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Assessing the suitability of habitat for the Endangered pygmy bluetongue lizard

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ABSTRACT: Species that have undergone range declines due to human activities present an unstable species-environment relationship and require integrative approaches for their conservation. The pygmy bluetongue lizard Tiliqua adelaidensis occupies vacant spider burrows and is found on private grazing properties. Without active conservation management, the species faces climate-induced extinction. Translocation to more southerly environments is considered a mitigation strategy. We used predictive distribution modelling (MaxEnt) combined with field surveys to identify potential translocation sites. Two species were potentially reliable indicators of suitable habitat: brush wire grass Aristida behriana and trapdoor spider Blakistonia aurea. Habitat suitability maps produced using these species found that, despite the importance of *B. aurea* burrows for lizard occupancy, A. behriana was a more suitable indicator species. Our field surveys compared habitat quality at lizard-occupied sites to potential translocation sites but did not reveal factors associated with higher lizard occupancy at current sites. Spider burrow availability was lower at potential translocation sites but a higher proportion were high-quality trapdoor spider burrows. Only one potential translocation site was found to be suitable; attributed to having trapdoor spider burrows, grasshoppers, and sheep grazing management. This site was a private property listed for sale during the surveys. Given that translocations of pygmy bluetongues are inevitable and this is the first suitable translocation site identified, we highlight the necessity of long-term measures to safeguard potential translocation sites on private property before any planned translocation occurs. Integrating conservation strategies with agricultural practices offers a chance to safeguard private properties so wildlife and agriculture may co-exist.

KEY WORDS: Habitat suitability \cdot Translocation \cdot MaxEnt \cdot Species distribution model \cdot Indicator species \cdot Private land conservation \cdot Grassland \cdot Tiliquini

1. INTRODUCTION

In modern times, the global loss of biodiversity has accelerated substantially and has been labelled the sixth mass extinction (Ceballos et al. 2015, Shivanna 2020). Human actions have caused irreversible damage to biodiversity globally and continue to drive this trend through habitat transformation (i.e. conversion to agriculture), overexploitation of natural resources, introduction of invasive species, and climate change (Banks-Leite et al. 2020). The multi-faceted problem of climate change alongside large-scale anthropogenic degradation and fragmentation of habitat poses some of the greatest threats to natural systems (Naeem et al. 2019, Howard et al. 2020, Sage 2020, Baral et al. 2023).

Agricultural land use has expanded globally, with more than one-third of the world's land surface occupied by some form of livestock (Denmead et al. 2015). Intensification and expansion of agriculture in addition to lost or altered historical disturbance regimes have inevitably contributed to the decline of grassland biodiversity (Bardgett et al. 2021, Nugent et al. 2023). Reptiles inhabiting natural grasslands or converted agroecosystems are inherently more vulner-

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able than more vagile taxa to anthropogenic disturbances and habitat loss (Mizsei et al. 2020). For shortrange endemic reptiles whose habitats are natural grasslands, translocation to a more favourable site may be the only solution to mitigate the combined threats of climate change and habitat degradation (Griffith et al. 1989, Buckley et al. 2012, Delean et al. 2013, Paaijmans et al. 2013, Mert & Kirac 2019). Translocation science has advanced considerably over the last few decades and is an important conservation tool (Bradley et al. 2023).

Species distribution modelling is increasingly being used as a conservation tool to assess the impacts of climate change and to identify potential translocation sites for vulnerable species (Wiens et al. 2009, Porfirio et al. 2014, Baral et al. 2023). Maximum entropy (Max-Ent) is a species distribution model that can be used to provide more accurate habitat suitability predictions where presence-only data exists for smaller data sets (Phillips et al. 2006, 2017, Mert & Kirac 2019), as is common for endangered and data-deficient species. Max Ent uses machine learning to build models consisting of entropy, the distribution most uniform throughout the study area, and constraints, the environmental variables across the study area (Phillips et al. 2004, Elith et al. 2011). Although MaxEnt does not directly estimate the probability of occurrence (Royle et al. 2012, Fitzpatrick et al. 2013) but rather the relative occurrence rate (Fithian & Hastie 2013, Renner & Warton 2013, Merow & Silander 2014), it produces a habitat suitability index (Phillips et al. 2006) that is valuable for identifying new areas for potential translocations.

Habitat suitability predictions may be improved through approaches that combine remote environmental data and targeted field observations (Hawlitschek et al. 2011, Mert & Kirac 2019). This is particularly true when investigating potential habitats where no established population of the species of interest is present, as field data may unveil habitat attributes that cannot be assessed remotely (Paraskevopoulou et al. 2022). For example, MaxEnt successfully predicted that 8 out of 11 potential new habitat sites were inhabited by an endangered viperid species in southeastern Europe (Mizsei et al. 2016). However, only field data, not remotely captured data, successfully differentiated the microhabitats that the vipers inhabited at each site in that study.

