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Invasive earthworm damage predicts occupancy of a threatened forest fern: Implications for conservation and management

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ABSTRACT

Adequate detection and monitoring of threatened, endangered, and sensitive species presents a challenge to forest managers seeking to balance management activities with conservation and forest health. This is especially true for rare, cryptic plant species that are difficult to detect, like goblin fern (*Botrychium mormo*), which is small and does not emerge from the duff layer of the rich hardwood forests it inhabits every year, even when present. Imperfect detection of this species makes it difficult to monitor, because lack of plants detected at a specific site does not necessarily indicate that the species has been extirpated there. In this study, 80 historic locations of *B. mormo* were surveyed for occurrence over three consecutive years to assess probability of occupancy and environmental factors expected to impact occupancy, including earthworm damage and canopy closure, while accounting for detectability. We found that probability of occurrence is most strongly related to earthworm damage and were able to identify levels of earthworm damage at which the species is more likely to remain present or be extirpated. These results suggest that use of a simple metric for quantifying ecological impact of earthworm damage can be used during monitoring to assess the likelihood that *B. mormo* is still present. With this information, forest managers can prioritize sites for habitat preservation and better shape policy and management decisions to protect and enhance habitat for this species. In addition, our study demonstrates the utility of occupancy modeling for management and conservation of rare and elusive plant species.

1. Introduction

Consideration of management impacts to threatened, endangered, and sensitive (TES) species is critical to forest management, especially for agencies that are under regulatory obligation to protect them (USDA). Developing appropriate policies and management strategies related to TES and other vulnerable species depends on precise knowledge of population locations, population viability, and factors contributing to any population declines. Inventory and monitoring programs are often designed to answer questions related to where populations are and how they are trending, but typically cannot identify causal agents of those trends (Elzinga et al., 1998). Further, traditional inventory and monitoring approaches may not be sufficient to adequately address any of these issues for elusive species that are difficult to detect.

Increasingly, imperfect detectability is recognized as an important factor to account for in research and monitoring (Kéry and Schmid, 2004). The assumption that a species is not present or has been extirpated at a site where it isn't detected often does not hold, since populations or individuals can be missed during surveys (Kéry, 2004). Detectability estimates are now routinely incorporated into analysis of occupancy and abundance data for animals (MacKenzie et al., 2017). Although this approach has been used less frequently in plants and other sessile organisms (but see: Alexander et al., 2009; Berberich et al., 2016; Emry et al., 2011), detectability in plant surveys is typically less than one, and therefore important to account for (Chen et al., 2013). Without perfect detection, "false zeros" recorded when a species is present but not detected in a monitoring dataset can lead to biased under-estimates of occupancy (and over-estimates of extirpation) and misleading or incorrect results regarding the factors influencing these occupancy states (Chen et al., 2013; MacKenzie et al., 2017). Thus, using inferences from analyses based on inventory and monitoring programs that don't incorporate detectability to inform policy and management decisions may be highly problematic (Guillera-Arroita et al., 2014). Utilizing occupancy models that incorporate presence/absence data, while accounting for imperfect detectability and factors contributing to heterogeneity in detectability and occupancy, can be a valuable tool for overcoming these common obstacles in analysis of rare plant monitoring data.

Botrychium mormo (goblin fern), is an example of a rare species of

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conservation concern that is of particular importance to forest management in the Great Lakes states, but is difficult to monitor due to its small size and the fact that it does not emerge from the duff every year. It is endemic to the rich northern hardwoods of the western Great Lakes region including Minnesota, Wisconsin, and Michigan in the USA, and has one recorded occurrence in Quebec, Canada (USDA NRCS, 2018; Wagner Jr. and Wagner, 1993). The majority of known extant populations are concentrated in north-central Minnesota within the Chippewa National Forest, which overlaps 90% of the Leech Lake Reservation (Johnson-Groh and Lee, 2002; Mortensen and Mortensen, 1998). In the Chippewa National Forest, the rich northern hardwood forests this species depends on account for approximately less than 1% of forested land. Surveyors have searched this habitat extensively over the past twenty years for *B. mormo*, and over 600 element occurrences have been documented in the Chippewa National Forest. The species has state conservation ranks assigned to it throughout its entire range designating it as vulnerable to extirpation in Minnesota, imperiled in Michigan, and critically imperiled in Wisconsin and Quebec (NatureServe, 2017). It has not been reviewed for the Endangered Species Act in the United States, but is listed as endangered in Wisconsin (WI DNR, 2017) and threatened in Minnesota and Michigan (Michigan Natural Features Inventory, 2017; MN DNR, 2013).

The life history of *B. mormo* makes it particularly difficult to detect and verify occupancy. It is a small perennial fern that is known to lie dormant or fail to emerge from the duff in some years, likely due to unsuitable conditions such as drought (Wagner and Wagner, 1981), even when there is a thriving population of immature and mature plants below-ground (Johnson-Groh, 1998). Because of this, abundance of above-ground plants can vary significantly from year to year, which does not necessarily correlate with total abundance of above- and below-ground plants, and individuals or all plants from a population may fail to emerge in a given year (Johnson-Groh and Lee, 2002; Johnson-Groh et al., 2002). Thus, monitoring above-ground plant number, especially for only one year, would yield poor estimates of abundance and underestimates of occupancy. Incorporating detectability estimates derived from surveys over multiple years into occupancy models is one way to obtain unbiased estimates of probability of occurrence within sites, and can also be used to assess the effect of factors that may influence probability of occurrence (MacKenzie et al., 2017).

