**Methods**

*Study Area*

Guam (13.45° N, 144.78° E; 540 km2) is the largest and southernmost island of the Mariana Island Archipelago in the Western Pacific. Rota (14.15° N, 145.21° E; 85 km2) is located 76 km north of Guam and has less than 2% of the population of Guam. Both islands have large areas of native limestone forest with similar species compositions. These islands contain a variety of habitat types, but limestone forests were chosen as the focus of this study because they contain a larger variety of native and endemic tree species relative to other habitats, such as savannah or ravine forest (Fosberg 1960, Raulerson and Rinehart 1991).

*Effect of ungulates on seedling survival*

To assess ungulate effects on seedling mortality, we set up paired plots in limestone forest sites in northern Guam and in Rota. We selected eight sites in northern Guam and seven sites in Rota. At each site, we erected a 1.8-m tall chicken-wire fence around one plot, and left the adjacent plot unfenced, allowing ungulate access. The fenced and unfenced plots were placed in areas with similar in canopy cover, rockiness, and surrounding vegetation. Each seedling plot covered an area of about 3.5 m x 5.5 m (Figure 1).

We selected six species for this experiment, that encompassed a variety of common native and introduced trees found in limestone forests of the Mariana Islands: *Carica papaya*, *Morinda citrifolia*, *Neisosperma oppositifolia*, *Aglaia mariannensis*, *Premna obtusifolia*, and *Psychotria mariannensis*. For each species, we collected seeds from at least five trees and at least four different sites to minimize maternal effects and effects of local adaptation. The date of seed collection and subsequent outplanting was staggered by species, in part due to differences in fruiting phenology. After seeds were collected, fleshy fruit was removed, and they were planted in a mixture of 50% perlite and 50% peat moss, and fertilized using Osmocote®. All seeds were planted on the island from which they were collected. The seed trays were placed under XX% shadecloth at a nursery in Guam and at a nursery in Rota, and allowed to grow in these conditions until they had fully rooted and grown their first true leaves. At this point, the seedlings were transported to the exclosure sites for outplanting.

Seedlings of each species were brought to each site and out-planted in the control and treatment areas on the same day. Seedlings were placed at least 0.3 m apart from each other, and at least 0.5 m away from the fences in fenced treatments (Figure XX). On Guam, 14 seedlings of each species were planted in each treatment at each site, except for *N. oppositifolia*, which had only 7 seedlings planted per treatment. This gave a total of 1232 seedlings planted in Guam. In Rota, nine seedlings of *N. oppositifolia*, 11 seedlings of *C. papaya*, and 14 seedlings of the other four species were planted in each treatment at each site, a total of 1190 seedlings. We were not able to get 14 seedlings for every treatment at every site on each island due to seed availability in the wild, The seedlings planted during drier months (*C. papaya, M. citrifolia,* and *N. oppositifolia*) were watered regularly during the first few weeks after transplantation to ensure they successfully established. After transplanting, seedlings were monitored monthly for survival until \_\_ date. Any evidence of ungulate-caused damage or mortality was recorded. It was also noted whether seedlings appeared clipped, dug up, and whether mortality did not seem to be caused by ungulates.

Seedlings were placed at least 0.3 m apart from each other, and at least 0.5 m away from the fences in fenced treatments (Figure 1). Seven seedlings of *N. oppositifolia* and 14 seedlings of the other five species were planted in each treatment at each site, a total of 1232 seedlings in Guam. In Rota, nine seedlings of *N. oppositifolia*, 11 seedlings of *C. papaya*, and 14 seedlings of the other four species were planted in each treatment at each site, a total of 1190 seedlings. The seedlings planted during drier months (*C. papaya, M. citrifolia,* and *N. oppositifolia*) were watered regularly during the first few weeks after transplantation. After transplanting, seedlings were monitored monthly for mortality.

*Germination from scats*

We collected scats from *R. marianna* and *S. scrofa* from limestone forest sites to determine if either ungulate dispersed viable seeds via endozoochory. Location and date were recorded for each collection. We collected throughout the year, through both rainy and dry season. Collection was focused on four different sites in northern Guam where abundant scats from both deer and pigs provided high numbers for minimal sampling effort.