To adequately address the conservation management of short-range endemic species that are also habitat specialists, it is necessary to take an approach that combines predictive distribution models with targeted field investigations (Draper et al. 2019, Parker et al. 2023, Elliott et al. 2024). The Endangered pygmy bluetongue lizard *Tiliqua adelaidensis* (Fenner et al. 2018) is a short-range endemic and habitat specialist, occupying vacant wolf spider (Lycosidae spp.) and trapdoor spider *Blakistonia* spp. burrows (Milne et al. 2003). It is restricted to fragmented temperate grasslands used for livestock grazing in the Mid North region of South Australia (Hutchinson et al. 1994, Bull & Hutchinson 2018). Under current climate change trajectories, pygmy bluetongues are anticipated to undergo further range contraction, and habitat suitability is predicted to project south of their current known range (Delean et al. 2013). Modelled translocation scenarios to mitigate pygmy bluetongue extinction have been proposed (Fordham et al. 2012), but only one potential translocation site has been investigated so far. That site, Mokota Conservation Park, is within the current pygmy bluetongue range but does not have a population of the species and is likely unsuitable for the lizards due to a lack of deep spider burrows (Souter et al. 2007).

We aimed to identify potential pygmy bluetongue translocation sites using an integrative approach: (1) modelling future habitat suitability using the known plant indicator species Aristida behriana and testing the usefulness of the spider B. aurea as another indicator species and (2) assessing habitat suitability of potential translocation sites with field data. The model predictions were used to improve our ability to identify potential translocation sites that may be suitable in the long term for the lizards and that require minimum ongoing artificial supplementation. Additionally, the targeted field observations should allow us to assess habitat suitability within identified climatically suitable regions and improve our current understanding of pygmy bluetongue habitat associations within areas that are currently occupied by the lizards.

2. MATERIALS AND METHODS

2.1. MaxEnt analysis

We modelled future habitat suitability for pygmy bluetongues using MaxEnt to identify potential translocation sites appropriate for field assessments. We selected MaxEnt modelling as it is useful for presence-only occurrence data (Phillips et al. 2006), can incorporate categorical variables (Phillips et al. 2006), is recommended for use when data represents a random sample across the distribution of the species (Merow & Silander 2014), and because we were most interested in producing a habitat suitability index. We fit a direct approach MaxEnt model using *Tiliqua* adelaidensis occurrence data and key environmental variables. We also fit an indicator species model by first fitting models on each of the indicator species (Aristida behriana, Blakistonia aurea) and subsequently using the model outputs to fit a model with T. adelaidensis occurrence data. A. behriana is a known indicator species, but it may not account for important biological processes such as the obligate dependence pygmy bluetongues have with burrowing spider species (Delean et al. 2013). Given the reliance of pygmy bluetongues on B. aurea burrows, it was previously suggested as a proxy for the pygmy bluetongue but was not used due to sparse distribution data (Delean et al. 2013). We chose to test *B. aurea* as an indicator species by including additional records from the literature and personal observations from the field.

A total of 25 environmental variables were used as predictor variables. Nineteen bioclimatic variables were downloaded from the WorldClim CMIP6 data set modelled for 2081-2100 (representative concentration pathway 4.5). This concentration pathway represents an intermediate scenario in which emissions are predicted to peak in 2040 with an average temperature increase of 1.4°C (Zuza et al. 2021). Six landscape variables were also included because integrating landscape structure with bioclimatic variables has the potential to improve predictions for reptiles (Mulhall et al. 2022). Occurrence data and environmental data sources are described in Text S1 in Supplement 1 at www.int-res.com/articles/suppl/n055p315 supp1. pdf. All variables were resampled at 0.5 min spatial resolution (~1 km²). We used ArcGIS Pro v.3.0 and R v.4.3.1 (R Core Team 2023) to process the environmental variables and MaxEnt v.3.4.4 for maximum entropy modelling. To reduce the effect of multicollinearity among bioclimatic variables, we used a Pearson correlation coefficient to identify variables with >0.80 correlation (Do et al. 2022). We then excluded correlated variables that had the lower jackknife score from a preliminary MaxEnt model that included all 25 environmental variables. This process was repeated for the 3 species of interest; the remaining variables used for model testing for each species can be found in Table S1 in Supplement 1. For the indicator species model, we used the resulting output for A. behriana and B. aurea (and no other environmental variables) as an input for the pygmy bluetongue because MaxEnt only allows for one species to be modelled at a time.

We used 75% of the presence data as training data and the remaining 25% as test data along with crossvalidation replication, and otherwise used the default settings that provide robust results for presence-only data sets (Phillips & Dudik 2008, Smith et al. 2012). The area under the curve (AUC) of the receiver operator curve was used to evaluate model performance, as it provides a single measurement of model performance independent of other thresholds (Phillips et al. 2006). When using presence-only data, the AUC cannot achieve a maximum of 1; therefore, each species modelled held an independent maximum AUC value. An AUC value of 0.5 correlates to random prediction (Phillips et al. 2006). Previous studies have used a value greater than 0.7 (Mert & Kirac 2019) or 0.75 (Smith et al. 2012) to indicate that the model is an appropriate explanatory model and accurately predicts species distribution. Therefore, we assigned an AUC value of 0.75 or greater as indicating an acceptable model.