One factor known to negatively impact *B. mormo* is the invasion of non-native earthworms in the northern hardwood forests that harbor this species. *Botrychium mormo* requires a thick duff layer and organic soil horizon over mineral soil that is well to moderately well-drained and has loamy to silty texture (Casson et al., 2001). Personal field observation (Henderson) and Natural Resource Conservation Service (NRCS) soils mapping further indicate goblin fern hot spots typically have well-drained fine sandy loam to fine loamy sand soil textures, and are nearly level to undulating with 1–8 percent slopes (Soil Survey Staff). Within the humus layer, this species is dependent on associations with mycorrhizal fungi to obtain nutrients and water, as are other *Botrychium* species (Berch and Kendrick, 1982; Whittier, 1984; Winther and Friedman, 2007). Earthworms, especially *Lumbricus* spp., rapidly reduce organic soil layers to the detriment of *B. mormo* and other plants dependent upon intact soil O horizons in northern hardwood systems (Gundale, 2002; Hale et al., 2005a, 2006, 2008; Mortensen and Mortensen, 1998). While we know that earthworms have a significant adverse effect on this species, the level of worm damage necessary to make habitat unsuitable for *B. mormo* has not yet been quantified. This information would be highly valuable for managers to help determine when the species is likely extirpated from sites, as not finding plants on a given monitoring visit does not necessarily mean the species is absent.

In addition to soil characteristics, another factor that may influence *B. mormo* occupancy is canopy closure. This species has only been observed in forests with a relatively closed canopy (Casson et al., 2001). Shade is hypothesized to be important to *B. mormo* spores, which only germinate in the dark (Whittier, 1973), and to maintain cool, moist conditions in the duff layer (Casson et al., 2001). *Botrychium mormo* is known to be sensitive to drought conditions, and can fail to emerge during drought years (Wagner and Wagner, 1981). An open canopy can create or exacerbate dry conditions on the forest floor, impacting species dependent on moisture in the duff layer (Harpole and Haas, 1999; Semlitsch et al., 2009). Factors such as earthworm invasion and canopy closure may contribute to heterogeneity in occupancy probability, and thus are important to include in occupancy models for this species.

During this three-year study, data on *B. mormo* occupancy and covariates, including earthworm invasion stage and canopy closure, were collected from historic *B. mormo* sites to answer the following three questions: (1) How do various levels of earthworm damage influence probability of occupancy? We expect that as earthworm damage increases, occupancy probability will decrease. (2) Does canopy closure influence probability of occupancy, and if so, how? We expect that decreased canopy closure may have a negative relationship with occupancy probability. (3) Do earthworm damage and canopy closure interact? We expect that the negative effects of earthworm damage may be buffered by increased canopy closure or may be exacerbated by decreased canopy closure. Results are discussed in the context of using occupancy modeling, including detectability, in monitoring efforts to inform conservation and forest management.

2. Methods

2.1. Data collection

To assess factors that may influence occurrence of *B. mormo*, locations where the species was historically documented were monitored in 80 randomly selected sites within the Leech Lake Reservation and Chippewa National Forest. For this experiment, a “site” is defined as the area where a population of a species has been documented. Site selection was stratified by choosing 20 random sites within four time periods of original date of detection (1994–1997, 1998–2002, 2003–2007, 2008–2011; Fig. 1). Because historical sites were used, our results apply to the population of sites where this species has been documented rather than the population as a whole. Sites were divided between two observers experienced in *Botrychium* surveys, who visited the same sites once per year over three years to increase likelihood of detection if the species was indeed occupying the site. Sites were relocated from original records based on GPS coordinates or coordinates inferred from field notes that identified sites based on unique features and bearings and distances from specific features (for records that originated before GPS was widely used). During initial relocation visits, a hybrid spiral/random meaner method was used for sites with coordinates. If historic occurrence sites had inferred coordinates, or had GPS coordinates but were not initially located through this method, a parallel transect method was used to more thoroughly search sites. Initial relocation efforts ranged from 1 to 6 h per site, with an average of 4 h per site (shorter times are associated with sites where the species was easily found near given coordinates). Surveys occurred between July and August, the time period during which above-ground abundance and individual plant size peak (Johnson-Groh and Lee, 2002).

During surveys, the following variables were recorded: presence/absence of above-ground *B. mormo*, abundance of *B. mormo* plants if detected, Invasive Earthworm Rapid Assessment Tool (IERAT) stage (Loss et al., 2013), and canopy closure (foliar volume measured with a densiometer). The IERAT stage was determined using a protocol developed by the Natural Resources Research Institute Great Lakes Worm Watch program, which takes into account several facets of worm damage to forest understories, including loss of duff layer and organic soil horizons, plant community diversity, and direct evidence of earthworms including castings, middens, and earthworms themselves, to rank ecological impact of worm invasion on a site (1–5, with 5 indicating the greatest impact; Loss et al., 2013). Canopy closure was

