Habitat preferences of the Critically Endangered greater Bermuda land snail *Poecilozonites bermudensis* in the wild

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Abstract The endemic, Critically Endangered greater Bermuda land snail *Poecilozonites bermudensis* is known from only two relict subpopulations. Little is known of its habitat preferences in the wild. Observations of released zoo-reared *P. bermudensis* suggested an affinity for limestone, which we investigated on Port's Island. Previous qualitative observations on Port's Island suggested an aversion to the litter of the invasive tree *Casuarina equisetifolia*, which we examined. Additionally, we hypothesized that snail abundance would increase with elevation, distance from the sea, and with increased plant species diversity. During 2 May–14 June 2018, we found 558 live *P. bermudensis* at 70 sites across Port's Island. We found no correlation between the number of live snails at a site and either the number of plant species, elevation or distance from the shoreline, but snails were significantly less abundant at sites dominated by *C. equisetifolia*. Significantly more snails were found around limestone features, indicating future reintroductions and searches for any undiscovered subpopulations should focus on limestone features where *C. equisetifolia* is absent.

Keywords Bermuda, habitat preference, land snail, *Poecilozonites bermudensis*, protected species recovery, reintroduction, terrestrial snail survey

Islands around the world support assemblages of endemic land snails (Chiba & Cowie, 2016), many of which have been driven to near extinction and rely on intensive management and captive breeding for their survival (Coote & Loève, 2003; Outerbridge et al., 2019). *Poecilozonites* is a genus of land snails endemic to Bermuda, with two extant species (Outerbridge & Sarkis, 2018). Both are categorized on the IUCN Red List as Critically Endangered (Ovaska & Outerbridge, 2019a,b). The greater Bermuda land snail *Poecilozonites bermudensis* (Plate 1) was believed extinct by the 1990s as a result of the introduction of invertebrate predators, primarily the rosy wolf snail *Euglandina rosea* and the flatworm *Bipalium vagum* (Ovaska & Outerbridge, 2019a). In 2014 a subpopulation was rediscovered in an urban alley and ex situ breeding was started (Outerbridge et al., 2019). A second subpopulation was found in 2017 on Port's Island, a 6.7 ha island unconnected to the rest of the archipelago (Fig. 1). An unmanaged, invasive-dominated, secondary growth forest covers 83% of Port's Island, and the remainder is a camping ground. The rediscovery of this wild subpopulation offered the opportunity to study the habitat preferences of the species, to aid identification of suitable release sites for captive-bred snails and to guide searches for additional wild subpopulations.

Observations of zoo-reared *P. bermudensis* released in 2016 indicated an affinity for limestone (Outerbridge et al., 2019). Chiba (2010) suggested that invasion by *Casuarina* species can reduce densities of native snails on islands under certain conditions. *Casuarina equisetifolia*, native to Southeast Asia, northern Australia and Pacific islands, is invasive on Bermuda's coasts, and previous anecdotal observations suggest *P. bermudensis* has an aversion to its leaf litter. In this study on Port's Island, we investigated whether *P. bermudensis* was more abundant around limestone features and less abundant where *C. equisetifolia* was present. Additionally, we expected *P. bermudensis* abundance to be higher at sites with greater plant species diversity, and for snail counts to increase with distance from the coast and altitude.

To test our hypotheses we used two approaches to identify sites: (1) random sites were chosen to assess differences in snail abundance and plant diversity at various altitudes and distances from the sea, and (2) targeted surveys to assess if the snails preferred limestone features and avoided *C. equisetifolia* trees.

The contours of Port's Island were used to stratify 30 random sites by randomly selecting locations along the contour lines using *ArcGIS 10.2.1* (Esri, Redlands, USA), ensuring sites were chosen from sea level to the peak of the island at 24 m altitude (Fig. 1). Targeted surveys at 10 *C. equisetifolia* trees and 10 limestone features were conducted opportunistically when we encountered appropriate features. Limestone features included natural outcrops, ledges, overhangs and rubble piles. An effort was made to select features from different parts of Port's Island (Fig. 1). A paired control site for each feature was selected by moving 3 m away from

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PLATE 1 Two adult-sized greater Bermuda land snails *Poecilozonites bermudensis* on Port's Island, Bermuda (Fig. 1).

the feature in a random direction. Control sites were a similar distance from the sea and within the same plant community, but without rocks, *C. equisetifolia* trees or large amounts of *C. equisetifolia* litter.