Scat samples were layered on top of a 50% perlite and 50% peat moss soil mix in germination trays. The trays were outdoors at a nursery, under shadecloth. *R. marianna* pellets were mixed in at the surface, and *S. scrofa* scats were broken up to mix at the surface. Trays were watered regularly before and after any seedlings emerged from scats. Seedlings were then identified and counted. Because the nursery was open air, species that were known to be wind-dispersed or that were common to all germination trays in the nursery (including from other experiments) were not counted.

*Vegetation transects and scat counts*

We conducted vegetation surveys to gather community composition data about limestone forest sites across Guam and Rota. We surveyed vegetation at each site using two 50-m by 1-m transects (see Figure 2), covering a total of 100 m2. At sites with exclosures, transects ran from opposite corners of the exclosure. At sites without exclosures, they were placed end to end. All plants within these belt transects were identified and recorded. Height was approximated for each adult plant, seedlings were identified and counted, epiphytes were identified on adult plants, and plants were categorized as vines, trees, ferns, or herbs.

In addition to vegetation surveys, scats from both *S. scrofa* and *R. marianna* were counted to estimate relative abundance for each across the sites. After surveys at the initial sites, seven additional sites in Guam were added to increase variation in ungulate abundance, as well as to cover more limestone forest area after gaining access to Andersen Air Force Base, which has extensive limestone forest habitat. One exclosure site, Racetrack Fragment, was excluded, as it did not provide enough forest area to run 100 m2 of transect line. A total of 14 sites were surveyed in Guam.

Because animal tracks are almost impossible to see in limestone forest terrain, and the detectability of other sign such as trails and grazing vary widely even in similar habitats, we determined scat to be the most reliable indicator of ungulateabundance (Schreiner 1997). Scats from *S. scrofa* and *R. marianna* were counted along the vegetation transects. A GPS device was used to walk roughly 400 meters in a square around the vegetation transect area (see Figure 2), covering an area of about 800 m2. Scats were identified to ungulate species and counted in a 2-meter belt.

*Statistical analyses*

Seedling survival was compared between fenced and unfenced plots, for each species planted. This was done using generalized linear models with mixed effects in R statistical software (R Development Core Team 2011). In these linear models, the fixed effects were fencing, species, and island, and site was a random effect. Factors were sequentially removed to determine their importance to giving the simplest fitted model. We determined that species had a strong effect on survival, and that it interacted with fencing to affect survival (Table 2). Therefore, we analyzed species separately with island and fencing treatment as fixed effects and site as a random effect. Factors were considered to have a significant effect on seedling survival if they improved AIC scores by more than -2 (Burnham and Anderson 2004) when included in a linear model.

The identity and abundance of all species that germinated from scats are presented in Table 4. Analysis focused on germination from pig scats, which had significantly more frequent germination when compared to deer scats. This was determined using a binomial test in R statistical software (R Core Development Team 2011). Selectivity was estimated using indices prescribed in Manly *et al.* (1993). This was done using the abundances of species that germinated from scat compared to their abundances in nature represented by data from vegetation surveys.

To determine if pigs exhibited selectivity for either native or exotic species that germinated from scat, we collected scat from two sites and compared them to vegetation surveys from those same sites. Although scat was collected from other sites, the majority of them were found from two sites, and the higher number of scats provided a more reliable basis for preference analysis. The two sites were “Ritidian Gate” on the National Wildlife Refuge, and “Anao North” in the Anao Conservation Area. We calculated the Manly Selectivity Index (Manly *et al.* 1993) for native and for exotic species at each site

*B = wi / ∑ wj*,

where *wi* is the selection probability determined by

*wi = ui / πi,*

where *u*i is the proportion of seedlings from scat of either native or exotic seedlings amongst all seedlings from scats from that site, and *πi* is the proportion of native or exotic adult plants at the site. Because nearly all ferns disperse their spores by wind (Tryon 1970), they were excluded from this analysis. Upper and lower confidence intervals for *wi* were calculated by using

*se(wi) =1.96 √{(1 - πi) / (utotπi)}.*

We used linear regression to correlate ungulate scat abundance to forest characteristics that we measured on vegetation transects. We could not combine pig and deer abundances because sign counts are not comparable due to potentially different and unknown rates of defecation and decomposition. Therefore, we used deer abundance and pig abundance as separate independent factors and forest characteristics as dependent factors. The forest characteristics that we investigated were total seedling abundance, native seedling abundance, exotic seedling abundance, vine abundance, and Shannon diversity (Shannon 1948). We tested for outliers using Bonferroni outlier tests. P-values for studentized residuals less than 0.05 would be considered outliers. The most extreme values all had Bonferroni P-values greater than 0.05, and so all data were included in analyses. R2 and P-values for each regression were determined using linear models in. To select the best-fit linear models, F-statistics and P-values were also calculated. All calculations and tests were performed using R statistical software (R Core Development Team 2011).