2.2. Field surveys

For our field assessments, we chose 4 sites within the Adelaide region that scored highly in the indicator species model and compared these to 4 sites with current pygmy bluetongue populations (Fig. 1). We assessed 2 public grassland reserves (Hallett Headland and Dry Creek), 1 airport (Parafield Airport), and 1 private property as potential translocation sites. However, preliminary field assessments revealed that one of the public grassland reserves, Hallett Headland, would not be a suitable candidate for translocation due to a lack of spider burrows, and it was excluded from further surveys. We chose 4 pygmy bluetongue populations to also be assessed at 1 southern (Kulpara), 2 central (Burra and Jamestown), and 1 northern property (Peterborough). All pygmy bluetonque sites surveyed occurred on properties used for sheep grazing. Further descriptions of each site can be found in Table 1.

Habitat assessments were conducted in April 2022 (Hallett Headland, Burra, Kulpara) and March– April 2023 (Dry Creek, Parafield Airport, private property, Kulpara, Jamestown, Peterborough). We used a minimum of 2 and a maximum of 6 plots $(30 \times 30 \text{ m})$ at all sites except Dry Creek, where only 1 plot was used due to size constraints. Plots were situated in areas of confirmed pygmy bluetongue occupancy or areas where spider burrows were found at potential translocation sites. Plots were spaced a minimum of 100 m apart. Our field surveys investigated spider burrow availability, invertebrates as a prey resource, and soil structure at all sites; we



private property

also compared vegetation structure at 2 of the pygmy bluetongue sites. Our primary criterion for evaluating the suitability of a potential translocation site was the availability of spider burrows, which are essential for the pygmy bluetongues' survival. We used the results of the remaining surveys to compare the potential translocation sites to current pygmy bluetongue sites, combined with an assessment of the general site location and current management to assess the suitability of a site for potential translocation (see Table S2 in Supplement 1).

2.3. Spider burrows

We investigated suitable spider burrow availability at all sites except Jamestown. Jamestown was excluded due to a dense vegetation cover making it difficult to ascertain if all spider burrows had been found. Within all plots outlined above, spider burrows were found by walking along 1 m wide transects north-south and east-west until all areas within the plots were examined in each of the directions and all burrows were marked. Spider burrow surveys tended

Site	Description	Approximate size of survey area (ha)	Management
Hallett Headland	Public conservation reserve. Grassland interspersed with walking tracks and rocks along the coastline	17.5	Slashing
Dry Creek	Site where lizards were last observed in the 1950s. Grassland in an industrial area with walking tracks, connected to wetland, ar surrounded by infrastructure such as a highway	0.5 nd	Unknown
Parafield Airport	Saltbush grassland outside the landing zone. Some restoration efforts	92	Slashing
Private property	Agricultural grassland. Approximately half of the property is us for viticulture and the remaining is used for sheep grazing	sed 267	Sheep grazing
Kulpara	Bottom portion of property used for cropping. High vegetation cover in pygmy bluetongue area	40	Sheep grazing
Burra	'Tiliqua' property owned by the Nature Foundation. Moderate vegetation cover	85	Sheep grazing
Jamestown	Bottom portion of property used for cropping. High vegetation cover in pygmy bluetongue area	175	Sheep grazing
Peterborough	Entire property used for sheep grazing. High bare ground and rock cover	350	Sheep grazing

Table 1. Site descriptions

to take place in the early morning or late evening hours to maximise cloud cover, which facilitated observers finding burrows. All burrows that were large enough to fit the fibre optiscope (minimum 6 mm in diameter) were measured for their entrance width, total depth, and faunal occupancy. A suitable burrow for lizard occupancy was determined to be a minimum of 6 mm wide, at least 150 mm deep, with no fauna occupying the burrow. We used 150 mm as the minimum depth because an adult lizard can retreat completely within this depth, despite pygmy bluetongues preferring deeper burrows (300 mm deep) (Milne & Bull 2000), as preferred burrow depths are not always available. We compared average burrow availability between potential translocation sites and current pyqmy bluetongue sites using a Student's *t*-test.