Sites were surveyed during 2 May–14 June 2018. Nested quadrat surveys were conducted at the 30 random sites, using a 0.5 × 0.5 m (0.25 m²) quadrat for snail counts within a 2 × 2 m (4 m²) quadrat for vegetation. In each 4 m² quadrat, plants were identified to species and counted, except for species of *Calophyllum*, which were only identified to genus. Each 0.25 m² quadrat was searched down to the soil surface, with leaves, rocks and twigs checked for snails. Live and dead *P. bermudensis* were counted and photographed. Since the invasive-dominated plant assemblage is relatively recent, and the differential preservation of shells in microhabitats is not understood, only live snails were used in data analysis. As it was the dry season, aestivating snails were only dislodged if obscuring others. An SM200 soil moisture sensor with HH2 moisture meter (Delta-T Devices, Cambridge, UK) was used at the centre of the 0.25 m² quadrat once it was cleared. The altitude and distance to the nearest coast were later determined with *ArcGIS*. Targeted surveys at *C. equisetifolia* trees, limestone features and paired control sites used the same sized quadrat, soil sensor and methods as for the random sites, but no vegetation survey was performed.

The live snail counts in the 30 random quadrats were analysed with a generalized linear model using the quasi Poisson error distribution, with plant species richness, distance to coast, altitude and soil moisture as predictor variables. Non-significant terms were dropped from the model using the backward elimination method, with probability values estimated from the type II Wald χ^2 test. The experimental paired design was used to analyse the response of the number of live snails to the presence of *C. equisetifolia*, limestone or neither habitat feature (control site). The non-parametric Mann–Whitney test, the paired t test or the paired non-parametric Wilcoxon signed-rank test were used depending on whether the data was normally distributed or not. All tests were conducted in *R 3.5.1* (R Core Team, 2018).

On Port's Island, 558 live and 1,179 dead *P. bermudensis* were found at 70 sites. Our linear model found no significant effect of plant diversity on the number of live snails ($\chi^2 = 0.51$, df = 1, P = 0.48), suggesting that plant diversity is not an important predictor of snail abundance on Port's Island. Similarly, we found no effect of soil moisture ($\chi^2 = 0.66$, df = 1, P = 0.42), elevation ($\chi^2 = 2.92$, df = 1, P = 0.09) or distance from the coast ($\chi^2 = 2.45$, df = 1, P = 0.12). *Poecilozonites bermudensis* were found in both coastal habitat and interior woodlands. The hypothesis

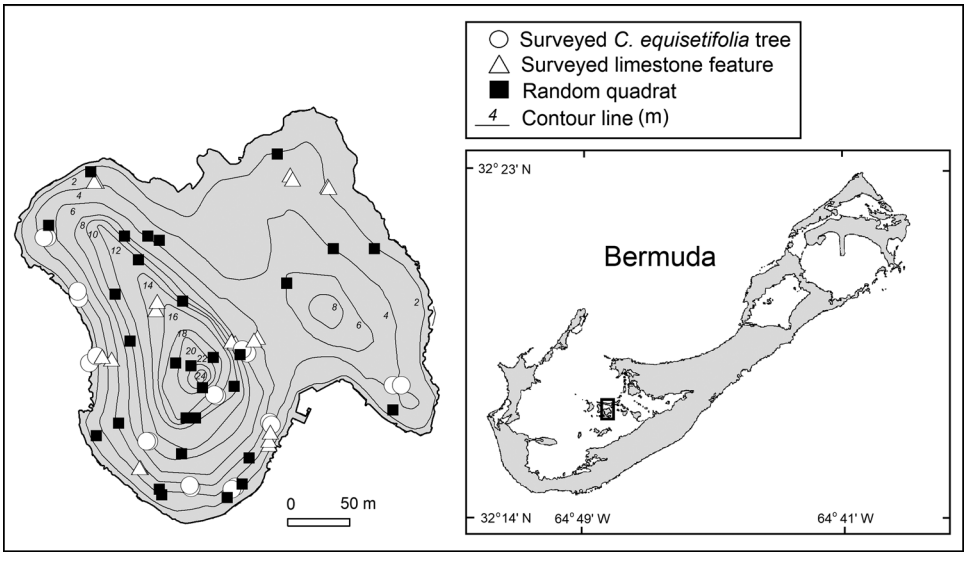

FIG. 1 Port's Island in the Bermuda archipelago, indicating locations surveyed for *Poecilozonites bermudensis*.