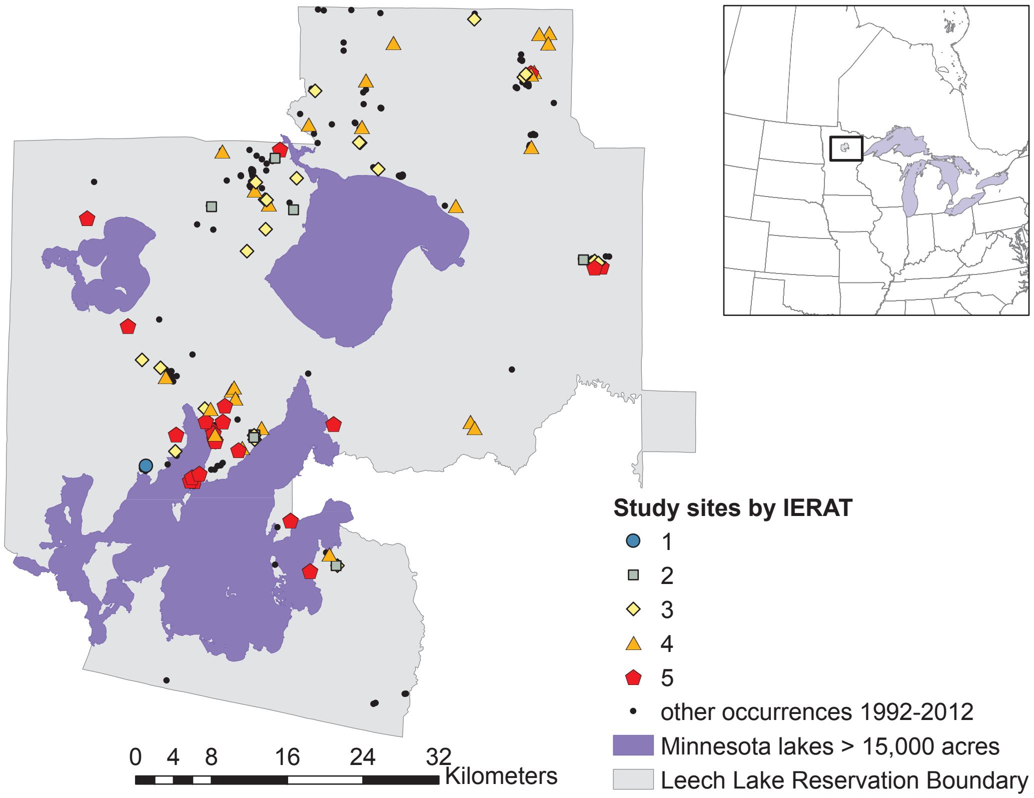

Fig. 1. Map showing general location of Leech Lake Reservation within Minnesota, USA and *Botrychium mormo* study sites by Invasive Earthworm Rapid Assessment Tool (IERAT) stage (stages: 1 – blue circle; 2 – green square; 3 – yellow diamond; 4 – orange triangle; 5 – red pentagon), with additional historic occurrences documented from 1992 to 2012 (black dots). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

measured using a model C concave spherical crown densiometer, with a cross-shaped grid of 24 quarter-inch squares.

2.2. Statistical analysis

Single-season occupancy models were used to model detectability of *B. mormo* and test effects of covariates on site occupancy (MacKenzie et al., 2017). Because *B. mormo* is a perennial plant that can have up to five years' worth of pre-formed leaf buds (Wagner, 1998), it was assumed that over a three-year period the occupancy state for this species is closed within survey sites (MacKenzie et al., 2017). After initial data exploration, several observations were removed from the final analyzed dataset. The lone site with an IERAT stage of one had *B. mormo* detected in all three surveys and was removed from analysis due to insufficient sampling of sites of this quality, which are rare across the landscape (Zuur et al., 2010). In addition, all 22 stage 5 sites were excluded from occupancy modeling, as *B. mormo* was not detected at any of these sites during any survey over the three-year survey period. Because no plants were observed at any site or visit for IERAT stage 5, analysis of this stage would yield inestimable standard errors for its occupancy probability estimate of 0. However, to ensure that excluding these observations did not impact results for stages 2–4, we also ran the analysis including all stage 5 observations. Results for model selection and beta coefficients of covariates were qualitatively the same, and results for occupancy estimates and their standard errors were the same, as the results when these observations were excluded. For this reason, we focus on occupancy analysis and results for IERAT stages 2–4 for the rest of the paper. Two additional sites were removed due to missing data for site-specific covariates. The final dataset included presence/absence and covariate data for 55 sites with the following distribution of IERAT stages: 2–7 sites, 3–22 sites, 4–26 sites.