**Results**

*Do ungulates affect forest recruitment?*

Seedling survival in seedling plots varied depending on species, island where they were planted, and fencing treatment. Treatment effects, or exposure to ungulates by planting in unfenced plots, decreased seedling survival significantly in four out of the six species planted (Table 3.2, Figure 5). Survival was significantly lower in unfenced or ungulate-accessible plots for *C. papaya*, *M. citrifolia*, *P. obtusifolia*, and *P. mariannensis*. Treatment had no significant effect on *A. mariannensis* or *N. oppositifolia*. Treatment and island had a significant interaction for *A. mariannensis*, although there was no difference between treatments for this species. Treatment and island also had a significant interaction for *P. mariannensis*, which had lower survival in ungulate plots in Guam, but no difference between treatment plots in Rota.

*Do ungulates act as seed-dispersers?*

We collected a total of 20 deer scats (pellet groups) and 31 pig scats from a number of limestone forest sites. Because very few seedlings emerged from the deer scats, analysis was focused on pig scats, and germination from deer scats is thus only briefly described here. Only four of the 20 deer scats collected had seedlings that appeared to have germinated after endozoochory. One scat had eight *C. papaya* seedlings and one scat had one *Passiflora suberosa* seedling. The other species that germinated were and one *Vitex parviflora*, and one *Mikania micrantha*, whose seed may have blown into the seedling tray (ISSG 2012). Alternatively, the seed could have been ingested accidentally, as this species does not have an edible fruit.

A greater abundance and diversity of seedlings emerged from collected pig scats. To determine if germination was significantly different between pig and deer scats, a binomial test was run between the total number of seedlings (11) from the 20 deer scats and the total number of seedlings (802) from 20 randomly selected pig scats. P < 0.001 for k = 11 successes.

Of the 31 pig scats collected from four different sites, 25 scats had seedlings (80.6%), with a total of 1657 seedlings successfully germinating. The species that germinated from pig scats were the native trees *M. citrifolia*, and *Ficus prolixa,* the introduced trees *C. papaya* and *Leucaena leucocephala*,and the introduced vines *Passiflora* spp.*, Chromolaena odorata* and *Coccinia grandis* (Table 3.2). All of these except for *C. odorata* and *L. leucocephala* have edible, fleshy fruits.

In this analysis, the stark differences in selectivity *B* between native and exotic species for both sites (Figure 3.2), and high significance (Table 3.3) indicate strong selection for native species dispersal. This should not be interpreted as an actual dietary preference that the pigs themselves have, but the result of a combination of factors that include the pigs’ dietary preferences, the abundance of seeds per fruit and fruit per tree, and the likelihood of survival after being digested. Considering all of this, the *w* and *B* values strongly indicate selection for native species at both sites (Table 3.3). All have fleshy and abundant fruits on each plant, and both *M. citrifolia* and *C. papaya* have large numbers of seeds per fruit.

*How does ungulate abundance relate to forest community composition?*

We used scat counts for deer and pigs to estimate relative abundance amongst sites. Deer scat counts varied greatly between the fourteen sites surveyed in Guam, indicating a wide variation in deer abundance between the sites. Deer scat counts in Rota varied less amongst the seven sites surveyed, all of which had scat counts smaller than the maximum value in Guam (Figure 3.3).

Strong negative loglinear relationships were detected between the following forest characteristics and deer abundance: total seedling abundance (R2 = 0.710, P < 0.001), native seedling abundance (R2 = 0.648, P < 0.001), exotic seedling abundance (R2 = 0.770, P < 0.001), and vine abundance (R2 = 0.751, P <0.001). No associations were detected between these community characteristics and deer abundance in Rota, and no associations were detected between these characteristics and pig abundance. Shannon diversity *H’* had a significant quadratic relationship with deer abundance only in Guam (R2 = 0.516, P = 0.019) (Figure 3.7). There were no significant associations between pig abundance and plant diversity in either Guam or Rota.