2.4. Invertebrates

We trialled 3 methods of capturing invertebrates (pitfall trap, sweep net, sticky traps) at 3 current sites (Jamestown, Kulpara, Peterborough) to compare with 3 potential translocation sites (Dry Creek, Parafield Airport, private property). We were unable to access Burra during the invertebrate survey period. Our aims were to determine which method was the most efficient at detecting grasshoppers, a key prey item of pygmy bluetongues (Nielsen & Bull 2020), and ascertain if the potential translocation sites had suitable prey availability. Each invertebrate capture trial took place within an 8 h diurnal period per site during March-April 2023. Invertebrate trials took place along 3 single-line 30 m transects at each site except for Dry Creek, at which only two 30 m transects could be obtained due to size constraints, with each transect located a minimum of 100 m apart. For pitfall trapping, we dug holes to fit a 45 mm wide trap to be level with the ground surface (55 mm deep, half-filled with 70% ethanol) every 6 m along the 30 m transect. At each of the pitfall traps, we also erected a marker to tie a double-sided yellow sticky trap $(101 \times 173 \text{ mm})$ above ground level (approximately 20 cm high) to prevent accidental captures of terrestrial vertebrates. Sweep netting was conducted 3 times during the day at each transect (i.e. morning, afternoon, and late afternoon) by walking the transect continuously for 2 min and sweeping backwards and forwards (mesh size: 0.9×0.3 mm, funnel tapered) at the mid-story level (halfway up grass tussocks). We identified captured invertebrates to order level except for Hymenoptera, which we divided into Formicidae (ants) and other hymenopterans (bees and wasps) (Souter et al. 2007). We used the many generalised linear model ('manyGLM') function in the 'mvabund' package (Wang et al. 2012) to identify particular species of interest and determine trap effect on invertebrate community assemblages. The manyGLM fits a model to each species, accounting for lower sampling effort at one site. Inference was carried out using 999 bootstrap sampling iterations. We conducted post hoc pairwise comparisons to identify invertebrate community assemblage differences between sites. We also used a Wilcoxon test to determine whether grasshopper abundances differed between pygmy bluetongue areas and potential translocation sites.

2.5. Soil

Following invertebrate capture trials and on the same day, separate soil samples were taken at ground surface level (0 cm), 10 cm and 20 cm deep at each sticky trap marker along each transect. Grain sizes of soil samples were determined by laser diffraction using a particle size analyser (Malvern Mastersizer 2000). Average values for grain size fractions from each site were entered into the GRADISTAT program (v.9.1) (Blott & Pye 2001) to obtain the percentage contribution of each sediment type and particle size $(D90-D10 \ \mu m)$. A subset $(n = 9 \ site^{-1})$ of these soil samples was dried in an oven at 65°C for a minimum of 4 h until a stable weight was reached and then incinerated at 450°C for 4 h. The percentage of organic matter within each of those soil samples was calculated as the loss of weight post incineration. We assessed differences in sediment grain sizes and organic matter between sites (fixed factor, 6 levels) and the 3 soil depths (fixed factor, 3 levels) using a permutational ANOVA (PERMANOVA) on untransformed data based on Euclidean distances. We also used Pearson correlation to examine if there was any correlation between soil organic matter and average spider burrow depth. At Jamestown, we used a previous survey from 2022 prior to vegetation overgrowth to determine average spider burrow depth.

2.6. Vegetation at pygmy bluetongue-occupied sites

Previous research has been unable to detect any plant community associations that indicate suitable habitat at current pygmy bluetongue sites (Souter et al. 2007). However, we recently found that lizards at lower-quality sites (i.e. Peterborough) exhibit distinct microhabitat associations (Michael et al. 2024). Therefore, we aimed to understand whether an association was present between vegetation structure and lizard density by assessing a higher- and lowerquality site. We chose Jamestown and Peterborough, as these sites are geographically close (~50 km) but dissimilar in vegetation structure visually. Jamestown is a high-quality habitat with a higher density of lizards (approx. 14 lizards ha⁻¹) and Peterborough is a lower-quality habitat and has a low to moderate density of lizards (approx. 8 lizards ha^{-1}). However, these estimates of lizards per hectare are likely an underestimate. We used 6 plots (30 × 30 m) on each property, and all plots were located within paddocks that were winter-grazed. Vegetation structure was assessed in April 2023.

We surveyed at an intermediate scale using 3 linepoint intercepts within the six 30×30 m plots on each property. Walking north, at every 3 m the observer recorded what was directly beneath the measuring tape (e.g. bare ground, vegetation, leaf litter, rock, moss, lichen). Along the middle transect, the basal inter-tussock space was measured from the edge of the plant base, where a plant must be rooted in the soil and a minimum of 30 mm wide or 30 mm high. For statistical analysis, we created an inter-tussock space complexity score (adapted from Brown et al. 2011 and Howland et al. 2016) as ln(variance in inter-tussock space × average inter-tussock space) and calculated the percentage of vegetation gaps within 3 categories (0-1, 2-30, and >31 cm). Grassland structural complexity was measured at the fine scale within one 1 m² quadrat randomly placed within each plot on both properties. We identified every plant species within the survey quadrat to the lowest taxonomic level practicable (usually to species) (Mid North Grasslands Working Group 2006), assigned an estimate cover to each species, and measured the height of each species. For statistical analyses, we grouped identified plant species into the functional groupings of ground covers (low-lying creeping plants; e.g. clovers Trifolium spp.), tussocks (native tussocks; e.g. A. behriana), and exotic plants (standalone exotics; e.g. thistle Silybum spp.). We used PERMANOVA+ for Primer (v.7.0.13) to analyse vegetation structural differences between the sites (fixed factor, 2 levels: Jamestown, Peterborough) using PERMANOVA and SIMPER analysis to identify the main contributing functional group to differences and used a canonical analysis of principal coordinates modelled approach to identify any vegetation structure associations with higher lizard densities.