Factors hypothesized to impact *B. mormo* occupancy were used to build a general occupancy model in PRESENCE (v. 12.7; Hines, 2006) to model detection probability. The initial general occupancy model included IERAT stage (IER; categories 2–4), canopy closure (CC), an interaction between IER and CC, and the original detection date (categories 1–4 based on dates site selection was stratified by). Canopy closure was transformed by dividing by 100 to improve model convergence, as suggested in the PRESENCE manual (Hines and MacKenzie). During initial model runs, original detection date was dropped from analysis because large standard errors, standard errors that were not estimable, and poor resolution on maximum likelihood estimates indicated that the model was likely over-parameterized given the sample size. In addition, there was no indication based on AIC that original detection date was a useful parameter for modeling occupancy, and original detection date is a difficult variable to interpret since it contains no information on how long the species has actually occupied a site, or what the population history (extirpation, colonization) has been at the site since the original detection. The final general model for occupancy was:

$$logit(\psi_i) = \alpha_0 + \alpha_1 IER_i + \alpha_2 CC_i + \alpha_3 IER_i x\ CC_i$$

This general model was used to assess factors that may influence *B. mormo* detectability. Four models were compared including one indicating whether *B. mormo* had been previously detected at a site (PD), the year of the survey (YR), and the minimum number of plants recorded in the original detection (ODA, standardized by subtracting each value from the mean and dividing by the standard deviation to generate Z scores) (Table 1). The variable indicating whether the species had been previously detected was important to include because, since the same surveyor visited each site every year, the assumption of independent site visits was violated. Including a variable indicating

Table 1
Summary of detectability model results for goblin fern (*Botrychium mormo*) data. ΔAIC is the difference in AIC between the model with lowest AIC (top ranked model), *w* is the AIC model weight, *Npar* is the number of parameters in the model, and −*2ll* is the −2 log likelihood of the model. For all models the global occupancy model ψ(IER + CC + IERxCC) was used, where IER is the Invasive Earthworm Rapid Assessment Tool (IERAT) stage and CC is canopy closure.

Model	ΔAIC	*w*	*Npar*	−*2ll*
p(PD)	0.00	0.6667	7	145.43
p(ODA)	2.89	0.1572	7	148.32
p(YR)	3.57	0.1119	9	145.00
p(•)	4.68	0.0642	7	150.11

PD = species detected during previous survey; ODA = abundance from original detection; YR = year of survey.

whether the species had been previously detected allows this dependency to be modeled for detectability (MacKenzie et al., 2017). Combinations of variables with PD were modeled, but removed from the candidate model set because there was not sufficient data to model multiple detection parameters (as indicated by the same problems discussed above for modeling original detection date). The final general detectability model, best supported by AIC was:

$$logit\,(p_{ij}) = \beta_0 + \beta_1 PD_i$$

Using the above detectability model, all combinations of the general occupancy model were tested, including a model holding occupancy constant across sites. However, before testing simpler models, the global model (IER + CC + IERxCC as site covariates and PD as covariate on detectability) was used to assess model fit using 5000 bootstrap samples (X^2 = 3.58, *P*-value = 0.8204, $\hat{c}$ = 0.4267). Results of the goodness-of-fit test show that the model is a reasonable fit for the data, that standard errors for model parameters do not require adjustment, and that simpler models derived from the global model will also be adequate to describe the data (MacKenzie and Bailey, 2004; MacKenzie et al., 2017). Final model selection was done using AIC to select the best supported model.

3. Results

Botrychium mormo was observed during at least one of three surveys at 27 out of 80 sites (33.75%), and the proportion of sites differed by IERAT stage. Of the randomly selected sites, 27.5% were assessed as IERAT stage 5, 32.5% stage 4, 27.5% stage 3, 8.75% stage 2, and 1.25% stage 1 (Table 2). *Botrychium mormo* was observed in at least one year at all sites with IERAT stages of 1 or 2, at 59% of sites with IERAT stage 3, 19% of sites with IERAT stage 4, and none of the sites with IERAT stage 5. For sites where the species was detected, all possible detection histories were represented, including plants being observed in 1/3 years, 2/3 years, and 3/3 years (9 sites, 12 sites, and 6 sites respectively).

Abundance across all sites was relatively low with 42 plants observed across all sites in the first year of surveys, 62 plants in the second year, and 54 plants the third year. Several sites had only one plant observed, the maximum number observed at a site was 12, and the mean was three (Table 2). Number of plants observed also fluctuated among years at the same site, for example, at one site three plants were observed the first year, 9 the next, and six in the third year. As another example, no plants were observed at one site the first year, one was observed the second year, and five the last year. Plants were not observed in any of the five sites with the greatest original abundance (three sites with over 100 and two with over 500 plants minimum originally observed). Two of these sites were scored as stage 4 and three as stage 5 on the IERAT scale. Minimum abundance from the historical detection was not correlated with abundance during any of the three years of surveys for this study, whether all sites were considered (2015: *r* = −0.07, *P* = 0.580; 2016: *r* = −0.10, *P* = 0.394; 2017: *r* = −0.11, *P* = 0.355) or only those where plants were found during that year (2015: *r* = 0.31, *P* = 0.277; 2016: *r* = −0.12, *P* = 0.623; 2017: *r* = −0.19, *P* = 0.410).

For sites included in occupancy analysis (IERAT stage 2–4), there was no significant difference in canopy closure between sites where *B. mormo* was observed and not observed. Canopy closure ranged from 63% to 98% in sites where *B. mormo* was observed and 62–96% at sites where *B. mormo* was not detected. Mean canopy closure did not differ between sites where the species was observed compared to sites where the species was not observed (86% vs 81%; *t* = 1.39, *P* = 0.17).

The occupancy model best supported by AIC model selection modeled occupancy based solely on IERAT stage, and detectability based on whether the species had been previously found at a given site (Table 3). Although CC was in the second-best ranked model, and this model was within two AIC points of the top-ranked model, this variable is considered uninformative. Akaike's Information Criterion penalizes models by increasing AIC 2 units for each additional model parameter (Johnson and Omland, 2004). Thus, if a model has one additional parameter and is within 2 units of the top model, as in this case, that additional parameter is considered uninformative (Arnold, 2010).

