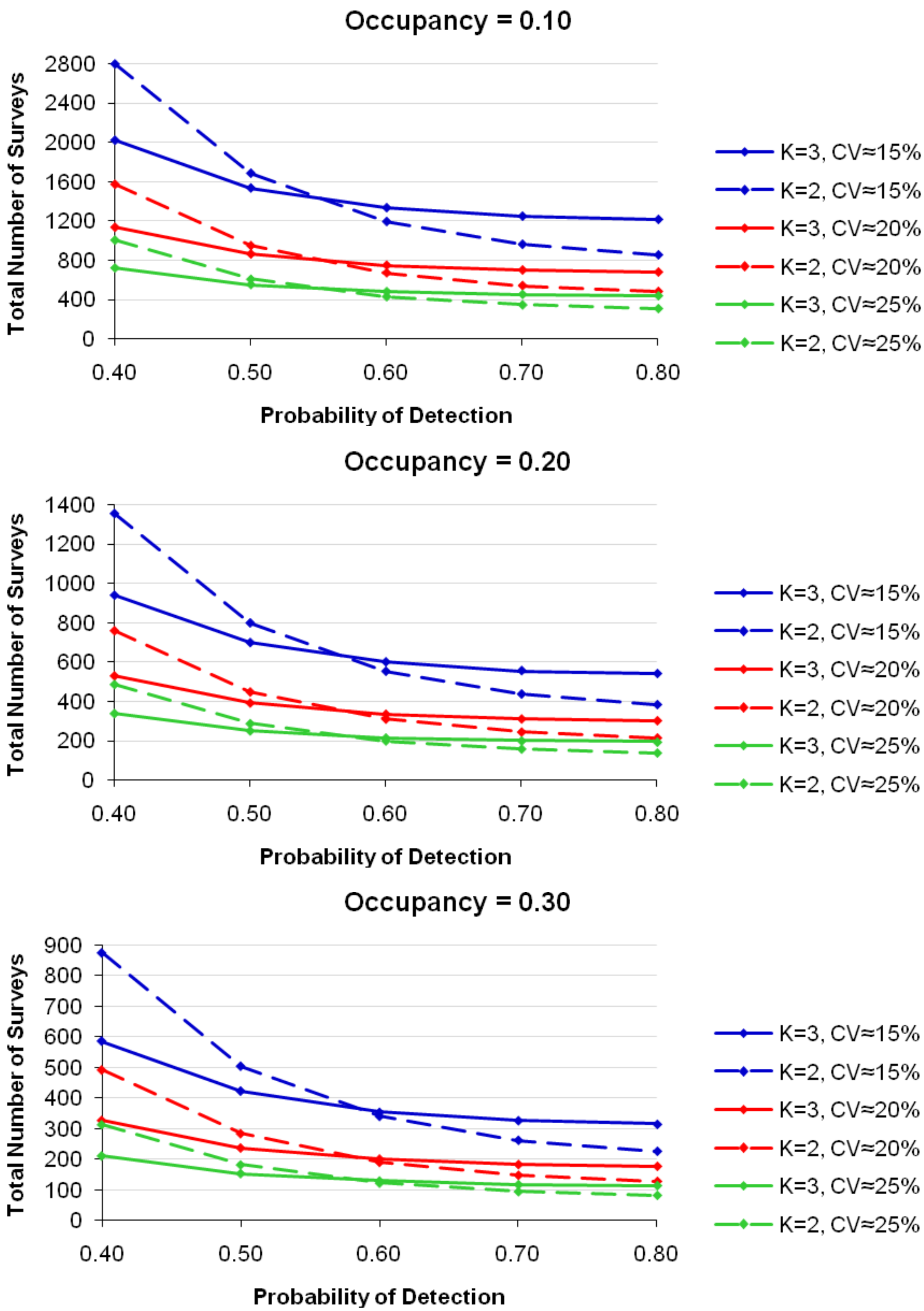

Figure 3. Estimated total number of surveys that would need to be completed in Michigan and Ohio for Copperbelly Water Snake given two or three visits to each site and several levels of occupancy (0.10-0.30), detectability (0.40-0.80), and precision (coefficient of variation).

DISCUSSION

Model Assumptions

There are several analytical methods, including distance sampling (Buckland et al. 2001), mark-recapture studies, and repeat-survey models (e.g., MacKenzie et al. 2002, 2003; Royle and Nichols 2003; Royle 2004), available to estimate population parameters, such as occupancy, detectability, abundance, and extinction and colonization probabilities. Each method has assumptions that must be considered when designing monitoring programs and conducting and interpreting analyses. Below we provide the key assumptions of the occupancy models used in this report and distance sampling used by Attum et al. (2009). We note situations in which violation of assumptions are possible based on our understanding of the species. Violations of assumptions can result in biased parameter estimates (MacKenzie et al. 2006); however, when using methods that do not account for detection probability (e.g., raw counts), stronger assumptions are made, such as constant detection probability across time, sites, and habitats or perfect detection (Mazerolle et al. 2007). Mazerolle et al. (2007) suggest that even when assumptions are violated, estimation methods that incorporate detection probability typically yield estimates with smaller biases than those of ad hoc methods.