3. RESULTS

3.1. Habitat suitability model of the indicator species brush wire grass and trapdoor spider

The training data for *Aristida behriana* obtained an AUC of 0.938 and the test data AUC value was 0.891. For *Blakistonia aurea*, the training data AUC was 0.994 and the test data AUC was 0.873. These AUC

values indicate that both models accurately predicted species distribution (i.e. >0.75). The variable with the highest gain when used in isolation for *A. behriana* was precipitation of the driest month, and the variable that decreased the gain the most when omitted from the analysis was mean clay percent at 30-60 cm depth. The variable with the highest gain when used in isolation for *B. aurea* was land use, and this variable also decreased the gain most when omitted from the analysis. Although *A. behriana* and *B. aurea*

occur throughout the study area, there were distinct differences in the habitat suitability maps of each species. Habitat suitability for *A. behriana* was predicted to occur throughout the entire 300 km study area (i.e. from the northernmost pygmy bluetongue region to the Adelaide region) (Fig. 2), with better-predicted conditions in the Adelaide region. *B. aurea* habitat suitability was predicted to occur clustered within a restricted area of the Adelaide region (Fig. 3).



Fig. 2. (a) Jackknife analysis of environmental variable importance for the *Aristida behriana* model. Green bars: model gain when each variable was used in isolation; red bar: total gain of the model using all variables. (b) Habitat suitability map of *A. behriana* where green indicated a more suitable habitat



Fig. 3. Same as Fig. 2, but for the Blakistonia aurea model



3.2. Habitat suitability model of the pygmy bluetongue

The direct approach pygmy bluetongue model did not incorporate any indicator species and only used the uncorrelated environmental variables. The training data had an AUC of 0.993 and the test data AUC



was 0.936. Land use was the variable with both the highest gain when used in isolation and that decreased the gain the most. This model showed a reduction in predicted habitat suitability area, concentrating in small areas within the pygmy bluetongue range and did not forecast the Adelaide region to be suitable (Fig. 4).

The pygmy bluetongue indicator species model used the previous A. behriana and B. aurea resulting 'ascii' files as the only environmental variables. The model had a training data AUC of 0.955 and test data AUC of 0.921. The variable with the highest gain when used in isolation and that decreased the gain most when omitted was A. behriana. This habitat suitability map followed a similar distribution as A. behriana, with predicted high suitability throughout the study region with best conditions south of the current pygmy bluetongue and into the Adelaide region (Fig. 5).

3.3. Spider burrows

Average availability of suitable spider burrows (i.e. unoccupied burrows that were a minimum of 6 mm wide and at least 150 mm deep) did not significantly differ between pygmy bluetongue sites and potential translocation sites (Student's *t*-test = -1.179, df = 4, p = 0.304). We found the greatest availability of spider burrows at pygmy bluetongue sites to be at Burra and at potential translocation sites to be at Parafield Airport (Fig. 6). Although there was greater burrow availability at Parafield Airport, burrows were of poor quality, as they were constructed in cracked soil and therefore did not remain intact upon inspection. In comparison, we found that the majority of spider burrows at both Dry Creek and the private property were high-quality trapdoor spider burrows with trapdoor spiders occupying them. We found the average number of total burrows within a plot to be greatest at Dry Creek and Burra, but Kulpara had the greatest average number of lizards found within a plot. Our main criterion for assessing the suitability of a potential translocation site was spider burrow abundance. Although spider burrow abundance was lower at the potential translocation sites than pygmy bluetongue sites, there was a higher proportion of high-quality trapdoor spider burrows at Dry Creek and the private property.

3.4. Invertebrates

Invertebrate community assemblages differed strongly between trap methods (dev. = 205.3, p = 0.001). Sweeping caught the greatest number of grasshoppers and the greatest diversity of invertebrates overall (see

180 9 pbts 4.5 pbts □ Available 160 ■ Unavailable Average total burrows 140 120 10 pbts 100 80 60 40

20

0

Dry Creek

Table S3 in Supplement 2 at www.int-res.com/articles/ suppl/n055p315 supp2.xlsx). Ants were most abundant within pitfall traps, flies and thrips were most abundant in sticky traps, and grasshoppers, spiders, and damsel bugs were most abundant within the sweep net samples. Post hoc pairwise comparisons showed that invertebrate community composition did not significantly differ between pygmy bluetongue areas and potential translocation areas ($F_1 = 1.49$, p = 0.24). We also found no strong evidence that grasshopper abundances differed between pyqmy bluetongue areas and potential translocation sites (Wilcoxon test, W = 30, p = 0.354).

3.5. Soil

All 6 sites were mainly composed of >70% sand (but varied between fine, medium, and coarse sand) among all 3 depths (Fig. 7) except at Dry Creek, where the 20 cm depth averaged 66.9% sand. However, we also found that Jamestown and Parafield Airport were not composed of a consistent sand particle size throughout the soil at 0, 10, and 20 cm depths (see Tables S4-S9 in Supplement 1). There was no significant correlation between average organic matter percentage and average spider burrow depth ($r_4 = 0.42$, p = 0.4).