The naïve overall occupancy estimate, not incorporating detectability, for sites at IERAT stages 2, 3, or 4 was 0.45. Modeled occupancy probability for each site differed based on the IERAT stage of that site (Fig. 2). Sites at IERAT stage 2 had a probability of 1 that the species is still occupying the site. As IERAT stage increases, estimated probability that the site is still occupied decreases to 0.68 (95% CI 0.42–0.86) for sites at IERAT stage 3 and to 0.22 (0.09–0.44) for sites at IERAT stage 4. Using these estimates to predict the proportion of extant sites within each IERAT stage, and assuming that the species still exists at the few sites with IERAT stage 1 and is extirpated on all IERAT stage 5 sites, we estimate that the proportion of extant sites across all IERAT stages is 38% (25–49%). When previous detection history is taken into account (whether the species was found during at least one survey over the

Table 2
Summary data for all sites, excluding one site with missing data for IERAT stage, including abundance from historical occurrence records, abundance recorded across three-year study, and number of historical sites where species was or was not observed in at least one year during three-year study, by IERAT stage. Note historical abundance is reported from original detection records and is a minimum estimate of population size at the time of the observation.

	Historical Data			Data from current three years of surveys					
	abundance			abundance					
IERAT stage	min	mean	max	min	mean	max	# sites species observed	# sites species not observed	Total # sites
1	12	12	12+	1	3	4	1	0	1
2	1	5	10+	0	3	9	8	0	8
3	1	10	50+	0	1	12	13	9	22
4	1	28	500+	0	< 1	5	5	21	26
5	2	23	500+	0	0	0	0	22	22

Table 3
Summary of occupancy models fit to goblin fern (*Botrychium mormo*) data. ΔAIC is the difference in AIC between the model with lowest AIC (top ranked model), *w* is the AIC model weight, *Npar* is the number of parameters in the model, and −*2ll* is the −2 log likelihood of the model. For all models the detection model *p* (PD) was used, where PD indicates whether the species was detected at a site on a previous visit.

Model	ΔAIC	*w*	*Npar*	−*2ll*
ψ(IER)	0.00	0.4827	4	147.53
ψ(IER + CC)	0.15	0.4479	5	145.68
ψ(IER + CC + IERxCC)	3.90	0.0687	7	145.43
ψ(CC)	14.38	0.0004	3	163.91
ψ(•)	14.54	0.0003	2	166.07

IER = Invasive Earthworm Rapid Assessment Tool (IERAT) stage, CC = canopy closure, ψ = probability of occupancy, ψ(•) = model with no covariates

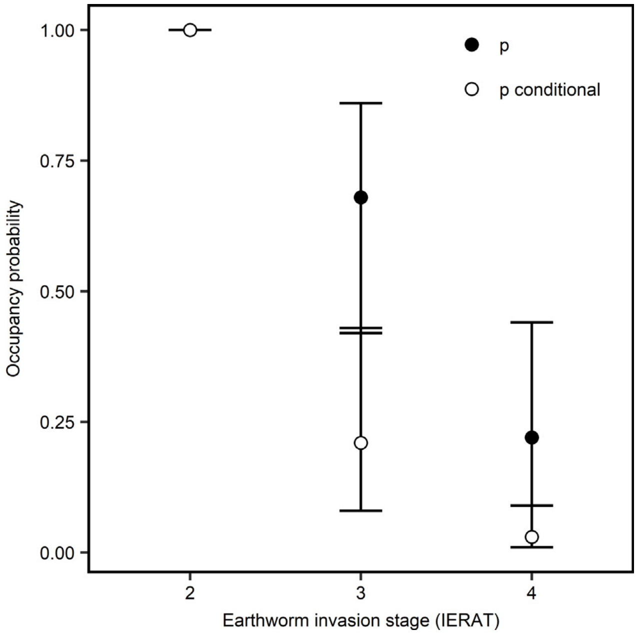

Fig. 2. Probability of occurrence for *B. mormo* by Invasive Earthworm Rapid Assessment Tool (IERAT) stage, showing both probability (p) and probability conditional on having found the species at least once at a site. Bars represent 95% confidence intervals. Note p and p conditional are the same at IERAT stage 2.

three-year study period), these occupancy probabilities decrease for sites where the species was not observed. For sites at IERAT stage 3 where *B. mormo* was not observed during any surveys over three years, estimated probability of occupancy dropped to 0.21 (95% CI: 0.08–0.43), and for sites at IERAT stage 4, estimated probability of occupancy plummets to 0.03 (0.01–0.09).

Detection probability in the first year, and for sites where the species had not yet been observed during the study period, was 0.50. Once the species was observed at a site (by the same observer), mean detection probability increased to 0.73 (95% CI 0.55–0.86).