Occupancy Models

MacKenzie et al. (2006) listed the following assumptions underlying the single-season (MacKenzie et al. 2002) and multiple-season (MacKenzie et al. 2003) models: (1) occupancy status at each site does not change during the survey season, or the sites are "closed" to changes in occupancy; (2) there is no unmodeled heterogeneity in rate parameters (occupancy, detection, colonization, and extinction probabilities); (3) species are not falsely detected; and (4) species detection and detection histories at each site are independent. Surveys both within and among sites need to be independent for these models (MacKenzie et al. 2004a). MacKenzie et al. (2004a) noted that movement of animals among sites produces dependence among surveys and reduces the effective sample size, making estimates less precise than indicated (i.e., standard errors are greater than estimated). Many of the survey sites used in our analyses were located close together and some were within the same wetland complex. Roe et al. (2003, 2004) found that Copperbelly Water Snakes often moved among several wetlands within the same season and moved greater distances and used larger areas compared to Northern Water Snake, so it is likely that our estimates are biased and should be viewed with caution. Kendall (1999) investigated the effects of closure violations on parameter estimates from mark-recapture studies, which are closely related to occupancy models. If movement into and out of the study area occurs randomly, Kendall (1999) found population estimates remained unbiased. However, if movement in and out of the sample unit is not random, occupancy estimates may be biased (Kendall 1999, MacKenzie et al. 2006).

The two models (i.e., Royle and Nichols 2003, Royle 2004) that we used to estimate Copperbelly Water Snake abundance have similar assumptions. Both models assume that occupancy status at a site does not change during the season (i.e., site are closed), and that animals are distributed across the study area randomly (i.e., abundance follows the Poisson distribution). Detection probability at a site is assumed to be a function of the species' inherent detection probability and site abundance under the Royle and Nichols (2003) models; whereas the Royle (2004) model considers the probability of detecting *n* animals at a site as a binomial trial of how many animals are actually at that site. Furthermore, it is assumed that detection probability is constant and detections of individuals are independent during a given survey under the Royle (2004) model.

Distance Sampling

We provide the assumptions of distance sampling because it has been used previously to estimate Copperbelly Water Snake abundance (Attum et al. 2009), and could be incorporated into a long-term monitoring program. Buckland et al. (2001) explained the critical assumptions that must be met when using distance sampling to estimate population parameters (i.e., population density, detection probability): (1) objects (i.e., snakes) occurring directly on the transect are always detected; (2) objects are detected at their initial location prior to any movement in response to the observer; and (3) distances are measured accurately or correctly placed in the proper distance interval. When implementing distance sampling, transects or points used for surveys should be randomly placed, and objects should be uniformly distributed with respect to perpendicular distance from the transect or point (Buckland et al. 2001). If Copperbelly Water Snakes tend to occur near wetland shorelines where survey routes are located, then density/population estimates produced by distance sampling could be biased (Attum et al. 2009). Herbert (2003) found that Copperbelly Water Snakes often used the peripheries of open wetlands. This issue could be remedied by altering survey methodology, such as using randomly selected transects placed perpendicular to the shoreline. Buckland et al. (2001) suggest that at least 60-80 detections (e.g., snake observations) are needed to reliably estimate the detection function and average density within a study area. The number of Copperbelly Water Snake observations documented in previous studies has been below this threshold. For example, only 38 snake observations were documented during multiple surveys of 105 wetlands in 2005, and Attum et al. (2009) only recorded 19 observations in their intensive 2006 study.

Application of Parameter Estimates

We produced several population parameter estimates for Copperbelly Water Snake and demonstrated the utility of occupancy analyses in monitoring animals. However, these models generally assume that sites are selected using a probabilistic design, which would produce a sample of sites representative of the study area. Given that the sites used in this project were selected based on recent or historic observations of Copperbelly Water Snakes, the parameter estimates should not be applied beyond the sites surveyed (i.e., estimates could be biased for the entire population). Because the abundance estimates we produced are based on the estimated mean number of snakes per site, this estimator should not be applied beyond the sites surveyed due to the manner in which sites were selected.

We noted above that our occupancy estimates may be biased (i.e., overestimated) due to the movement of snakes among wetlands and violation of the closure assumption. These estimates may be unbiased if we assume the movement of snakes occurred randomly. MacKenzie et al. (2006) suggest that in these cases, the occupancy estimator should be viewed as the proportion of sites used by the target species, and detection probability as the probability the species is present at the time of survey and detected at the used sites.