Parafield Airport Fig. 6. Average total number of spider burrows found within a plot at each site. Available burrows were considered suitable for pygmy bluetongue occupancy (i.e. unoccupied burrows that were a minimum of 6 mm wide and at least 150 mm deep). Average number of pygmy bluetongues (pbts) found within a plot are indicated above Burra, Kulpara, and Peterborough

Butta

Pitvate property

Peterborough

Kullpara

Fig. 7. Soil texture triangle (classification according to the USDA classification system, based on relative fractions of clay, silt, and sand) where coloured dots represent soil texture from averaged 0, 10, and 20 cm depths

3.6. Vegetation at pygmy bluetongue-occupied sites

We found that only spear grasses *Austrostipa* spp. were common among all plots at Peterborough, whereas brush wire grass A. behriana, wild oat Avena barbata, hop clover Trifolium campestre, and narrowleaved clover T. angustifolium were all common among plots at Jamestown. The SIMPER analysis clearly separated vegetation structure at the 2 sites. The greatest contributors to vegetation structure at Peterborough were bare ground and leaf litter cover. Species richness, vegetation cover, and exotic species height were the greatest contributors to vegetation structure at Jamestown. Furthermore, the canonical analysis of principal coordinates analysis did not reveal any significant association between pygmy bluetongue presence and specific vegetation structure at either site (trace statistics $tr(Q_m'HQ_m)$: 0.201, p = 0.561 with 999 permutations).

4. DISCUSSION

Animal translocations are being increasingly used as a conservation measure to mitigate land modification and climate-induced habitat change (Griffith et al. 1989, Germano & Bishop 2009, Berger-Tal et al. 2020). Integrative approaches that combine field surveys with species distribution modelling to assess habitat suitability for a translocation should continually improve conservation outcomes (Mizsei et al. 2016, Draper et al. 2019, Mert & Kirac 2019). In this study, we tested the spider *Blakistonia aurea* as an indicator species of habitat suitability for the Endangered pygmy bluetongue against a known plant indicator species. B. aurea did not perform as well as the native grass Aristida behriana in the MaxEnt model. However, the presence of *B. aurea* burrows at potential translocation sites indicated higher habitat suitability at 2 sites: Dry Creek and the private property.

4.1. The use of indicator species in MaxEnt

To overcome the constraints of estimating habitat suitability for short-range endemic species, other species may be used as indicators of habitat suitability for species distribution modelling (Nally & Fleishman 2002, 2004), where the indicator species distribution reflects environmental conditions of conservation interest (Landres et al. 1988, Björklund et al. 2020). An indicator species approach can be particularly useful, since models that use a direct approach tend to not project beyond known areas of occupancy (Molloy et al. 2017). We found this to be the case for our direct approach model, as habitat suitability was restricted to the current range of the pygmy bluetongue. In comparison, our indicator species approach was able to project beyond the known range, and thus contribute more guidance to conservation planning.

We found that the best predictive habitat suitability map was generated by using both indicator species, although B. aurea contributed less towards the model. The limited contribution of *B. aurea* may be because these spiders occur in a greater geographic area than pygmy bluetongues (Delean et al. 2013); therefore, the model did not associate spiders with lizard occurrence, despite the known importance of spider burrows of this species for the lizards (Bull & Hutchinson 2018). Including multiple species of trapdoor spiders as an indicator for the pygmy bluetongue would likely change the predictive abilities of our model and would be warranted, as other species of trapdoor species have been found within population distributions of pygmy bluetongues (Clayton 2018). This inclusion would also boost occurrence records of indicator species available for modelling (Delean et al. 2013). In comparison, the native grasslands in which A. behriana occurs are much more limited in spatial area and have a greater association with pygmy bluetongue habitat. Our results were congruent with the habitat suitability map produced using A. behriana by Delean et al. (2013) and reinforce the use of A. behriana as a suitable indicator species of pygmy bluetongue habitat. Our results also highlight that incorporating landscape structure variables is important to promote more accurate predictive models, as landscape configuration drives the distribution of reptiles and access to resources (Mulhall et al. 2022). We acknowledge that the MaxEnt modelling used in this study produces a habitat suitability index and does not directly estimate probability of occurrence (Phillips et al. 2006, Bradley et al. 2012, Fitzpatrick et al. 2013). Thus, we interpret our models as indicators of future climatically suitable areas to guide the selection of potential translocation sites that should then be subjected to appropriate field assessments.

4.2. Field survey: spider burrows

On average, there were greater numbers of spider burrows found and burrows available for lizard occupancy at the currently occupied sites. However, the majority of these burrows were wolf spider burrows that are of lower quality for occupancy by the pygmy bluetongue. Given that pygmy bluetongues prefer trapdoor versus wolf spider burrows (Clayton 2018), the higher proportion of trapdoor spider burrows at 2 of the potential translocation sites (Dry Creek and the private property) indicates that both these sites are suitable. The higher proportion of trapdoor spider burrows in the Adelaide region also corroborates with our *B. aurea* indicator species model.