Negative coefficients for IERAT stages 3 and 4, relative to IERAT stage 2, show decreasing log odds that *B. mormo* occupies a site as earthworm damage progresses (Table 4). The positive intercept term indicates that the log odds of occupancy are high at sites with IERAT stage 2. As IERAT stage increases to 3 and 4, the magnitude of negative coefficients with relatively small standard errors indicates an enormous negative effect on occupancy. This results in odds ratios near 0 (i.e. 3.5×10^{-13} for IERAT stage 3), which indicates occupancy is much less likely in sites with IERAT stages greater than 2. The log odds presented here are relatively large for logistic regression, and their magnitude is driven by comparison with IERAT stage 2, which had comparatively few observations (sites of this quality are rare on the landscape), and positive species detection within all sites in at least one year of surveys. Because of this, the beta coefficients comparing log odds with IERAT stages that are less likely to be occupied in this analysis are inflated. Nevertheless, these results make sense biologically, and we feel they are important to report and document due to the increasing rarity of high quality sites on the landscape and the striking effect of degradation of habitat suitability beyond this level of worm infestation.

4. Discussion

4.1. Earthworm effects and canopy closure

Botanists and ecologists have long known that earthworm damage to northern hardwood forest floors degrades habitat for species like *B. mormo*, a threatened plant of particular relevance to forest management in the Great Lakes states. This species depends on a relatively thick organic soil horizon and duff layer, which earthworms, especially *Lumbricus* spp., rapidly destroy in a matter of years after invasion (Gundale, 2002; Hale et al., 2006, 2008; Mortensen and Mortensen, 1998). Our results agree with other work showing that non-native earthworms strongly drive ecosystem changes resulting in declines and extirpation of *B. mormo* populations (Gundale, 2002). Further, we identify levels of worm damage most likely to negatively impact *B. mormo* occupancy and show that a simple, quick protocol, the Invasive Earthworm Rapid Assessment Tool, can be effectively used to describe and monitor worm damage to assess *B. mormo* habitat.

Most importantly, our results from three years of sampling allow us to identify a threshold in habitat quality after worm invasion for *B. mormo* (Question 1). Over 90% of sites within the Leech Lake Reservation, the stronghold for occurrences within this species' range, have degraded habitat such that *B. mormo* no longer occupies historic sites or is in peril (IERAT ≥ 3). At IERAT stage 2, there is a high probability that *B. mormo* still occupies sites. This stage is characterized by invasion of *Dendrobaena octaedra* (Loss et al., 2013), an epigeic species that lives and feeds in leaf litter without reducing forest floor thickness (Hale et al., 2005b), and does not negatively impact *B. mormo* (Gundale, 2002). Sites at IERAT stage 3 still have a relatively high occupancy probability, but between stages 3 and 4 probability of occupancy decreases below 50%, to a mean of 0.22. Especially for sites categorized as IERAT 4, it is very unlikely that *B. mormo* still occupies a

Table 4
Summary of regression coefficients (SE) for occupancy models fit to goblin fern (*Botrychium mormo*) data; *w* is the AIC model weight.

Model	*w*	Intercept	IER3	IER4	CC	IER3xCC	IER4xCC
ψ(IER)	0.48	29.40 (2.70)	−28.67 (2.70)	−30.66 (2.71)	–	–	–
ψ(IER + CC)	0.45	30.46 (2.90)	−33.88 (2.77)	−35.74 (2.77)	4.83 (3.08)	–	–
ψ(IER + CC + IERxCC)	0.07	16.49 (5.19)	−21.90 (5.93)	−20.58 (5.56)	18.85 (7.83)	−11.62 (9.59)	−15.42 (8.09)
ψ(CC)	0.00	−3.43 (2.40)	–	–	4.23 (2.88)	–	–
ψ(•)	0.00	0.09 (0.31)	–	–	–	–	–

IER = Invasive Earthworm Rapid Assessment Tool (IERAT) stage, CC = canopy closure, ψ = probability of occupancy, ψ(•) = model with no covariates holding occupancy constant across sites.

site if it hasn't been observed during three consecutive years of surveys by an experienced surveyor. At IERAT stage 5, we find no evidence that sites can continue to support *B. mormo*. This tipping point corresponds to invasion of *Lumbricus rubellus,* an epi-endogeic species which rapidly consumes litter and organic soil horizons (Hale et al., 2005b) at IERAT stage 3. The final stages of invasion correspond to initial introduction and peak of *L. terrestris* at IERAT stages 4 and 5 (Loss et al., 2013). *Lumbricus terrestris* is an anecic species that invades sites after the forest floor has been impacted by other species, such as *L. rubellus* (Hale et al., 2005b). It burrows deep within the soil and can consume all litter each year, preventing the forest floor and organic soil horizon from recovering after earthworm invasion (Hale et al. 2005b). Our results support Gundale's (2002) work showing invasion of *L. rubellus* is associated with decline in *B. mormo* populations; however, our results suggest that *L. terrestris* invasion, or a combination of species with *L. terrestris,* at stages 4 and 5 is most detrimental to *B. mormo.* Alternatively, the existing populations at stage 3 may represent a lag effect on extirpation if sites inevitably proceed to stage 4. Although earthworm species present in earlier IERAT stages can facilitate invasion of species associated with later stages (Hale et al., 2005b), introduction of those species to sites is still necessary (Holdsworth et al., 2007). At the current level of infestation, our study suggests that 60% of historical *B. mormo* sites are already unsuitable and that the species is either extirpated or in immediate peril at sites with IERAT stages 5 and 4 respectively. Continued monitoring of these sites, which is planned for the next 10 years, will help to elucidate how invaded sites progress in terms of IERAT stage and provide more precise information on the conditions that lead to population extinction, especially within IERAT stages 3 and 4.