Inconsistencies in the way the 2005 and 2006 data were collected and small sample sizes for some analyses may have reduced the precision of our parameter estimates. Because the timing of surveys was not consistent, missing observations were common in the data sets. Although the occupancy models we used are robust to missing observations, precision of the estimates decreases as the number of missing observations increases (MacKenzie et al. 2002, 2003). Given the limited amount of 2006 data available at the time of analysis, we caution the use of parameter estimates for the 2006 single-season and 2005-2006 multiple-season models. The sample sizes for these analyses were low compared to the 2005 data set. There were also only a few sites surveyed during the first survey window of 2006. We observed greater coefficients of variation for the occupancy and detectability parameters in the 2006 single-season and multiple-season models compared to 2005 single-season models, and the colonization and extinction probabilities from our best-approximating multiple-season model had coefficients of variation of 47% and 34%, respectively.

Monitoring Design Recommendations

Site Selection

The goals and objectives of a possible long-term Copperbelly Water Snake monitoring program have not yet been finalized; however, we have demonstrated how occupancy and detection probability estimates can inform the design of a program. Program partners will need to define the extent of the sample frame based on the goals of the program (e.g., tracking trends at known sites vs. entire distribution). Additional decisions about site definition (e.g., individual wetland vs. wetland complex) and stratification (e.g., based on previous occupancy status) will need to be made. Regardless of the sample frame, we suggest using a probabilistic design (e.g., simple random, stratified random, generalized random tessellation sampling) to facilitate application of the results beyond the sites surveyed. We add that focusing a monitoring program on recently or historically occupied sites could produce results and trends not representative of the entire population. Not only could monitoring sites with a greater occupancy rate than the overall population bias the estimates themselves, but MacKenzie et al. (2006) demonstrated that this type of sampling can indicate apparent trends in estimates over time when no trend actually exists for the entire population. Although sampling sites with a greater proportion of sites occupied could provide better estimates of extinction probabilities, a better approach may be to stratify the sites into samples of known and unknown historic occupancy status (MacKenzie et al. 2006).

As discussed above, closure of sites to changes in occupancy status is an assumption of occupancy models. During past surveys, sites were often placed within the range of distances that Copperbelly Water Snakes have been observed moving within a season (or in some cases within a day; see Roe et al. 2004). Roe et al. (2003) documented Copperbelly Water Snakes using several wetlands and making multiple movements to/from wetlands within a given season. Given the ecology of this species, it seems unlikely that many past sites were closed to changes in occupancy. We suggest a future monitoring program select sites in a way that minimizes the risk of violating the closure assumption, such as using a minimum distance to separate sites based on previous studies of Copperbelly Water Snake movements and area use. For example, Roe et al. (2004) estimated a mean total usage area (sexes combined) of 15.8 ha. If we translate this area to a circle, it would have a diameter of approximately 450 m. Males had an average total usage area of 18.9 ha (Roe et al. 2004), which converts to a circle with a diameter of about 490 m. Using these rough calculations, one could use a minimum separation distance of approximately 450-500 m to separate wetland monitoring sites and reduce the potential effects of snake movements on population estimates.

Survey Design

When designing a monitoring program, several related decisions need to be made about how to arrange surveys (e.g., standard design vs. removal sampling), the number of surveys to conduct, and the number of sites to survey. There are many ways to conduct repeated surveys that permit the estimation of occupancy and detection probability, such as standard repeat surveys (all sites surveyed *K* times), removal sampling (surveys stop at a site once the target species is detected or *K* surveys are completed), double sampling (a subset of sites are surveyed *K* times, whereas all others are surveyed once), and double-observer surveys (two independent observers survey the same sites). The selection of the "best" design depends on a variety of factors, including project goals, estimated occupancy and detectability rates, acceptable levels of precision, and resource and associated sample size limitations. Although MacKenzie and Royle (2005) found that removal sampling is often a more efficient design than standard repeat surveys, they observed the standard design to be more efficient than removal sampling for species with low occupancy levels (i.e., <0.3). When costs of initial and subsequent surveys are equal, MacKenzie and Royle (2005) found little advantage to double sampling compared to the standard design. Double-observer surveys would likely be difficult to implement in the field (e.g., the presence of observer 1 could disturb snakes prior to survey by observer 2), and probably would not be feasible given budgetary limitations. Based on our preliminary occupancy and detection probability estimates and the recommendations of MacKenzie and Royle (2005), we believe the standard repeat survey design would be most appropriate for a Copperbelly Water Snake monitoring program. However, the survey design may need to be adjusted in the future as parameter estimates change due to alterations to the sample frame and/or changes in population status.

