**Introduction**

The extent of human influence is so pervasive that the earth today is comprised mostly of novel ecosystems (Seastedt et al. 2008). Novel ecosystems have species compositions and relative abundances that have not been previously observed, and usually are the result of anthropogenic changes (Hobbs et al. 2009). Species introductions are major drivers in the creation and maintenance of novel ecosystems both by adding new species and by removing native species (Seastedt et al. 2008). Novel ecosystems may be severely degraded and comprised of non-native species, but many still harbor species that serve important ecological functions, and many are good candidates for restoration. However, effective conservation in these systems requires better knowledge of the species that comprise them – both the remnant native and the introduced species – and their current ecological roles.

Most conservation goals focus on restoring native species to historical abundances, but restoring ecological functions could be more beneficial and feasible for degraded ecosystems (Hobbs et al. 2010). Conservation efforts that have focused on removing invasive species and reintroducing native species have yielded many positive results, however, removing invasive species could have negative consequences if these species play important ecological roles in the novel system (Savaleta et al. 2001). Likewise, reintroducing reduced or extirpated native species can prove unsuccessful or detrimental if persisting threats and current conditions are poorly understood (Hobbs et al. 2011, Godefried et al. 2011).

Although the negative impacts of introduced species are extensive, some introduced species have beneficial roles. Introduced species can be good candidates for restoring severely degraded habitats. For example, planting non-native trees in abandoned pastures in Puerto Rico facilitated the return of native plant communities where the native plants would not have originally colonized (Lugo 1997). Some introduced species may be providing desirable ecological functions such as seed dispersal or food sources for native species (Goodenough 2010). Introduced Japanese white-eyes (*Zosterops japonica*) in Hawaii are seed dispersers for native plants that previously relied on now extinct or rare native birds (Foster and Robinson 2007). Finally, invasive species can act to slow or reverse negative ecological effects from other anthropogenic impacts. For example, cascade effects from overfishing in Cape Cod salt marshes are being reversed by green crabs (Carcinas maenas), which are normally considered a harmful invasive (Bartness and Coverdale *in press*).

Feral ungulates are components of novel ecosystems around the world, and although their negative effects are well-documented (Nogueira-Filho et al 2009, Rooney & Waller 2003, Ickes et al. 2001), there are a few examples where they play beneficial roles (Desbeiz et al. 2011, O’Connor and Kelly 2012). Ungulate eradication is an important restoration tool, especially in island environments where ungulates are considered destructive invasive species (Kessler 2002, Spear and Chown 2009). However, invasive ungulates may also fill missing ecological roles in highly degraded island systems; this complexity makes management of ungulates challenging. Pigs have been documented as dispersers of native plant species on islands where they have been introduced (O’Connor and Kelly 2012) and other ungulates such as cattle and sheep effectively control exotic plant species in island habitats (Klinger et al. 1994). If ungulates have been part of a system for a long time, they may play considerable functional roles, such that their removal results in unintended consequences; these include the release of invasive plant species (Coomes et al. 2001, Cabin et al. 2000) or reduced seed-dispersal (citation- New Zealand paper?). Natural resource managers would be aided by discerning which non-native species serve important functions, and which species are solely destructive before implementing conservation actions in a novel ecosystem.

The islands of Guam and Rota in the Mariana Archipelago, Western Micronesia, as with many islands around the world, have had a long history of species introductions (Fritts and Rodda 1998). Perhaps the most famous invasive species is the brown treesnake (*Boiga irregularis*), which was unintentionally introduced to Guam and is responsible for the extinction of most of Guam’s native birds between 1945 and 1985 (Savidge 1987). The nearby island of Rota has retained more pristine examples of limestone forest habitats than Guam (Falanruw et al. 1989), however, both islands have experienced many species introductions, including ungulates, which have been established for centuries (Wiles et al 1999, Safford 1905, Conry 1989). Common wisdom and the small number of studies on deer and pigs in the Marianas have shown them to have negative effects on the forest. Philippine deer (*Rusa* *mariannae*) density in Guam has been correlated with reduced seedling recruitment in some species of native trees (Wheeler 1979, Schreiner 1997). Feral pigs (*Sus scrofa*) are thought to affect seedling recruitment through physical disturbance of the forest floor and through seed predation (Conry 1989, Ickes et al. 2001). However, these impacts are occurring within unique novel rather than pristine ecosystems, therefore a more thorough examination of the role of each species within the larger ecological context is needed to make appropriate management decisions.

We investigate the ecological role of invasive ungulates in a highly degraded novel ecosystem and in a less degraded system with similar biological history. Because Guam’s forests have effectively lost all ecological services provided by avifauna, we were able to discern the relative magnitude of impacts from introduced ungulates between Guam and Rota. In the Mariana Islands, birds are especially important for seed dispersal of a number of native forest species (Haldre? pers comm.) Here, we examine the impact of feral pigs and deer on seedling survival, seed dispersal, and overall plant community composition. We contrast their role in the bird-free forests of Guam to that in the nearby forests of Rota which still maintain avian populations.

**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.

**Discussion**

Deer and pigs can, but may not always, be important engineers of novel ecosystems. Seedling plot experiments on the islands of Guam and Rota revealed selective browsing on a sample of common forest species by deer. Deer abundance in native forest showed strong negative loglinear relationships with overall seedling abundance, including both native and exotic seedling abundance, and vine abundance. We also found that overall plant diversity is greatest at intermediate levels of deer abundance, consistent with the intermediate disturbance hypothesis (IDH) (Connell 1978). We did not detect these relationships with pig abundance, suggesting that deer may have a stronger role in determining species abundance and dispersal in these forests. However, pigs did appear to have a strong role in seed dispersal in Guam, selectively dispersing native seedlings.