Aside from an intact forest floor, canopy closure is hypothesized to be important to providing necessary darkness and moisture to *B. mormo* plants, but this factor was uninformative for predicting occupancy in this study (Question 2). Similarly, there was no evidence for a significant interaction between canopy closure and IERAT on occupancy (Question 3). It is critical to realize that while canopy closure didn't provide any explanatory power beyond IERAT for these sites, that does not mean canopy closure isn't important for this species. This species has only been found in sites with relatively high canopy closure (Casson et al., 2001). The randomly selected sites in this study ranged from over 60% to over 90% canopy closure, which did not differ between sites that were and were not occupied over the three years of this study. Further, models that included canopy closure had large positive regression coefficients suggesting a positive relationship between canopy closure and occupancy. Taken together, these results suggest that the range of canopy closure for all sites in this study is within the range that defines suitable habitat for this species. This is an important issue as it impacts forest management decisions, for example, buffer size around plants of conservation concern (Casson et al., 2001). While more research is needed to determine the effect of harvest on *B. mormo* populations, anecdotal evidence suggests that clearcutting results in extirpation of populations while selective harvests that don't directly impact plants, or the duff layer and organic soil horizons they depend on, may allow populations to persist (Casson et al., 2001; Sather et al., 1998). In addition, our results provide no insight into the potential importance of different forest strata to providing canopy coverage, which can be patchy to continuous in the mesic hardwood forests where *B. mormo* is found (MN DNR, 2003).

4.2. Occupancy and abundance

This study illustrates the importance of designing monitoring efforts based on species' life history attributes and including detectability in analysis of monitoring data for plants and TES species. For *B. mormo*, managers cannot count on a single survey by a naïve observer to determine whether this species is present during one monitoring visit in a single year. Our results show that even for very experienced observers, it is just as likely that plants will be found when present than that they will not, unless the same observer has already found *B. mormo* at a given site. In addition, for a majority of sites where the species was documented as present during the three years of sampling, plants were not observed in all years. This underscores the importance of monitoring this species over several years to determine whether a site is occupied, and using habitat information, especially IERAT, to assess the likelihood that the species is still present in sites that were historically occupied for making management decisions. Our results also highlight the importance of modeling detection probability for this species in assessing site occupancy, because, at best, experienced botanists who have already found the species at a site have a probability less than 0.75 of detecting the species, which will lead to biased underestimates of occupancy if detectability is not incorporated in analysis.

While this study focused on occupancy, some notes can be made regarding population abundance. Observed abundance within populations during the three years of sampling was relatively low and variable, which agrees with other research and reports (Casson et al., 2001; Johnson-Groh and Lee, 2002). Since *B. mormo* was first officially recorded as a Regional Forest Sensitive Species (RFSS) within the Chippewa National Forest (CNF) in 1992, over 600 historic records have been documented, most of which were recorded between the late 1990′s and 2012. The overwhelming majority of these historical observations had small population counts, typically ranging between 1 and 8 individuals (CNF, unpublished data), which is similar to abundances observed in our three years of contemporary surveys. In one report from the CNF in 2001 (Casson et al., 2001), 79% of 116 populations were documented as having 20 or fewer above-ground plants. Large fluctuations in countable population size from year to year and lack of correlation inhibits making inferences about abundance for this species. More sophisticated analysis of abundance would be valuable, but given the life-history of *B. mormo,* existing statistical approaches may not be valid. For example, the Royle-Nichols abundance model assumes a demographically closed population across the study period, which likely does not hold over three years, even for this perennial species (Denes et al., 2015). Also, sampling was not standardized to occur during peak population size in mid-July (Johnson-Groh and Lee, 2002), which could bias results. A more sophisticated approach may be possible (Dail and Madsen, 2011), but we don't have precise estimates of the demographic rates, especially for underground plants (Berlin et al., 1998), necessary to model abundance to overcome the assumption of a closed population between, or even within, seasons.

Greater abundance does not appear to have buffered the species from extirpation at sites with high worm impacts; the species appears to be extirpated from all of the sites with the largest historical populations, which were documented as having over 100 individuals. Sites that originally harbored these large populations are now heavily impacted by worms and are at IERAT stage 4 or 5. This point highlights the importance of historical data in this study. Without knowing that the species had once occurred, and as large populations, in sites that now have IERAT stages of 4 or 5, we may have inferred, for example, that *L. terrestris* and *B. mormo* potentially inhabit different habitat niches. Several hypotheses may help to explain the complete loss of *B. mormo* from the sites in this study that historically had large populations. Large populations may have always been rare, making up just 3% of occurrences with at least 1 verified plant between 1992 and 2012 on the Chippewa National Forest (Chippewa National Forest, unpublished data). One hypothesis is that the area where most of these populations occurred on Ottertail Point, a large peninsula that juts into Leech Lake from the north, experienced earthworm invasions relatively early (possibly through release or escape of worms used for fishing bait) and are showing the result of prolonged and successive damage. This hypothesis is supported by documentation of *Lumbricus* spp. on the peninsula for over 20 years and evidence from tree rings of earthworm fronts on the peninsula dating to the 1960s (Hale et al., 2005a; Larson et al., 2010). Further, data from this area show that below-ground abundance of *B. mormo* decreases following decrease in above-ground abundance in response to worm invasion (Johnson, 2015). A second hypothesis is that the unique rich hardwood forests in this area contained exceptional habitat characterized by a deep duff layer that facilitated both large populations of *B. mormo* in the past and explosive growth in the worm population after invasion (Curry, 1998). Third, plant-soil feedbacks, positive or negative, could influence population abundance through potential mechanisms such as build-up of pathogens in the soil or changes to mycorrhizal communities *Botrychium* depend on to obtain nutrients (Bennett et al., 2017; Klironomos, 2002).