We estimated that between 2-4 surveys would need to be conducted at each site in a Copperbelly Water Snake monitoring program based on our estimates of site occupancy and probability of detection and the guidelines of MacKenzie and Royle (2005). Field et al. (2005) stated that 2-3 surveys appeared to be sufficient for most species, unless occupancy levels were high or detection probability low. A decision on how many surveys to conduct needs to be made in the context of the number of sites to be visited, desired precision levels for estimates, total survey effort, and budgetary and personnel limitations. MacKenzie and Royle (2005) suggested that for rare species one should survey more sites less intensively. When conducting fewer visits per site, more sites will be needed to achieve the same level of precision. We estimated that conducting two visits per site would require more sites to be visited compared to a standard design with three visits, but that total survey effort decreased when detection probability was greater than about 0.55-0.60. Although this finding suggests that a Copperbelly Water Snake monitoring program could use a design with two visits to allow surveys at more sites, MacKenzie and Royle (2005) recommended that three visits be considered the minimum when detection probability is greater than 0.50. Given this recommendation and the preliminary nature of our estimates, we suggest conducting three visits per site in a Copperbelly Water Snake monitoring program.

Considering the budgetary constraints associated with any monitoring program, the number of sites to be surveyed will likely be determined by available funding rather than desired levels of precision on population estimates. However, assuming Copperbelly Water Snake occupancy of about 0.20 and detectability of 0.50-0.60, we estimated between 110 and 230 sites would be needed to achieve moderate levels of precision. An option to increase the total sample size for a monitoring program is to use a rotating panel design in which smaller groups of sites are surveyed each season on a rotating basis over a number of years rather than surveying the same sites every season (Urquhart and Kincaid 1999). Panel designs have obvious logistical benefits, and provide a much larger sample size than could be accomplished by surveying the same sites every year. However, MacKenzie (2005) noted that in simulations of various designs in occupancy studies, precision of trend estimation in occupancy was similar for rotating panel designs and standard designs in which the same units were surveyed each year. The most important factor influencing trend estimation appeared to be the number of sites surveyed in a given season rather than the total number of sites surveyed (MacKenzie 2005). MacKenzie (2005) argued against the use of panel designs due to the possibility of spatial and temporal changes in occupancy becoming confounded. MacKenzie et al. (2006) noted that the time frame in which trends need to be evaluated will influence the required survey effort, with more sampling required if precise information is needed in a short time frame. Therefore, if the number of sites visited each season is limited by resource constraints, then a longer amount of time will be needed to provide trend information. Based on the recommendations of MacKenzie (2005), we suggest using a design in which the maximum number of sites possible is surveyed each year.

Other Design Considerations

We recommend several other aspects of a Copperbelly Water Snake monitoring program be standardized, such as the timing of survey periods, number of observers, pattern of site visits, and survey methods. If a repeat survey approach is used, it will be essential that the timing of the surveys be consistent among all sites. The survey period should be selected to minimize the movement of snakes into or out of sites (i.e., minimize likelihood of violating closure assumption). Once the survey season is determined, survey windows need to be identified for each replicate visit, so that surveys are done at approximately the same time at all sites. For example, using a standard design with three surveys, visits one, two, and three would occur at all sites during the same time frames. Every effort should be made to visit each site during each survey window to minimize missing observations. To reduce possible heterogeneity in detection probabilities, we also suggest standardizing the number of observers conducting surveys (e.g., one surveyor per site). MacKenzie et al. (2004a) suggest rotating observers among all sites to maximize the independence of surveys. We also think rotating the order in which the sites are surveyed could reduce possible confounding effects of survey site and time of day on detectability. Care should be taken to ensure that the methods (e.g., survey routes, observation points) used to survey snakes are consistent among all sites.

Future Analyses

We feel the multi-season model developed by MacKenzie et al. (2003) would have utility for a long-term Copperbelly Water Snake monitoring program as it would provide estimates of site occupancy, detectability, and extinction and colonization probabilities. This model also permits inclusion of site and survey covariates that might influence occupancy and detection probability estimates. We suggest partners identify potential site and survey covariates that might be important to determining Copperbelly Water Snake occupancy and detection, so these data could be collected consistently by observers during surveys. By including covariates in future modeling efforts, we could learn what variables appear to greatly affect occupancy and detection probability, which could inform recovery efforts and possible modifications to the monitoring design. We believe the multiple-season occupancy model could be augmented by single-season abundance models (e.g., Royle and Nichols 2003, Royle 2004) that allow population indicators (e.g., estimated number of snakes per site) to be estimated and tracked over time along with occupancy. Lastly, we suggest exploring other occupancy models, such as staggered-entry (relaxes closure assumption) and multiple-species (MacKenzie et al. 2004b) models to learn more about the status and ecology of Copperbelly Water Snakes.

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