Deer affect the community composition and size class in forest communities in Guam. The negative relationships we found between forest characteristics and deer abundance (Figure x) demonstrate that forests are heavily impacted, and about equally so, across all except extremely low deer abundances. This mirrors studies in other systems, such as in North America where population densities of native white-tailed deer (*Odocoileus viginianus*) only slightly higher than historic levels controlled forest regeneration of once common forest species (Alverson et al 1988). Therefore, only drastic reductions or elimination of deer are likely to lead to noticeable changes in forest communities.

Deer also had important effects on overall forest community diversity, with the greatest forest diversity at intermediate levels of deer abundance (Fig x). This pattern of plant diversity is consistent with the intermediate disturbance hypothesis (IDH) (Connell 1978). The IDH proposes that at low levels of disturbance, as is caused by ungulate herbivory, a few competitive dominants rule, while at high levels of disturbance, only disturbance-tolerant species survive. In contrast to our results, many studies about ungulate effects on biodiversity show a pattern of monotonic decline (Stockton *et al.* 2005, Spear and Chown 2009). In fact, a review by Milchunas *et al.* (1988) concluded that grazing as a disturbance often failed to support IDH because of concomitant changes in modes of competition among plant species with increasing disturbance. One of the few studies of herbivory that did support the IDH model involved deer, and was restricted to ground-cover in a temperate forest habitat. However, the pattern was only evident when other environmental factors were controlled (Suzuki *et al.* 2012). Our study, in contrast, showed an IDH pattern in forest community diversity as a response to deer herbivory, even with other environmental factors at play.

Just as deer in other systems selectively browse plant species, we found deer in Guam and Rota to be selective amongst tested species of seedlings (Figure x). With the high population densities of deer suspected in Guam (Knutson and Vogt 2002), this could have strong implications for species composition of forests with deer, especially combined with the loss of avian ecological functions in Guam. Very few studies have looked at species ingested by deer in the Marianas (Wheeler 1979), further stomach-content analyses could reveal which species are selectively browsed by deer and in what quantities. Stomach content analyses and long-term vegetation monitoring could shed light on whether ungulate-impacted forests with few to no birds dispersing seeds will begin shifting to a community composition favoring species that are resistant to deer herbivory and species not reliant on avian seed dispersal.

Feral pigs are notorious for having detrimental effects on plant communities in different ecosystems, so we were surprised that we could not detect relationships between forest characteristics and pig abundances. This may have to do with our indicator of abundance – scat counts may not be an accurate portrayal of relative pig abundances across sites (Andersen and Stone 1994). Pigs do, however, appear to play role in seed dispersal. Studies in Hawaii have implicated pigs not only of inhibiting forest regeneration (Katahira 1980, Nogueira-Filho et al 2009), but of indirectly affecting native plants by selectively dispersing invasive plants (Aplet et al 1991). In contrast, although some invasive species were found in scats collected in Guam, pigs selectively dispersed native species like *Morinda citrifolia* and *Ficus prolixa*. With the loss of avian and fruit bat seed dispersal in Guam due to the invasive brown treesnake and overhunting, dispersal by pigs may be the major remaining mode of dispersal for fruit trees in Guam.

Forests of the two adjacent islands had different responses to ungulate herbivory. Unlike in Guam, we did not observe any association between seedling abundance and deer abundance in Rota. The continued presence of avian seed dispersal in Rota may have obscured any associations between seedling abundance and deer abundance there. Other studies have observed complementary roles of herbivory and seed dispersal (Henry and Dubost 1999, Vellend et al. 2006), but focus on one species responsible for both herbivory and dispersal. We speculate that multiple species may be determining forest composition through a combination of herbivory and seed dispersal. Seedling mortality was higher in Rota for both fenced and unfenced treatments (Figure x), suggesting that an additional factor unimportant in Guam might be affecting seedling survival in Rota. The cause of this greater mortality is unclear, but we speculate that slug herbivory is responsible. We noticed a particularly high abundance of exotic slugs (Veronicellidae) at our sites in Rota. Slugs extensively damaged native plants in some areas of Hawaii (Joe and Daehler 2008).

Multiple ecological factors such as avian loss, invasive plants, and invertebrate herbivores make it difficult to predict the results of ungulate management, but this study provides a better understanding of their role and the likely impacts of various management options. Whether their roles are interpreted as positive or negative, ungulates are major parts of Guam and Rota’s ecosystems, and their removal or control is likely to have complicated results. Information from this study is important for formulating ecosystem management plans in the Marianas. For example, deer suppression would have to first reach a level low enough to elicit responses from plant communities, so low-level hunting pressure does not appear to be an effective tool for controlling deer herbivory. Enhanced suppression or eradication would be needed for recovery if deer control were deemed a priority for recovering native plant species. If deer suppression is achieved, invasive vines might become a problem when released from herbivory. Pig control or eradication could also have unwanted effects by disrupting what is likely the only vertebrate-facilitated seed dispersal in Guam. Ungulates and snakes might never be eradicated, but ecosystems can still be managed to maintain some native species and functions based on our developing knowledge of novel ecosystem interactions such as these.