4.3. Conservation and management recommendations

In light of the severe decline in occupancy observed in this study, we recommend a conservative approach to management within the vicinity of known *B. mormo* populations and conservation of high quality habitat. Human mediated transport of worms is the most important factor influencing earthworm spread across the landscape (Cameron et al., 2007; Hale, 2008). Use of worm species, especially *Lumbricus* spp., as fishing bait is a major vector of earthworm introduction, and cabin and boat landing locations are associated with spread of these species (Holdsworth et al., 2007). Vehicles and equipment, including ATVs and logging equipment (Hale et al., 2009), transport both live worms and cocoons, and distance to roads is a significant predictor of earthworm presence, especially for *Lumbricus* spp. (Holdsworth et al., 2007). Within the Chippewa National Forest, recommendations currently restrict forest management activity within a 250′ buffer around known occurrences and limit harvest activity beyond 250′ to winter harvest (Casson et al., 2001). Earthworm eggs and cocoons can survive temperatures below freezing (Holmstrup, 1994), so even if the ground is not disturbed, earthworm cocoons inadvertently picked up and transported by equipment during winter could pose a threat to *B. mormo* populations. Once a site has been invaded, worms on their own can spread 5–10 m per year, meaning that it would take approximately 7–15 years to overcome the 250′ buffer (Hale, 2008). However, with continuous facilitated spread and introduction, for example along an ATV trail or road, this can occur much more rapidly (Hale et al., 2009). Thus restricting motor vehicle and equipment use, practicing stringent equipment cleaning within and around sites harboring habitat not yet significantly impacted by worms, and educating foresters and other users of forest roads regarding earthworm damage and spread may help prevent further destruction to northern hardwood forests (Hale et al., 2009).

In addition to addressing spread of worms through management, we recommend identifying and protecting sites of high quality habitat for *B. mormo*. First, an inventory of high quality mesic northern hardwood and *B. mormo* sites should be conducted to identify areas in early IERAT stages. Ideally, this information would be collected each time a northern hardwood stand is surveyed, which would allow decoupling the effects of worm damage and management, and detecting potential interactions between those factors. Based on that information, we recommend designating and protecting areas that are the appropriate native plant community (in Minnesota primarily MHn35 and MHn47, Northern Mesic Hardwood Forest and Northern Rich Mesic Hardwood Forest; J. Almendinger, personal communication; Casson et al., 2001; MN DNR, 2003) and have an IERAT stage of 2 or less as “critical habitat” for *B. mormo*. Such sites could be buffered by ecological unit rather than distance to help prevent earthworm spread. For example, management could be limited to stands buffered from *B. mormo* sites or critical *B. mormo* habitat by other plant communities that may be less prone to supporting large worm populations, including fire-dependent sites with mor humus and dry, sandy soil that is relatively poor in organic matter, or acidic wetlands (Curry, 1998; Edwards and Bohlen, 1996; Parkinson and McLean, 1998). Protecting suitable habitat, even if not yet occupied, may be extremely important to *B. mormo*’s persistence on the landscape in the future, because animals are considered the primary dispersal agent for spores (Wagner and Wagner, 1993). If deposited on suitable habitat, even a single *B. mormo* spore has the potential to germinate and grow into a reproducing plant, since gametophytes produced from individual spores produce both sperm and eggs, and this species reproduces almost exclusively via self-fertilization in this manner (Farrar and Johnson-Groh, 1990).

4.4. Conclusions

Our results showing that goblin fern is extirpated from over 50% of historic sites, and in peril at another 40% of sites, underscores the importance and urgency of implementing conservation measures for this species. Reduction in probability of occupancy is best described by earthworm damage to sites and we show that IERAT can be used as a simple and fast method of assessing habitat quality for this species. Human mediated transport of earthworms, especially at boat landings and along roads and trails, is the primary means of their dispersal and spread across the landscape, rather than natural expansion of worm fronts (Hale, 2008). Because natural earthworm spread is slow and some areas are not yet impacted by earthworms, or at least the most destructive species, policies, regulations, and education aimed at restricting movement of earthworms and their cocoons are the most likely ways in which we can protect sites from infestation for a very long time. Such policies are especially important to implement now, to prevent infestation of additional, and more destructive species of Asian worms that are beginning to invade hardwood forests in the Great Lakes (Qiu and Turner, 2017). These efforts will help protect the future integrity of the northern hardwood ecosystem as a whole, which supports many ecologically and culturally important species, including sugar maple and wildlife species, that are also negatively impacted by earthworm invasion (Hale 2004; Loss and Blair, 2011; Loss et al. 2012). Finally, our study underscores the importance of appropriate monitoring and analysis techniques to assess occupancy, even for sessile species, that take life history into account.

Declarations of interest

None.

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