Our results indicate that a greater number of spider burrows within a plot area does not necessarily correlate to higher burrow availability, and it is more likely that the occupancy of a spider burrow by a pygmy bluetongue is a complex interaction between finding an available burrow and the quality of the burrow (Michael et al. 2024). Further, pygmy bluetongues will accept artificial burrows constructed of a hollowed wooden dowel hammered vertically into the ground (Souter et al. 2004). It is likely artificial burrows will be used in the initial translocation of lizards, and more artificial burrows can be installed to supplement burrow availability if required. However, artificial burrow installation and ongoing maintenance will be at a cost; therefore, the rationale of spider translocations should be considered. Ultimately, a population of burrowing spiders will be necessary at any potential translocation site.

Spider burrows are a keystone resource for the pygmy bluetongue (Milne & Bull 2000), and our aim was to identify a potential translocation site that would require as minimal intervention as possible. Compared with current pygmy bluetongue areas, none of the potential translocation sites had similar or higher abundances of naturally occurring spider burrows. We recommend further research be conducted on burrowing spiders and their potential to be translocated as well. This research would also likely benefit spiders, as a dispersal-limited group (Mason et al. 2013). Despite their ecological importance, spiders are underrepresented in conservation programs but are likely to be essential for the future of vertebrates like the pygmy bluetongue (Milano et al. 2021).

4.3. Field survey: invertebrates

Invertebrate community composition remained similar among sites, which indicated that the potential translocation sites all have suitable prey availability. Grasshopper abundance did not differ between pygmy bluetongue areas and potential translocation sites with the exception of Dry Creek, where no grasshoppers were caught. Local abundance of grasshoppers may vary due to various factors, including vegetation availability, seasonality, and weather events (Walls 1983, Jonas & Joern 2007). These factors, coupled with a short survey duration, may have resulted in the lack of detection of grasshoppers in our study. Given that pygmy bluetongues are opportunistic ambush predators and their diet changes seasonally (Fenner et al. 2007), low abundance or lack of grasshoppers does not necessarily exclude Dry Creek as a potential translocation site. Pygmy bluetongues consume a greater proportion of grasshoppers early in their activity season (i.e. October-December) (Nielsen & Bull 2020), whereas our surveys took place later in the season (March-April). The manyGLM analysis revealed that sweep nets captured the greatest species diversity and grasshopper abundance. We suggest sweep net surveys should be conducted earlier in the activity season at Dry Creek and other sites to better reflect grasshopper availability during peak grasshopper consumption.

There was variability in the type of invertebrates caught by each trap method. Ants were most abundant in pitfall traps and, given that we did not attempt to negate the 'digging in' effect (i.e. temporary increase in catches due to disturbance when placing traps in the ground) (Jiménez-Carmona et al. 2019), the high abundance of ants caught is not surprising. In contrast, yellow sticky traps are designed to attract flying invertebrates, predominantly dipterans, and other target species like grasshoppers are possibly less well represented, as they may only accidentally encounter the sticky trap. Sweeping allows the observer to move throughout the landscape, and the sweeping motion will stir up invertebrates (Doxon et al. 2011) such as grasshoppers that can then be caught within the net. We recommend that future invertebrate surveys employ sweep net surveys because this method caught the greatest number of grasshoppers. Sweeping may also better reflect invertebrate abundance and diversity within the landscape (Bakker et al. 2022).

4.4. Field survey: soil analysis

We investigated whether soil composition at a site may be an indicator of habitat suitability for burrowing spiders, and therefore pygmy bluetongues. Soil composition did not significantly differ among the sites, and sand contributed the most to the soil composition at all sites. Spider species display behavioural plasticity and can construct different burrows in response to substrate characteristics (De Simone et al. 2019). For example, other Australasian trapdoor spiders have been found to burrow in both clay and sandy soil (Mason et al. 2013, Smith et al. 2021). Burrowing spiders may be able to inhabit a variety of soil types due to the relatively stable moist microhabitat within their constructed burrow (Mason et al. 2013, Smith et al. 2021). Furthermore, burrow construction in sandier soils is a lower energetic cost to a spider, which may result in deeper burrows (Suter et al. 2011). However, burrow resilience to livestock trampling is greater in vegetationrich soils (Clayton & Bull 2015). Therefore, we also investigated whether organic matter contributed to greater burrow integrity. Although there was no positive correlation of higher organic matter contributing to deeper spider burrows, Peterborough had the shallowest spider burrows and lowest organic matter. Further investigation is warranted to understand if there is a link between burrow depth and the percent of organic matter. A very low percentage (<5%) of organic matter in the soil profile may impede deeper burrow construction (Řezáč et al. 2018). Burrows that are occupied by fauna, including pygmy bluetongues and spiders, have been found to be more resistant to weather-related destruction than unoccupied burrows (Ebrahimi et al. 2012, Nielsen & Bull 2017); thus, fauna occupancy may contribute to burrow integrity more than soil composition. Soil particle size may not be an accurate indicator of suitable habitat for burrowing spiders. Therefore, we recommend field surveys to ensure that a population of burrowing spiders occurs at a potential translocation site as opposed to only examining soil characteristics. Overall, understanding what areas may be suitable for these spiders based on soil characteristics is still an open question.