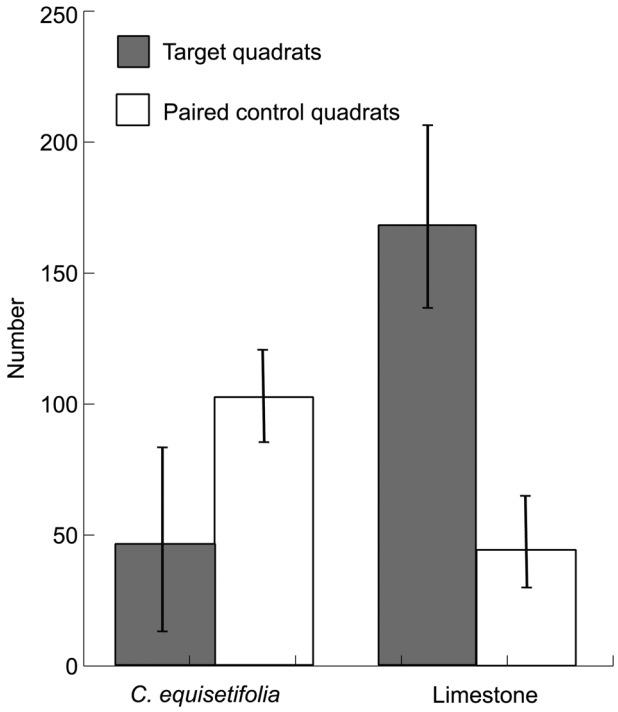

FIG. 2 Mean ± SE number of live *P. bermudensis* found in 10 targeted quadrats and 10 paired control quadrats in areas with *Casuarina equisetifolia* and with limestone.

that vegetation diversity contributes to higher abundance of snails inland (as fewer plant species tolerate the salt exposure at the coast) can be rejected. Furthermore, a Spearman's rank correlation of the number of plant species in the 30 quadrats against their distance from the coast showed no significant correlation ($r = 0.17$, $df = 30$, $P = 0.36$). As most of the island is covered by invasive plants, this is unsurprising. The number of individual plants in the 4 m^2 quadrats was 9–105 (mean 31), with a total of 27 species.

If *P. bermudensis* is differentially drawn to or repelled by limestone and *C. equisetifolia*, differences in live snail counts would be expected between the two sets of 10 quadrats; a Mann–Whitney test demonstrated a significant difference in medians between them ($U = 17.17$, $N = 20$, $P = 0.006$; Fig. 2). A Wilcoxon matched pairs test showed the number of live *P. bermudensis* found on limestone was significantly greater than at the control sites ($V = 2.10$, $N = 20$, $P = 0.025$), and the number was significantly less beneath *C. equisetifolia* trees than at the control sites ($t = -4.04$, $df = 9$, $P = 0.001$).

Our findings suggest that *P. bermudensis* is not more abundant in places with higher plant diversity and at sites away from the coast, but is more abundant on limestone and less abundant where the invasive tree *C. equisetifolia* is present. The dry season timing of this survey probably contributed to the strength of the association with limestone, as snails were using overhangs and damp crevices as refugia. Snails concentrated around limestone were possibly seeking calcium for their egg shells. In breeding tanks, large *P. bermudensis* have been observed eating limestone (R. Marirea, pers. comm., 2018). The breeding season of *P. bermudensis* in the wild has not been documented, but we found eggs on Port's Island on 4 May, shallowly buried in the soil.

Availability of suitable, protected habitat is critical to the success of snail reintroductions (Coote & Loève, 2003). Our finding that *P. bermudensis* is common at random sites throughout the invasive-dominated woodland of Port's Island is encouraging, given that all of Bermuda's protected areas are now dominated by invasive plant species. If *P. bermudensis* does not associate strongly with native plants and therefore requires a managed habitat; the number of potential reintroduction locations is greater. The use of specific indigenous and invasive plant species by *P. bermudensis* for food and shelter at Port's Island requires further study. Coastal sites above the storm surge zone can be considered as potential reintroduction sites if they are not dominated by *C. equisetifolia*. At least one shaded limestone feature should be present, preferably a stacked stone wall, rubble pile, or similar rough surfaced feature, to provide refuge in hot, dry weather.

Searches for other wild subpopulations of *P. bermudensis* are called for in the species recovery plan (Outerbridge & Sarkis, 2018). As *P. bermudensis* uses habitats close to the sea, low elevation islands and coastal sites should be included in future searches.

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Conflicts of interest None.

Ethical standards This protected species was handled with permission of the Bermuda Government, and no specimens were collected. The research otherwise abided by the *Oryx* guidelines on ethical standards.

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