4.5. Field survey: vegetation structure at pygmy bluetongue-occupied sites

We investigated whether an association between vegetation structure and lizard density occurred, as previous research has been unable to identify any associations (Souter et al. 2007). Our canonical analysis of principal coordinates found that pygmy bluetongues will inhabit sites of high bare ground (Peterborough) as well as sites of dense vegetation cover with high exotic plant height (Jamestown). However, we did not identify any associations indicating higher suitability for lizards in any specific vegetation structure. Pygmy bluetongues exhibit microhabitat associations, specifically occupying burrows closer to vegetation and with greater vegetation cover at sites of high bare ground like Peterborough (Michael et al. 2024). Livestock alter vegetation structure and the associated microhabitat (Kay et al. 2017), and pygmy bluetongues have shown both negative and positive responses to livestock (Pettigrew & Bull 2012, Nielsen & Bull 2020). The maintenance of native grasslands in the pygmy bluetongue range is undertaken through livestock grazing, and an appropriate land management strategy will be required at any potential translocation site. Therefore, further research is required to identify vegetation structural factors associated with high pygmy bluetongue habitat quality to inform land management decisions at potential translocation sites (Clayton & Bull 2016, Gardner 2024). Furthermore, we suggest assessing microhabitat availability surrounding natural spider burrows at potential translocation sites. Manipulating the microhabitat surrounding naturally occurring and installed artificial burrows may be sufficient to prevent lizard dispersal during the initial translocation phase (Ebrahimi & Bull 2013, 2014).

4.6. Habitat suitability requirements for future translocations

Habitat quality at translocation sites is positively associated with the translocation success of animals (Griffith et al. 1989, Parker et al. 2023). Population models have indicated that translocations to more suitable climates will mitigate climate-induced extinction of the pygmy bluetongue (Fordham et al. 2012). We investigated habitat suitability at current lizard sites to gain a further understanding of factors influencing habitat guality and to compare those sites with potential translocation sites identified through MaxEnt modelling in their historical range of Adelaide. Our key findings are that (1) the native grass A. behriana versus the spider B. aurea (that provides high-quality burrows) is a more appropriate indicator species to produce habitat suitability maps for pygmy bluetongues; and (2) a comprehensive approach to field surveys, such as our investigations of spider burrows, invertebrates, soil, and vegetation structure, is required to assess the viability of potential translocation sites for pygmy bluetongues and potentially burrowing spiders. This is the first study to investigate potential translocation sites south of the current pyqmy bluetongue range. Overall, we found that 2 of the potential translocation sites, Dry Creek and the private property, had higher suitability for pygmy bluetongue translocation. This suitability was mainly attributed to the abundance of high-quality trapdoor spider burrows at both sites. However, when coupled with additional site information such as size and location within an urbanized capital city in addition to an

abundance of grasshoppers, only 1 site had practical potential to be used for future translocations. The private property is a large agricultural area where half of the property (approx. 267 ha) is used for sheep grazing and thus has the most similar land management regime as current pygmy bluetongue areas. In contrast, Dry Creek is a small (approx. 0.5 ha) grassland that is degraded, connected to a freshwater wetland, and surrounded by infrastructure. We therefore recommend that the private grazing property be considered for future pygmy bluetongue translocation research and highlight the necessity of integrating field surveys with species distribution models.

A longer-term strategy for the persistence of species such as the pygmy bluetongue is the purchase of suitable private properties and their perpetual conservation as reserves (Souter et al. 2007). This strategy also aligns with the IUCN (2013, p. 14) guidelines, which state that

'the release area and essential habitat for the translocated organisms should be secure from incompatible land use change before the conservation goal is reached, and, ideally, in perpetuity'.

The pyqmy bluetongue presents a complex conservation case where it is currently known to only occur on private properties, and we have only identified one suitable site for potential translocation that is also a private property. Access was granted to survey the private property in Adelaide by the landowner at the end of 2022 and the property was listed for sale in early 2023. Although the property was not sold during the time of our surveying, this highlights the need for longterm agreements in advance of any planned activities, as well as legal protections. Native and unploughed grasslands are a rarity (Gibson-Roy 2023), particularly in an urbanised capital city such as Adelaide. Thus, legal measures are crucial for preserving both current native inhabitants and potential translocated species like the pygmy bluetongue (Parker et al. 2023).

Ensuring habitat suitability at translocation sites underlies translocation success. The most common approach is to base the assessment of translocation site quality on the translocated species' biology (McCoy et al. 2014). However, like many endangered species, conservation of the pygmy bluetongue will benefit other species such as burrowing spiders. Here, we have provided a comprehensive framework to guide habitat suitability assessments for the pygmy bluetongue. For the pygmy bluetongue and burrowing spiders, integrating conservation strategies with agricultural practices and land management offers a chance for wildlife and agricultural use to co-exist (Velten et al. 2021, Hardie Hale et al. 2022, Gardner 2024). *Data availability.* Data is available upon reasonable request from the corresponding author.

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